Chromatography bioseparation technologies and in-silico modelings for continuous production of biotherapeutics

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ABSTRACT

The potential of continuous bioprocessing is hindered by the bottlenecks of chromatography processing, which continues to be executed in batch mode. Highlighting the critical drawbacks of batch chromatography, this review underscores the transition that the industry has made by implementing continuous upstream process without devising a working model for downstream chromatography operations. Even though multitude of process development initiatives have commenced, the review emphasizes the first principle models of chromatography on which these initiatives are built. Various models of continuous chromatography, which are essential, but not limited to multi-column systems, employed to congeal a unified process are reviewed. Advancements made by several mechanistic models and simulations to maximize productivity and performance are described, in an attempt to provide the integral tools. The modeling tools can be used for development of a strong model based control strategy and can be embedded into the continuous chromatography framework. The review addresses the limitations and challenges of the current modeling methods for development of robust mechanistic modeling and efficient unit operation platform in continuous chromatography.

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1. Genesis of continuous bioprocessing

For a few decades, bioprocessing research in both academia and industry has been investigating the utility and applications of continuous bioprocessing as a refined alternative to batch bioprocessing [1–3]. These investigations, which began as an attempt to adopt the continuous operations of automobile, food and pharmaceutical industries, have led to the conceptualization of simpler, faster and economical operability within bioprocesses [4–6]. While these concepts of continuous bioprocessing are better in principle, their transition to implementation has been historically protracted, due to multitude of challenges spanning across short-term flexibility to long-term operability. The earliest implementation of continuous bioprocessing was observed around 1980's when perfusion culture emerged as a viable alternative for production of several labile proteins largely due to the inadequacies of the fed batch model [7]. Since then, the evolutionary transition of utilization of continuous bioprocessing, towards intensified, miniaturized, maximum capacity processing batches, has been gradual [8,9].

However, the recent emphasis of the Food and Drug Administration (FDA) on incorporating continuous processing into biopharmaceutical manufacturing, highlighting the quality being intrinsically built into the product, has promoted the industry to move toward an empirical and preemptive approach of drug development and manufacturing [10,11]. The early initiatives of continuous bioprocessing have gained momentum driven by several key factors. To begin with, the continuous operations are more suitable for automation, Quality by Design (QbD) and Process Analytical Technology (PAT) [3,12], making the continuous processing approach appealing from a regulatory standpoint. The rise of untimely epidemics has further pushed the industry to invent effective solutions to provide quick measures in a timely manner. Increased regional demands of various bio therapeutics have called for localized manufacturing facilities. Flexible facilities have been advancing to serve local markets and concurrently abide by the standard regulatory compliance [13,14]. Further, the dawn of personalized medicine and variety of equally potent drugs have drastically reduced the output per drug, ultimately increasing the cost pressure on production [15,16]. A continuous platform in a closed environment that can be transported portably or built locally in the affected areas is the evident way to go, but has been a long eluding challenge for the scientific community on account of production efficiency, product safety, regulatory compliance and protection of intellectual property.

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1.1. Challenges in continuous chromatography process

The bioprocessing industry has witnessed a disorderly momentum in recent times with the introduction of a working model of continuous operation in upstream bioprocessing [17,18]. The transition from perfusion to easily scalable WAVE bioreactors or disposable technology has achieved sizeable protein titer in lower volumes of bioreactor [18,19]. Moreover, an unprecedented leap in cell biology generating stable and high performance cell lines, and growth media have enormously contributed to the protein titers [19–24]. The inherent limitations of chromatography batch operations as explained in the section below, increase the footprint of the company and call for huge investment of time and infrastructure, translating into long technology transfers and validation at multiple scales, rendering purification as a rate limiting step [25]. However, the recent developments have attempted to remove the bottleneck of traditional batch operations. There have been slow but successful working models in continuous chromatography operation [26–31], especially in purification through chromatography. Precisely, purification is progressing towards integrated bioprocessing, which consists of connected continuous unit operations with a smaller footprint in the upstream and downstream processes, for higher productivity, accelerated scale-up and enhanced flexibility.

1.2. Characteristics of batch chromatography

Though batch chromatography remains the industry standard for most chromatographic operations, the drawbacks of these colossal batch columns have compelled the industry to explore alternatives. With limited flexibility of continuous upstream operations, batch chromatography has been a bottleneck at the capture step.

- The batch chromatography process of the current industry operations do not allow complete utilization of the resin in the lower sections of the column. This **"resin under-utilization"** remains a critical issue, lowering the dynamic binding capacity of the column, as the loading stops at 0.9% breakthrough as shown in Fig. 1.
- **Column Size is** proportional to the mass of the protein to be purified and the dynamic binding capacity of the resin. The column size used during the process development stage is very small with a fixed binding capacity. However, for manufacturing operations, the columns need to be larger to process high process load as the dynamic binding capacity is constant. These limitations are further magnified due to the inherent mass transfer and diffusion effects, steadily reducing the column efficiency. With proportional increase in column width and multiple purification runs, the cost and time constraints involving batch operations also increases. Consider a column to be split in three parts as shown in Fig. 1. As loading step progresses, the top portion of the column is saturated first, followed by the middle section. The bottom portion is highly underloaded when breakthrough occurs.
- As the column size is dependent on the batch and the purification is essentially processed as batches, any **"batch variability"** poses additional challenges to the process. The variation in the quality attributes of the protein from batch to batch have historically scalded the column chromatography operations, by imparting inconsistency in purity and standardization of the process, hindering improvements.
- These inherent issues involving column capacity and batch variations, delay the **"processing of high titer load"**. The loading could occur intermittently during a batch process, with extended hold times, which have shown to undesirably af-
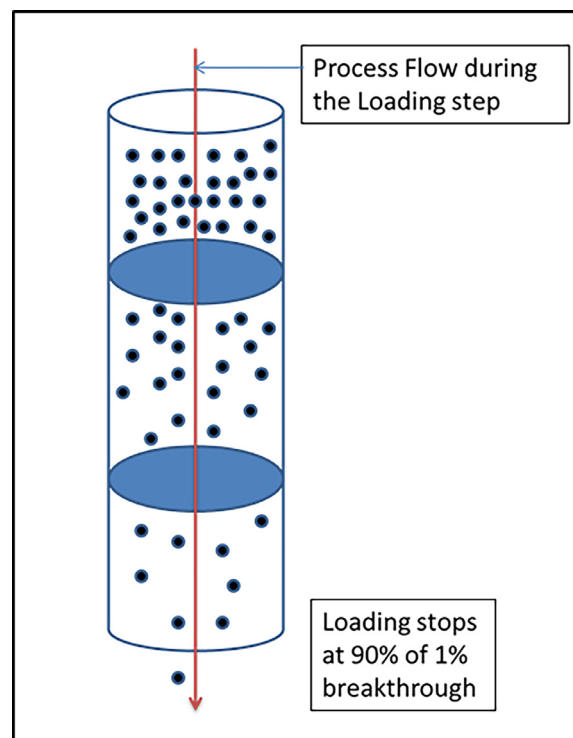

Fig. 1. A schematic representation of an underloading of column in batch chromatography process.

fect the protein stability. Further, due to such extended operations the need for large hold tanks and columns also increases, further increasing the infrastructure requirements and time constraints.

Continuous chromatography has shown to maximize resin capacity and minimize column size [32]. This review also assesses suitable mechanistic models and corresponding technologies available until date, which are required for seamless integration of purification stages. The impact of physical and biochemical interactions between the resins and proteins in the chromatographic processes, which is different from the conventional small molecule separation, is discussed in this review. For simplicity and immediate application of continuous technology in the capture step, the review will be limited to affinity chromatography.

2. First principle models for continuous chromatography

The foundation of all preparative chromatography principles, be it affinity, hydrophobic interaction chromatography (HIC) [2,33], ion exchange chromatography (IEC) [9,34–36], mixed mode chromatography, etc. relies on basic mathematical models. While there exist numerous theories or variations of non-linear, non-ideal, multicomponent liquid chromatography principles, all of these have historically been simulated, optimized, validated and utilized based on three fundamental categories of equilibrium models [37–41], plate models [42,43] and rate models [44,45] of capture and purification. These models explain the mass transfer kinetics, diffusion phenomena, dispersion effects and adsorption-desorption mechanics of protein purification with varying complexities of parameter estimation [46,47]. A brief description of these fundamental principles is provided in Fig. 2 and various modifications of the three fundamental models are listed in Table 1. The Van Deemter plot shown in Fig. 2, although widely used, is only applicable to linear, ideal peaks for a batch chromatography process and cannot be applied to the overloaded non-linear peaks as is the case for con-
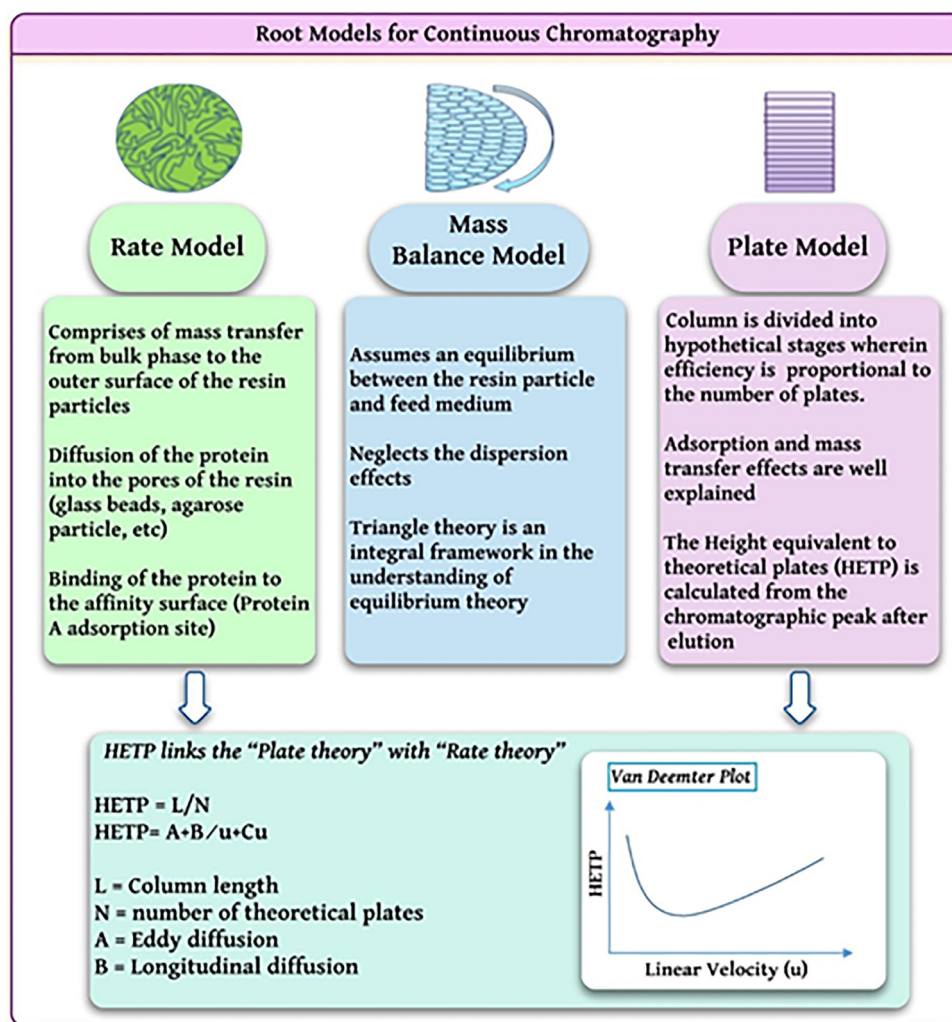
Fig. 2. Root models for continuous chromatography.

Table 1
Chromatography models and their characteristics.

Models available	Types of models	References	Characteristics
Plate model	Martin-Synge plate model	[47,48]	Continuous plate model
	Extended Martin-Synge model	[41,49,50]	Considers elution when column is overloaded and slow mass transfer kinetics
	Craig plate model	[51,52]	Iterative scheme based on distribution factors
Mass Balance model	Equilibrium model	[53,54]	Empirical model for fast mass transfer rate and assumes direct local equilibrium between mobile & stationary phase
	Equilibrium-Dispersive model	[47,55,56]	Relates apparent axial dispersion and column HETP
	Kinetic model	[56,57]	Explains the mass transfer kinetics
General rate model (GRM)	GRM for axial flow	[58–60]	Complex model which examines stationary phase surface phenomena, mobile phase and internal pore dynamics
	GRM with second order reaction kinetics	[56,61]	Considers reaction kinetics and size exclusion effects for affinity chromatography
	Power law model for elution	[43]	Explains asymmetric peak shapes
	Exponential model for elution	[62,63]	Provides relation to symmetric peak shapes

tinuous chromatography. However for an overloaded column, the shape of the elution peak in the Van Deemter plot is highly non-linear and requires complex modifications to the HETP calculations to explain the efficiency of the columns in continuous chromatography [48]. Such modifications are not feasible during manufacturing operations and hence newer modeling technologies are needed to determine the column integrity during continuous chromatography.

2.1. Significance of breakthrough curve

The mass transfer and binding kinetics together explain the protein adsorption/desorption phenomena. Protein adsorption on a resin is governed by binding conditions, protein size and biochemistry, resin bead characteristics (pore network, bead size and phase ratio) and the functional ligand properties (type and density) [49–51]. The shape and nature of BC are based on the Mass Transfer

(MT) kinetics of protein binding [52]. Adsorption isotherm answers the key question - how the protein molecules are distributed in the resin pores and mobile phase under equilibrium conditions. The adsorption isotherm also explains the equilibrium between the adsorbate in the solid phase and the liquid phase. Furthermore, the isotherms can generally be estimated by experimental approach. Different concentrations of adsorbate are contacted with a fixed volume of slurry until equilibrium is reached. The adsorbate concentration is then measured in the liquid phase to obtain the equilibrium isotherm curve. The type of adsorption isotherm at equilibrium determines the separation mechanism which aid in the optimization of the separation process. The binding which occurs at the surface active sites, is represented by the Langmuir model [53–55]. Other isotherm models include Freundlich isotherm, [56,57] Brunauer-Emmett-Teller (BET) isotherm, [58,59] Dubinin-Radushkevich (DR.) isotherm, [60,61] Radke-Prausnitz isotherm, [62,63] Redlich-Peterson isotherm [64,65] and Combination isotherm (Langmuir-Freundlich) isotherm [66,67]. Of these, Langmuir isotherm equations have been widely used to model the breakthrough curves (BCs) in chromatography [68–70]. The Langmuir isotherm is given by (1)–(5)

$$q = \frac{Q_{max} \ C}{K_d + C} \tag{1}$$

Here q is the antibody concentration in the solid phase i.e. within the pores while C is the protein concentration in the bulk phase. The maximum antibody uptake at specific conditions is given by Q_{max} and the equilibrium coefficient relating antibody concentration to the solid phase is given by K_d. Simultaneous load adsorption and desorption curves are used to evaluate the equilibrium conditions. Bi-Langmuir binding kinetics coupled with required transport phenomena is crucial for the entire breakthrough curve description until saturation. [68] Langmuir isotherm has been reported in literature to explain the Protein A resin performance over specific cycles for a few alkali resistant resins like Mabselect Sure and Mabselect Sure LX. [71] The resin performance over a number of cycles is imperative for the continuous loading setup. Offline qualification of column integrity measurements are less feasible if the column is overloaded. Other isotherm models have been reported in literature to explain the Protein A resin behavior [72]. The sigmoidal curve of BC is an indicator of the column capacity, linear flow rate, temperature and buffer concentration, which further explains the integrity of the stationary phase. [73,74] The fundamental parameters, namely breakthrough volume, equilibrium volume, retention volume and total volume characterize the curve [69,70,75–77]. Standard deviation method [78], direct method [79] and third derivative method [80] are available to evaluate the parameters. The standard deviation method provides a graphical approximation of BT volume, number of theoretical plates, equilibrium volume, capacity factor and recovery of the protein. The standard deviation and direct method have shown some inflexibility in parameter estimation as the latter requires the initial protein concentration and fails to consider the asymmetrical nature of BC. The third derivative method shows significant observations based on the troughs and crest in the third derivative graph using Savitsky-Golay smoothed data. [81,82]. Dynamic Binding Capacity (DBC) at a certain flow rate has been attempted to reach Static Binding Capacity (SBC) using multiple smaller columns. However, comparative analysis and performance of the smaller multiple columns with one traditional column necessitate further probing.

2.2. Characterization of elution profile

The batch elution profile is characterized with a Gaussian curve and dependence on Height Equivalent to Theoretical Plates (HETP) and plate number for the column integrity. Batch loading lies in the linear region of the Van Deemter curve which and hence allows for qualification of columns with HETP test. The HETP method of column qualification is based on the ideal peaks for an underloaded column during elution. In the case of continuous capture, the columns are overloaded and hence, the elution curves are nonlinear and have the form of a sharp leading and a broad tailing curve [83]. The traditional Gaussian curve determination is not applicable for the saturated columns. New methods of column qualification are thus required to determine the integrity of the column.

In addition, there is a need for understanding the resin performance to optimize the utilization in continuous chromatography. First principle models, which can focus on the column size determination, are described well in literature. The merits of these models rest in their utility to simulate the scale up process of purification, through computational modeling, before physically scaling up the process in a biopharmaceutical set up. The simulations are effective tools for saving time and resources in evaluating a large range of alternatives to arrive at the most lucrative options. High throughput experiments could then help to identify and validate the processes [47]. The roadmap for mechanistic modeling as an effective tool for scale-up is provided below. For example, the study on isotherm behavior affecting the thermodynamics of the chromatography column has greatly facilitated the scale-up in biopharmaceutical setup, increasing predictability in the column productivity [46]. However, the risk of failure and considerable cost concerns of developing and testing these models have placed these theoretical advancements in superior position over its actual execution. Nevertheless, several effective mechanistic models and their modifications are available to resolve the above described bottleneck issues around chromatography.

2.3. Scale-up considerations in MCC

Models and corresponding dimensionless numbers have been widely used in the scale-up of column design. Dimensionless numbers govern the scale-up and scale-down design considerations providing for coherent visualization as provided in Fig. 3. There are two methods to obtain dimensionless numbers – π theorem and use of partial differential equations. The π theorem identifies the important variables of the physical process; physical proper-
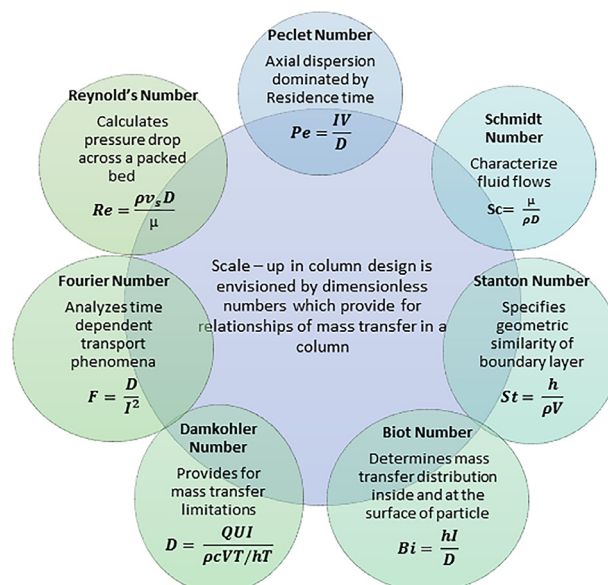

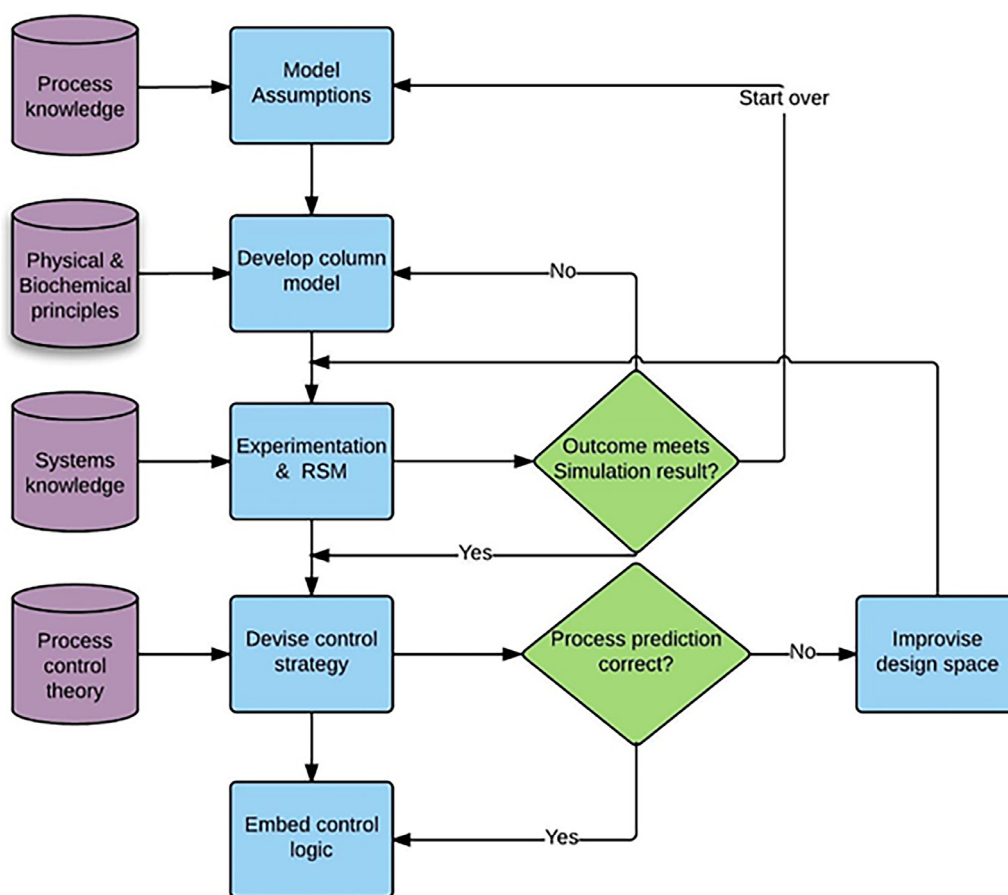**Fig. 3.** Significance of dimensionless numbers in Scale-up/Scale-down.

**Fig. 4.** Control strategy for continuous chromatography.

ties, geometry and flow variables, then setup a linear system and assign exponents to each variable which form the dimensionless numbers. The use of dimensionless numbers provides physical correlation to understand similarity among different scale of capture columns. The need for scale-up would arise to reduce the residence time or increase the feed concentration to make the capture process efficient or fast. A significant amount of experimental work can be replaced by utilizing dimensionless numbers during scale-up analysis. Dimensionless numbers provide the relationships of mass transfer in a column scale-up design as shown in Fig. 3. The Peclet and Biot numbers explain the mass transfer across the column. The Stanton and Damkohler numbers characterize the different column sizes. The Schmidt and Fourier numbers analyzes the fluid flow. The Reynold's Number calculates the pressure drop across a packed bed and signifies the characteristic length, which is important to assess the flow characteristics and thus sizing of the columns during scale-up operations.

A single column model is very well explained in the literature. A continuous process is actually a series of single column performing purification in a cyclic fashion. The cyclic column switching connects the single column to the multi-column dynamics. So the continuous chromatography can still be explained with the existing single column dynamics after addition of the column switching mechanism. The switching strategy is critical for a column, which undergoes loading until a specific target loading concentration. Most of the multi-column technologies explained further have found novel ways of performing column switching. The column switching strategy combined with the single column model used have generated various multi-column models as described below. Also, the breakthrough curve has been historically applied in single

column chromatography to assess the extent of loading wherein the loading stops based on the process economics model, typically about 80% of 10% of the breakthrough curve [84]. In multi-column chromatography, the breakthrough curve is imperative in the column performance. The extent of loading depends on the column switching criteria of the user [85]. We will review each of the technologies and how they have modeled the column switching dynamics. This review covers the underlying challenges for the Multi Column Chromatography (MCC) process, and discusses available solutions for path forward.

2.4. Control strategy for continuous chromatography processing

As the control strategy of continuous chromatography poses questions of varying complexity, a semi-empirical model wherein a mathematical framework is complimented with experimental study can effectively address the concern for process control. A control strategy for continuous chromatography is shown in Fig. 4. An extensive process knowledge is required to build model assumptions. The biological, biochemical and mass transfer knowledge will be required to setup a Protein A model. The dimensionless numbers can be utilized to develop the process model. Next, the column model parameters can be estimated with experiments. Systems knowledge is required to design the experiments and perform Response Surface Model (RSM) analysis. The semi-empirical model will then need to be validated with a different set of simulations and experiments. Once a validated model is developed and meets the simulation result, a control strategy can be devised based on the process control theory. The model simulation results then need to be assessed for accuracy and sensitivity after which
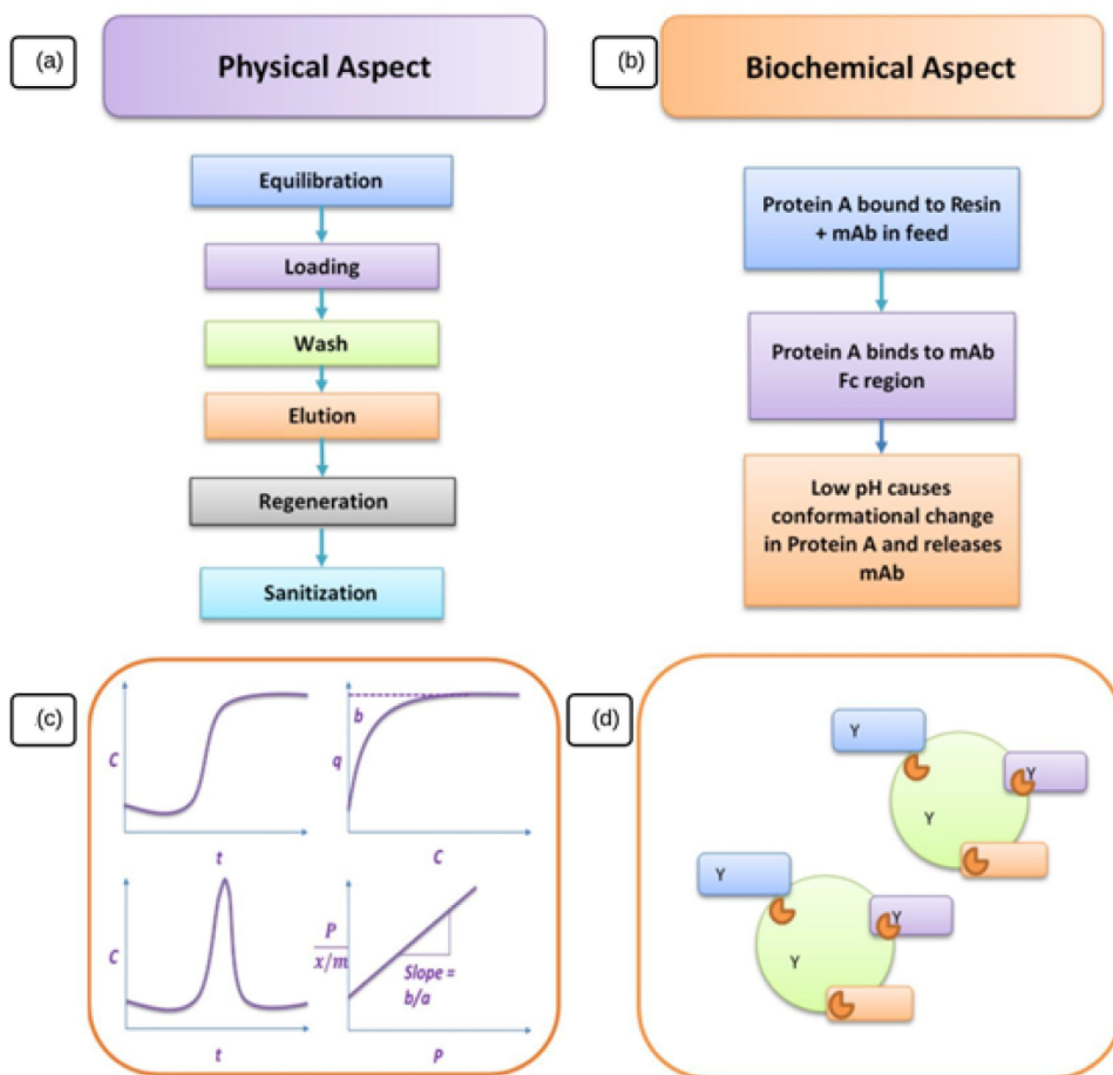
Fig. 5. Physical and biochemical aspect in Protein A chromatography. (a) Physical aspect, (b) biochemical aspect, (c) binding kinetics of resin and (d) binding of Protein A ligand to antibody.

the model could be used for research/industrial applications. The model thus developed can provide a theoretical framework for the experimental design. A shrinking core model was developed by the authors to study the impact of caustic on the Protein A ligand degradation [31] which will be critical for the column switching decision during continuous chromatography.

2.5. Biochemical aspect of continuous separation technology

With biopharmaceuticals, one prime challenge is to consider the additional biological behavior and biochemistry between the proteins and resins as shown in Fig. 5. The classical models show marginal predictability with the protein separation on account of the additional complexity at the structural level of the proteins and the aggregate formation with changes in ionic strength, pH, stress, etc. Protein A is a 42 kDa surface protein with an extended shape naturally found in the cell wall of *Staphylococcus aureus* (*S. aureus*). Protein A has high affinity towards immunoglobulin G (IgG) with varying degrees of specificity for the different types [86,87]. It has an isoelectric point of 5.1 and is made of single polypeptide chain. The stable protein has two sites available for IgG binding and the binding occurs via Fc region of the γ-chain [88,89]. Most commercial Protein A resins use a recombinant form which have increased

specific affinity to mAbs and resitance to NaOH deagradation. Protein A affinity chromatography makes use of the high binding affinity of Protein A towards IgG (monoclonal antibodies) as a capture step after harvest and depth filtration. The purification step shows > 95% purity and reduces the harvest volume considerably. As a result, the following polishing steps have to face lower volumes of feed.

3. Current chromatography technologies

Various multi-column process technologies have been evolved to facilitate the continuous capture step. Dynamic Binding Capacity (DBC) at a certain flow rate has been attempted to reach Static Binding Capacity (SBC) using multiple smaller columns. However, comparative analysis and performance of the smaller multiple columns with one traditional column necessitate further probing. Most technologies employ semi-continuous chromatography where instead of using a single large column, multiple smaller columns which can undergo loading and non-loading operations simultaneously. The columns undergo loading, wash, elution wherein the column which underwent the loading until a predefined saturation is then washed and eluted while the other column(s) undertakes the loading until it reaches saturation and then it is conse-
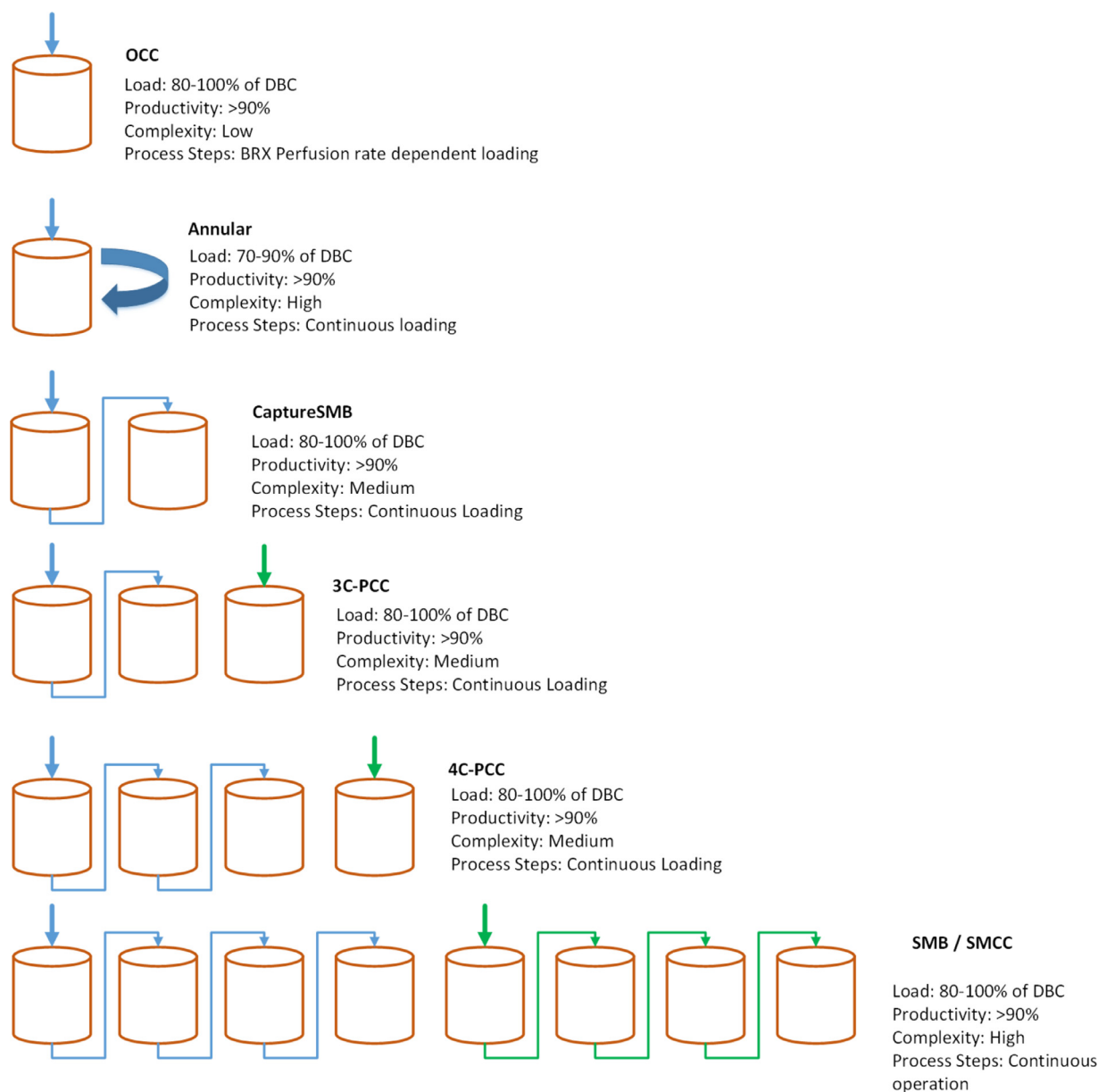
Fig. 6. Schematic of multi-column chromatography design.

quently washed, eluted and equilibrated to receive the next load [28,90]. The multi-column design works as a staggered process wherein one large column is split into multiple small columns. There are two columns always in the loading phase. Following are the multi-column chromatography designs which use different number of columns for the multi-column operations. A general schematic is shown in Fig. 6 and an overview of the current multi-column technologies is provided in Table 2. These technologies are based on mechanistic models which is explained in the following x section.

3.1. SMB technology for multi-column operation

Simulated Moving Bed (SMB) is based on the hypothetical True Moving Bed (TMB) process wherein the liquid and solid phase assume a countercurrent movement. At one point, two fluid streams (feed and elution buffer) enter in and two streams (extract and raffinate) flow out. Although SMB was conventionally developed for binary separation with 4–12 columns [91,92], several illustra-

tions are available for effective separation with reduced number of columns [93,94]. SMB has internal recycling of buffers. The equilibrium theory provides the feasible region for separation under specific constraints already set by the column and flow path design. As SMB technology utilizes the triangle theory for binary and multi-component separations, the linear and non-linear isotherms are employed during the modeling. The column switching is time-based wherein the flow switching occurs at fixed time intervals during the cyclic process. The switch time (t^*) is a relation between volumetric flow rate of the solid particle (antibody) (Q_s), particle porosity(ε_p), and column volume (V) [55]. Switch time is defined as the interval between two subsequent switches of the inlet and outlet ports:

$$t^* = \frac{V(1 - \epsilon)}{Q_s} \qquad (2)$$

wherein,

$$Q_s = U_s A(1 - \epsilon)t^* = \frac{V_e}{Q_j} = (1 + (1 - \varepsilon_p)) * \left(\varepsilon + m_j(1 - \varepsilon)\right) \qquad (4)$$

Table 2

Overview of all chromatography technologies available.

Chromatography technology	Number of columns needed	Advantages	Disadvantages	Applications
Simulated Moving Bed technology (SMB)	4–12	Internal recycling of buffers available, faster flow rates suitable for labile or unstable proteins	Column switching is time-based and needs more columns for recycling of buffers	IEX - AEX/CEX, Mixed Mode, Affinity
Sequential Multi-column technology (SMCC)	2–8	DBC remains constant at higher flow rates	No internal recycling of buffers	IEX - AEX/CEX, Mixed Mode, Affinity
Three Column Periodic Chromatography (3C-PCC)	3–4	Simple process design	No internal recycling of buffers, low DBC at high flow rates	IEX - AEX/CEX, Mixed Mode, Affinity
Two Column Chromatography CaptureSMB (2C-PCC)	2	Variable flow rate during interconnected and parallel mode, flexibility with pump speed	No internal recycling of buffers, low DBC at high flow rates	IEX - AEX/CEX, Mixed Mode, Affinity
One column Continuous Chromatography (OCC)	1	Loading dependent on perfusion rate, simple process design	No internal recycling of buffers, low DBC at high flow rates	IEX - AEX/CEX, Mixed Mode, Affinity
Annular Chromatography	1	Improved specific binding due to moving annulus	High complexity due to rotating column, No internal recycling of buffers, low flow rate	Affinity

where Q_j is the volumetric flow rate, V_e is the column volume, m_j is the dimensionless flow rate where j is the number of columns, ε is the column void fraction given by column void fraction (ε_b) and particle porosity(ε_p).

$$\varepsilon = \varepsilon_b + \varepsilon_p(1 - \varepsilon_b) \tag{5}$$

3.2. Sequential multi-column chromatography (SMCC)

The sequential MCC process is an open loop process and has been demonstrated with 2–8 columns [95,96]. The open loop SMCC differs from open loop SMB [94] wherein the wash of one column is recycled to the next column. Open loop SMB requires more columns, larger footprint and thus more costs. SMCC depends on transport dispersive model with variable lumped isotherm. The flow rate which inversely affects the DBC of the resin is decoupled so that even with higher flow rates, the DBC remains constant. SMCC design accounts for optimized resin volume, mAb concentration, flow rate of load step, buffer concentration, buffer volume, buffer pH, elution buffer concentration for IEX, etc. SMCC promotes operations in a state of control encouraged by the regulatory authorities and reduces the processing time with few or no holdups. However, SMCC has no capability of internal buffer transfer. Protein A adsorbent has been characterized and the column connections and periodic switching of inlets and outlets have been optimized in literature [95,96].

3.3. Three column periodic counter-current chromatography (3C-PCC)

A minimum of two columns are required for continuous loading in a PCC column wherein two columns are always in the loading phase while the remaining column(s) undergo wash, elution and sanitization. 3C-PCC encompasses maximum usage of resin capacity, efficient use of resin lifetime and reduction in losses during wash step. This technology is similarly demonstrated for 4 column operation as well. Langmuir isotherm kinetics is considered to study the effect of change of feed concentration on the separation efficiency. However, the BC model assumes a fully saturated column at a finite flow rate which tends to make the process very slow. The PCC system utilizes a ratio of static binding capacity to the feed concentration to determine the residence time and the number of columns wherein higher ratio translates into shorter residence times and lesser columns. As PCC is based on an empirical model, further research in the mechanistic modeling could provide an enhanced understanding of the system. PCC has been demonstrated for an integrated continuous process such that the outlet flow of the harvest is controlled and a constant protein load concentration is used during the loading phase. (3C PCC) employs

long residence times, which may or may not be favorable for labile and unstable proteins [52,73,90].

3.4. Capture SMB - Two Column periodic counter-current chromatography (2C-PCC)

CaptureSMB is a twin-column 2C-PCC process which involves two columns running in parallel and inter-connected mode in a cyclic fashion. The system has a start-up phase in which the second column is fully saturated at initialization. This allows the process to reach cyclic state of control promptly. There is interchange between the parallel and interconnected phase, which defines the cyclic process and is repeated until the feed is exhausted. This process considers minimizing the mass transfer effects during the interconnected loading phase by permitting faster loading and broader breakthrough curves. The process aims to reduce the pressure drop during elution by eluting at faster rates with a single column. The 2C-PCC process decreases on company footprint while aiming for similar or higher yields as batch processes [97–99].

3.5. One-column continuous chromatography (OCC)

This process involves a single column for continuous integrated downstream applications with continuous upstream processes. The method applies a combination of perfusion rate and loading flowrate to control the column loading and non-loading steps. It employs a surge tank to collect the perfusate during the non-loading steps. Equal time for loading and non-loading steps allows for minimization of surge tank volume and maximization of resin capacity. This method efficiently maximizes the Protein A resin usage and allows for minimum facility change during shift from batch to the OCC method albeit with thorough process optimization. The complexity of the process control is reduced as compared to the multicolumn operations wherein only one column needs to be monitored [100]. The column switching decision, due to any complication or at the end of resin lifetime, calls for future process development work. Real time process performance monitoring will be crucial in this method to enable smooth process control. A mathematical modeling framework needs to be developed for robust process optimization.

3.6. Annular chromatography

The resin in Continuous Annular Chromatography (CAC) is packed in the annulus between two concentric cylinders. The feed and buffer streams enter the annulus at the top from fixed inlets and after the state of control is achieved, leave the annulus

through established outlets in a helical pattern. The annulus is rotated at a specific speed and the angle between the inlet and outlet streams depends on the adsorption rate of protein ligand to the resin, i.e. the retention time, wherein strongly bound ligands elute farther from the vertical head while the loosely bound protein/debris elute close to the vertical head. The mass balance is determined using Langmuir-type isotherm at isothermal steady state like conditions and assuming a local equilibrium between the feed and resin phase [101]. Preparative-Continuous Annular Chromatography (P-CAC) was demonstrated by Giovannini et al. in Protein A annular chromatography [102]. The flow rates used were nearly half to that of the batch column chromatography due to column pressure constraints. Steady state like conditions were achieved after one revolution of the annulus.

4. Future challenges and solutions in continuous chromatography process

The existing control strategy is inadequate to ensure process robustness for the continuous operations. For future continuous processes, fundamental models based on thermodynamics and/or kinetics [103,104] will be required for better understanding of the process and thus leading to superior control strategy for online process monitoring [105,106]. With further increase in impetus for online process monitoring, precise measurement and real time adaptive control will be essential requirements in order to monitor and control product quality, stability & purity [107,108]. The control strategy to enable the future processes for continuous chromatography process is shown in Fig. 4. The current scenario, where the in-process tests are offline, PAT in its infancy and process variations causing sizable inconsistencies, a comprehensive model coupled with robust experimental validation will be critical for future advancements. Integration of different unit operations with respect to process controls should be addressed with effective controls. For instance, following capture step, viral inactivation [109,110] and inline buffer conditioning should be recognized as a standard practice. All process variations should be addressed with rapid actions and risk management would be critical in any event of contamination, for which corrective measures should be established in advance. Design space analysis by performing Design of Experiments (DOE) is one of the promising solutions for risk management. [19,110,111] All personnel should be well trained with respect to all the above competences, with focus on developing key skills involving advanced process controls. [112] With the use of smaller columns for extended period of time, offline qualification tools have shown to be inadequate. The column switching decision has been surfaced with the cyclic continuous chromatography process and needs further research [27,52,113–117]. Although in-house tools have been developed within several industries for on-line measurements, generic predictive tools will need to be developed for the larger community with the aid of mechanistic modeling and simulations.

5. Conclusion

A review of the current chromatography processes capable of performing continuous capture operations is provided. The review emphasizes the first principle models of chromatography on which the processes are designed, and reveals the processes that nevertheless require mechanistic modeling platform for a robust process. Changing from batch to continuous processing has shown a manifold of advantages but the most prominent benefit is to describe the chromatography operation as a state of control process to ensure consistent quality. The progressive reduction in the number of columns required to achieve a continuous cyclic process has been demonstrated well in the literature and demands further study.

The continuous chromatography process thus calls for simple models which can be conveniently integrated in the process controls in contemplation of maximizing the degree of adaptive control. Building mechanistic models and visualizing the simulations afford a safety net for the furtherance of experiments, with model assumptions bearing a pivotal role. However, the imminent challenges of real-time control, risk management, etc. must be overcome to pave way for the next generation bioprocessing.

Authors statement

Ketki Behere: Methodology, Data curation, Formal analysis, Writing - original draft, Writing - review & editing; **Seongkyu Yoon**: Supervision, Funding acquisition, Conceptualization, Methodology, Data curation, Writing - review & editing.

Declaration of Competing Interest

None.

Acknowledgment

References

[1] R. Allmendinger, A.S. Simaria, S.S. Farid, Multiobjective evolutionary optimization in antibody purification process design, Biochem. Eng. J. 91 (0) (2014) 250–264.

[2] R. Bochenek, et al., Evaluating the performance of different multicolumn setups for chromatographic separation of proteins on hydrophobic interaction chromatography media by a numerical study, J. Chromatogr. A 1301 (2013) 60–72.

[3] A. Xenopoulos, A new, integrated, continuous purification process template for monoclonal antibodies: process modeling and cost of goods studies, J. Biotechnol. 213 (2015) 42–53.

[4] D. Reay, C. Ramshaw, A. Harvey, Process Intensification: Engineering for efficiency, sustainability and flexibility, Butterworth-Heinemann, 2013.

[5] H. Thomas, Batch-to-continuous – coming out of age., Chem. Eng. 805 (2008) 38–40.

[6] A. Kumar, et al., Model-based analysis of high shear wet granulation from batch to continuous processes in pharmaceutical production – a critical review, Eur. J. Pharm. Biopharm. 85 (3) (2013) 814–832 Part B.

[7] W.W. Su, E.C. Asali, A.E. Humphrey, Springer, 2012, pp. 1–8. Editor.

[8] A.J.P. Martin, R.L.M. Synge, A new form of chromatogram employing two liquid phases. 1. A theory of chromatography. 2. Application to the micro-determination of the higher monoamino-acids in proteins, Chromatogr.Amino Acids 151 (1941) 1358–1368.

[9] D. Åsberg, et al., Fast estimation of adsorption isotherm parameters in gradient elution preparative liquid chromatography. I: the single component case., J. Chromatogr. A 1299 (2013) 64–70.

[10] S. Chatterjee, FDA perspective on continuous manufacturing, IFPAC Annual Meeting (Baltimore, MD, 2012 http://www.fda.gov/downloads/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/UCM341197.pdf.

[11] A.C. Fisher, et al., The current scientific and regulatory landscape in advancing integrated continuous biopharmaceutical manufacturing, Trends Biotechnol. 37 (3) (2019) 253–267.

[12] M. Li, Y.X. Qiu, A review on current downstream bio-processing technology of vaccine products, Vaccine 31 (9) (2013) 1264–1267.

[13] Rios, M., Minimizing costs and process times with local biomanufacturing, in bioprocess international. 2010.

[14] J. Walther, et al., The business impact of an integrated continuous biomanufacturing platform for recombinant protein production., Integr. Contin. Biomanuf. 213 (2015) 3–12.

[15] J. Strube, et al., Process development and design of downstream processes, Chemie Ingenieur Technik 83 (7) (2011) 1044–1065.

[16] M.W. Jornitz, D. Szarafinski, T. Peuker, in: Single-Use Technology: Opportunities in Biopharmaceutical Processes, in Biopharmaceutical Production Technology, Wiley-VCH Verlag GmbH & Co. KGaA, 2012, pp. 793–816.

[17] Y. Genzel, T. Vogel, J. Buck, I. Behrendt, D.V. Ramirez, G. Schiedner, I. Jordan, U. Reichl, High cell density cultivations by alternating tangential flow (ATF) perfusion for influenza A virus production using suspension cells, Vaccine 32 (24) (2014) 2770–2781.

[18] E. Jain, A. Kumar, Upstream processes in antibody production: evaluation of critical parameters, Biotechnol. Adv. 26 (1) (2008) 46–72.

[19] A.A. Shukla, U. Gottschalk, Single-use disposable technologies for biopharmaceutical manufacturing, Special Issue 31 (3) (2013) 147–154.

[20] N.M.G. Oosterhuis, et al., Second Edition, Academic Press, Burlington, 2011, pp. 249–261. Editor.

[21] C.A. Knaack, A.M.J. Hawrylechko, A systematic approach to the validation of monoclonal antibody manufacturing processes, Pharm. Sci. Technol. Today 1 (7) (1998) 300–308.

[22] B.L. Rellahan, et al., Second Edition, Academic Press, Burlington, 2011, pp. 363–375. Editor.

[23] M.E. Rodrigues, et al., Wave characterization for mammalian cell culture: residence time distribution, New Biotechnol. 29 (3) (2012) 402–408.

[24] J. Zhu, Mammalian cell protein expression for biopharmaceutical production, Biotechnol. Adv. 30 (5) (2012) 1158–1170.

[25] M. Holzer, H. Osuna-Sanchez, L. David, Multicolumn chromatography: a new approach to relieving capacity bottlenecks for downstream process efficiency, BioProcess Int. 6 (8) (2008) 74–84.

[26] C.K.S. Ng, et al., Design of high productivity sequential multi-column chromatography for antibody capture, Adva. Biosep. Food Bioprocess. 92 (2) (2014) 233–241.

[27] V. Warikoo, et al., Integrated continuous production of recombinant therapeutic proteins, Biotechnol. Bioeng. 109 (12) (2012) 3018–3029.

[28] E. Mahajan, A. George, B. Wolk, Improving affinity chromatography resin efficiency using semi-continuous chromatography, J. Chromatogr. A 1227 (2012) 154–162.

[29] A.T. Hanke, M. Ottens, Purifying biopharmaceuticals: knowledge-based chromatographic process development, Trends Biotechnol. 32 (4) (2014) 210–220.

[30] J.H. Chon, G. Zarbis-Papastoitsis, Advances in the production and downstream processing of antibodies, Antibodies 28 (5) (2011) 458–463.

[31] K. Behere, B. Cha, S. Yoon, Protein a resin lifetime study: evaluation of protein a resin performance with a model-based approach in continuous capture, Prep. Biochem. Biotechnol. 48 (3) (2018) 242–256.

[32] V. Warikoo, et al., Integrated continuous production of recombinant therapeutic proteins, Biotechnol. Bioeng. 109 (12) (2012) 3018–3029.

[33] S. Ghose, Y. Tao, L. Conley, D. Cecchini, Purification of monoclonal antibodies by hydrophobic interaction chromatography under no-salt conditions, In MAbs, Vol. 5, Taylor & Francis, 1994, pp. 795–800.

[34] N. Jakobsson, et al., Using computer simulation to assist in the robustness analysis of an ion-exchange chromatography step, J. Chromatogr. A 1063 (1) (2005) 99–109.

[35] K. Kaczmarski, et al., Comparative modeling of breakthrough curves of bovine serum albumin in anion-exchange chromatography, J. Chromatogr. A 925 (1–2) (2001) 1–17.

[36] L. Lapidus, N.R. Amundson, Mathematics of adsorption in beds. VI. The effect of longitudinal diffusion in ion exchange and chromatographic columns, J. Phys. Chem. 56 (8) (1952) 984–988.

[37] K. Horváth, et al., Martin-Synge algorithm for the solution of equilibrium-dispersive model of liquid chromatography, J. Chromatogr. A 1217 (52) (2010) 8127–8135.

[38] P. Sá Gomes, A.E. Rodrigues, Simulated moving bed chromatography: from concept to proof-of-concept, Chem. Eng. Technol. 35 (1) (2012) 17–34.

[39] P. Sá Gomes, A.E. Rodrigues, Simulated moving bed chromatography: from concept to proof-of-concept, Chem. Eng. Technol. 35 (1) (2012) 17–34.

[40] R.J. Silva, R.C. Rodrigues, J.P. Mota, Relay simulated moving bed chromatography: concept and design criteria, J. Chromatogr. A 1260 (2012) 132–142.

[41] A. Toumi, et al., Efficient optimization of simulated moving bed processes, Chem. Eng. Process. 46 (11) (2007) 1067–1084.

[42] J. Baeza-Baeza, M. García-Álvarez-Coque, A theoretical plate model accounting for slow kinetics in chromatographic elution, J. Chromatogr. A 1218 (31) (2011) 5166–5174.

[43] W.-C. Lee, G.-J. Tsai, G.T. Tsao, Analysis of chromatography by plate theory, Sep. Technol. 3 (4) (1993) 178–197.

[44] G. Sandoval, B.A. Andrews, J.A. Asenjo, Elution relationships to model affinity chromatography using a general rate model, J. Mol. Recognit. 25 (11) (2012) 571–579.

[45] E. von Lieres, J. Andersson, A fast and accurate solver for the general rate model of column liquid chromatography, Comput. Chem. Eng. 34 (8) (2010) 1180–1191.

[46] G. Guiochon, Preparative liquid chromatography, J. Chromatogr. A 965 (1) (2002) 129–161.

[47] A. Susanto, et al., High throughput screening for the design and optimization of chromatographic processes: automated optimization of chromatographic phase systems, Chem. Eng. Technol. 32 (1) (2009) 140–154.

[48] C.A. Lucy, P.W. Carr, Analysis of the separability of plate height into overload and intrinsic contributions using the kinetic model of non-linear chromatography, J. Chromatogr. A 556 (1–2) (1991) 159–168.

[49] R. Hahn, et al., Comparison of protein a affinity sorbents: II. Mass transfer properties., J. Chromatogr. A 1093 (1) (2005) 98–110.

[50] S. Ghose, B. Hubbard, S.M. Cramer, Binding capacity differences for antibodies and Fc-fusion proteins on protein A chromatographic materials, Biotechnol. Bioeng. 96 (4) (2007) 768–779.

[51] S. Ghose, et al., Use and optimization of a dual-flowrate loading strategy to maximize throughput in protein-A affinity chromatography, Biotechnol. Prog. 20 (3) (2004) 830–840.

[52] R. Godawat, et al., Periodic counter-current chromatography–design and operational considerations for integrated and continuous purification of proteins, Biotechnol. J. 7 (12) (2012) 1496–1508.

[53] R.A. Latour, The langmuir isotherm: a commonly applied but misleading approach for the analysis of protein adsorption behavior, J. Biomed. Mater. Res. Part A 103 (3) (2015) 949–958.

[54] A. London, An isotherm equation for adsorption on fractal surfaces of heterogeneous porous materials, Langmuir 5 (6) (1989) 1431–1433.

[55] M. Mazzotti, Equilibrium theory based design of simulated moving bed processes for a generalized Langmuir isotherm, J. Chromatogr. A 1126 (1) (2006) 311–322.

[56] C. Ng, et al., Freundlich adsorption isotherms of agricultural by-product-based powdered activated carbons in a geosmin–water system, Bioresour. Technol. 85 (2) (2002) 131–135.

[57] C. Sheindorf, M. Rebhun, M. Sheintuch, A Freundlich-type multicomponent isotherm, J. Colloid Interface Sci. 79 (1) (1981) 136–142.

[58] R.J. Aguerre, C. Suarez, P.E. Viollaz, New BET type multilayer sorption isotherms.Part II: modelling water sorption in foods, Lebensm. _Wiss. a. Technol. 22 (1989) 192–195.

[59] A. Ebadi, J.S. Soltan Mohammadzadeh, A. Khudiev, What is the correct form of BET isotherm for modeling liquid phase adsorption? Adsorption 15 (1) (2009) 65–73.

[60] S. Chen, R. Yang, Theoretical basis for the potential-theory adsorption-isotherms-the Dubinin-Radushkevich and Dubinin-Astakhov equations, Langmuir 10 (11) (1994) 4244–4249.

[61] U. Huber, F. Stoeckli, J.-P. Houriet, A generalization of the Dubinin-Radushkevich equation for the filling of heterogeneous micropore systems in strongly activated carbons, J. Colloid Interface Sci. 67 (2) (1978) 195–203.

[62] K. Foo, B. Hameed, Insights into the modeling of adsorption isotherm systems, Chem. Eng. J. 156 (1) (2010) 2–10.

[63] K.V. Kumar, K. Porkodi, Relation between some two-and three-parameter isotherm models for the sorption of methylene blue onto lemon peel, J. Hazard. Mater. 138 (3) (2006) 633–635.

[64] F.-C. Wu, et al., A new linear form analysis of Redlich–Peterson isotherm equation for the adsorptions of dyes, Chem. Eng. J. 162 (1) (2010) 21–27.

[65] K.V. Kumar, Optimum sorption isotherm by linear and non-linear methods for malachite green onto lemon peel, Dyes Pigments 74 (3) (2007) 595–597.

[66] R.J. Umpleby, et al., Characterization of molecularly imprinted polymers with the Langmuir– Freundlich isotherm, Anal. Chem. 73 (19) (2001) 4584–4591.

[67] G.P. Jeppu, T.P. Clement, A modified Langmuir-Freundlich isotherm model for simulating pH-dependent adsorption effects, J. Contam. Hydrol. 129 (2012) 46–53.

[68] F. Gritti, G. Guiochon, Analytical solution of the ideal model of chromatography for a bi-Langmuir adsorption isotherm, Anal. Chem. 85 (18) (2013) 8552–8558.

[69] M. Jozwik, K. Kaczmarski, R. Freitag, Investigation of the steric mass action formalism in the simulation of breakthrough curves on a monolithic and a packed bed column, J. Chromatogr. A 1073 (1) (2005) 111–121.

[70] S. Dimartino, C. Boi, G.C. Sarti, Influence of protein adsorption kinetics on breakthrough broadening in membrane affinity chromatography, J. Chromatogr. A 1218 (26) (2011) 3966–3972.

[71] P. Thillaivinayagalingam, K. Reidy, A. Lindeberg, A.R. Newcombe, Revisiting protein A chromatography, Bioprocess Int. 10 (3) (2012).

[72] E. Müller, J. Vajda, Routes to improve binding capacities of affinity resins demonstrated for Protein A chromatography, J. Chromatogr. B 1021 (2016) 159–168.

[73] G. Carta, E.X. Perez-Almodovar, Productivity considerations and design charts for biomolecule capture with periodic countercurrent adsorption systems, Sep. Sci. Technol. 45 (2) (2010) 149–154.

[74] L. Bian, Q. Li, X. Ji, Binding of angiogenesis inhibitor kringle 5 to its specific ligands by frontal affinity chromatography, J. Chromatogr. A 1401 (2015) 42–51.

[75] E. Santacesria, et al., Separation of xylenes on Y zeolites. 2. Breakthrough curves and their interpretation, Ind. Eng. Chem. Process Des. Dev.;(United States) 21 (3) (1982).

[76] N. Raghavan, D. Ruthven, Numerical simulation of a fixed-bed adsorption column by the method of orthogonal collocation, AIChE J. 29 (6) (1983) 922–925.

[77] D. Garg, D. Ruthven, Theoretical prediction of breakthrough curves for molecular sieve adsorption columns—I asymptotic solutions, Chem. Eng. Sci. 28 (3) (1973) 791–798.

[78] M.A. Nelson, A. Moser, D.S. Hage, Biointeraction analysis by high-performance affinity chromatography: kinetic studies of immobilized antibodies, J. Chromatogr. B 878 (2) (2010) 165–171.

[79] N.W. Chan, et al., Frontal affinity chromatography–mass spectrometry assay technology for multiple stages of drug discovery: applications of a chromatographic biosensor, Anal. Biochem. 319 (1) (2003) 1–12.

[80] B. Morelli, Determination of a quaternary mixture of vitamins B6, B1, and B12 and uridine 5′-triphosphate, by derivative spectrophotometry, J. Pharm. Sci. 84 (1) (1995) 34–37.

[81] J. Staggs, Savitzky–Golay smoothing and numerical differentiation of cone calorimeter mass data, Fire Saf. J. 40 (6) (2005) 493–505.

[82] S.R. Delwiche, J.B. Reeves, III, A graphical method to evaluate spectral preprocessing in multivariate regression calibrations: example with Savitzky–Golay filters and partial least squares regression, Appl. Spectrosc. 64 (1) (2010) 73–82.

[83] A. Seidel-Morgenstern, Experimental determination of single solute and competitive adsorption isotherms, J. Chromatogr. A 1037 (1) (2004) 255–272.

[84] B.D. Bowes, A.M. Lenhoff, Protein adsorption and transport in dextran-modified ion-exchange media. II. Intraparticle uptake and column breakthrough, J. Chromatogr. A 1218 (29) (2011) 4698–4708.

[85] A. Rajendran, G. Paredes, M. Mazzotti, Simulated moving bed chromatography for the separation of enantiomers, J. Chromatogr. A 1216 (4) (2009) 709–738.

[86] S. Hober, K. Nord, M. Linhult, Protein A chromatography for antibody purification, J. Chromatogr. B 848 (1) (2007) 40–47.

[87] A. Forsgren, J. Sjöquist, Protein A" from S. aureus I. Pseudo-immune reaction with human γ-globulin, J. Immunol. 97 (6) (1966) 822–827.

[88] M. Freiherr von Roman, S. Berensmeier, Improving the binding capacities of protein A chromatographic materials by means of ligand polymerization, J. Chromatogr. A 1347 (2014) 80–86.

[89] B. Nilsson, et al., A synthetic IgG-binding domain based on staphylococcal protein A, Protein Eng. 1 (2) (1987) 107–113.

[90] J. Pollock, et al., Optimising the design and operation of semi-continuous affinity chromatography for clinical and commercial manufacture, J. Chromatogr. A 1284 (2013) 17–27.

[91] G. Sharma, et al., Control-relevant multiple linear modeling of simulated moving bed chromatography, IFAC-PapersOnLine 48 (8) (2015) 477–482.

[92] N. Wagner, et al., A Separation-integrated cascade reaction to overcome thermodynamic limitations in rare-sugar synthesis, Angewandte Chemie 127 (14) (2015) 4256–4260.

[93] K. Buhlert, M. Lehr, A. Jungbauer, Construction and development of a new single-column simulated moving bed system on the laboratory scale, J. Chromatogr. A 1216 (50) (2009) 8778–8786.

[94] J.M. Araújo, et al., Chiral separation by two-column, semi-continuous, open-loop simulated moving-bed chromatography, J. Chromatogr. A 1217 (33) (2010) 5407–5419.

[95] C.K. Ng, et al., Design of high productivity sequential multi-column chromatography for antibody capture, Food Bioprod. Process. 92 (2) (2014) 233–241.

[96] M. Ballerstein, et al., A theoretical study of continuous counter-current chromatography for adsorption isotherms with inflection points, Comput. Chem. Eng. 34 (4) (2010) 447–459.

[97] Muller-Spath, T., et al., Affinity capture of F (ab') 2 fragments: using twin-column countercurrent chromatography. 2015.

[98] M. Angarita, et al., Twin-column CaptureSMB: a novel cyclic process for protein A affinity chromatography, J. Chromatogr. A 1389 (2015) 85–95.

[99] T. Müller-Späth, et al., Model simulation and experimental verification of a cation-exchange IgG capture step in batch and continuous chromatography, J. Chromatogr. A 1218 (31) (2011) 5195–5204.

[100] M.-H. Kamga, M. Cattaneo, S. Yoon, Integrated continuous biomanufacturing platform with ATF perfusion and one column chromatography operation for optimum resin utilization and productivity, Prep. Biochem. Biotechnol. 48 (5) (2018) 383–390.

[101] A. Jungbauer, Continuous downstream processing of biopharmaceuticals, Trends Biotechnol. 31 (8) (2013) 479–492.

[102] R. Giovannini, R. Freitag, Isolation of a recombinant antibody from cell culture supernatant: continuous annular versus batch and expanded-bed chromatography, Biotechnol. Bioeng. 73 (6) (2001) 522–529.

[103] H. LIANG, J. Zhenbin, Non-equilibrium thermodynamic separation theory of nonlinear chromatography II. The 0-1 model for nonlinear-mass transfer kinetic processes, Chin. J. Chromatogr. 25 (6) (2007) 785–798.

[104] J. Zhu, A. Katti, G. Guiochon, Comparison of various isotherm models for predicting competitive adsorption data, J. Chromatogr. A 552 (1991) 71–89.

[105] F. Yi, et al., Experimental verification of multiinput seismic control strategies for smart dampers, J. Eng. Mech. 127 (11) (2001) 1152–1164.

[106] M. Bauer, I.K. Craig, Economic assessment of advanced process control–a survey and framework, J. Process Control 18 (1) (2008) 2–18.

[107] R. Karlsson, A. Michaelsson, L. Mattsson, Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system, J. Immunol. Methods 145 (1–2) (1991) 229–240.

[108] M. Krättli, F. Steinebach, M. Morbidelli, Online control of the twin-column countercurrent solvent gradient process for biochromatography, J. Chromatogr. A 1293 (2013) 51–59.

[109] D. Low, R. O'Leary, N.S. Pujar, Future of antibody purification, J. Chromatogr. B 848 (1) (2007) 48–63.

[110] A.A. Shukla, et al., Downstream processing of monoclonal antibodies—application of platform approaches, J. Chromatogr. B 848 (1) (2007) 28–39.

[111] B. Kelley, Very large scale monoclonal antibody purification: the case for conventional unit operations, Biotechnol. Prog. 23 (5) (2007) 995–1008.

[112] G. Salvendy, Design of Work and Development of Personnel in Advanced Manufacturing, John Wiley & Sons, 1994.

[113] A.K. Dutta, et al., Purification of monoclonal antibodies from clarified cell culture fluid using Protein A capture continuous countercurrent tangential chromatography, J. Biotechnol. 213 (2015) 54–64.

[114] M. Bisschops, L. Frick, S. Fulton, T. Ransohoff, Single-use, continuous-countercurrent, multicolumn chromatography, BioProcess Int. 7 (6) (2009) 18–23.

[115] V. Girard, et al., Large-scale monoclonal antibody purification by continuous chromatography, from process design to scale-up, J. Biotechnol. 213 (2015) 65–73.

[116] X. Gjoka, et al., A straightforward methodology for designing continuous monoclonal antibody capture multi-column chromatography processes, J. Chromatogr. A 1416 (2015) 38–46.

[117] F. Steinebach, T. Müller-Späth, M. Morbidelli, Continuous counter-current chromatography for capture and polishing steps in biopharmaceutical production, Biotechnol. J. 11 (9) (2016) 1126–1141.