Accelerating Vaccine Manufacturing Development through Model-Based Approaches: Current Advances and Future Opportunities

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Accelerating vaccine manufacturing development through model-based approaches: current advances and future opportunities

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This review highlights the importance of model-based approaches in accelerating vaccine manufacturing process development. The challenges of scaling up from laboratory to commercial processes are addressed through the adoption of Process Analytical Technology frameworks and Quality by Design principles. The application of various modeling approaches beyond downstream and upstream processes in vaccine production is discussed in detail. These *in silico* process simulation approaches enable deeper understanding of manufacturing dynamics, identification of critical process parameters, and the development of well-defined design spaces, ultimately leading to accelerated vaccine development and improved product quality. The authors stress the significance of an integrated modeling platform for vaccine manufacturing, exemplified by the Inno4Vac project. This initiative seeks to develop a comprehensive computational platform for vaccine manufacturing and stability testing, with a particular focus on stakeholder engagement and collaboration with regulatory bodies to ensure the acceptance and implementation of the platform.

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Introduction

Vaccine administration is widely recognized as one of the most valuable healthcare interventions for routine immunization and outbreaks management [1]. The recent COVID-19 pandemic has underscored the critical importance of developing efficient and effective pharmaceutical manufacturing processes to produce vaccines that are affordable, available at scale, and widely accessible in a short amount of time.

Despite the remarkable advancements in vaccine discovery science since its discovery in the early 19th century [2], the task of effectively scaling up laboratory-based processes for commercial manufacturing continues to pose a significant challenge [3]. The manufacturing process plays a crucial role in preserving the essential

properties identified during vaccine discovery, enabling the production of substantial quantities under tightly controlled conditions for clinical trials and subsequent market supply. The complexity and inherent variations in the manufacturing process, coupled with stringent regulatory requirements governing the biological and chemical components, manufacturing processes, testing, release procedures, and determination of the product expiry period, contribute to the lengthy and costly nature of vaccine production. Process development alone can account for up to 30% of the time needed for successful market introduction of a vaccine [4]. The three COVID vaccine approvals within a year of the pandemic's start highlight technology's accelerating role and regulatory adoptability in emergent situations.

To enhance the efficiency of pharmaceutical manufacturing, regulatory bodies such as the U.S. Food and Drug Administration (FDA) and European Medicine Agency (EMA) have encouraged the adoption of innovative methodologies through Process Analytical Technology (PAT) frameworks and implementation of Quality by Design (QbD) principles [5]. These frameworks underscore the importance of knowledge-based tools that enable manufacturers to better understand, predict, and assure the quality of their products during development process and manufacturing. In this context, emerging modeling tools such as hybrid modeling [6,7], machine learning [8,9], and digital twins [10,11] are evaluated for their potential benefits. These approaches rely on *in silico* process simulation, which is based on mathematical representation of the manufacturing process. By simulating the manufacturing process, one can gain a deeper understanding of the underlying dynamics and identify critical process parameters (CPPs) that have a significant impact on the quality of the final product, and their relationship to critical quality attributes (CQAs). With a well-defined design space, processes can be optimized without requiring additional approvals [12]. Thus, accurate and reliable process simulation models can enhance the development of robust vaccine manufacturing processes, ensuring product quality and stability, and reducing development time.

Model-based approaches have been widely applied in diverse biomanufacturing domains to enhance the efficiency of production [13,14]. However, in the context of vaccine biomanufacturing, the adoption of these methods has not kept pace, despite the existence of novel platform technologies for upstream and downstream processes in the biopharmaceutical industry [15–17]. This disparity can be attributed, in part, to the ever-evolving landscape of vaccine types, ranging from whole organisms to purified macromolecules, combined antigens, recombinant vectors, synthetic peptides, DNA, or RNA [2]. Regulatory concerns regarding the impact of models on CPPs further contribute to this constraint [18]. However, the application of modeling tools in vaccine manufacturing is emerging as the biotechnology sector increasingly embraces the QbD principles.

The aim of this publication is to provide an overview of recent advances in model-based approaches for the purpose of improving process development in vaccine manufacturing, and to give an outlook on how an integrated model-based approach can influence vaccine manufacturing in the future.

Vaccine biomanufacturing process development (state-of-the-art)

Vaccine manufacturing

Vaccine production, related to the active component or the antigen, involves a sequence of unit operations designed to transform starting materials to a final product of the required purity. The specific sequence and composition of the components vary depending on the characteristics of the vaccines. Figure 1 outlines a generic flowsheet diagram for vaccine manufacturing and shows an example of the key stages for an *E. coli*-expressed antigen process. The process involves upstream cell cultivation (fermentation) and isolation through centrifugation or filtration, followed by product recovery (lysing) and downstream purification through centrifugation (or sequential membrane filtration) and chromatographic steps to reach the desired purity. The purified drug/substance can then be used for the formulation/stabilization process and the final fill and finish stage. A more detailed description of the specific stages for certain vaccine types can be found elsewhere [1].

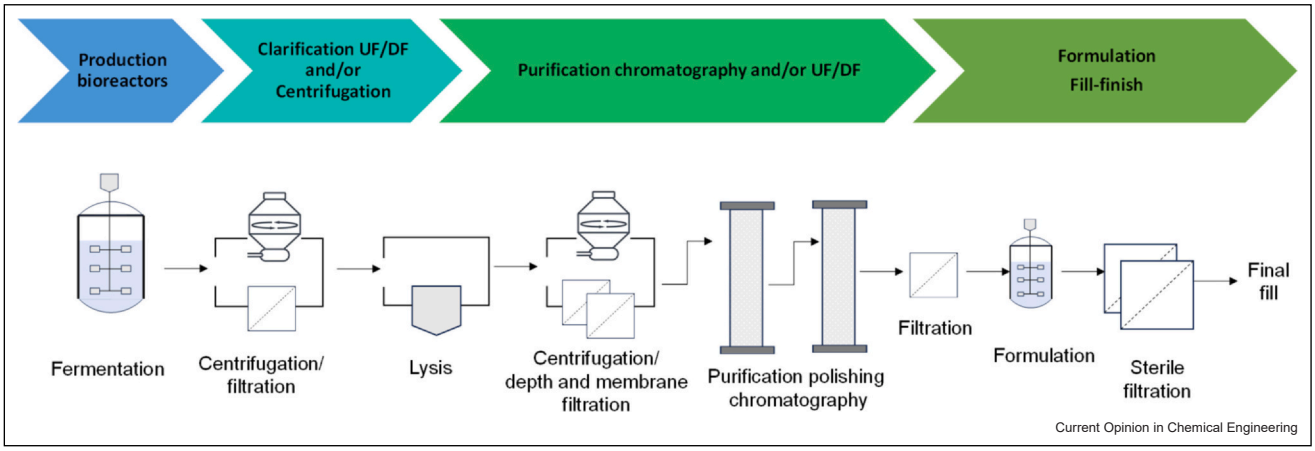
Vaccine manufacturing is a complex process influenced by several factors, including the intricate nature of underlying processes and unit operations, as well as the diverse range of equipment setups at different scales and for different vaccine types. Additionally, the dynamic operation modes at various production stages, such as fed-batch fermentation and batch purification, contribute to the complexity. These complexities are currently being addressed in process modeling research and development, as outlined in this review.

Process development

The objective of vaccine manufacturing process development is to identify an optimum design that can consistently achieve production and purity targets with limited costs and time, while adhering to regulatory standards.

In line with the PAT framework and QbD principles, process development begins with heuristic risk analysis and qualitative assessment to screen parameters. This is succeeded by scale-down multivariate experiments, utilizing statistical methods to define the design space. Subsequent scale-up studies, encompassing monitoring

Figure 1



Generic flow diagram for vaccine antigen (upper diagram) and an example of an *E. coli*- expressed antigen production (lower flowsheet). UF: ultrafiltration, DF: diafiltration.

and control, ensure replicable and consistent process performance at commercial scales.

The upstream process involves fermentation of starting material, which, in most cases, is generated based on cell bank derivation and characterization guidelines (ICH Q5D [19]). The objective of upstream process development is to increase product yield and expression rate while maintaining quality by evaluating and optimizing cell metabolism and fermentation process conditions [20]. This is typically done using a scale-down approach, where the impact of commercial-scale dynamic conditions on cell metabolism is studied in laboratory setups and the information is used for scale-up optimization and validation [21].

Downstream process development, with chromatography as the main step, aims to achieve a target purification level and involves evaluation of optimal resin type, buffer conditions that allow optimal binding of proteins to resins, optimal elution conditions, and process robustness [22]. The design space is typically explored using empirical approaches involving laboratory experiments [23].

The scale-down experimentation approach (for both upstream and downstream processes) enables the use of advanced high-throughput experimentation (HTE) methods to screen a larger number of process parameters using design of experiments (DoE) and response surface model [24,25]. However, this approach requires significant experimental efforts, offers limited process understanding during the development phase, and might even lead to a suboptimal process design as the scale-down setups might lack accurate representation of conditions in the large-scale. Moreover, while the complexity of the purification process is greatly influenced by cell cultivation conditions, it is common practice to optimize upstream and downstream processes separately [23], which further contributes to a suboptimal design. Considering the highly dimensional parameter space across multiple unit operations, the experimental studies must be complemented with more advanced model-based tools to generate deeper process understanding, and efficiently explore the design space.

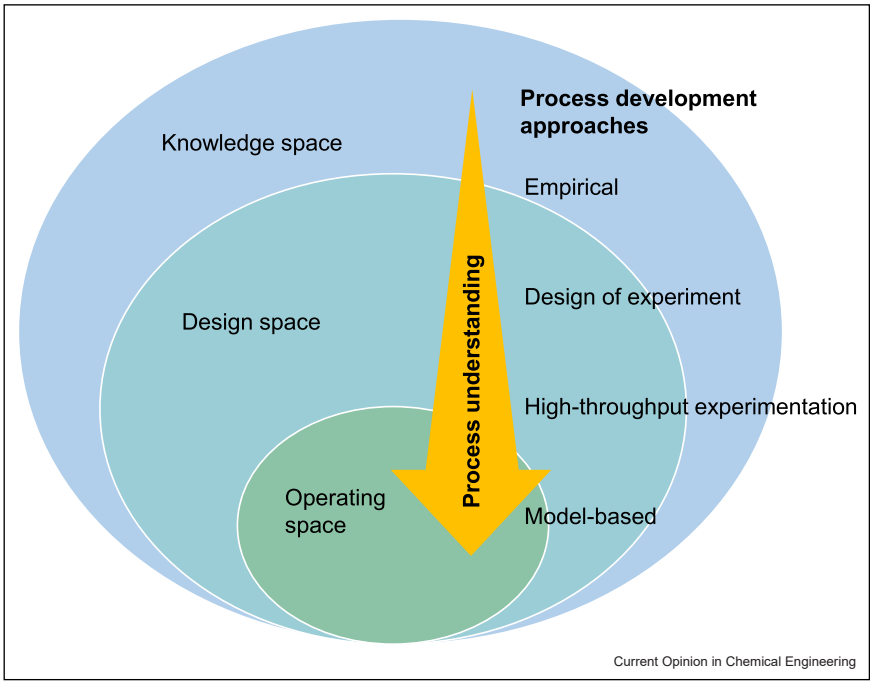
Figure 2 illustrates the various approaches employed throughout process development, emphasizing the necessity of process understanding when transitioning from the knowledge space to the operating space. In this context, validated models serve as valuable tools for bridging the gap between theoretical insights and practical implementation.

Modeling for process development in vaccine manufacturing

Potential benefits of modeling

Modeling offers several distinct advantages in the context of process development. It provides a robust framework for understanding the underlying mechanisms of the process. By capturing the relationships between CPPs and CQAs, models offer valuable insights into the system dynamics and behavior [26]. Additionally, modeling serves as a valuable aid in planning and designing experimental setups [27] and in evaluating the value propositions and risks associated with new products and processes under different scenarios [28]. Moreover, validated models can be used for the development of advanced control strategies in later stages of process development [29]. In recent years, models are also increasingly used to predict environmental impact of (parts of) a process [30].

Figure 2



Process development approaches for vaccine production to establish the design and operating space.

Models have far-reaching implications beyond process development, particularly in the manufacturing stage. They are the key elements of digital twin and real-time model-based control strategies and can significantly improve the efficiency of manufacturing processes when integrated with real-time data [10]. However, currently, there are limitations in applying models for online monitoring and control of biopharmaceuticals as they become subject to FDA and EMA software regulations due to their direct impact on CPPs [18].

Modeling approaches

Modeling approaches span a wide range, including mechanistic models based on fundamental principles [12,15,20], empirical models derived from experimental observations as well as some mechanistic assumptions [23,31], and data-driven models that utilize statistical methods or machine learning algorithms to establish relationships or identify patterns from large datasets [32,33]. Hybrid modeling, which combines elements from different approaches (e.g. incorporating data-driven components into mechanistic models), is an emerging modeling approach for biopharmaceutical processes [6,7].

The degree to which a mechanistic or data-driven modeling approach can be applied for process development depends on the extent of knowledge about the underlying processes and the availability of data. While mechanistic models offer high predictability, they can pose computational challenges in describing complex processes mathematically and entail difficulties in determining key parameters. Consequently, the development of mechanistic models requires extensive calibration and validation efforts, as well as labor-intensive improvements when new knowledge about the mechanism arises.

Data-driven models, on the other hand, while circumventing the described challenges of mechanistic models, rely heavily on experimental data for their development and training. Therefore, they are restricted to predicting within the boundaries of experimental observations and their accuracy and reliability highly depend on both the quality and the representativeness of the data [34]. Additionally, interpretability is a challenge as they lack explicit physical explanations. There is however an increasing interest in developing interpretable and explainable artificial intelligence [35]. To address these limitations, hybrid modeling has emerged as an effective approach combining the predictive capabilities of mechanistic models with the efficiency of data-driven models. This integration allows for minimizing the amount of data needed for model development while ensuring the inclusion of physical relevance to key processes [7,36].

In the context of bioreactor modeling, various scales and degrees of complexity — from macro to micro and

extracellular to intracellular — are considered to study and predict scale-up-related mechanisms such as hydrodynamics, population heterogeneity, cell metabolism, and biological adaptation. At the molecular level, highly predictive mechanistic models such as metabolic network models and flux balance analysis are employed to capture the influence of various operating conditions (e.g. temperature, pH, dissolved oxygen, agitation, perfusion rate, and media supplements) on cellular physiology, productivity, and/or expression [20]. To address the complexity of metabolic models with regard to parameterization, lumping and pooling techniques are developed [14]. The simplest cell models are the black box (unstructured) models, such as Monod-based kinetic models, which do not consider intracellular kinetics. These models have constant yields and may not accurately predict fed-batch or batch processes as compared with the metabolic (structured) models. To account for population heterogeneity (i.e. cell-to-cell variations such as mass, age, and internal metabolism), population balance models are mainly used [21]. To describe CQAs such as glycosylation patterns, hybrid kinetic models are utilized [37]. These hybrid kinetic models combine known mechanistic knowledge with machine learning methods to identify data-driven functional representations of kinetics and cell regulation. Constructing these models from data necessitates well-prepared and unified data collections along with computationally demanding training procedures [36]. To model large-scale bioreactors, computational fluid dynamics (CFD) models are coupled with (metabolic) kinetic models to predict process yield and productivity in the presence of environmental heterogeneities [38]. To address computational demands, CFD-based compartment models have been developed [39]. CFD and compartment models can be used for regime analysis to design more representative scale-down setups in simulating large-scale conditions in the laboratory [40].

Chromatography modeling encompasses both empirical and mechanistic approaches. Empirical methods rely on conducting experiments using DoE and HTE techniques [23]. On the other hand, mechanistic modeling delves into the physical chemistry principles underlying the process. This includes studying transport phenomena at the column and system levels and considering diffusion, adsorption kinetics, and equilibrium at the particle level [41]. Additionally, process configuration factors such as valve switching times and elution gradients are incorporated into the models [42]. To investigate flow distribution in chromatography columns at commercial scales, high-definition CFD models are developed [43,44]. In chromatography modeling, the adsorption behavior is essential, capturing the adsorption difference between impurities and the product. Adsorption description must capture the separation in a multidimensional space, including parameters such as feed and buffer compositions, ligand and resin properties, temperature, pH, salt, and so on. During the initial stages of downstream processing, particularly in centrifugation and capture, the feed and processes are complex and therefore descriptions need to be simplified. However, as we progress to later stages, such as polishing, detailed adsorption of small quantities of process and product-related impurities becomes essential.

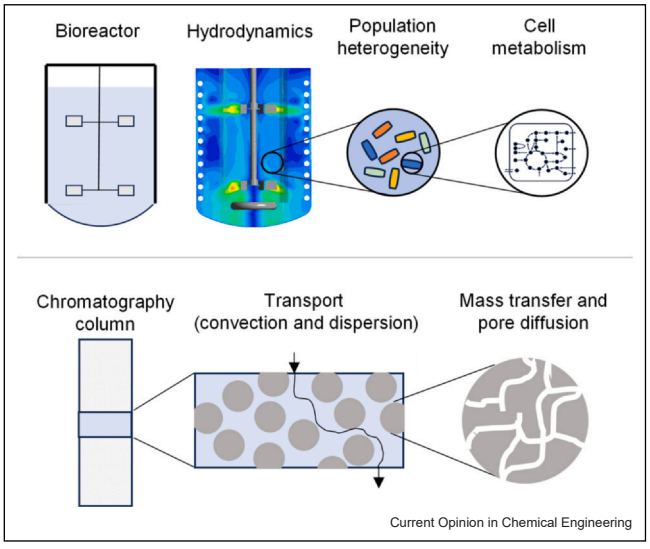
Later in vaccine development, expiration periods are determined based on guidelines such as ICH Q1 and 5C [45]. These guidelines, initially designed for small molecules, are now unfit for rapid vaccine development as they reply on simple linear models and demand extensive historical data on vaccine stability. In contrast, modern modeling methodologies such as Bayesian hierarchical models [46], or advanced kinetic models integrating covariates of several sources of variability [47], allow to get a better understanding of the product's long-term stability and enable accelerated stability studies.

Employing diverse modeling approaches across process development stages can effectively characterize the multiscale, multidisciplinary mechanisms in vaccine manufacturing (see Figure 3). Mechanistic models allow driving process development from early stages reducing timeline and costs associated with large-scale activities. Subsequently, more data-dependent and surrogate models (e.g. hybrid and reduced mechanistic models) can play a significant role during later stages of development, primarily for optimization, monitoring, and control purposes (e.g. development of soft sensors [48]). Hybrid modeling can significantly reduce the number of experimental iterations required throughout process development. This is particularly relevant for vaccine process development, given the complexity of the processes involved and the resource-intensive nature of data generation.

Available simulation software

As a result of advances in algorithms and computer technology, advanced modeling approaches have been applied in the biotech industry, leading to the development of several commercial and noncommercial simulators for modeling biopharmaceutical processes. Commercial tools such as SuperPro Designer and BioPro Designer by Intelligen, Inc. (https://www.intelligen.com/), BioProcess Simulator and Aspen Chromatography by Aspen Technology (https://www.aspentech.com/), GoSilico by Cytiva (https://www.gosilico.com/), Ypso-Ionic by YpsoFacto (https://www.ypsomed.com/en/), BioSolve Process by BioPharm (https://www.biopharmservices.com/), BioContinuum by Merck (https://www.merckgroup.com/), and gPROMS by Siemens process systems engineering (PSE) (https://www.psenterprise.com/) have been designed to enable experimentalists to conduct simulations or flowsheet

Figure 3



Overview of multiscale and multidisciplinary nature of the underlying mechanisms in bioreactors (upper illustration) and chromatography columns (lower illustration).

simulations without requiring programming expertise. These tools are primarily used for screening different processing schemes based on feasibility and profitability. In contrast, academic tools, although requiring some programming knowledge, enable researchers to explore diverse modeling approaches and address specific research questions. For instance, the open-source tool CADET (https://cadet.github.io) [49] serves as a powerful solver for various models, such as column transport, adsorption isotherms, and chemical reactions. It also provides functionalities for process analysis, parameter estimation, and optimization.

Outlook: toward an integrated modeling platform

The life science industry has experienced a shift toward open-sourceness and collaboration, with biotech and biopharma companies actively partnering with research institutes to foster open-source model development initiatives. There is a growing emphasis on adopting non-proprietary software and establishing a common platform for early-stage model development to facilitate effective communication among experts and researchers, while intellectual property rights are still maintained for modeling activities with significant business impact.

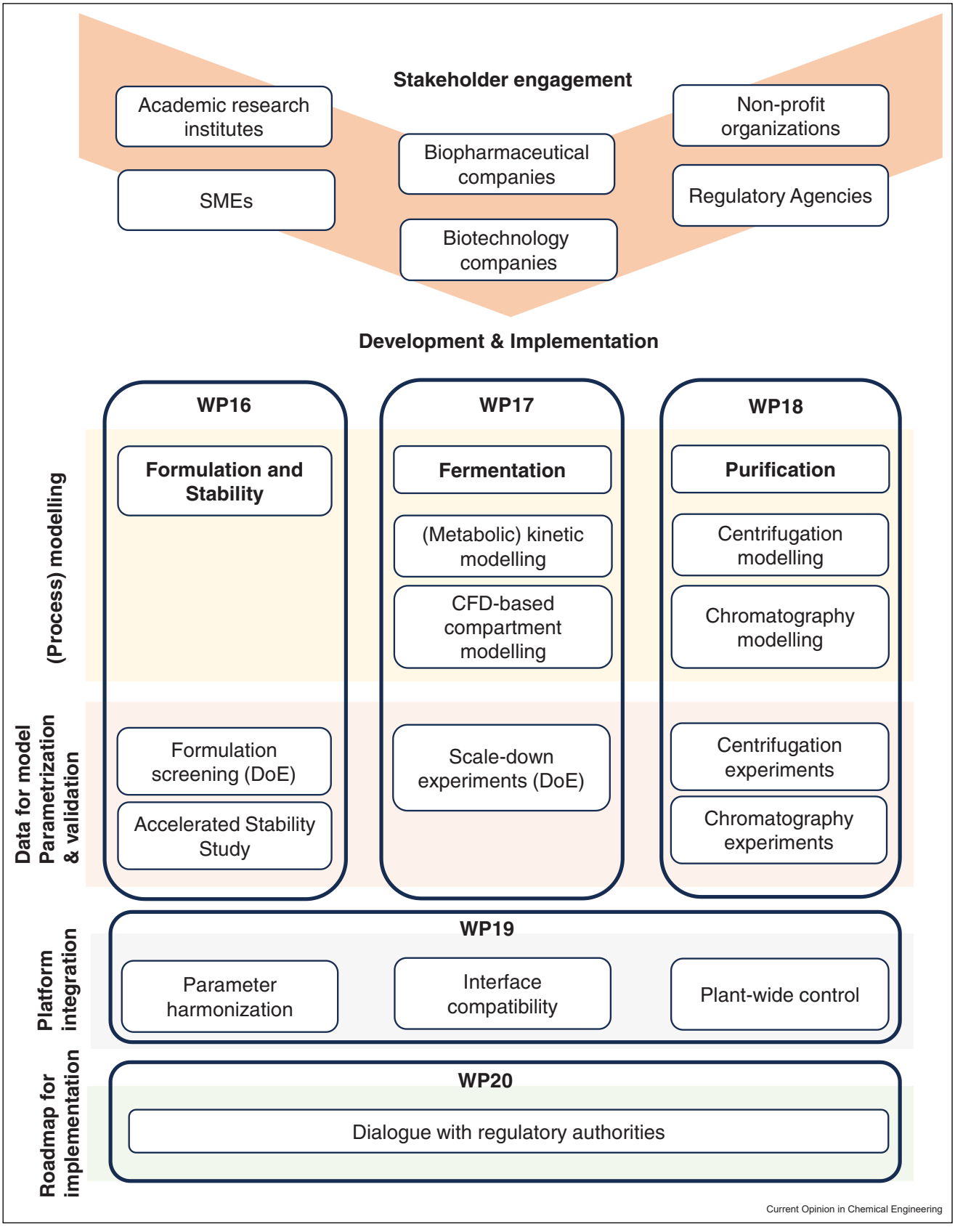
This trend is reflected in the area of bioprocess modeling, particularly in bioreactor/metabolic modeling and purification (mainly chromatography), which has evolved from an academic setting to commercial and open-source solutions. However, the integration of models remains a substantial challenge because a) the modeling approaches to individual operations were designed to help develop process understanding of those unit operations, not for whole bioprocess modeling, b) the system complexity is high, leaving the bioreactor requiring understanding of the cell population and how it will interact with the operations before chromatographic purification, such as cell lysis and solid–liquid separation. This complexity makes fully mechanistic modeling impractical, especially when the purpose is to predict formation of impurities. Consequently, some level of empirical evidence is required, which may arise from activities related to process development and/or measurement taken throughout the process. The latter area is growing steadily [50] with the PAT initiative given prominence by the FDA nearly 20 years ago.

Having a connected approach to modeling a bioprocess is critical not only for the concept of a digital twin but also for the QbD philosophy. The material complexity during harvest introduces substantial batch-to-batch variation, making it essential to understand CPPs. To achieve an integrated model, a systematic and holistic approach is necessary for harmonization of the unit operations. This includes interface compatibility, availability and robustness of parameter outputs and inputs, production scales, as well as modes of operation, for example, feed strategies in bioreactors and specific sequences of operations in a series of chromatography columns. In fact, failure to couple the unit operations together in a simulation model is considered to be one of the main risks of bioprocess modeling, because models for bioreactors and downstream operations typically focus on completely different sets of variables. Thus, in order to mitigate the potential failures in developing an integrated model-based platform for vaccine manufacturing, it is essential to comprehensively assess the issues related to heterogeneity, inconsistency, and varying model accuracy across unit processes.

These aspects have been addressed by the ongoing Inno4Vac project (www.inno4vac.eu) [51]. The project's acronym stands for Innovations to accelerate vaccine development and manufacture. While encompassing various relevant topics, including effective vaccine epitope prediction tools and the development of novel nonanimal and human infection models, a key focus of Inno4Vac in subtopic 4 (ST4) is the establishment of a modular open-source computational platform for *in silico* modeling of protein subunit vaccine biomanufacturing and stability testing. The ST4 consists of five work packages (WP16–20, see Figure 4) to achieve the following objectives:

- WP16: Develop stability prediction models for vaccine manufacturing using linear and nonlinear equations and integrate them into a global biomanufacturing platform.

Figure 4


Stakeholder engagement
Academic research institutes
SMEs
Biopharmaceutical companies
Biotechnology companies
Non-profit organizations
Regulatory Agencies
Development & Implementation
WP16
WP17
WP18
(Process) modelling
Formulation and Stability
Fermentation
Purification
(Metabolic) kinetic modelling
CFD-based compartment modelling
Centrifugation modelling
Chromatography modelling
Data for model Parametrization & validation
Formulation screening (DoE)
Accelerated Stability Study
Scale-down experiments (DoE)
Centrifugation experiments
Chromatography experiments
Platform integration
WP19
Parameter harmonization
Interface compatibility
Plant-wide control
Roadmap for implementation
WP20
Dialogue with regulatory authorities
Current Opinion in Chemical Engineering

Overview of elements in establishing an integrated modeling platform within ST4 of Inno4vac project [51]. See text for further information on the objectives for each work package. (CFD; computational fluid dynamics, SMEs; small-to-medium enterprises).

- WP17: Establish a cloud-based platform to assess the performance and robustness of biomanufacturing processes, with a specific emphasis on scaling up and down in the production of vaccines using *E. coli*.
- WP18: Create digital twins for key purification units, apply advanced *in silico* analysis and design tools, and enable real-time control to optimize downstream processing, especially for scale changes.
- WP19: Validate the predictive capabilities of *in silico* models for vaccine stability, unit operations in protein subunit vaccine manufacturing, and control modules developed in WP18.
- WP20: Initiate a regulatory dialog to engage with authorities, paving the way for the future inclusion of predictive modeling in chemistry, manufacturing, and control (CMC) dossiers for vaccines.

Figure 4 illustrates the key elements involved in each work package of ST4 in the Inno4vac project in establishing an integrated modeling platform for vaccine manufacturing process development. This involves stakeholder engagement, process modeling, parameterization and validation with experimental and real-world data, integration of all models, and a regulatory roadmap.

Platform development

By integrating upstream processes such as hydrodynamic and metabolic bioreactor modeling, with downstream processes such as centrifugation and chromatography, the platform provides comprehensive insights into vaccine production. One of the key objectives is to establish robust interfaces among the packages, ensuring seamless data flow from upstream models into downstream models. Furthermore, the platform encompasses an overall control strategy as well as a module to facilitate the prediction of pharmaceutical product stability, thus aiming to offer a holistic solution for the optimization of vaccine manufacturing processes. The infrastructure of Inno4Vac includes a JupyterHub as a versatile cloud-based platform to integrate various modeling packages in CADET (https://cadet.github.io). It enables code execution in multiple languages, such as Python, C++, and R, within an interactive environment. The platform also provides user management features, facilitating collaboration among multiple users. Inno4Vac serves as a demonstration of the potential of *in silico* modeling in vaccine manufacturing, offering a roadmap for further developments.

Stakeholder engagement

Stakeholder involvement is crucial to the development and acceptance of effective models that support the design, manufacture, and testing of innovative vaccines, while simultaneously accelerating their availability to the populations who need them the most. To achieve this ambitious goal, the Inno4Vac project was designed to foster public–private collaboration with an engagement strategy of involving key stakeholders of vaccine development, manufacturing, and approvals as official project partners. The main project stakeholders comprise prominent global pharmaceutical companies, small- and medium-sized enterprises, academic institutions, nonprofit organizations, and regulatory agencies: all of whom play a vital role in shaping the project's outcomes.

Early dialog between model developers and regulators is essential to achieving the highest probability of project success. Currently, regulators are expanding their knowledge and understanding of general modeling principles, model abilities to conform to regulatory expectations (including dossier requirements), and their utility to inspections (e.g. link to Good Modeling Practice information and change management under the companies' Pharmaceutical Quality System). A common concern regarding model implementation is the potential for added regulatory burdens, particularly due to limited awareness at the National Regulatory Agencies (NRAs). This is especially relevant during product registration and lifecycle management, including global registration. The Quality Innovation Group Listen and Learn focus group recently discussed this concern in relation to topics such as continuous and decentralized manufacturing [52]. In the field of stability prediction of pharmaceutical products, several groups at Coalition for Epidemic Preparedness and Innovations, The European Federation of Pharmaceutical Industries and Associations, and Biotechnology Industry Organization currently work on integration of stability modeling in international guidelines, leveraging dialog between industry, regulators, and institutions.

In this emerging field, ongoing developer–regulator dialog is crucial to clarify model objectives, data expectations for regulatory files, and enhance mutual understanding [53]. To boost regulatory awareness among modelers and facilitate regulators' grasp of model use in vaccine development, Inno4Vac organized a dedicated regulatory workshop with key NRAs and is currently planning a follow-up to share further progress in this area. Furthermore, continuous external regulatory monitoring identifies opportunities and landscape changes for future external utilization of the platform/models. External experts are engaged proactively to address potential future regulatory hurdles during the development

process. Communication, documentation, and test datasets are included in the strategy to enhance accessibility and usage of the resulting platform.

Conclusions

Model-based approaches offer significant opportunities for enhancing vaccine production. Current advances in modeling biopharmaceutical processes can address the multiscale and multidisciplinary nature of the underlying mechanisms. They provide powerful tools that can significantly reduce the cost and time in the development stage and facilitate scaling-up and transfer of bioprocesses to other manufacturing sites.

The value of developing an integrated modeling platform for biopharmaceutical manufacturing is exemplified by the Inno4Vac (www.inno4vac.eu) project. While focusing on model development and integration for upstream and downstream processes, the project emphasizes stakeholder engagement and proactive regulatory dialog as essential elements in facilitating the implementation of model-based approaches. To ensure regulatory acceptance, novel guidelines and internationally harmonized protocols are necessary as digitalization of manufacturing advances. Efforts by regulatory bodies such as EMA and FDA to address modeling use and deployment are underway (e.g. [52]).

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships that may be considered as potential competing interests: Krist V. Gernaey reports financial support was provided by Innovative Medicines Initiative 2 Joint Undertaking. This project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 101007799. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA (see also www.inno4vac.eu) All authors have an ongoing collaboration in the frame of this grant agreement, and the paper can be considered as one of the scientific outputs of the project.

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