Recent advances to accelerate purification process development
A review with a focus on vaccines

Keulen, Daphne; Geldhof, Geoffroy; Bussy, Olivier Le; Pabst, Martin; Ottens, Marcel

DOI
10.1016/j.chroma.2022.463195

Publication date
2022

Document Version
Final published version

Published in
Journal of Chromatography A

Citation (APA)
Keulen, D., Geldhof, G., Bussy, O. L., Pabst, M., & Ottens, M. (2022). Recent advances to accelerate purification process development: A review with a focus on vaccines. *Journal of Chromatography A*, *1676*, Article 463195. https://doi.org/10.1016/j.chroma.2022.463195

Journal of Chromatography A 1676 (2022) 463195

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Recent advances to accelerate purification process development: A review with a focus on vaccines

Daphne Keulen[a], Geoffroy Geldhof[b], Olivier Le Bussy[b], Martin Pabst[a], Marcel Ottens[a,*]

[a] Department of Biotechnology, Delft University of Technology, Van der Maasweg 9, Delft 2629, the Netherlands
[b] GSK Vaccines, Technical Research and Development – Microbial Drug Substance, Rue de l'Institut 89, Rixensart 1330, Belgium

ARTICLE INFO

Article history:
Received 7 April 2022
Revised 24 May 2022
Accepted 1 June 2022
Available online 2 June 2022

Keywords:
Downstream processing
Vaccine purification processes
Chromatography
Model-based high throughput process development
Artificial intelligence

ABSTRACT

The safety requirements for vaccines are extremely high since they are administered to healthy people. For that reason, vaccine development is time-consuming and very expensive. Reducing time-to-market is key for pharmaceutical companies, saving lives and money. Therefore the need is raised for systematic, general and efficient process development strategies to shorten development times and enhance process understanding. High throughput technologies tremendously increased the volume of process-related data available and, combined with statistical and mechanistic modeling, new high throughput process development (HTPD) approaches evolved. The introduction of model-based HTPD enabled faster and broader screening of conditions, and furthermore increased knowledge. Model-based HTPD has particularly been important for chromatography, which is a crucial separation technique to attain high purities. This review provides an overview of downstream process development strategies and tools used within the (bio)pharmaceutical industry, focusing attention on (protein subunit) vaccine purification processes. Subsequently high throughput process development and other combinatorial approaches are discussed and compared according to their experimental effort and understanding. Within a growing sea of information, novel modeling tools and artificial intelligence (AI) gain importance for finding patterns behind the data and thereby acquiring a deeper process understanding.

1. Introduction

The COVID-19 pandemic has engulfed the world, which has already cost over millions of lives and is still infecting hundreds of thousands of people every day, one and a half year after the first outbreak in December 2019. More than ever the world is aware of the value of vaccination, contributing to improved public health, reduced healthcare costs, economic growth, travel safety and prolonged life expectancy [1,2]. In general, vaccination is estimated to prevent 2–3 million childhood and almost 6 million adult deaths annually [1,3]. Recently, the WHO published an action plan making vaccination available to everyone in the world and promoting innovation within the vaccine industry [4].

The downstream process plays a key role in reducing contaminant concentrations in vaccines to very low values. This prevents for example high reactogenicity and unwanted immune responses, and guarantees the safety and efficacy of the vaccine. Designing a vaccine purification process is accompanied with many decisions, such as type and sequential order of purification techniques, conditions, costs, and other performance measurements [5]. Additionally, optimization of a single unit operation and overall purification sequence is important, whereas small variations of conditions in one step may affect the subsequent unit operation performance. High safety and purity demands lead to increased complexity of the vaccine purification process. This, often along with a low productivity and process capability, makes the downstream process very expensive in both costs and time [6,7]. One of the main challenges in developing vaccine purification processes is the separation of critical impurities closely related to the product, such as host cell proteins (HCPs) to a protein-antigen vaccine or genomic DNA or RNA to a DNA or RNA-based vaccine, respectively. Another challenge is the preservation of the antigen structure during the purification process, as well as the antigen stability, as most antigens are vulnerable to temperature, pH or salt concentration changes.

Fast vaccine process development is of utmost importance in light of infectious outbreaks and pushing competitive market, which highly depends on its design strategy for the purification

* Corresponding author.
E-mail address: m.ottens@tudelft.nl (M. Ottens).

https://doi.org/10.1016/j.chroma.2022.463195

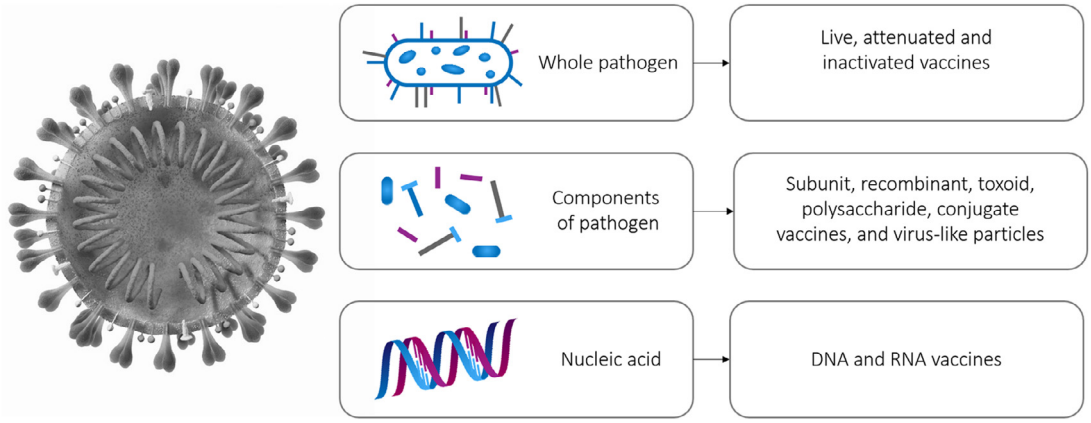

Fig. 1. Types of vaccines classified in whole pathogen, antigenic components of pathogen and nucleic acid vaccines [6,129–131].

process [6]. Traditionally, vaccines are developed within 10 – 15 years, hence pharmaceutical companies desire to reduce the process development time drastically in every aspect. One of the reasons the first SARS-CoV-2 vaccines could be developed within 12 years, is the employment of an accelerated development timeline due to parallelization of phases instead of sequential development [8]. Additional reasons for such a quick development are the application of previous knowledge and production processes from related viruses and existing vaccines (i.e. platform knowledge), and widely available government funding enabling parallelization, risk-taking and fast regulatory reviewing [9].

The 'quality by design' (QbD) paradigm [10,11] made the pharmaceutical industry shift from a trial-and-error approach towards a more comprehensive, systematic, and efficient approach, with the purpose to increase process understanding and process control [12–16]. The implementation of high-throughput process development (HTPD) approaches contributes to faster and more efficient process developments, additionally decreasing material consumption and improving cost-effectiveness [16]. HTPD is a combinatorial approach of both high throughput experimentation (HTE) and modeling techniques. Recently Sao Pedro et al. [17] outlined the areas of major problems (e.g. cell culture, filtration and analytical tools) within HTPD, along with suggested solutions (microfluidics, modeling and Process Analytical Technologies (PAT)) for the purpose of integrated and continuous bio manufacturing. Although this review is not focused on continuous biomanufacturing, the current limitations of HTPD are likewise applicable to the vaccine purification process development.

Vaccine purification processes can differ enormously from each other as they depend strongly on the type of vaccine and crude starting material/host organism (e.g. fertilized eggs, bacterial-, mammalian-, and insect cells). Carvalho et al. [18] pointed out the influence of vaccine types on downstream process strategies and described into detail each vaccine purification step with a focus on influenza vaccines. A general overview of vaccine types is shown in Fig. 1, being classified either as whole pathogen (inactivated or attenuated), antigenic components (subunit) of pathogen or nucleic acid vaccines, though slightly different classifications have also been reported.

In order to preserve the genetic stability of live and inactivated vaccines, the downstream process consist of only a few steps. The purification of protein recombinant or subunit vaccines involves a complex purification challenge because of the presence of HCPs closely-related to the target protein [6]. Recently Jones et al. [19] pointed out the concerns of high-risk HCPs and recommended a strategy for monitoring and eliminating the known impurities. Despite the great variance between different protein subunit vaccine downstream processes, the generic order of purification steps is similar as shown in Fig. 2. If the antigen (product of interest) is produced intracellular the purification process requires a cell lysis step, while this step is not needed if the antigen is produced extracellular. Detailed purification schemes for certain vaccine types are outside the scope of this paper and can be found elsewhere. For example, Josefsberg and Buckland [20] described the production process of several virus-based conjugate and DNA vaccines, while Abdulrahman and Ghanem [21] summarized the most recent advances in the purification of plasmid DNA vaccines. In the book of Wen et al. [6], viral vaccines purification [22] and protein subunit vaccines purification [23] are described into more detail.

Most of the current vaccine development approaches are based on design of experiments (DoE), in which multiple factors are changed simultaneously to evaluate the underlying interactions, thereby obtaining a multidimensional model that correlates the effects of various factors on the critical quality attributes (CQA), which is an essential aspect within QbD guidelines [14,24]. However, the existing vaccine process development strategy requires high experimental effort and little process understanding is gained through it. Moreover, the sequential determination of purification steps and individual process optimizations might lead to a suboptimal process design with respect to the objective, such as yield or costs [25–27]. A standardized approach, also known as platform process, as established for monoclonal antibodies (mAbs) [28] is yet missing, mainly due to the large diversity between vaccine types. Even when considering only protein subunit vaccines, already a very diverse range of proteins can be found due to a variety of expression systems.

A platform process for specific vaccine types would be highly beneficial in terms of process development time, knowledge, resources, costs and regulatory aspects [7]. Another often complicated task is the precise quantitatively measurement and characterization of virus or bacterial particles, further complicated by the lack of rapid analytical technologies [7,22]. A trend within the QbD initiative is the use of PAT, allowing real-time measurements to ensure consistent product quality and performance, besides providing a better understanding of the process [14]. Mechanistic models rely on physical processes occurring during a certain separation step and can therefore be of great merit to the process understanding, but also decrease experimental effort and allow to perform processes on different scales *in silico*. The use of AI techniques could eliminate shortcomings within the modeling area and bring modeling techniques to a higher level of applicability and usability.

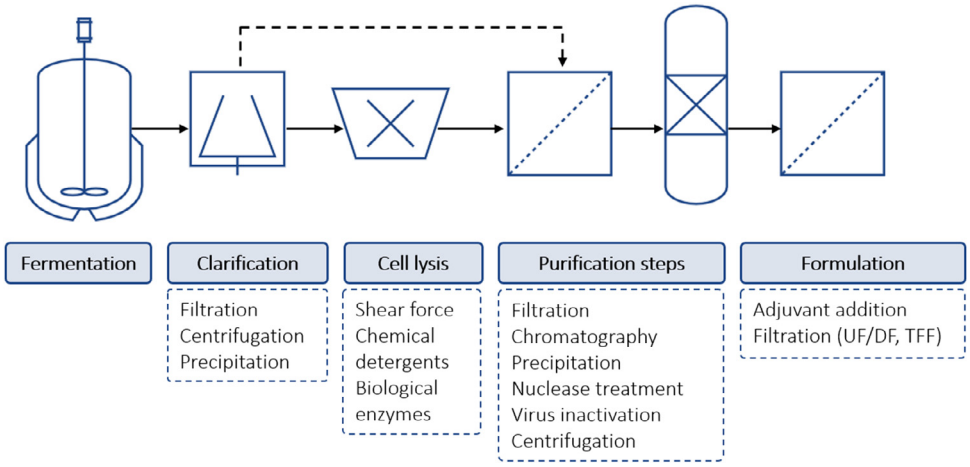

Fig. 2. General process flowsheet for vaccines including the upstream and downstream part, from fermentation to the last formulating steps. The optional processing techniques for different types of vaccines are given below each unit operation. The solid line represent a purification process in which the antigen is produced intracellular, including the cell lysis. The dashed line shows a purification process for extracellular products excluding cell lysis.

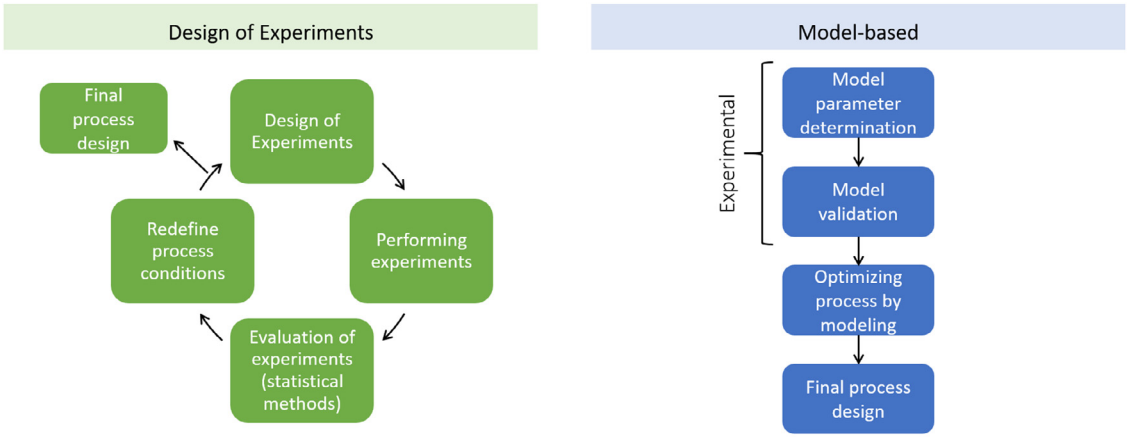

Fig. 3. Overview of two different process development approaches. Left: Design of Experiments (DoE) approach, which performs experiments based on statistical tools and evaluating the results by statistical analysis. This approach is commonly applied within biopharmaceutical industry. Right: Model-based process development approach, which employs targeted experiments to determine model input parameters such as isotherm parameters and column parameters. The model has to be validated before performing the optimization.

This review presents modern and future downstream process development approaches and their application in (bio)pharmaceutical industry with a focus on chromatography, as this is the main purification technique for protein subunit vaccines. This paper aims to show the evolvement of model-based high throughput process development approaches through the use of more advanced modeling techniques, such as empirical, mechanistic and hybrid modeling. The applicability and benefit using these methods are supported by case studies from industry and academia.

2. Downstream process development methods

The overall goal of process development is to design the optimal purification process, by means of achieving purity targets at minimum costs and time efforts, while at the same time adhering to all regulatory requirements. Currently, vaccine development employs mostly DoE-based methods, though it could benefit from more advanced model-based process development approaches, which are already used in other biopharmaceutical branches, such as for the purification of mAbs. Fig. 3 shows two types of process development approaches, the DoE-based method and a modeling-based method. In the following section, process development approaches are described briefly. More comprehensive reviews on this topic can be found elsewhere [16,29].

2.1. Experimental driven downstream process development

2.1.1. One-factor-at-a-time and design of experiments

One-factor-at-a-time (OFAT) is a more traditional approach in which one factor is changed during a series of experiments while the other factors are kept constant. In this method dependencies between factors are neglected and therefore discovery of the optimum is rather difficult and quite inefficient [30]. For that reason, the biopharmaceutical industry shifted more than a decade ago to the statistics-based DoE approach to design and analyze experiments, thereby obtaining more valuable information by conducting less experiments. The classical DoE-method is factorial design. Experiments are performed on all possible combinations of factors with the purpose to identify effects of each factor as well as interactions between factors on the response. An improvement on the classical DoE-screenings is definitive screening design, which estimates the curvature effects and enables separation of factors having a significant impact on the response from the factors having negligible effects. Oher methods offering a three level multifactorial design are for example Box-Behnken [31] or central composite designs. Hibbert D.B. extensively described the most commonly used DoE methods with a focus on chromatography [32,33]. Various DoE software are available nowadays, such as Design-Expert, Modde and JMP, though other statistical software,

like R, SPSS and various Python packages, can also be used for DoE purposes.

2.1.2. Parameter acquisition for modeling purposes

An alternative experimental strategy is to determine parameters that serve as input for mechanistic or physical models. The use of mechanistic models has been established decades ago and is nowadays widely adopted by chemical industry, where some processes are even designed entirely *in silico* [34]. Only recently, biopharmaceutical and vaccine industry initiated this strategy into their process development, in which the major challenge is often the complex feed mixture containing the product of interest (e.g. antigen) together with thousands of proteins and impurities [23]. This is probably why mechanistic modeling together with parameter acquisition has not been widely adopted yet, as it is nearly impossible to experimentally determine and model thousands of proteins and impurities. However, HTE made it worthwhile to determine model parameters even for more complex mixtures [35–37]. Noteworthy, a validated model increases process understanding and enables to optimize the process *in silico*, resulting in time, material and costs savings [38]. For chromatographic purposes, as this is the main purification technique in protein subunit vaccines, the adsorption isotherm parameters describing the binding behavior of components to the solid phase, are of utmost importance. Experimental determination of adsorption equilibria is required to establish the isotherm parameters and can be obtained by batch adsorption experiments [36,39–42], frontal analysis, isocratic elution or linear gradient elution [41,43,44] or by making use of inverse techniques, which minimize the difference between experimental and simulated elution profiles by tuning certain parameters [36,44,45]. Besides isotherm determination, column and resin characteristics must also be obtained in order to acquire a validated model, however these are more straightforwardly obtained [41].

2.1.3. High throughput screening (HTS)

The introduction of liquid handling stations (LHS), about two decades ago, allowed the acceleration of conducting experiments, also known as HTE or HTS. Due to automation, miniaturization and parallelization it became viable to create large data sets while using a reduced amount of sample volume and resources within a shorter time-frame [46,47]. Another benefit of automation is the lowered variability and superior precision [48]. Nowadays, LHS is a widely applied technique in both academia and industry and reduces the process development time significantly [49–51]. As LHS allows to screen more conditions, it is more feasible to find optimal conditions for a purification process. Apart from the system's benefits there are certainly also some disadvantages pointed in literature [52,53]. For example, the LHS's limitation in accurately mimicking the flow distributions of process columns [49]. HTS requires high understanding of efficient experimental design in order to make optimal use of the system, therefore it is rather a tool to be used than an approach on its own.

2.2. Expert-knowledge driven downstream process development

2.2.1. Universal

Rules of thumb, available knowledge and experience of previous processes are the basis for expert knowledge or heuristic approaches to design new production processes [29,54]. Using expert insights is easy to apply and can speed up the process design by eliminating combinations of unit operations with less promising results [55]. Lienqueo and Asenjo [54,56] developed an expert system focused on downstream protein processes; this software uses databases consisting of expert knowledge on universal process designs (heuristics) to support and accelerate decision-making for the selection of a sequence of unit operations. Several handbooks, like Hagel et al. [57] and GE healthcare [58], outline general design heuristics extensively. Most vaccine purification processes are also based upon heuristics, as for example the purification of hepatitis A virus from mammalian cell cultures, in which the first step involves a low-cost anion-exchange chromatography to capture the product and remove a substantial amount of impurities and the last step of the downstream process a polishing and desalting step using size-exclusion chromatography [22,57,59]. A general example that is almost entirely based on knowledge are platform processes as explained into more detail in the next paragraph.

2.2.2. Platform process

Platform processes are used as 'templates' for designing an entire purification sequence for a specific type of molecule, utilizing a pre-established series of unit operations [29]. The platform instructions provide details of the operating conditions for each unit operation, corresponding to the overall purification process. One of the key advantages is a reduced process development time, regulatory aspect and resources for similar molecules and accordingly decreased time-to-market and validation effort [57]. Moreover, the platform documents can be shared and aligned among not only different departments, but also across different manufacturing sites, serving as a site-independent process [60]. The platform process approach is most suited for biopharmaceuticals with similar characteristics and thus purification steps [28,57]. For example, mAbs are relatively well defined and platform processes are used to establish similar purification processes for new mAbs variants. Detailed information about process-related contaminants such as persistent HCPs and other impurities for the corresponding cell culture, i.e. CHO and hybridoma are known [60]. The order of purification steps includes protein A chromatography, low pH viral inactivation, IEX chromatography polishing steps, viral filtration, and ultrafiltration/diafiltration. Only small changes are required in the purification process conditions to determine a new mAb variant purification process. Other potential candidates for platform approaches could be pDNA vaccines and influenza vaccines, both having similar properties and purification steps [21,57]. However, mAbs are relatively similar to each other in their properties, while protein subunit vaccines vary greatly in their appearance, making it more difficult to standardize the purification process.

2.3. Model-based downstream process development

In process engineering models play an important role, they aim to represent a real system in an abstracted mathematical format [61,62]. Bézivin and Gerbé [63] defined a model as "a simplification of a system built with an intended goal in mind. The model should be able to answer questions in place of the actual system". The intended goal related to process engineering could be for example, control, simulation, design, monitoring or optimization. Depending on the goal, different models can be appropriate [64]. Models help to understand complex problems and could provide potential solutions if the model is an adequate representation of the modeled system's features of interest [65]. Running the model with a given set of parameters is a simulation and hence an inexpensive and safe way to run a virtual experiment [66]. For that reason, the number of experiments in laboratory can be reduced and/or designed more efficiently, thereby reducing time and material consumption. Although, using models sounds attractive and promising, it does cost time, effort and knowledge to develop decent models that are able to fulfill the desired purposes. Moreover, there is a lack of educated people in this area that can develop and maintain scientific-, and engineering software. Within the near future, it is expected that more process engineers or scientist are familiar with modeling, because most technical related studies provide programming and data-processing courses nowadays. In or-

Table 1
Overview of the main advantages and disadvantageous of different modeling approaches.

	Advantages	Shortcomings
Data-driven models	- Requires no or little process understanding in advance - Takes less effort/time to develop the model - Easy to use and understand	- Only valid in a predefined measured region - Extrapolation generally not applicable - Parameters have often no physical meaning - Data-collection might be an issue for the application and generalization of data-driven models in biomanufacturing industry
Mechanistic models	- Allows extrapolation and exploration of conditions beyond measured results - Acquires process understanding- Parameters have a physical meaning	- Requires process understanding in advance - Complex to develop and hence time and effort - Determination of model parameters can be difficult
Hybrid models	- Eliminate drawbacks of certain modeling approaches - Improved model accuracy and extrapolation properties- Less data is required compared to purely data-driven models	- Requires additional effort, time and knowledge to develop hybrid models - Data-collection can be challenging

der to build a model two main resources are essential, knowledge of the process, translated into laws of nature, and the collection of data obtained from the real system [66]. In process engineering, a distinction can be made between first-principles, mechanistic or knowledge-driven models and data-driven or empirical models, respectively known as transparent white-box and less transparent black-box models [61]. A combination of both is named hybrid semi-parametric models. An overview of the main advantages and disadvantages is given in Table 1.

2.3.1. Data-driven models

Data-driven or empirical models attempt to describe the input-output relation based upon observed experiments within a predefined design space, such as artificial neural networks (ANN), statistical and regression models [64]. The biopharmaceutical industry often makes use of statistical models, either by executing a predefined set of experiments using DoE and an appropriate statistical data analysis method, such as response surface methodology (RSM), or by employing a multivariate data analysis using an existing dataset [67]. RSM is a well-known empirical model and describes the relation of a response between different tested factors within a DoE, and produces a model describing the mathematical relationship [32]. This statistical (black-box) model solely observes the factor-to-response correlation without gaining fundamental mechanistic (physiochemical) understanding of the estimated parameters. By making use of DoE and regression analysis through first- and second order polynomials the optimum input combination can be estimated [68]. However, fitting data to second order polynomials is a major drawback of RSM, as frequently not all curvatures within the systems can be described by the second order polynomial [69]. DoE in combination with empirical modeling has been widely applied to design downstream purification processes in biopharmaceutical industry and academia [70–72]. The effect of high-salt solution on RNA precipitation and pDNA recovery was investigated using DoE and linear regression models [71]. And more recently, Chiang et al. [73] evaluated the impact of chromatographic parameters on virus clearance when switching from a single to multicolumn operation utilizing DoE. A major limitation of data-driven models is that they are merely valid in a defined region of measured variables and only able to predict variables within that region, making extrapolation generally highly inaccurate. Moreover, little process knowledge can be extracted, because the parameters are often just correlations [74]. On the other hand, data-driven modeling requires no process understanding in advance and is less time consuming compared to mechanistic modeling [74].

2.3.2. Mechanistic models

Mechanistic, first-principle, or knowledge-driven models attempt to describe the inner mechanisms and phenomena occurring in a process or system based upon knowledge about the process. These models consist of material and/or energy balances together with transport and thermodynamic phenomena and have a fixed structure, meaning the parameters might have a physical interpretation [74]. The model parameters are estimated by experimental data or physical correlations. The physical processes occurring during a purification process can be translated into mathematical simulation models. A validated mechanistic model allows to explore various conditions *in silico* and therefore enables to acquire optimum operating conditions efficiently [75]. The phenomena taking place inside a chromatographic column are well described in literature, Ruthven [76] extensively outlined the dynamics and adsorption processes. Kinetic or rate models are most common in practice, including dispersive factors, like mass transfer and dispersion effects, and equilibrium factors, such as adsorption isotherms, ionic dissociation and intermolecular association [41]. The three most prominent kinetic models are lumped kinetic model, lumped pore model, and general rate model, which are listed in order of complexity. The main difference between these models is the degree of covering pore diffusion effects [77]. However, it applies for all mechanistic models that isotherm parameters are crucial as explained previously in Section 2.1.2, for which numerous binding models exist, such as linear, Langmuir [78], steric mass action [43] and mixed-mode [39]. The utilization of chromatographic models varies from process synthesis, optimization and control [79–85] to scale-up, resin selection and robustness checks [86–89]. One step further is the simulation of a combination of integrated chromatography and other conditioning steps to find the overall optimum purification process [5,25,90–93]. Nowadays, various commercial software of chromatographic mechanistic models are available, e.g. GoSilico (now part of Cytivia, and formally known as ChromX) [94], Aspen Chromatography, DelftChrom, CADET [95] and ChromaTech [96].

Alternative *in silico* methods for adsorption experiments have been investigated for several years. Molecular dynamics simulations attempt to describe the interaction between resin-proteins on a detailed atomic level [97–99]. Quantitative structure-activity relationships (QSAR) combine molecular properties with empirical modeling to find correlations among retention behavior and protein surface properties [100–102]. This kind of molecular modeling can be used to predict the retention behavior of proteins on resins to reduce process development times [103]. However, often detailed information is required about each component, such as amino acid sequence or crystal structure and also a large amount of experiments [75].

Mechanistic models can explore conditions over a wide range and even beyond the observed measured results, possessing an increased extrapolation capability compared to data-driven models [74]. This contributes to process understanding, which is line with the QbD initiative, although mechanistic modeling also re-

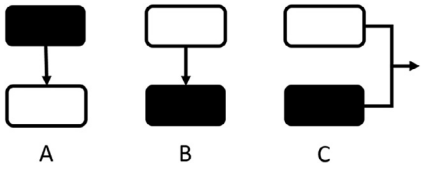

Fig. 4. Hybrid modeling configurations, white-boxes represent mechanistic/first-principle models and black-boxes represent data-driven models [61]. Serial approach (A, B) and parallel mode (C).

quires physical understanding. The major drawback of knowledge-based models is their complexity, hence requiring more development time compared to data-driven models.

2.3.3. Hybrid (semi-parametric) models

Hybrid (semi-parametric) modeling combines parametric (i.e. first principle-, mechanistic-, and knowledge-based models) with nonparametric (i.e. data-driven models) in order to eliminate drawbacks of individual approaches and get the best out of both [61]. Von Stosch et al. [61] extensively reviewed the hybrid semi-parametric modeling framework and the various applications in (bio)chemical engineering concerning process monitoring, control, optimization, model-reduction and scale-up**.** The parametric and nonparametric models can be configured in series or parallel, depending on the scope of the model. Usually a parallel mode is recommended when the mechanistic (white-box) model performance is limited or insufficiently accurate and the addition of a nonparametric (black-box) model may improve the estimations, Fig. 4c. A serial approach is often utilized for reducing complexity of mechanistic models by determining parameters using nonparametric models, Fig. 4a, or when the results of mechanistic models function as an input for nonparametric models, Fig. 4b [61].

The usefulness of hybrid modeling lies within its capability to cost-effectively and efficiently solve a complex problem and develop a model. Other advantages are an improved model accuracy, transparency and extrapolation properties, besides gaining a broader process understanding [74]. However, the challenge is knowing in what manner different type of models can be combined to develop a hybrid model. Therefore, thorough understanding on both data-driven and mechanistic models is desired, as well as knowledge to acquire the correct data. Hybrid modeling is gaining more interest in both industry and academia, and seems to be a promising approach to overcome deficiencies in data-driven-, and mechanistic models.

3. High throughput process development

3.1. Single or double purification steps

Hybrid process development approaches combine experimental and modeling tools to design a process. After the introduction of the LHS, hybrid approaches gained a special interest as LHS enabled experimentation in high throughput manner. Utilizing HTE in relation to process design is known as HTPD, combining HTS/HTE with empirical or mechanistic modeling is named model-based HTPD [16,38]. The implementation of HTPD pursues the QbD paradigm in terms of process and product understanding, hence contributing to high and stable product quality as well as process robustness [47]. The establishment of HTPD arose about 15 years ago [51,55,104,105] and evolved ever since as an efficient and cost-effective method broadly acknowledgement by industry [15]. (Model-based) HTPD can be applied in various development stages and for different purposes, like resin and solubility screenings, design-space definition, risk-assessment, process robustness and control. In the review of Baumann and Hubbuch [29], several commercial miniaturized HT-suitable systems in both up and downstream process development are described. The technical review of Lacki [52] outlined the most frequently used chromatography HT equipment, such as microtiter filter plates, prefilled pipette tips and robocolumns, nowadays ranging from 50 to 600 µL. Here, HTPD research from academic and industrial researchers are discussed. One can find more details on these and other HTPD approaches in Table 2. Depending on the purpose of the research a different HTDP approach is suitable, for example resin selection usually goes together with the use of empirical models while mechanistic modeling is preferred for an overall process design including multiple purification steps.

Bhambure and Rathore [50] proved a tremendous increase in productivity (170x higher) utilizing a HTPD platform (2 and 6 µL resin volume) against the traditional laboratory scale (0.5 mL resin volume) for defining the characterization space of an ion exchange chromatography step using DoE. A more practical and general HTPD workflow was developed by Welsh et al. [106] involving a multistep approach of HT chromatography techniques as a guidance for defining the operating space. No detailed modeling tools were implemented as accurate performance predictions were not the aim, only isotherm models to regress the partitioning coefficient and maximum binding capacity were used. Weigel et al. [107] applied a similar method as Welsh et al. to investigate the effectiveness of hydrophobic interaction chromatography (HIC) as a final purification step for a cell culture-derived influenza A and B virus. 96-well filter plate experiments were used for screening various resins and salt concentrations, followed by conventional lab-scale columns for dynamic binding capacity characterization. However, the major reason for choosing a rational stepwise method over mathematical modeling was the lack of available virus purification data by HIC to be able to determine model parameters. As vaccine platform processes are barely available yet, Ladd Effio et al. [108] initiated a capture step as first part of a generic purification platform process for virus-like particles (VLP). Ladd Effio et al. [108] established a one-step removal of HCPs and DNA from a complex VLP feedstock with an anion-exchange membrane capture step by making use of HTE and mechanistic modeling for *in silico* optimization purposes. Although equilibrium and binding capacities of membrane chromatography are often limited, at high flowrates membrane chromatography outperforms conventional packed bed chromatography in terms of productivity and for short residence times also in bed utilization [109]. It is expected that in the near future membrane materials with higher binding capacities will become available and therefore could overcome the restriction on surface area per unit volume of resin. The advancement in membrane chromatography technology is definitely interesting to the biopharmaceutical industry.

Even though chromatography is one of the main purification techniques for biopharmaceuticals and vaccines, other downstream process techniques are also HT-suited. Precipitation is a well-known technique to isolate a desired component such as a protein, DNA or virus and proven to be HT-suited [110,22]. This separation technique depends on the physical and/or chemical interaction between the precipitating agent (e.g. calcium chloride, ammonium sulfate or PEG) with one or several of the components in which solubility is the most critical thermodynamic property [111]. Aqueous two-phase systems (ATPs) could also be an alternative to chromatography as it is based on liquid-liquid extraction employing two immiscible phases to separate components from mixtures. HT techniques in combination with statistical [112,113] or mathematical/thermodynamic [114] models are a convenient method for characterizing these systems.

Analytics to monitor the process are just as important as the purification techniques itself. Analytics, however, remain a bottle-

Table 2
Overview of HTPD approaches which have been applied in industry and/or academia using HT and/or modeling techniques.

Purpose of research	Experimental Method			Modeling method	Application, unit operation and stage of development	Refs.
	HT	DoE	Lab-scale	Empirical or Mechanistic		
Defining characterization/operating space	Robocolumns for condition screening	Full factorial DoE, varying two process variables, pH and buffer molarity.	None	Empirical: Regression analysis by Least square fitting and optimization using contour profiler.	- GCSF - IEX - Early	[50]
	Batch adsorption experiments for isotherms and Robocolumns breakthrough experiments (DBC)	None	Lab-scale columns, validation experiments	Mechanistic: Regression of partitioning coefficient and maximum binding capacity, Langmuir isotherm model.	- mAb - IEX, MMC - Early	[106]
Resin selection and operating conditions	Batch binding and condition screening for resin-protein interactions	None	Lab-scale columns, comparison of results between lab-scale columns and HTS filter plate results	Calculation of partition coefficient and the separation factor	- mAb IgG1 from CHO - HIC - Early	[125]
	Batch binding and conditions screening for resin-protein interactions	None	None	Empirical: Partition coefficient of the product fitted to a response surface model (ANOVA) of pH and total chloride concentration	- mAb IgG1 from CHO - CEX, AEX - Early	[126]
Resin selection and salt concentration	Batch adsorption experiments for condition screening	None	Lab-scale column, breakthrough experiments (DBC – 10%)	None	- Virus, influenza A, B - HIC - Early	[107]
Design bind-and-elute membrane process	Batch binding and buffer screenings	Buffer screenings	Lab-scale column, membrane characterization, breakthrough experiments	Mechanistic: General rate model for radial flow chromatography Regression and chromatogram fitting for estimating isotherm and model parameters	- Virus Like Particles - AEX, membrane chromatography - Early and late	[108]
Resin selection, optimization and defining operation window	Robocolumns for resin screening and optimization screenings	Definitive screening designs for resin screening Central composite designs for optimization screenings	Lab-scale column for model verification	Empirical: Multivariate data analysis and usage of multi-criteria decision-making techniques. Process parameter optimization and Robustness analysis	- Highly aggregate antibody solution. - CEX - Early	[127]
Resin selection, multiple unit optimization	Robocolumns, bind-elute mode, resin and operating condition screening	None	None	Empirical: Multi-objective mixed integer nonlinear programming model. Adopted ε-constraint method solved by Dinkelbach's algorithm	- Recombinant Fc Fusion protein - CEX - MMC - Early	[86]
Flowsheet optimization, resin selection, design of process	Batch adsorption experiments (HT) for isotherm determination and resin selection.	None	Lab-scale column experiments for validation and acquisition of molecular properties	Mechanistic: Flowsheet optimization top-to-bottom approach using chromatographic mechanistic models including adsorption isotherm models	- mAb from hybridoma cell culture - CEX, AEX, HIC, SEC - 4-steps - Early and late	[87,90,128]
	Robocolumns to determine isotherm parameters Batch-uptake experiments for determining maximum binding capacities	None	Lab-scale column experiments for validation	Mechanistic: Flowsheet optimization, global optimization along with ANN and Local optimization along with Mechanistic models, including isotherm models	- mAb IgG1 from CHO - CEX, MMC, HIC, UF/DF - 4-steps - Early and late	[5,36]
	None	None	Lab-scale breakthrough column experiments.	Mechanistic: Flowsheet optimization using mechanistic models, including isotherm models.	- applied to three model proteins - CEX, AEX - 2-steps - Early and late	[25]

neck during HTE, and consequently slow down experimentation considerably. Konstantinidis et al. [115] provided a strategic assay deployment that helps selecting appropriate analytical methods, while preserving data quality. Nonetheless, finding innovative ways to accelerate the analytical throughput would be of great merit.

3.2. Overall purification process

The studies described in the previous paragraph focused mainly on applying HTPD to one or two sequential purification steps, but thereby do not consider the overall purification workflow. Designing a downstream process by optimizing each unit operation individually could lead to a suboptimal process design, as small variations in one-unit operation may affect the performance of subsequent following purification steps. The combination of HT and model-based optimization approaches for a sequence of unit operations has seldom been studied. Nfor et al. [90] established a systematic approach to rationally define the protein purification process utilizing a top-to-bottom approach. The least promising flowsheets were eliminated at each tree-diagram level by means of flow-sheet selection with the aim of keeping a minimum number of purification units. Instead of sequential optimization, which might generate a suboptimal process [25,26], Huuk et al. [25] presented a simultaneous two-step ion exchange chromatography process flowsheet optimization, including salt-gradient shapes and cut-points for fraction collection. Pirrung et al. [5] even proved the feasibility of simultaneous optimization of an integrated process consisting of three chromatographic steps (e.g. cation exchange, hydrophobic interaction and mixed-mode), including buffer exchange steps in between (e.g. ultra- and diafiltration) applied to a complex biological feedstock purification. First the isotherm parameters were acquired utilizing HT techniques as illustrated in more detail in previous work [36], hence other parameters were obtained by conventional lab-scale experiments. The use of ANN for finding suitable starting conditions for the local optimization using mechanistic models enabled circumvention of speed-limitations [5,27]. These examples to optimize an overall downstream process require a comprehensive combination of modeling and experimental methods. If more HTPD approaches are established that combine efficiently all available technologies (e.g. LHS, modeling-, analytical-, and data-processing tools), this optimization strategy could become more interesting.

4. Artificial intelligence in process development

The interest in HTPD raised after the introduction of HT technologies, having the major benefit to generate more data while consuming less material. Nevertheless, these arising technologies still face a number of hurdles. Experimentally transferring every item into HT mode, including analytics, remains a burden and although more data is being produced, processing and handling these data efficiently is still challenging. Modeling is a promising tool to close this gap. Further advancements of modeling are discussed in the following paragraph.

While complex mechanistic models attempt to describe the mechanisms and thermodynamic phenomena, determining certain parameters is rather difficult. Simplifying models could avoid certain difficulties, however, oversimplifications may cause inaccurate predictions and meaningless results. The optimal model should be as simple as possible while still gaining high or sufficient understanding. Moreover, a trade-off between accuracy versus speed has to be made especially when running optimizations. This led to the question; how to reduce the computational time effort or simplify complex models while retaining a similar level of accuracy and/or detail.

Although ANNs were already used in the late 90 s to predict retention times in chemical chromatography [116,117]. Due to the generation of larger data-sets and better computer systems in recent years, the use of AI gained popularity in various technology fields, likewise within the biotechnology area. In 1992 Psichogios and Ungar [118] presented the first hybrid neural network-first principles approach applied to model a fed-batch bioreactor. This hybrid model used a neural network model to estimate unknown process parameters serving as an input to a first principle model, resulting in an improved inter- and extrapolating capability, and understanding over merely “black-box” neural networks. Von Stosch et al. [61] extensively reviewed the hybrid semi-parametric modeling framework, as explained in 2.3.3., and the various applications in (bio)chemical engineering concerning process monitoring, control, optimization, model-reduction and scale-up. Nagrath et al. [119] established an optimization framework using a serial white- and black-box configuration (Fig. 4) to find the optimal design for a chromatographic process applied to a binary and tertiary mixtures. After obtaining the physical model parameters experimentally, numerous simulations were performed under various conditions using the physical model (i.e. white-box) for training the neural network. Finally, the optimal operating conditions for several purity levels were identified by using the neural network to accelerate the computation. Likewise, Pirrung et al. [5,27] used an ANN to accelerate a flowsheet optimization consisting of three chromatography and UF/DF units. However, here the ANN was used to find adequate starting conditions during the global optimization to be used for the local optimization, which was performed together with a mechanistic model in order to assure realistic and accurate results. A speed improvement of 70% was found, including training of the networks, compared to using solely mechanistic models for the optimization. Reducing the computational cost was the main objective for these latter two examples (Nagrath et al. and Pirrung et al.), and therefore using ANNs was advantageous. However, the data-driven model, here ANNs, depends on the accuracy of the mechanistic model and so limits the predictive power of ANNs. Recently, Nikita et al. [120] showed a novel approach making use of reinforcement learning (RL) to increase computational efficiency during a continuous chromatography process optimization. Each mechanistic model simulation is rewarded according to a RL-method and consequently the optimization criteria (design space) are adjusted to accelerate the convergence of optimization. The optimal flowrate, directly related to yield and purity demands, was found three times faster using the RL based optimization method compared to conventional trial and error methods. However, thorough understanding of the RL-principle and mechanistic modeling is required to develop this RL-method. Apart from using hybrid semi-parametric modeling for optimization intentions, other research showed the usefulness of black-box modeling to estimate certain white-box model parameters that are hard to determine. Wang et al. [121] used neural networks to directly derive mass transfer, isotherm and characteristic charge parameters from experimental chromatograms, after which these parameters served as input for the mechanistic model. In this way, time-consuming experimental methods for determining these parameters were circumvented. However, this approach requires still a considerable number of experiments. In mechanistic filtration models the flux is a key parameter, but predicting this parameter accurately might be quite complex. Therefore Krippl et al. [122] used an ANN to determine the flux using transmembrane pressure, cross-flow velocity and concentration as input parameters. Placing the hybrid model in series enabled to perform a multistep ahead prediction of the concentration over time. In general, data-driven models combined with white-box models can be advantageous in terms of prediction accuracy, computing and

model development efficiency and enhanced extrapolation properties [61].

With an eye on the future more applications of hybrid modeling approaches are expected, in both industry and academia. In order to realize this prospective, more experts in modeling are needed to develop and maintain these software applications. Moreover, the modeling techniques utilized in the HTPD can also be used for process control and optimization in later development stages and manufacturing processes. One step ahead is industry 4.0, known as the latest revolution and aiming to digitalize the whole manufacturing process. From process control to decision-making, all monitored data is efficiently collected, which in turn is also valuable for process development [123]. In order to realize Industry 4.0, digital twins are highly essential, defined as a virtual counterpart of the physical process and their interconnection [124].

5. Summary and conclusion

Vaccination protects millions of people from infectious diseases and, because a high product quality is pivotal, the downstream processing is likewise as important. Downstream process operations in manufacturing have a direct influence on time-to-market, product quality and cost of goods. Therefore, modernizing the strategies for developing processes could be of great merit. The urge to decrease the process development timeline of vaccines has raised, as well as the need for deeper process understanding as stated by the QbD guideline.

The introduction of HT technology accelerated experimental data generation and allowed to investigate the influence of parameters more thoroughly and systematically. However, HT also required to enhance data-processing and modeling techniques. Mechanistic models provide insights on the inner working mechanism of unit operations and are being increasingly adopted by industry in recent years, proving they add deeper process understanding and greater application possibilities. The combination of HT and modeling techniques led to HTPD approaches, acquiring and using data in a more efficient and purposeful way, thereby also enabling standardized process development approaches. The future direction in process development is to design and optimize the overall downstream process *in silico*, for which only a limited number of model calibration and validation experiments are needed. Hybrid (semi-parametric) modeling can help to ease the model development or improve the accuracy by making optimal use of both mechanistic and data-driven models. Recent research has shown the potential of artificial neural networks in addition to mechanistic models for circumvention of computational speed limitation or estimation of parameters.

With these emerging new technologies, it will now be possible to standardize process development workflows, provided that a proficient combination of experimenting and modeling techniques is utilized. Creating a generic process development workflow will enhance process development time and shared knowledge among different departments and manufacturing sites.

Declaration of Competing Interest

All authors have declared the following interests: Geoffroy Geldhof and Olivier Le Bussy are employees of the GSK group of companies. The other authors declare no conflict of interests.

CRediT authorship contribution statement

Daphne Keulen: Conceptualization, Writing – review & editing, Writing – original draft. **Geoffroy Geldhof:** Writing – review & editing. **Olivier Le Bussy:** Writing – review & editing. **Martin Pabst:** Writing – review & editing. **Marcel Ottens:** Conceptualization, Writing – review & editing.

Acknowledgment

This study was funded by GlaxoSmithKline Biologicals S.A. under cooperative research and development agreement between GlaxoSmithKline Biologicals S.A. (Belgium) and the Technical University of Delft (The Netherlands). The authors thank the colleagues from GSK Vaccines and Technical University of Delft for their valuable input.

References

[1] F.E. Andre, R. Booy, H.L. Bock, J. Clemens, S.K. Datta, T.J. John, B.W. Lee, S. Lolekha, H. Peltola, T.A. Ruff, M. Santosham, H.J. Schmitt, Vaccination greatly reduces disease, disability, death and inequity worldwide, Bull. World Health Organ. 86 (2) (2008) 140–146, doi:10.2471/BLT.07.040089.
[2] C.M.C. Rodrigues, S.A. Plotkin, Impact of vaccines; health, economic and social perspectives, Front. Microbiol. 11 (1526) (2020), doi:10.3389/fmicb.2020.01526.
[3] J. Ehreth, The global value of vaccination, Vaccine 21 (7) (2003) 596–600, doi:10.1016/S0264-410X(02)00623-0.
[4] N. Arora, Y. Al Mazrou, A. Cravioto, et al., Assessment Report of the Global Vacinne Action Plan (2014).
[5] S.M. Pirrung, C. Berends, A.H. Backx, R.F.W.C. van Beckhoven, M.H.M. Eppink, M. Ottens, Model-based optimization of integrated purification sequences for biopharmaceuticals, Chem. Eng. Sci. X 3 (2019) 100025, doi:10.1016/j.cesx.2019.100025.
[6] E.P. Wen, R. Ellis, N.S. Pujar, Vaccine Development and Manufacturing, Wiley, 2014.
[7] M. Zhao, M. Vandersluis, J. Stout, U. Haupts, M. Sanders, R. Jacquemart, Affinity chromatography for vaccines manufacturing: finally ready for prime time? Vaccine 37 (36) (2019) 5491–5503, doi:10.1016/j.vaccine.2018.02.090.
[8] F. Krammer, SARS-CoV-2 vaccines in development, Nature 586 (7830) (2020) 516–527, doi:10.1038/s41586-020-2798-3.
[9] P. Ball, The lightning-fast quest for COVID vaccines - and what it means for other diseases, Nature 589 (2021) 16–18, doi:10.1038/d41586-020-03626-1.
[10] ICH, ICH Harmonised Tripartite Guideline: Pharmaceutical Development Q8 (R2), ICH, 2009.
[11] FDA, PAT guidance for industry - a framework for innovative pharmaceutical development, manufacturing and quality assurance, 2004. www.fda.gov/regulatory-information/search-fda-guidance-documents/pat-framework-innovative-pharmaceutical-development-manufacturing-and-quality-assurance.
[12] L.X. Yu, Pharmaceutical quality by design: product and process development, understanding, and control, Pharm. Res. 25 (4) (2008) 781–791, doi:10.1007/s11095-007-9511-1.
[13] A.S. Rathore, Roadmap for implementation of quality by design (QbD) for biotechnology products, Trends Biotechnol. 27 (9) (2009) 546–553, doi:10.1016/j.tibtech.2009.06.006.
[14] A.S. Rathore, Quality by design (QbD)-based process development for purification of a biotherapeutic, Trends Biotechnol. 34 (5) (2016) 358–370, doi:10.1016/j.tibtech.2016.01.003.
[15] K.M. Lacki, High throughput process development in biomanufacturing, Curr. Opin. Chem. Eng. 6 (2014) 25–32, doi:10.1016/j.coche.2014.08.004.
[16] A.T. Hanke, M. Ottens, Purifying biopharmaceuticals: knowledge-based chromatographic process development, Trends Biotechnol. 32 (4) (2014) 210–220, doi:10.1016/j.tibtech.2014.02.001.
[17] M.N. São Pedro, T.C. Silva, R. Patil, M. Ottens, White paper on high-throughput process development for integrated continuous biomanufacturing, Biotechnol. Bioeng. (2021) n/a(n/a), doi:10.1002/bit.27757.
[18] S.B. Carvalho, C. Peixoto, M.J.T. Carrondo, R.J.S. Silva, Downstream processing for influenza vaccines and candidates: an update, Biotechnol. Bioeng. (2021) n/a(n/a), doi:10.1002/bit.27803.
[19] M. Jones, N. Palackal, F. Wang, G. Gaza-Bulseco, K. Hurkmans, Y. Zhao, C. Chitikila, S. Clavier, S. Liu, E. Menesale, N.S. Schonenbach, S. Sharma, P. Valax, T. Waerner, L. Zhang, T. Connolly, High-risk host cell proteins (HCPs): a multi-company collaborative view, Biotechnol. Bioeng. (2021) n/a(n/a), doi:10.1002/bit.27808.
[20] J.O. Josefsberg, B. Buckland, Vaccine process technology, Biotechnol. Bioeng. 109 (6) (2012) 1443–1460, doi:10.1002/bit.24493.
[21] A. Abdulrahman, A. Ghanem, Recent advances in chromatographic purification of plasmid DNA for gene therapy and DNA vaccines: a review, Anal. Chim. Acta 1025 (2018) 41–57, doi:10.1016/j.aca.2018.04.001.
[22] B. Kalbfuss-Zimmermann, U. Reichl, Viral vaccines purification, Vaccine Dev. Manuf. (2014) 97–180, doi:10.1002/9781118870914.ch5.
[23] Y.-p. Yang, T. D'Amore, Protein subunit vaccine purification, Vaccine Dev. Manuf. (2014) 181–216, doi:10.1002/9781118870914.ch6.
[24] V. Kumar, A. Bhalla, A.S. Rathore, Design of experiments applications in bioprocessing: concepts and approach, Biotechnol. Prog. 30 (1) (2014) 86–99, doi:10.1002/btpr.1821.

[25] T.C. Huuk, T. Hahn, A. Osberghaus, J. Hubbuch, Model-based integrated optimization and evaluation of a multi-step ion exchange chromatography, Sep. Purif. Technol. 136 (2014) 207–222, doi:10.1016/j.seppur.2014.09.012.
[26] B. Otero, M. Degerman, T.B. Hansen, E.B. Hansen, B. Nilsson, Model-based design and integration of a two-step biopharmaceutical production process, Bioproc. Biosyst. Eng. 37 (10) (2014) 1989–1996, doi:10.1007/s00449-014-1174-9.
[27] S.M. Pirrung, L.A.M. van der Wielen, R.F.W.C. van Beckhoven, E.J.A.X. van de Sandt, M.H.M. Eppink, M. Ottens, Optimization of biopharmaceutical downstream processes supported by mechanistic models and artificial neural networks, Biotechnol. Prog. 33 (3) (2017) 696–707, doi:10.1002/btpr.2435.
[28] A.A. Shukla, J. Thommes, Recent advances in large-scale production of monoclonal antibodies and related proteins, Trends Biotechnol. 28 (5) (2010) 253–261, doi:10.1016/j.tibtech.2010.02.001.
[29] P. Baumann, J. Hubbuch, Downstream process development strategies for effective bioprocesses: trends, progress, and combinatorial approaches, Eng. Life Sci. 17 (11) (2017) 1142–1158, doi:10.1002/elsc.201600033.
[30] V. Czitrom, One-factor-at-a-time versus designed experiments, Am. Stat. 53 (2) (1999) 126–131, doi:10.1080/00031305.1999.10474445.
[31] G.E.P. Box, D.W. Behnken, Simplex-sum designs: a class of second order rotatable designs derivable from those of first order, Ann. Math. Stat. 31 (4) (1960) 838–864, doi:10.1214/aoms/1177705661.
[32] D.B. Hibbert, Experimental design in chromatography: a tutorial review, J. Chromatogr. B 910 (2012) 2–13, doi:10.1016/j.jchromb.2012.01.020.
[33] S.L.C. Ferreira, R.E. Bruns, E.G.P. da Silva, W.N.L. dos Santos, C.M. Quintella, J.M. David, J.B. de Andrade, M.C. Breitkreitz, I.C.S.F. Jardim, B.B. Neto, Statistical designs and response surface techniques for the optimization of chromatographic systems, J. Chromatogr. A 1158 (1) (2007) 2–14, doi:10.1016/j.chroma.2007.03.051.
[34] J.J. Siirola, Industrial applications of chemical process synthesis, in: Advances in Chemical Engineering, Academic Press, 1996, pp. 1–62, doi:10.1016/S0065-2377(08)60201-X.
[35] A.T. Hanke, E. Tsintavi, M.D.R. Vazquez, L.A.M. van der Wielen, P.D.E.M. Verhaert, M.H.M. Eppink, E.J.A.X. van de Sandt, M. Ottens, 3D-liquid chromatography as a complex mixture characterization tool for knowledge-based downstream process development, Biotechnol. Prog. 32 (5) (2016) 1283–1291, doi:10.1002/btpr.2320.
[36] S.M. Pirrung, D.P. da Cruz, A.T. Hanke, C. Berends, R.F.W.C. van Beckhoven, M.H.M. Eppink, M. Ottens, Chromatographic parameter determination for complex biological feedstocks, Biotechnol. Prog. 34 (4) (2018) 1006–1018, doi:10.1002/btpr.2642.
[37] L.J. Benedini, D. Figueiredo, J. Cabrera-Crespo, V.M. Gonçalves, G.G. Silva, G. Campani, T.C. Zangirolami, F.F. Furlan, Modeling and simulation of anion exchange chromatography for purification of proteins in complex mixtures, J. Chromatogr. A 1613 (2020) 460685, doi:10.1016/j.chroma.2019.460685.
[38] B.K. Nfor, P.D.E.M. Verhaert, L.A.M. van der Wielen, J. Hubbuch, M. Ottens, Rational and systematic protein purification process development: the next generation, Trends Biotechnol. 27 (12) (2009) 673–679, doi:10.1016/j.tibtech.2009.09.002.
[39] B.K. Nfor, M. Noverraz, S. Chilamkurthi, P.D.E.M. Verhaert, L.A.M. van der Wielen, M. Ottens, High-throughput isotherm determination and thermodynamic modeling of protein adsorption on mixed mode adsorbents, J. Chromatogr. A 1217 (44) (2010) 6829–6850, doi:10.1016/j.chroma.2010.07.069.
[40] J. Chen, S.M. Cramer, Protein adsorption isotherm behavior in hydrophobic interaction chromatography, J. Chromatogr. A 1165 (1) (2007) 67–77, doi:10.1016/j.chroma.2007.07.038.
[41] G. Carta, A. Jungbauer, Protein chromatography: process development and scale-up, 2010.
[42] M. Moreno-González, V. Girish, D. Keulen, H. Wijngaard, X. Lauteslager, G. Ferreira, M. Ottens, Recovery of sinapic acid from canola/rapeseed meal extracts by adsorption, Food Bioprod. Process. 120 (2020) 69–79, doi:10.1016/j.fbp.2019.12.002.
[43] C.A. Brooks, S.M. Cramer, Steric mass-action ion exchange: displacement profiles and induced salt gradients, AlChE J. 38 (12) (1992) 1969–1978, doi:10.1002/aic.690381212.
[44] A. Osberghaus, S. Hepbildikler, S. Nath, M. Haindl, E. von Lieres, J. Hubbuch, Determination of parameters for the steric mass action model—a comparison between two approaches, J. Chromatogr. A 1233 (2012) 54–65, doi:10.1016/j.chroma.2012.02.004.
[45] D. Saleh, G. Wang, B. Müller, F. Rischawy, S. Kluters, J. Studts, J. Hubbuch, Straightforward method for calibration of mechanistic cation exchange chromatography models for industrial applications, Biotechnol. Prog. (2020) e2984 n/a(n/a), doi:10.1002/btpr.2984.
[46] M. Wiendahl, P. Schulze Wierling, J. Nielsen, D. Fomsgaard Christensen, J. Krarup, A. Staby, J. Hubbuch, High throughput screening for the design and optimization of chromatographic processes – miniaturization, automation and parallelization of breakthrough and elution studies, Chem. Eng. Technol. 31 (6) (2008) 893–903, doi:10.1002/ceat.200800167.
[47] R. Bhambure, K. Kumar, A.S. Rathore, High-throughput process development for biopharmaceutical drug substances, Trends Biotechnol. 29 (3) (2011) 127–135, doi:10.1016/j.tibtech.2010.12.001.
[48] N. Singh, S. Herzer, Downstream processing technologies/capturing and final purification, in: New Bioprocessing Strategies: Development and Manufacturing of Recombinant Antibodies and Proteins, Springer International Publishing, Cham, 2018, pp. 115–178, doi:10.1007/10_2017_12.
[49] A.S. Rathore, D. Kumar, N. Kateja, Recent developments in chromatographic purification of biopharmaceuticals, Biotechnol. Lett. 40 (6) (2018) 895–905, doi:10.1007/s10529-018-2552-1.
[50] R. Bhambure, A.S. Rathore, Chromatography process development in the quality by design paradigm I: establishing a high-throughput process development platform as a tool for estimating “characterization space” for an ion exchange chromatography step, Biotechnol. Prog. 29 (2) (2013) 403–414, doi:10.1002/btpr.1705.
[51] M. Bensch, P. Schulze Wierling, E. von Lieres, J. Hubbuch, High throughput screening of chromatographic phases for rapid process development, Chem. Eng. Technol. 28 (11) (2005) 1274–1284, doi:10.1002/ceat.200500153.
[52] K.M. Lacki, High-throughput process development of chromatography steps: advantages and limitations of different formats used, Biotechnol. J. 7 (10) (2012) 1192–1202, doi:10.1002/biot.201100475.
[53] T. Bergander, K.M. Lacki, High-throughput process development: chromatography media volume definition, Eng. Life Sci. 16 (2) (2016) 185–189, doi:10.1002/elsc.201400240.
[54] M.E. Lienqueo, J.A. Asenjo, Use of expert systems for the synthesis of downstream protein processes, Comput. Chem. Eng. 24 (9) (2000) 2339–2350, doi:10.1016/S0098-1354(00)00590-1.
[55] B.K. Nfor, T. Ahamed, G.W.K. van Dedem, L.A.M. van der Wielen, E.J.A.X. van de Sandt, M.H.M. Eppink, M. Ottens, Design strategies for integrated protein purification processes: challenges, progress and outlook, J. Chem. Technol. Biotechnol. 83 (2) (2008) 124–132, doi:10.1002/jctb.1815.
[56] E.W. Leser, J.A. Asenjo, Rational design of purification processes for recombinant proteins, J. Chromatogr. B Biomed. Sci. Appl. 584 (1) (1992) 43–57, doi:10.1016/0378-4347(92)80008-E.
[57] L. Hagel, G. Jagschies, G. Sofer, Handbook of Process Chromatography, Development, Manufacturing, Validation and Economics, 2008.
[58] G.H.L. Sciences, Recombinant protein purification handbook, principles and methods, 2012.
[59] A. Hagen, J. Aunins, P. DePhillips, C.B. Oswald, J.P. Hennessey Jr, J. Lewis, M. Armstrong, C. Oliver, C. Orella, B. Buckland, R. Sitrin, Development, preparation, and testing of VAQTA®, a highly purified hepatitis a vaccine, Bioprocess Eng. 23 (5) (2000) 439–449, doi:10.1007/s004499900157.
[60] A.A. Shukla, B. Hubbard, T. Tressel, S. Guhan, D. Low, Downstream processing of monoclonal antibodies—application of platform approaches, J. Chromatogr. B 848 (1) (2007) 28–39, doi:10.1016/j.jchromb.2006.09.026.
[61] M. von Stosch, R. Oliveira, J. Peres, S.F. de Azevedo, Hybrid semi-parametric modeling in process systems engineering: past, present and future, Comput. Chem. Eng. 60 (2014) 86–101, doi:10.1016/j.compchemeng.2013.08.008.
[62] P.-.A. Muller, F. Fondement, B. Baudry, B. Combemale, Modeling modeling modeling, Softw. Syst. Model. 11 (3) (2012) 347–359, doi:10.1007/s10270-010-0172-x.
[63] J. Bezivin, O. Gerbe, Towards a precise definition of the OMG/MDA framework, in: Proceedings of the 16th Annual International Conference on Automated Software Engineering (ASE), 2001, pp. 273–280.
[64] D. Bonvin, C. Georgakis, C.C. Pantelides, M. Barolo, M.A. Grover, D. Rodrigues, R. Schneider, D. Dochain, Linking models and experiments, Ind. Eng. Chem. Res. 55 (25) (2016) 6891–6903, doi:10.1021/acs.iecr.5b04801.
[65] B. Selic, The pragmatics of model-driven development, IEEE Softw. 20 (5) (2003) 19–25, doi:10.1109/MS.2003.1231146.
[66] L. Ljung, T. Glad, Modeling of Dynamic Systems, Englewood Cliffs (N.J.) : Prentice-Hall1994.
[67] A.S. Rathore, S. Mittal, M. Pathak, A. Arora, Guidance for performing multivariate data analysis of bioprocessing data: pitfalls and recommendations, Biotechnol. Prog. 30 (4) (2014) 967–973, doi:10.1002/btpr.1922.
[68] J.P.C. Kleijnen, in: Response Surface Methodology, Handbook of Simulation Optimization, Springer New York : Springer, New York, NY, 2015, pp. 81–104, doi:10.1007/978-1-4939-1384-8_4.
[69] D. Baş, İ.H. Boyacı, Modeling and optimization I: usability of response surface methodology, J. Food Eng. 78 (3) (2007) 836–845, doi:10.1016/j.jfoodeng.2005.11.024.
[70] C. Anirban Roy, B. Paramita, S.P. Gandham, Development of suitable solvent system for downstream processing of biopolymer pullulan using response surface methodology, PLoS One (2013).
[71] A. Eon-Duval, K. Gumbs, C. Ellett, Precipitation of RNA impurities with high salt in a plasmid DNA purification process: use of experimental design to determine reaction conditions, Biotechnol. Bioeng. 83 (5) (2003) 544–553, doi:10.1002/bit.10704.
[72] M. Toueille, A. Uzel, J.-.F. Depoisier, R. Gantier, Designing new monoclonal antibody purification processes using mixed-mode chromatography sorbents, J. Chromatogr. B 879 (13) (2011) 836–843, doi:10.1016/j.jchromb.2011.02.047.
[73] M.-.J. Chiang, M. Pagkaliwangan, S. Lute, G. Bolton, K. Brorson, M. Schofield, Validation and optimization of viral clearance in a downstream continuous chromatography setting, Biotechnol. Bioeng. 116 (9) (2019) 2292–2302, doi:10.1002/bit.27023.
[74] D. Solle, B. Hitzmann, C. Herwig, M. Pereira Remelhe, S. Ulonska, L. Wuerth, A. Prata, T. Steckenreiter, Between the poles of data-driven and mechanistic modeling for process operation, Chem. Ing. Tech. 89 (5) (2017) 542–561, doi:10.1002/cite.201600175.
[75] S.M. Pirrung, M. Ottens, High Throughput Process Development, in: Preparative Chromatography for Separation of Proteins, John Wiley & Sons, Inc, 2017, pp. 269–292.

[76] D.M. Ruthven, Principles of Adsorption and Adsorption Processes, Wiley, New York, 1984.
[77] A. Felinger, G. Guiochon, Comparison of the kinetic models of linear chromatography, Chromatographia 60 (1) (2004) S175–S180, doi:10.1365/s10337-004-0288-7.
[78] I. Langmuir, The constitution and fundamental properties of solids and liquids. Part I. solids, J. Am. Chem. Soc. 38 (11) (1916) 2221–2295, doi:10.1021/ja02268a002.
[79] B.K. Nfor, J. Ripic, A. van der Padt, M. Jacobs, M. Ottens, Model-based high-throughput process development for chromatographic whey proteins separation, Biotechnol. J. 7 (10) (2012) 1221–1232, doi:10.1002/biot.201200191.
[80] L.K. Shekhawat, M. Chandak, A.S. Rathore, Mechanistic modeling of hydrophobic interaction chromatography for monoclonal antibody purification: process optimization in the quality by design paradigm, J. Chem. Technol. Biotechnol. 92 (10) (2017) 2527–2537, doi:10.1002/jctb.5324.
[81] M. Moreno-González, D. Keulen, J. Gomis-Fons, G.L. Gomez, B. Nilsson, M. Ottens, Continuous adsorption in food industry: the recovery of sinapic acid from rapeseed meal extract, Sep. Purif. Technol. 254 (2021) 117403, doi:10.1016/j.seppur.2020.117403.
[82] J. Gomis-Fons, H. Schwarz, L. Zhang, N. Andersson, B. Nilsson, A. Castan, A. Solbrand, J. Stevenson, V. Chotteau, Model-based design and control of a small-scale integrated continuous end-to-end mAb platform, Biotechnol. Prog. 36 (4) (2020) e2995, doi:10.1002/btpr.2995.
[83] K. Westerberg, N. Borg, N. Andersson, B. Nilsson, Supporting design and control of a reversed-phase chromatography step by mechanistic modeling, Chem. Eng. Technol. 35 (1) (2012) 169–175, doi:10.1002/ceat.201000505.
[84] N. Andersson, A. Löfgren, M. Olofsson, A. Sellberg, B. Nilsson, P. Tiainen, Design and control of integrated chromatography column sequences, Biotechnol. Prog. 33 (4) (2017) 923–930, doi:10.1002/btpr.2434.
[85] M.M. Papathanasiou, F. Steinebach, M. Morbidelli, A. Mantalaris, E.N. Pistikopoulos, Intelligent, model-based control towards the intensification of downstream processes, Comput. Chem. Eng. 105 (2017) 173–184, doi:10.1016/j.compchemeng.2017.01.005.
[86] S. Liu, S. Gerontas, D. Gruber, R. Turner, N.J. Titchener-Hooker, L.G. Papageorgiou, Optimization-based framework for resin selection strategies in biopharmaceutical purification process development, Biotechnol. Prog. 33 (4) (2017) 1116–1126, doi:10.1002/btpr.2479.
[87] B.K. Nfor, D.S. Zuluaga, P.J.T. Verheijen, P.D.E.M. Verhaert, L.A.M. van der Wielen, M. Ottens, Model-based rational strategy for chromatographic resin selection, Biotechnol. Prog. 27 (6) (2011) 1629–1643, doi:10.1002/btpr.691.
[88] E.J. Close, J.R. Salm, D.G. Bracewell, E. Sorensen, A model based approach for identifying robust operating conditions for industrial chromatography with process variability, Chem. Eng. Sci. 116 (2014) 284–295, doi:10.1016/j.ces.2014.03.010.
[89] S. Vogg, T. Müller-Späth, M. Morbidelli, Design space and robustness analysis of batch and counter-current frontal chromatography processes for the removal of antibody aggregates, J. Chromatogr. A 1619 (2020) 460943, doi:10.1016/j.chroma.2020.460943.
[90] B.K. Nfor, T. Ahamed, G.W.K. van Dedem, P.D.E.M. Verhaert, L.A.M. van der Wielen, M.H.M. Eppink, E.J.A.X. van de Sandt, M. Ottens, Model-based rational methodology for protein purification process synthesis, Chem. Eng. Sci. 89 (2013) 185–195, doi:10.1016/j.ces.2012.11.034.
[91] J. Schmölder, M. Kaspereit, A modular framework for the modelling and optimization of advanced chromatographic processes, Processes 8 (1) (2020) 65.
[92] A. Hamidi, H. Kreeftenberg, L. van der Pol, S. Ghimire, L.A.M. van der Wielen, M. Ottens, Process development of a new haemophilus influenzae type b conjugate vaccine and the use of mathematical modeling to identify process optimization possibilities, Biotechnol. Prog. 32 (3) (2016) 568–580, doi:10.1002/btpr.2235.
[93] A. Löfgren, M. Yamanee-Nolin, S. Tallvod, J.G. Fons, N. Andersson, B. Nilsson, Optimization of integrated chromatography sequences for purification of biopharmaceuticals, Biotechnol. Prog. 35 (6) (2019) e2871, doi:10.1002/btpr.2871.
[94] T. Hahn, T. Huuk, V. Heuveline, J. Hubbuch, Simulating and optimizing preparative protein chromatography with ChromX, J. Chem. Educ. 92 (9) (2015) 1497–1502, doi:10.1021/ed500854a.
[95] S. Leweke, E. von Lieres, Chromatography analysis and design toolkit (CADET), Comput. Chem. Eng. 113 (2018) 274–294, doi:10.1016/j.compchemeng.2018.02.025.
[96] K. Meyer, S. Leweke, E. von Lieres, J.K. Huusom, J. Abildskov, ChromaTech: a discontinuous Galerkin spectral element simulator for preparative liquid chromatography, Comput. Chem. Eng. 141 (2020) 107012, doi:10.1016/j.compchemeng.2020.107012.
[97] F. Dismer, J. Hubbuch, 3D structure-based protein retention prediction for ion-exchange chromatography, J. Chromatogr. A 1217 (8) (2010) 1343–1353, doi:10.1016/j.chroma.2009.12.061.
[98] S. Parimal, S. Garde, S.M. Cramer, Interactions of multimodal ligands with proteins: insights into selectivity using molecular dynamics simulations, Langmuir 31 (27) (2015) 7512–7523, doi:10.1021/acs.langmuir.5b00236.
[99] S. Banerjee, S. Parimal, S.M. Cramer, A molecular modeling based method to predict elution behavior and binding patches of proteins in multimodal chromatography, J. Chromatogr. A 1511 (2017) 45–58, doi:10.1016/j.chroma.2017.06.059.
[100] J. Kittelmann, K.M.H. Lang, M. Ottens, J. Hubbuch, An orientation sensitive approach in biomolecule interaction quantitative structure–activity relationship modeling and its application in ion-exchange chromatography, J. Chromatogr. A 1482 (2017) 48–56, doi:10.1016/j.chroma.2016.12.065.
[101] J. Woo, S. Parimal, M.R. Brown, R. Heden, S.M. Cramer, The effect of geometrical presentation of multimodal cation-exchange ligands on selective recognition of hydrophobic regions on protein surfaces, J. Chromatogr. A 1412 (2015) 33–42, doi:10.1016/j.chroma.2015.07.072.
[102] J. Kittelmann, K.M.H. Lang, M. Ottens, J. Hubbuch, Orientation of monoclonal antibodies in ion-exchange chromatography: a predictive quantitative structure–activity relationship modeling approach, J. Chromatogr. A 1510 (2017) 33–39, doi:10.1016/j.chroma.2017.06.047.
[103] J.F. Buyel, J.A. Woo, S.M. Cramer, R. Fischer, The use of quantitative structure–activity relationship models to develop optimized processes for the removal of tobacco host cell proteins during biopharmaceutical production, J. Chromatogr. A 1322 (2013) 18–28, doi:10.1016/j.chroma.2013.10.076.
[104] K. Rege, M. Pepsin, B. Falcon, L. Steele, M. Heng, High-throughput process development for recombinant protein purification, Biotechnol. Bioeng. 93 (4) (2006) 618–630, doi:10.1002/bit.20702.
[105] A. Susanto, E. Knieps-Grunhagen, E. von Lieres, J. Hubbuch, High throughput screening for the design and optimization of chromatographic processes: assessment of model parameter determination from high throughput compatible data, Chem. Eng. Technol. 31 (12) (2008) 1846–1855, doi:10.1002/ceat.200800457.
[106] J.P. Welsh, M.G. Petroff, P. Rowicki, H. Bao, T. Linden, D.J. Roush, J.M. Pollard, A practical strategy for using miniature chromatography columns in a standardized high-throughput workflow for purification development of monoclonal antibodies, Biotechnol. Prog. 30 (3) (2014) 626–635, doi:10.1002/btpr.1905.
[107] T. Weigel, R. Soliman, M.W. Wolff, U. Reichl, Hydrophobic-interaction chromatography for purification of influenza A and B virus, J. Chromatogr. B 1117 (2019) 103–117, doi:10.1016/j.jchromb.2019.03.037.
[108] C. Ladd Effio, T. Hahn, J. Seiler, S.A. Oelmeier, I. Asen, C. Silberer, L. Villain, J. Hubbuch, Modeling and simulation of anion-exchange membrane chromatography for purification of Sf9 insect cell-derived virus-like particles, J. Chromatogr. A 1429 (2016) 142–154, doi:10.1016/j.chroma.2015.12.006.
[109] C. Boi, A. Malavasi, R.G. Carbonell, G. Gilleskie, A direct comparison between membrane adsorber and packed column chromatography performance, J. Chromatogr. A 1612 (2020) 460629, doi:10.1016/j.chroma.2019.460629.
[110] B.K. Nfor, N.N. Hylkema, K.R. Wiedhaup, P.D.E.M. Verhaert, L.A.M. van der Wielen, M. Ottens, High-throughput protein precipitation and hydrophobic interaction chromatography: salt effects and thermodynamic interrelation, J. Chromatogr. A 1218 (49) (2011) 8958–8973, doi:10.1016/j.chroma.2011.08.016.
[111] R.E. Lovrien, D. Matulis, Selective precipitation of proteins, Curr. Protoc. Protein Sci. 7 (1) (1997) 4.5.1-4.5.36, doi:10.1002/0471140864.ps0405s07.
[112] S. Zimmermann, S. Gretzinger, M.-.L. Schwab, C. Scheeder, P.K. Zimmermann, S.A. Oelmeier, E. Gottwald, A. Bogsnes, M. Hansson, A. Staby, J. Hubbuch, High-throughput downstream process development for cell-based products using aqueous two-phase systems, J. Chromatogr. A 1464 (2016) 1–11, doi:10.1016/j.chroma.2016.08.025.
[113] S.A. Oelmeier, F. Dismer, J. Hubbuch, Application of an aqueous two-phase systems high-throughput screening method to evaluate mAb HCP separation, Biotechnol. Bioeng. 108 (1) (2011) 69–81, doi:10.1002/bit.22900.
[114] B.C. Bussamra, D. Sietaram, P. Verheijen, S.I. Mussatto, A.C. da Costa, L. van der Wielen, M. Ottens, A critical assessment of the flory-huggins (FH) theory to predict aqueous two-phase behaviour, Sep. Purif. Technol. 255 (2021) 117636, doi:10.1016/j.seppur.2020.117636.
[115] S. Konstantinidis, E. Heldin, S. Chhatre, A. Velayudhan, N. Titchener-Hooker, Strategic assay deployment as a method for countering analytical bottlenecks in high throughput process development: case studies in ion exchange chromatography, Biotechnol. Prog. 28 (5) (2012) 1292–1302, doi:10.1002/btpr.1591.
[116] J. Havel, J.E. Madden, P.R. Haddad, Prediction of retention times for anions in ion chromatography using artificial neural networks, Chromatographia 49 (9) (1999) 481, doi:10.1007/BF02467746.
[117] E. Marengo, M.C. Gennaro, S. Angelino, Neural network and experimental design to investigate the effect of five factors in ion-interaction high-performance liquid chromatography, J. Chromatogr. A 799 (1) (1998) 47–55, doi:10.1016/S0021-9673(97)01027-3.
[118] D.C. Psichogios, L.H. Ungar, A hybrid neural network-first principles approach to process modeling, AIChE J. 38 (10) (1992) 1499–1511, doi:10.1002/aic.690381003.
[119] D. Nagrath, A. Messac, W.B. B, M.C. S, A. Hybrid, Model framework for the optimization of preparative chromatographic processes, Biotechnol. Prog. 20 (1) (2004) 162–178, doi:10.1021/bp034026g.
[120] S. Nikita, A. Tiwari, D. Sonawat, H. Kodamana, A.S. Rathore, Reinforcement learning based optimization of process chromatography for continuous processing of biopharmaceuticals, Chem. Eng. Sci. (2020) 116171, doi:10.1016/j.ces.2020.116171.
[121] G. Wang, T. Briskot, T. Hahn, P. Baumann, J. Hubbuch, Estimation of adsorption isotherm and mass transfer parameters in protein chromatography using artificial neural networks, J. Chromatogr. A 1487 (2017) 211–217, doi:10.1016/j.chroma.2017.01.068.
[122] M. Krippl, A. Dürauer, M. Duerkop, Hybrid modeling of cross-flow filtration: predicting the flux evolution and duration of ultrafiltration processes, Sep. Purif. Technol. 248 (2020) 117064, doi:10.1016/j.seppur.2020.117064.

[123] J. Markarian, Industry 4.0 in biopharmaceutical manufacturing, Biopharm Int. (2018) 31.
[124] R.M.C. Portela, C. Varsakelis, A. Richelle, N. Giannelos, J. Pence, S. Dessoy, M. von Stosch, When is an *in silico* representation a digital twin? in: A Biopharmaceutical Industry Approach to the Digital Twin Concept, Digital Twins, Springer Berlin Heidelberg, Berlin, Heidelberg, 2020, pp. 35–55, doi:10.1007/10_2020_138.
[125] J.F. Kramarczyk, B.D. Kelley, J.L. Coffman, High-throughput screening of chromatographic separations: II. Hydrophobic interaction, Biotechnol. Bioeng. 100 (4) (2008) 707–720, doi:10.1002/bit.21907.
[126] B.D. Kelley, M. Switzer, P. Bastek, J.F. Kramarczyk, K. Molnar, T. Yu, J. Coffman, High-throughput screening of chromatographic separations: IV. Ion-exchange, Biotechnol. Bioeng. 100 (5) (2008) 950–963, doi:10.1002/bit.21905.
[127] C. Stamatis, S. Goldrick, D. Gruber, R. Turner, N.J. Titchener-Hooker, S.S. Farid, High throughput process development workflow with advanced decision-support for antibody purification, J. Chromatogr. A 1596 (2019) 104–116, doi:10.1016/j.chroma.2019.03.005.
[128] B.K. Nfor, T. Ahamed, M.W.H. Pinkse, L.A.M. van der Wielen, P.D.E.M. Verhaert, G.W.K. van Dedem, M.H.M. Eppink, E.J.A.X. van de Sandt, M. Ottens, Multi-dimensional fractionation and characterization of crude protein mixtures: toward establishment of a database of protein purification process development parameters, Biotechnol. Bioeng. 109 (12) (2012) 3070–3083, doi:10.1002/bit.24576.
[129] A.J. Pollard, E.M. Bijker, A guide to vaccinology: from basic principles to new developments, Nat. Rev. Immunol. 21 (2) (2021) 83–100, doi:10.1038/s41577-020-00479-7.
[130] R. Rappuoli, Bridging the knowledge gaps in vaccine design, Nat. Biotechnol. 25 (12) (2007) 1361–1366, doi:10.1038/nbt1207-1361.
[131] B. Donaldson, F. Al-Barwani, V. Young, S. Scullion, V. Ward, S. Young, Virus-like particles, a versatile subunit vaccine platform, Subunit Vaccine Deliv. (2014) 159–180, doi:10.1007/978-1-4939-1417-3_9.