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Quality by design and techno-economic modelling of RNA vaccine production for pandemic-response

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Abstract

Vaccine production platform technologies have played a crucial role in rapidly developing and manufacturing vaccines during the COVID-19 pandemic. The role of disease agnostic platform technologies, such as the adenovirus-vectored (AVV), messenger RNA (mRNA), and the newer self-amplifying RNA (saRNA) vaccine platforms is expected to further increase in the future. Here we present modelling tools that can be used to aid the rapid development and mass-production of vaccines produced with these platform technologies. The impact of key design and operational uncertainties on the productivity and cost performance of these vaccine platforms is evaluated using techno-economic modelling and variance-based global sensitivity analysis. Furthermore, the use of the quality by digital design framework and techno-economic modelling for supporting the rapid development and improving the performance of these vaccine production technologies is also illustrated.

Keywords: techno-economic modelling; Quality by Design (QbD) modelling; process development; RNA vaccine production.

1. Introduction

Process Systems Engineering tools have a lot to offer and are not applied to their full potential in vaccine and biopharmaceutical product-process development, and during production process operation. Over the past decades, substantial progress has been made in the field of computational modelling and mechanistic, dynamic, machine learning and hybrid models have been successfully implemented in various manufacturing fields, outside of vaccine and biopharmaceutical manufacturing. These digital tools have been used to create a digital replica (or digital twins) of the manufacturing process. Vaccine and biopharmaceutical production are lagging behind in digitalisation, because vaccines and biopharmaceuticals are conventionally produced using cell-based processes that, due to their inherent complexity and variability, have been challenging to model. In addition, vaccine manufacturing is highly regulated, and improvements are not implemented rapidly to avoid the risk of negatively impacting product quality, safety, and efficacy. Modelling of complex biological systems, digitalisation, real-time monitoring, process control, automation, and knowledge-rich regulatory submissions are hindered by the lack

of real-time or near-real-time hardware sensors for measuring vaccine and biopharmaceutical quality attributes. This is because several critical quality attributes (CQAs) and parameters are difficult, time-consuming, or expensive to measure or estimate in real-time, drastically limiting the information available for developing computational models. To overcome these limitations, software sensors are being developed.

To our knowledge, the quality of vaccines is currently assured without taking advantage of digitalisation and is currently tested after every production batch. Batches that fail to yield the product quality specifications are discarded, wasting valuable resources. Quality assurance could be improved by real-time product quality monitoring and by using model-predictive control. Assuring product quality with such digital tools would fit perfectly into the QbD framework (CMC-Vaccines Working Group, 2012). The use of the QbD framework is supported by regulatory authorities for systematic co-development of the vaccine product, vaccine production processes and of the process control strategies, based on sound science and quality risk management (ICH Expert Working Group, 2009). As far as we know, a full QbD framework has not been implemented for this purpose. The QbD framework combined with digital tools is also referred to as the Quality by Digital Design (QbDD) framework and this has the potential to replace quality by testing with assuring product quality by the design and operation of the production process.

Besides QbD modelling, techno-economic modelling also offers a valuable tool for assessing the productivity and cost profile of the holistic production process (Ferreira and Petrides, 2021; Ferreira et al., 2021; Kis et al., 2021a, 2021b, 2020; Pereira Chilima et al., 2020). Moreover, this process-cost modelling approach also helps to identify the production bottlenecks, and then de-bottlenecking approaches are evaluated to increase process performance. Techno-economic modelling is also used to evaluate various scenarios, for example different downstream configurations, at different production scales to identify the process configuration that leads to maximum productivity and lowest cost (Kis et al., 2021a, 2021b, 2020). Additionally, uncertainty and sensitivity analysis is performed combined with techno-economic modelling, to identify how the co-variation of many uncertainties would impact production throughputs and resource requirements (Kis et al., 2021b).

In this work, we showcase the use of the QbDD framework together with techno-economic modelling to guide the development and operation of new vaccine production platform technologies, such as the messenger RNA (mRNA), self-amplifying RNA (saRNA) and adenoviral vectored (AVV) vaccine platforms.

2. The AVV, mRNA and saRNA vaccine production platform technologies

The AVV production process was modelled based on the manufacturing of the replication-deficient chimpanzee adenovirus-vectored (ChAdOx1) vaccine which was co-developed by Oxford University and AstraZeneca plc (Kis et al., 2021b). The ChAdOx1 production process starts with preparing the HEK293 cell seed train and the adenovirus inoculum seed train. For this, the HEK293 cells are cultured at increasing volumes until the culture amounts required for the production bioreactor (commonly at 2000 L working volume) scale are obtained. These cells are then infected with the adenovirus which was genetically modified to express the SARS-CoV-2 spike protein. Following virus replication in HEK293 cells in the bioreactor, the virus culture and cell culture enter the downstream purification, whereby cells are initially lysed then the larger

impurities are removed using microfiltration. Next, tangential flow ultrafiltration/diafiltration is carried out, followed by an ion-exchange chromatography step. After this, the adenoviral vector solution is sterile filtered, and the buffer can be exchanged for the formulation buffer, using tangential flow ultrafiltration/diafiltration. Subsequently, the adenovirus vaccine drug substance (active ingredient) is formulated and filled into vials or other containers, often at a different facility / location (Kis et al., 2021b).

The mRNA and saRNA vaccines (collectively referred to as RNA vaccines) are synthesised using the T7 RNA polymerase based on a DNA template in the *in vitro* transcription reaction, which is usually completed in 2 hours, substantially faster than AVV production (Kis et al., 2021b). Following RNA synthesis, the plasmid DNA is digested using the DNase I endonuclease enzyme. Next, the RNA is purified out of the reaction mix using a series of conventional filtration- and chromatography-based unit operations (Kis et al., 2021a, 2020). These can include tangential flow ultrafiltration and diafiltration combined with one or two of the following chromatography techniques: ion-exchange, reverse-phase, oligo dT affinity, hydroxyapatite, hydrophobic interaction, multimodal hydrogen bonding and anion exchange, cellulose-based, and multimodal core-beads. After the RNA is purified out of the enzymatic reaction mix, the RNA is encapsulated in lipid nanoparticles. For this, the four lipid components contained in an ethanol stream are mixed with the RNA contained in an aqueous stream (e.g. in citric acid buffer). The mixing of the lipids with RNA can be achieved using a mixing device based on: microfluidics, T-junction, impingement jet, vortex, or pressurised stainless-steel tanks. Following formulation, the solution is sterile filtered and shipped to the fill-to-finish site for filling into glass vials or other containers (Kis et al., 2021b).

3. Techno-economic modelling of the AVV, mRNA and saRNA platforms

Rapid and global response to pandemics by mass vaccination is currently limited by the rate at which vaccine doses can be manufactured on a global scale. Here techno-economic modelling is presented for the AVV, mRNA and saRNA vaccine production platform technologies that were deployed during the COVID-19 pandemic. Unlike AVV and mRNA vaccines, several of which were approved by the regulatory authorities, the saRNA platform is not yet deployed at commercial scale for vaccines, with saRNA vaccines still undergoing clinical development. Herein, a combination of techno-economic modeling and variance-based global sensitivity analysis (GSA) is applied. This quantifies the performance of each platform in terms of their productivity and resource requirements, subject to key design and operational uncertainties, cf. Figure 1. GSA was carried out by interfacing SobolGSA with SuperPro Designer via MatLab and Excel Visual Basic for Applications (VBA). For GSA, 10,000 simulations were performed for each of the three platform technologies. For these simulations, model inputs were quasi-randomly sampled from a seven dimensional input space using Sobol sequences, as previously described (Kis et al., 2021b). These seven model inputs are: the scale of the production process, batch failure rate, titre/yield in the production bioreactor, cost of raw materials, cost of labour, drug substance amount per dose and cost of quality control (Kis et al., 2021b).

Cost and productivity results from the techno-economic modelling and GSA are shown below in Figure 2. The ranges and probability distributions of the number of drug substances and finished drug product doses that can be produced based on a one billion USD investment in operating expenses (OpEx) for the three platform technologies are

shown in the violin plots in Figure 2A. A one billion USD investment in OpEx will produce a median of 2.66 (IQR=2.44-2.83) billion AVV drug product doses, a median of 0.95 (IQR=0.74-1.23) billion mRNA drug product doses and a median of 2.48 (IQR=2.36-2.58) billion saRNA drug product doses. OpEx includes the annualised capital costs, however it is worth noting that investment in facilities must be made upfront, because constructing, equipping, validating and starting up production can take several years. The ranges and probability distribution of the cost per dose for the drug substance and finished drug product for the three platform technologies is shown in Figure 2B. The drug product manufacturing cost per dose is 0.38 (IQR=0.35-0.41), 1.05 (IQR=0.81-1.35), 0.4 (IQR=0.38-0.42) for AVV, mRNA and saRNA vaccines, respectively.

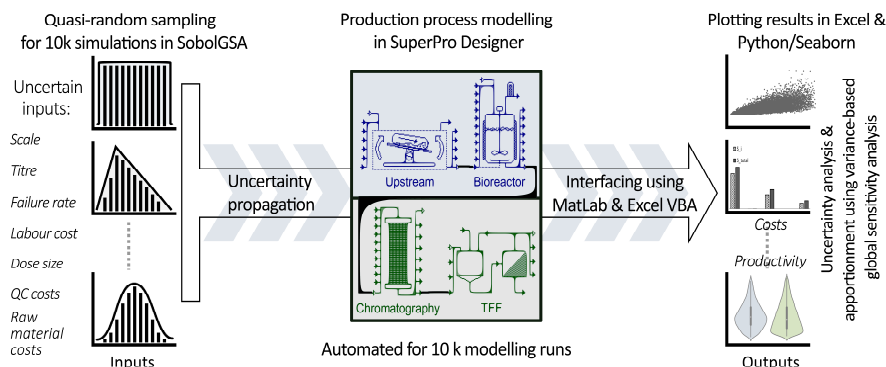


Figure 1. A computational framework for uncertainty quantification for AVV, mRNA and saRNA production. The uncertainty is propagated from the inputs via the model to the outputs. In addition, the sensitivity of the model output key performance indicators (KPIs) is attributed to the individual inputs, to determine the degree to which individual inputs impact the output KPIs. Modified from (Kis et al., 2021b).

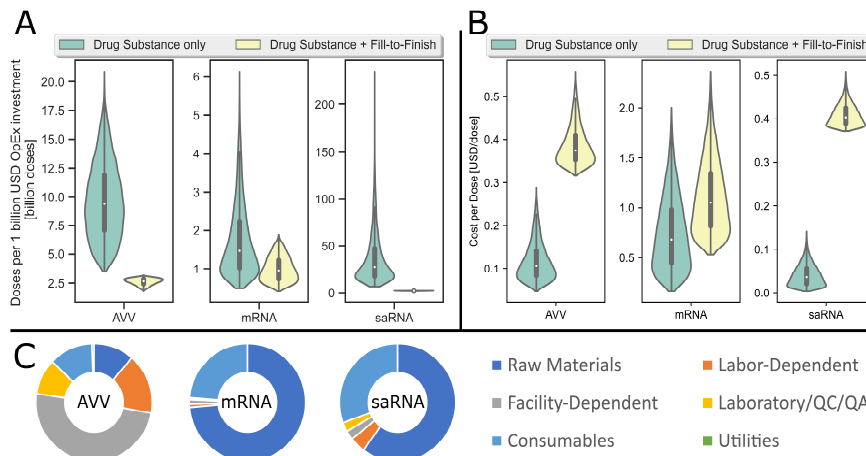


Figure 2. Cost distributions associated with AVV, mRNA and saRNA vaccine production. **A.** Violin plots showing the distribution of the estimated number of doses produced based on a 1 billion USD investment in operating expenses (OpEx). The OpEx contains the annualised facility costs. **B.** Violin plots showing the distribution of cost per dose values for AVV, mRNA and saRNA vaccine production. **C.** Doughnut charts showing the distribution of OpEx, the annualised capital costs are included in the facility-dependent costs.

In the centre of all the violin plots, box and whisker plots are shown with the median values indicated by the white dots; the 25th and 75th percentiles with the top and bottom of the boxes; and minimum and maximum values, excluding outliers, with the ends of the whiskers. The width of the violin plots represents the probability distributions. Figure 2C shows the breakdown of the annual production costs for the baseline scenarios (c.f. (Kis et al., 2021b)) for these three vaccine platform production technologies. Fixed costs dominate the AVV production costs, whereas mRNA and saRNA vaccines production is driven by variable costs. This implies that maintaining surge capacity based on the RNA platform will be more cost effective than based on the AVV platform. Fill-and-finish was modelled with 10-dose vials for AVV vaccines and 5-dose vials for mRNA and saRNA vaccines.

4. Integration of QbD and techno-economic modeling with the RNA platform

The mRNA vaccine production platform technology has been proven clinically successful during the COVID-19 pandemic. The cell-free nature and consequently the relative simplicity (compared to cell-based vaccine production) makes the RNA platform technology ideal for digitalisation and advanced automation with the QbDD framework. The integration of the QbDD framework with the RNA platform will accelerate product-process development, enhance production rates and production volumes, reduce costs, and assure high product quality. Moreover, the RNA vaccine production platform and the QbDD framework will form a powerful synergy as both tools are disease agnostic. This synergy will use prior platform knowledge, experimental data, clinical data, quality risk management and digital tools to accelerate product and process development. This will also accelerate and streamline the regulatory approval process based on knowledge-rich regulatory submissions and demonstrated product knowledge and process understanding.

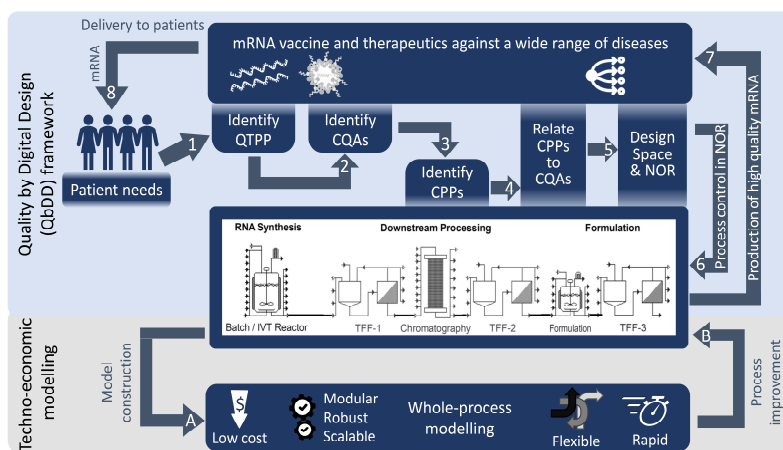


Figure 3. Integration of techno-economic and QbDD modelling with the RNA vaccine production process. Abbreviations: QbDD - Quality by Digital Design, QTPP - Quality Target Product Profile, CQAs – product Critical Quality Attributes for safety and efficacy, CPP - Critical Process Parameters, NOR - Normal Operating Range, within the design space.

The product specifications will be based on product performance instead of batch history, and the focus will shift from reproducibility to process-product robustness (Kis et al., 2020; van de Berg et al., 2021). In addition, the QbDD framework and the digital tools

will be used to automate RNA vaccine manufacturing, building quality into the design and operation of the process. These features of the QbDD framework will also support scale-up and technology transfer. In addition, techno-economic modelling will guide cost-reduction, de-bottlenecking and improved process performance. The interplay between the RNA vaccine platform technology, the QbDD framework and techno-economic modelling is shown above in Figure 3.

5. Conclusions

In conclusion, on top of having surge vaccine manufacturing capacity available for future outbreaks, modelling tools such as those presented here can further accelerate vaccine development and improve the performance of the vaccine manufacturing processes. The combination of vaccine platform technologies and these disease-agnostic modelling tools provides a powerful approach for rapid-response vaccine deployment against currently known and unknown diseases.

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References

- CMC-Vaccines Working Group, 2012. A-Vax: applying quality by design to vaccines.
- Ferreira, R., Petrides, D., 2021. Messenger RNA (mRNA) Vaccine Large Scale Manufacturing – Process Modeling and Techno-Economic Assessment (TEA) using SuperPro Designer. <https://doi.org/10.13140/RG.2.2.12780.28800>
- Ferreira, R.G., Gordon, N.F., Stock, R., Petrides, D., 2021. Adenoviral Vector COVID-19 Vaccines: Process and Cost Analysis. Processes. <https://doi.org/10.3390/pr9081430>
- ICH Expert Working Group, 2009. ICH harmonised tripartite guideline on pharmaceutical development Q8 (R2) (No. August), ICH guideline Q8 (R2) on pharmaceutical development. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), Geneva, Switzerland.
- Kis, Z., Kontoravdi, C., Dey, A.K., Shattock, R., Shah, N., 2020. Rapid development and deployment of high-volume vaccines for pandemic response. *J. Adv. Manuf. Process.* 2, e10060. <https://doi.org/10.1002/amp2.10060>
- Kis, Z., Kontoravdi, C., Shattock, R., Shah, N., 2021a. Resources, Production Scales and Time Required for Producing RNA Vaccines for the Global Pandemic Demand. *Vaccines*. <https://doi.org/10.3390/vaccines9010003>
- Kis, Z., Tak, K., Ibrahim, D., Papathanasiou, M.M., Chachuat, B., Shah, N., Kontoravdi, C., 2021b. Pandemic-response adenoviral vector and RNA vaccine manufacturing. medRxiv. <https://doi.org/10.1101/2021.08.20.21262370>
- Pereira Chilima, T.D., Moncaubeig, F., Farid, S.S., 2020. Estimating capital investment and facility footprint in cell therapy facilities. *Biochem. Eng. J.* 155, 107439. <https://doi.org/10.1016/j.bej.2019.107439>
- van de Berg, D., Kis, Z., Behmer, C., Samnuan, K., Blakney, A., Kontoravdi, C., Shattock, R., Shah, N., 2021. Quality by Design modelling to support rapid RNA vaccine production against emerging infectious diseases. *NPJ Vaccines* 6, 1–10. <https://doi.org/10.1038/s41541-021-00322-7>