Received: 9 June 2021 | Revised: 6 July 2021 | Accepted: 21 July 2021

DOI: 10.1002/btpr.3196

RESEARCH ARTICLE

BIOTECHNOLOGY PROGRESS

In silico process characterization for biopharmaceutical development following the quality by design concept

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Abstract

With the quality by design (QbD) initiative, regulatory authorities demand a consistent drug quality originating from a well-understood manufacturing process. This study demonstrates the application of a previously published mechanistic chromatography model to the in silico process characterization (PCS) of a monoclonal antibody polishing step. The proposed modeling workflow covered the main tasks of traditional PCS studies following the QbD principles, including criticality assessment of 11 process parameters and establishment of their proven acceptable ranges of operation. Analyzing effects of multi-variate sampling of process parameters on the purification outcome allowed identification of the edge-of-failure. Experimental validation of in silico results demanded approximately 75% less experiments compared to a purely wet-lab based PCS study. Stochastic simulation, considering the measured variances of process parameters and loading material composition, was used to estimate the capability of the process to meet the acceptance criteria for critical quality attributes and key performance indicators. The proposed workflow enables the implementation of digital process twins as QbD tool for improved development of biopharmaceutical manufacturing processes.

KEYWORDS
antibody purification, cation exchange chromatography, in silico process characterization, mechanistic chromatography modeling

1 | INTRODUCTION

The biopharmaceutical industry is under an unprecedented pressure to implement technologies for rapid process development. Main reasons are rising numbers of monoclonal antibodies (mAbs) in development[1,2] and strongly accelerated development timelines.[3] While achieving a short time-to-market timeline, mAb manufacturers have to ensure high product quality by following the quality by design (QbD) concept. The QbD concept demands a consistent product quality originating from an intrinsic quality built into the design and the control of the manufacturing process. In recent years, regulatory authorities and biopharmaceutical organizations formulated clear concepts for the implementation of QbD in pharmaceutical development.[4–6] Yu et al.[5] listed the key elements for a development strategy that complies with the QbD concept:

- A quality target product profile (QTPP) for the identification of critical quality attributes (CQAs) of the drug product
- Identification of critical material attributes (CMAs) and critical process parameters (CPPs) potentially effecting CQAs
- Measuring the effect of CPPs and CMAs on CQAs
- Development of a control strategy
- Process capability and continual improvement.

Development workflows comprising the above listed QbD elements make use of general process knowledge and statistical design of experiments (DoE) for the characterization of a unit operation.[7–9] For many process steps, including preparative chromatography, it is not feasible to include all controllable process parameters in a DoE study. Even on small-scale systems,[10] it is challenging to screen hundreds of process conditions when considering the subsequent analytical bottleneck. Therefore, process parameters have to undergo a risk-based criticality assessment considering their potential impact on CQAs before designing an experimental process characterization study (PCS).[7,8] Risk-based decision trees for process parameter classification are able to reduce the dimensionality of DoE studies, and thus reduce the experimental burden for process development. However, parameter criticality assessment can be influenced by subjective decision-making caused by the lack of experimental data at this development stage. As a result, incorrectly classified process parameters could lead to avoidable experimental effort, or worse, to a poorly understood control strategy. Further, PCS approaches based on DoE are limited to regression models correlating CPPs to CQAs with a limited amount of data points per CPP.

In the ICH Q8/Q9/Q10 (R2) documents,[11] regulatory authorities propose the use of mathematical models to support bioprocess development and manufacturing. These models include mechanistic models describing the physical phenomena within a unit operation, which can be used to predict process outcomes under varying conditions.[11] Digitalization initiatives in biopharma industry and academia identified mechanistic chromatography modeling as a promising tool for in silico development of downstream processes (DSP).[12–15] After overcoming the initial hurdle of model calibration,[16–20] mechanistic chromatography models show a broad applicability to bioprocess development, including process optimization,[21] robustness analysis,[22–24] or scale-up.[10,25] Recently, Andris et al.[26] developed a mechanistic model for the separation of antibody-drug conjugates. Their work allowed the characterization of a design space, revealing the relevance of digital process twins in the light of QbD. For ion exchange chromatography, Jakobsson et al.[24] used mechanistic modeling to design a robust pooling strategy under consideration of model uncertainty. A mechanistic modeling study performed by Close et al.[22] identified robust operating conditions for a hydrophobic interaction chromatography process, where resin and loading material had a considerable impact on process performance. Following the QbD concept, Rischawy et al.[27] used mechanistic modeling for the identification of CPPs for a cation exchange chromatography step applied to the polishing of a bispecific mAb. Shekhawat et al.[28] developed a model that improved understanding around resin fouling in Protein A chromatography. The here mentioned mechanistic modeling studies increased process understanding or solved specific problems regarding process robustness. However, as described earlier, regulatory authorities defined clear perspectives on the implementation of QbD in process development and the related tasks. To the best of our knowledge, it is still to be shown how mechanistic models could be applied to a PCS study addressing the essential QbD elements.

Our previous publications introduced a quality system for mechanistic chromatography modeling in biopharmaceutical process development. The selection of representative experiments for model calibration ensured adequate model certainty with minimal resources.[17] This mechanistic model was validated against data of multiple scales, including clinical manufacturing-scale.[29] As a sequel of this publication series, the mechanistic model is applied to the PCS of a cation-exchange chromatography (CEX) step. Simulations are performed at manufacturing-scale avoiding limitation of experimental scale-down model studies. The in silico strategy aims to fulfill the fundamental tasks of a PCS following the QbD concept. This includes criticality assessment of process parameters and measuring their effect on CQAs and key performance indicators (KPIs). Further, simulations provide the database to identify proven acceptable ranges (PARs) for process parameters as part of the control strategy. An experimental design is derived from mechanistic model predictions to reduce the experimental effort compared to wet-lab driven DoE approaches. As a last element, Monte-Carlo simulation allows the calculation of process capability under consideration of CPP, KPP, KMA, and CMA variances measured during clinical manufacturing. The presented methodology generates in-depth process understanding following the QbD concept, while debottlenecking experimental limitation of DoE approaches. Mechanistic modeling for in silico PCS can improve decision-making in DSP development, assuring product quality throughout the entire value chain.

2 | MODELING

Details about model discrimination, model parameters, the model calibration strategy, and scale-dependent considerations can be found in our previous publications.[17,29] Protein-specific model parameters are listed in Table 1. This section gives an overview on the mechanistic model and complementations necessary for model-guided scale-up. The one dimensional (1D) transport dispersive model was selected as column model, due to multiple successful case studies for the simulation of ion exchange chromatography systems.[16,20,30–32] Equation (1) describes the macroscopic transport of component *i* through the chromatography column. The change of the concentration c_i at position x in time t is a function of convective mass transport in the interstitial volume, peak broadening caused by axial dispersion D_{ax}, and mass transfer from the interstitial volume into the pore phase of the particle with the radius r_P. Further, mass transfer between the interstitial

TABLE 1 Protein specific model parameters for the pH-dependent SMA model. Details regarding the model calibration procedure are described in our previous publication.[17] For a clear representation of model parameters at pH 5.8, the pH was normalized to zero. pH 5.5 = −0.3, pH 5.8 = 0, pH 6.1 = 0.3

Parameter	APG	Main	BPG	HMW
$k_{eff0,i}$ [mm/s]	1.4E-3	1.4E-3	1.4E-3	1.2E-3
$k_{eff1,i}$ [−]	4.7E-05	4.7E-05	4.7E-05	3.3E-05
$\nu_{pH5.8,i}$ [−]	7.38	7.50	7.70	10.97
$\nu_{1,i}$ [−]	−1.44	−1.44	−1.44	−6.77
$k_{eq,pH\ 5.8,i}$ [−]	1.45	1.41	1.69	1.86
$k_{eq,1,i}$ [−]	−4.26	−4.26	−4.26	−5.39
$k_{eq,2,i}$ [−]	2.19	2.19	2.19	5.59
$k_{kin,i}$ [sM$^{\nu}$]	8.08E-06	1.00E-04	5.00E-04	3.4E-05
σ_i [−]	128.6	56.3	107.1	0

Abbreviations: APG, acidic; BPG, basic peak groups; HMW, high molecular weight species.

volume and the particle pores is affected by the interstitial porosity ϵ_{col} and the effective mass transfer coefficient $k_{eff,i}$. The accumulation of mass in the pore phase $c_{p,i}$ with the particle porosity ϵ_p and the stationary phase q_i is described in Equation (2). The Danckwerts' boundary conditions are given in Equations (3) and (4).

$$\frac{\partial c_i(x,t)}{\partial t} = -\frac{u}{\varepsilon_{col}}\frac{\partial c_i(x,t)}{\partial x} + D_{ax}\frac{\partial^2 c_i(x,t)}{\partial x^2} - \frac{(1-\varepsilon_{col})}{\varepsilon_{col}}\left(\frac{3}{r_p}k_{eff,i}\left(c_i(x,t)-c_{p,i}(x,t)\right)\right) \quad (1)$$

$$\frac{\partial c_{p,i}(x,t)}{\partial t} = \frac{3}{r_p}\frac{k_{eff,i}}{\varepsilon_p}\left(c_i(x,t)-c_{p,i}(x,t)\right) - \frac{1-\varepsilon_p}{\varepsilon_p}\frac{\partial q_i(x,t)}{\partial t} \quad (2)$$

$$\frac{\partial c_i}{\partial x}(0,t) = \frac{u(t)}{D_{ax}}\left(c_i(0,t)-c_{in,i}(t)\right) \quad (3)$$

$$\frac{\partial c_i}{\partial x}(L,t) = 0 \quad (4)$$

Linear flow rates ranged from 155 cm/h to 360 cm/h between investigated scales, demanding the introduction of flow dependencies for the axial dispersion coefficient D_{ax} and effective mass transfer parameter $k_{eff,i}$. The penetration correlation allowed the direct calculation $k_{eff,i}$ for monomer and high molecular weight (HMW) species at relevant flow rates, respectively.[33,34] Within the investigated range, flow dependencies for D_{ax} and $k_{eff,i}$ could be approximated using linear regression according to Equation (5) and (6).

$$D_{ax}(u) = D_{ax0} + uD_{ax1} \quad (5)$$

$$k_{eff,i}(u) = k_{eff0,i} + uk_{eff1,i} \quad (6)$$

Protein adsorption is simulated using the semi-mechanistic SMA adsorption model.[35] The multicomponent SMA model formulates the equilibrium binding behavior of the protein in consideration of the salt concentration in the pore phase c_s, the ionic capacity of the resin Λ, and the proteins characteristic charge ν_i. Equation (7) shows the kinetic form of the SMA isotherm modified by Hahn et al.,[31] where $k_{eq,i} = k_{ads,i}/k_{des,i}$ and $k_{kin,i} = 1/k_{des,i}$ describe adsorption and desorption rates of component i, respectively. In addition, the steric shielding parameter σ_i denotes the number of functional groups on the resin surface blocked by the protein.

$$k_{kin,i}\frac{\partial q_i}{\partial t} = k_{eq,i}(pH)\left(\Lambda - \sum_{j=1}^{k}\left(\nu(pH)_j + \sigma_j\right)q_j\right)^{\nu(pH)_i} c_{p,i} - q_i c_s^{\nu(pH)_i} \quad (7)$$

$$q_{salt} = \Lambda - \sum_{j=1}^{k}\nu_j q_j \quad (8)$$

The introduction of pH-dependent isotherm parameters is crucial for industrial applications. Equations (9) and (10) show the empirical pH dependencies of the characteristic charge ν_i and the equilibrium constant $k_{eq,i}$ developed by Hunt et al.[36] This model was found to be sufficient for the process relevant pH range of pH 5.8 ± 0.3 used in this study.[17]

$$k_{eq,i}(pH) = k_{eq0,i}e^{k_{eq1,i}pH + k_{eq2,i}pH^2} \quad (9)$$

$$\nu_i(pH) = \nu_{0,i} + pH\nu_{1,i} \quad (10)$$

3 | MATERIAL AND METHODS

3.1 | CEX unit operation

The investigated protein is an IgG1 mAb expressed in stably transfected Chinese hamster ovary cells (Boehringer Ingelheim GmbH & Co. KG, Biberach, Germany). The mAb was captured via Protein A affinity chromatography and further polished using anion exchange chromatography in flow-through mode. The presented mechanistic model describes the subsequent CEX unit operation

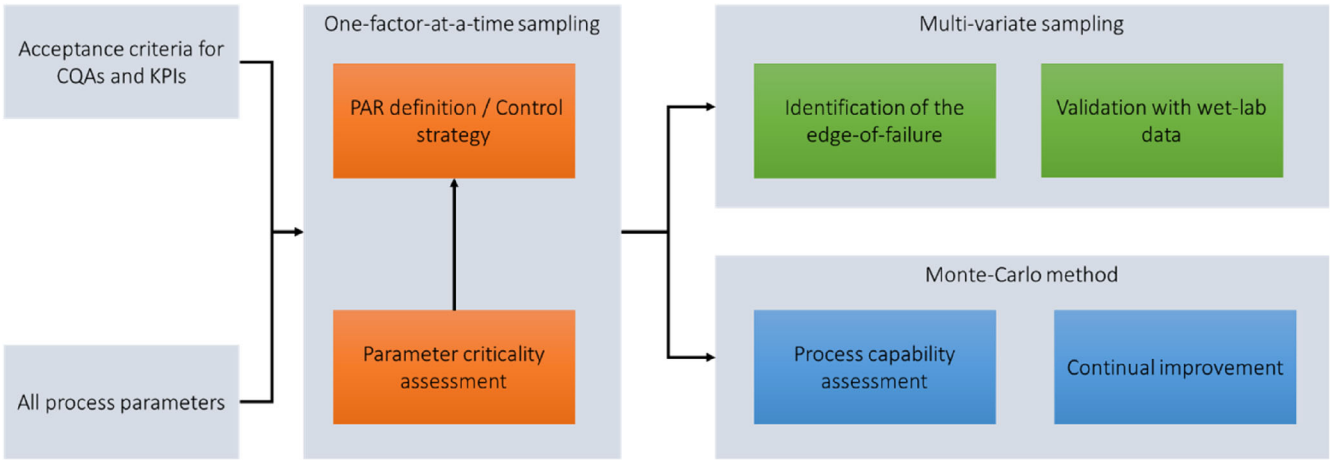

FIGURE 1 In silico process characterization of a unit operation for monoclonal antibody purification

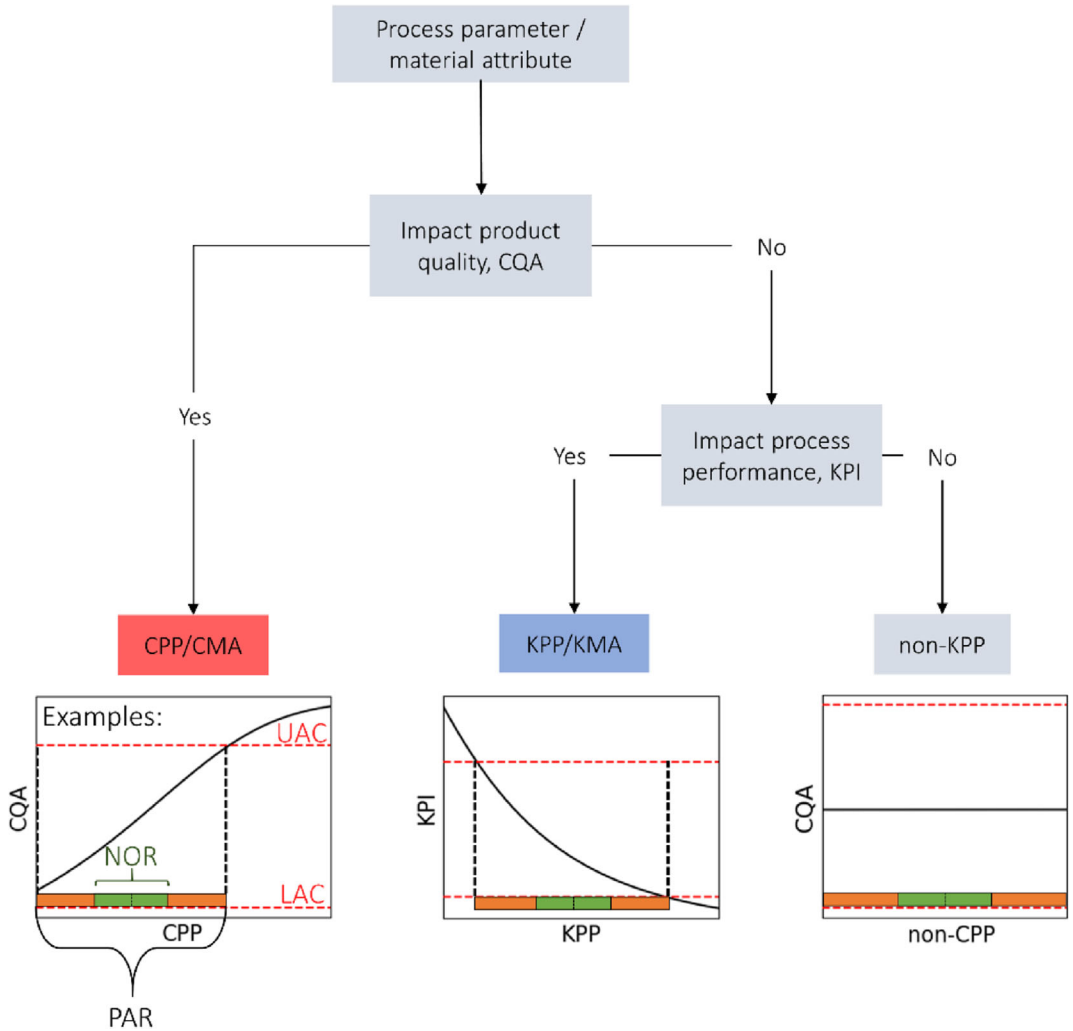

FIGURE 2 Decision tree for model-guided criticality assessment of process parameters and establishment of their proven acceptable ranges (PARs). LAC, lower acceptance criterion; UAC, upper acceptance criterion

using the strong CEX resin POROS 50 HS (Thermo Fisher Scientific, Waltham, USA). The process was performed at constant pH 5.8 in bind-elute mode and at a maximal load density of 45 g/L_{resin}. The column was equilibrated at a counter-ion concentration of 87 mM Na^+, with the same buffer applied to the wash phase after column loading. Subsequently, elution was induced at

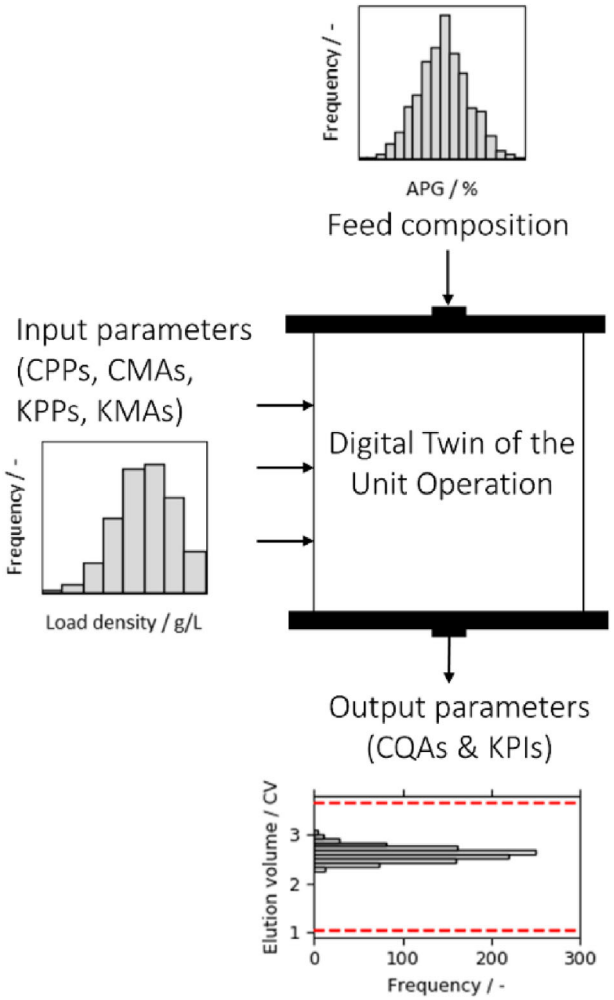

FIGURE 3 Monte-Carlo simulation for the calculation of process capability. The stochastic simulation procedure considered loading material compositions and input parameter distributions resulting in the calculation of process capabilities for six critical quality attributes (CQAs) and key performance indicators (KPIs). Exemplary input and output distributions are shown

a counter-ion concentration of 247 mM Na^+. For column regeneration and storage, 1 M and 0.1 M NaOH were applied respectively. Selected experiments from wet-lab PCS studies were used to validate the most critical relationships between process parameters and CQAs/KPIs. Bench-scale experiments were performed on an Äkta Avant 25 (Cytiva, Uppsala, Sweden) using an experimental scale down model (SDM) column with a bed height of 300 mm and an inner diameter of 10 mm.

Charge variant and HMW concentrations in the elution pool were quantified using analytical CEX chromatography and analytical size exclusion chromatography, respectively. Acidic (APG), neutral (Main) and basic peak groups (BPG), as well as HMW species were considered as CQAs. Process step yield and elution volume were defined as KPIs and quantified using protein concentration determined via absorbance at 280 nm and gravimetric volume measurement. Details of the model calibration and validation, as well as analytical chromatography methods, are presented in one of our previous publications.[17]

3.2 | In silico PCS workflow

This chapter describes the methodology of an in silico PCS following the QbD concept. The PCS workflow consisted of three major building blocks: (1) Process parameter criticality assessment and establishment of PARs; (2) Identification and validation of the edge-of-failure; (3) Calculation of process capabilities. Protein- and system-specific mechanistic model parameters were kept constant and were obtained from our previous publications.[17,29] Only process parameters were varied during in silico sampling. All simulations were performed at manufacturing-scale, under consideration of system and column dimensions. Before starting in silico experimentation, the applied mechanistic model was validated as digital representation of the real-world process. Model validation must consider the intended purpose of the model and its potential impact on the control strategy at manufacturing-scale. Small-scale experiments validated that the model captures the impact of process parameter variation on the purification outcome.[17] Model validation across scales showed that the model captures relevant system effects and proofed equivalence between the mechanistic model and manufacturing-scale.[29] Based on this previously published validation strategy, it is reasonable to use the model for in silico PCS.

Figure 1 depicts the three different parameter-sampling methods. Initially, a one-factor-at-a-time sampling (OFAT) scheme enabled criticality assessment of process parameters and definition of PARs. During OFAT sampling, one parameter was sampled in a wide range around its intended set point, while the other process parameters were kept constant. The loading density was sampled below its upper limit of 45 g/L_{resin}. Following the decision tree in Figure 2, process parameters were ranked as non-KPP, CPP, KPP, CMA or KMA based on their effect on CQAs and KPIs.

- non-KPP: Process parameter does not affect a CQA or KPP
- CPP: Critical process parameter affects at least one CQA
- KPP: Key process parameter affects at least one KPI and not affects CQAs
- CMA: Critical material attribute affects at least one CQA
- KMA: Key material attribute affects at least one KPI and not affects CQAs

Subsequently, the same data obtained from OFAT sampling allowed definition of PARs for all investigated process parameters. The establishment of PARs is a fundamental part of the control strategy and represents the main goal of a PCS. According to the European Medicines Agency (EMA) and ICH Q8 R2 guideline,[11,37] the PAR is defined as the operating range of a process parameter for which the unit operation will produce a drug substance meeting the relevant quality criteria. When all process parameters are kept constant, but one parameter varies within its PAR, all CQAs and KPIs measured in the

TABLE 2 Criticality assessment of in silico screened process parameters of the CEX unit operation. Process parameters were classified according to the decision tree depicted in Figure 2

Process parameter	Unit	Tested range	Effect on CQA	Effect on KPI	Classification
pH elution buffer	pH	5.5–6.1	Yes	Yes	CMA
Salt elution buffer	mM Na^+	230–265	Yes	Yes	CMA
Flow rate elution	cm/h	100–350	Yes	Yes	CPP
pH equilibration/wash buffer	pH	5.5–6.1	Yes	Yes	CMA
Loading density	g/L_{resin}	22.5–45	Yes	Yes	CPP
Salt equilibration/wash buffer	mM Na^+	74–99	No	Yes	KMA
Flow rate loading	cm/h	100–350	No	No	non-KPP
pH load	pH	5.5–6.1	No	No	non-KPP
Salt load	mM Na^+	62–85	No	No	non-KPP
Flow rate wash	cm/h	100–350	No	No	non-KPP
Column length	mm	270–330	No	No	non-KPP

Abbreviations: CEX, cation-exchange chromatography; CQA, critical quality attributes; KPI, key performance indicators.

elution pool must be located within their predefined acceptance criteria (AC). Thus, OFAT sampling of input parameters is a suitable method for the establishment of PARs. As presented in Figure 2, the intersection of ACs and the curve obtained via in silico sampling defined the lower and upper boundary of the PAR. If a process parameter did not cause CQAs or KPIs to violate the AC, the entire in silico screened parameter range of this process parameter was defined as PAR.

Process parameters ranked as CPPs and KPPs were analyzed in subsequent multi-parametric sampling studies. The multi-parametric sampling study represented the second building block of the in silico PCS. Here, CPPs and KPPs were varied jointly to study the worst-case operating scenarios. This procedure enabled the identification of the edge-of-failure under consideration of the AC. Historical wet-lab experiments at process conditions around the edge-of-failure were used to validate the in silico findings.

In a last step, the process capability of the unit operation was calculated based on stochastic simulation (Monte-Carlo simulation), as described in Figure 3. Therefore, probability functions of process parameters and loading material composition were calculated based on 20 chromatographic cycles at clinical manufacturing-scale. Subsequently, 1000 simulations were performed using random samples of the previously determined probability function as model input. The resulting CQA and KPI distributions were then plotted and compared to the AC. The standard deviations $\hat{\sigma}$ obtained from in silico generated CQA and KPI distributions enabled calculation of the corresponding process capabilities C_{pl} and C_{pu} for the lower and upper AC (LAC and UAC), respectively,

$$C_{pl} = \frac{Mean - LAC}{3\hat{\sigma}} \tag{11}$$

$$C_{pu} = \frac{UAC - Mean}{3\hat{\sigma}} \tag{12}$$

$$C_{pk} = \min\left(c_{pl}; c_{pu}\right). \tag{13}$$

For each CQA or KPI, the overall process capability C_{pk} was defined as the minimum of C_{pl} and C_{pu}. When only an LAC or an UAC was defined, the overall process capability could be simplified to $C_{pk} = C_{pl}$ or $C_{pk} = C_{pu}$.

4 | RESULTS AND DISCUSSION

In the following chapters, a previously published mechanistic chromatography model was applied to the in silico PCS of a CEX unit operation.[17,29] The multi-stage modeling workflow aimed to fulfill essential tasks of PCS following the QbD concept. This includes CPP identification and PAR definition. Multi-parametric effects on the purification outcome were identified and validated with wet-lab experiments. Monte-Carlo simulation allowed the determination of process capability under consideration of real CPP, KPP, CMA, and loading material composition variability.

4.1 | Parameter criticality assessment and control strategy

Before starting characterization of a unit operation, process parameters must be classified according to their impact on CQAs and KPIs. The mechanistic chromatography model enabled effect analysis of process parameters following an OFAT sampling scheme. Table 2 lists the results of the parameter criticality assessment following the decision tree in Figure 2. In silico investigation of one process parameter consisted of 50 simulations, with equidistant steps in a wide range around the set-point condition. While one parameter was varied, all other process parameters were kept on the set point. Five process parameters were ranked as CPPs or CMAs, showing effects on at least one CQA. Salt concentration in the equilibration/wash buffer was ranked as KMA, since it only affected the KPI step yield. All remaining process parameters were

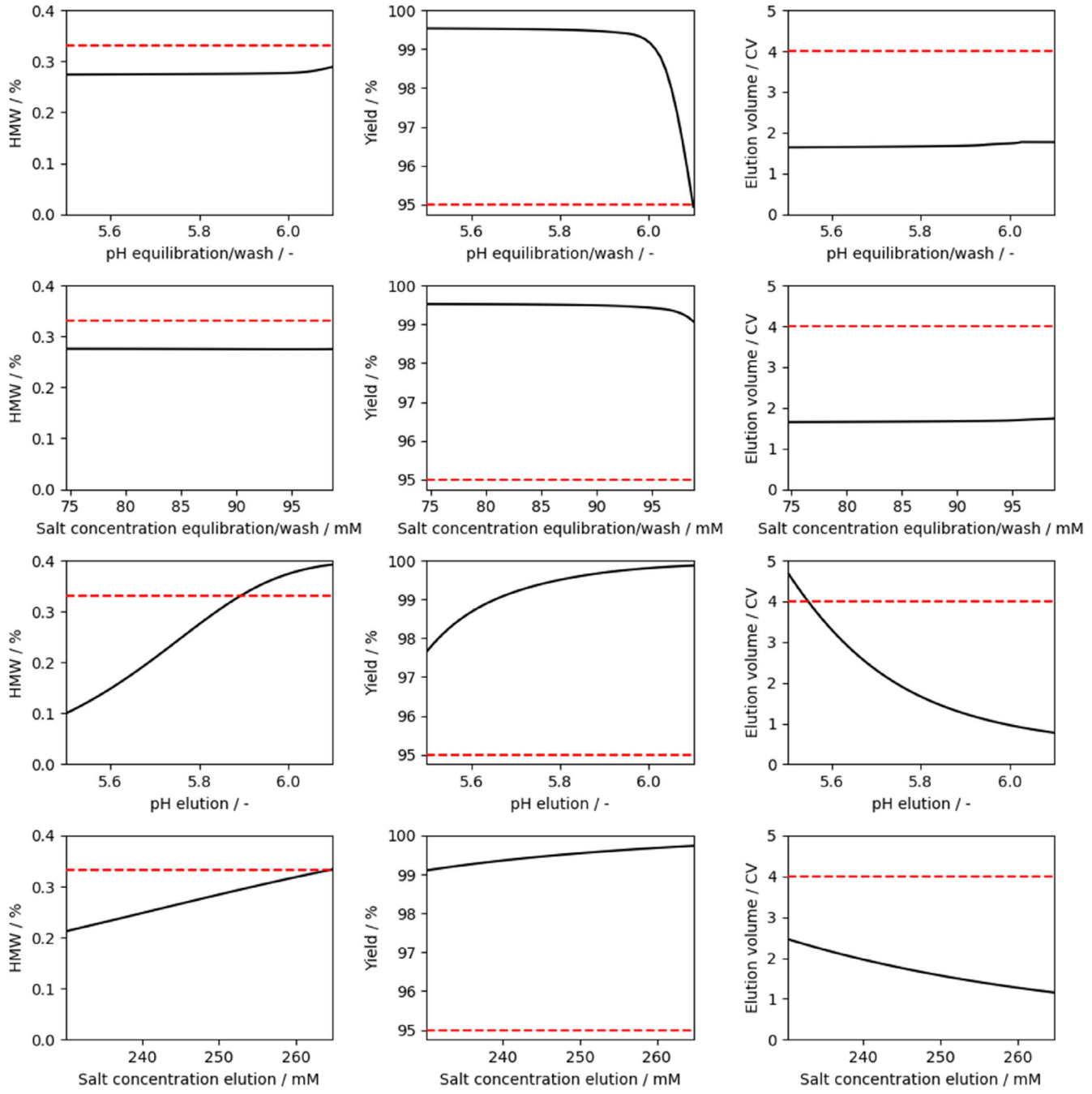

FIGURE 4 Criticality assessment of process parameters via in silico one-factor-at-a-time sampling (OFAT) sampling. The figure shows effects of mobile phase conditions during elution and wash phase on critical quality attributes (CQAs) and key performance indicators (KPIs). Each sub-figure contains the information of 50 simulations at varying process conditions (black line). Dashed red lines indicate the acceptance criteria for CQAs and KPIs

ranked as non-KPPs and did not affect CQAs or KPIs within the screened parameter ranges.

Mobile phase pH and salt concentrations were amongst the process parameters showing the strongest impact on CQAs and KPIs. Thus, Figure 4 highlights the effects of mobile phase conditions during equilibration/wash and elution on the purification result. The non-linear correlation between elution pH and HMW concentration was identified as the most considerable effect. The mechanistic model predicted that an elution buffer with pH above pH 5.9 results in HMW levels violating the upper AC. Typically, the initial criticality-assessment of process parameters is based on failure mode and effect analysis (FMEA). The FMEA allows a risk-ranking depending on initial experiments and available data from process development, historical knowledge from different mAbs at comparable process steps, and process understanding of subject matter experts. A validated mechanistic model could be used to support a

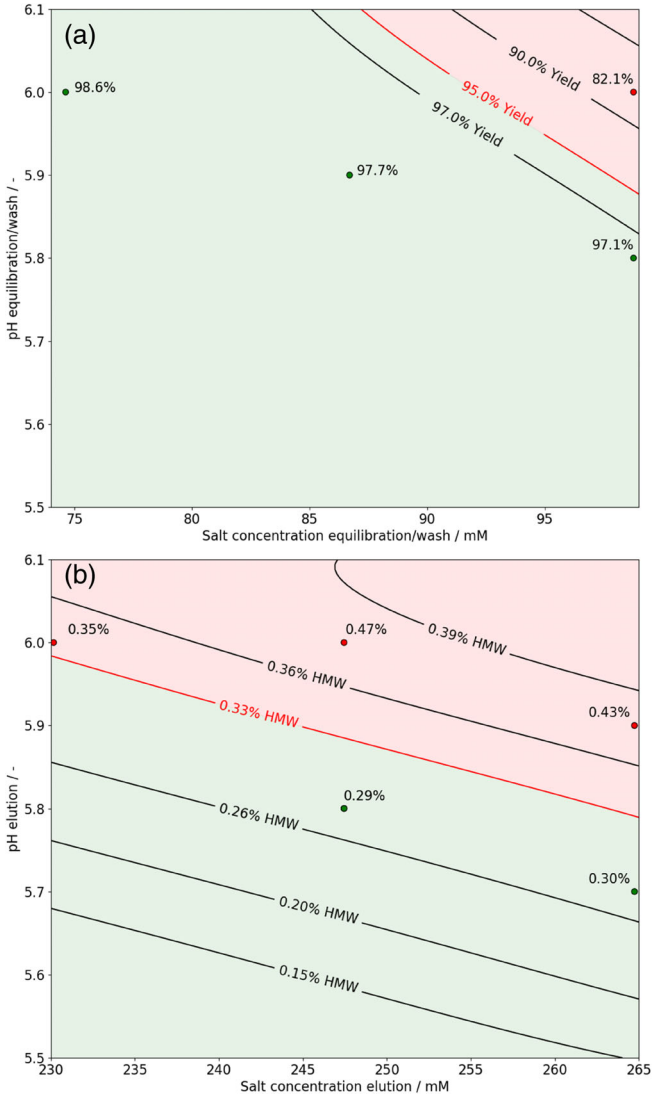

FIGURE 5 Effect of mobile phase conditions on step yield (a) and HMW removal (b). Scatter plots show the results of wet-lab experiments performed at process conditions close to the edge-of -failure (green = within AC, red = outside AC). Red contours represent the edge-of-failure, as the cutting line of model prediction and AC. Each contour plot is calculated based on 400 simulations at varying process conditions

knowledge-based FMEA. The effects of potential CPPs and KPPs identified via FMEA on CQAs and KPIs are then screened in a DoE approach. The in silico OFAT screening allowed a rationalized identification of critical input parameters without experimental limitations. Process understanding leveraged from 550 simulations was used to generate the parameter classifications given in Table 2. Such a number of experiments containing similar amount of information cannot be screened economically in wet-lab.

Following the methodology described in Section 3.2, upper and lower limits for PARs (not presented in numbers) were directly derived from the intersection of simulated data and predefined AC in Figure 4. PARs can be established using wet-lab data obtained from OFAT or DoE studies. Due to experimental limitations, data evaluation is often limited to first- or second-degree regression modeling. The data in Figure 4 reveals how the non-linear correlations between CQAs and CPPs affect the establishment of PARs. Simple regression modeling based on a small number of experiments would result in different PARs. The in silico established PARs could be used as part of the control strategy during commercial manufacturing. From a regulatory perspective, the process understanding obtained via OFAT sampling based on mechanistic modeling represents a Level 3 control strategy.[5] The process control assures product quality meeting the specifications when a single process parameter deviates within its PAR. Further, the effect of deviating process parameters on CQAs and KPIs is well-understood enabling the possibility to adapt controls upstream in the process chain. Potentially, the mechanistic model could be applied to a Level 1 control strategy, substituting traditional testing of the intermediate product. In this case, continual generation of in silico data would enable automated adjustment of process parameters assuring a consistent product quality within the AC.[5] Application of mechanistic models as soft-sensors in a Level 1 control strategy could be useful for continuous manufacturing,[38,39] when the adoption of process analytical technology (PAT) is not feasible.

The applied mechanistic model considered large-scale column dimensions and properties. Further, the model was validated against manufacturing-scale data. Consequently, the mechanistic model enabling in silico PAR definition was representative to the final manufacturing-scale. Traditional DoE approaches rely on scale-down experimentation. The ICH guidelines support the establishment of PARs using small-scale experimentation. However, all simplifications and assumptions made during SDM experimentation must be justified during approval process. A pure in silico PCS is currently not recommended if not all CQAs are fully covered by the mechanistic model. Therefore, the following section focusses on a minimal amount of wet-lab experiments for validating the relevant correlations between process parameters and CQAs and KPIs.

4.2 | Identification and validation of the edge-of-failure

In the previous section, in silico OFAT screening enabled classification of process parameters and establishment of PARs. This chapter aims to validate the identified effects of process parameters on CQAs and KPIs using experimental data obtained from previous wet-lab PCS experiments. Here, the minimal number of experiments demanded for validation of in silico results was compared to the experimental effort of an entirely wet-lab based PCS. The multi-variate sampling investigated effects on step yield and aggregate concentration.

Amongst the investigated parameters, mobile phase properties showed the strongest impact on HMW removal and step yield. Mechanistic model predictions showed that an increased counter ion concentration and mobile phase pH during the wash phase caused an early desorption of protein, negatively affecting step yield. Both process parameters, wash salt concentration and wash pH, were simultaneously varied in silico using a parametric sweep study

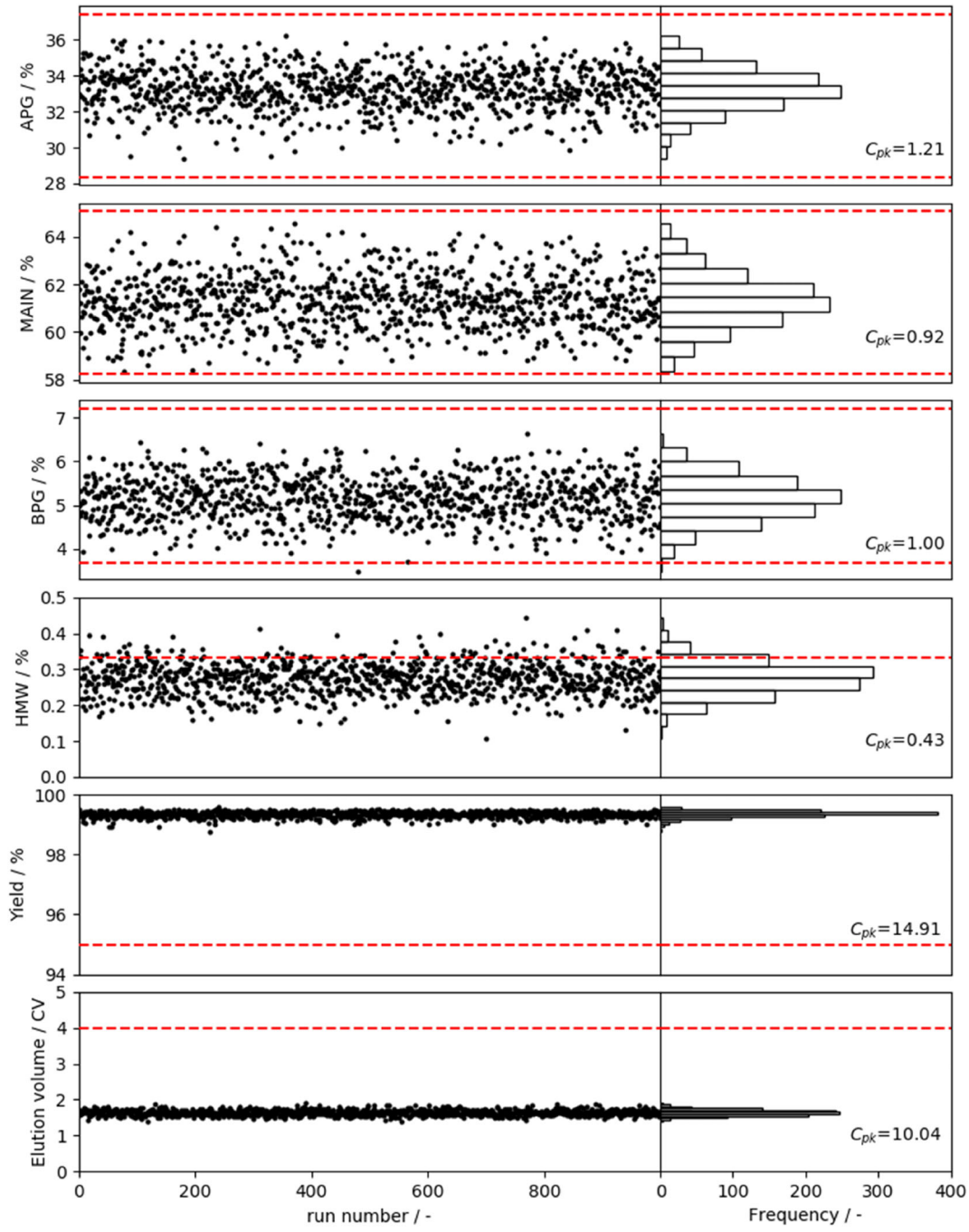

FIGURE 6 Monte-Carlo simulation of the CEX unit operation at pH 5.8 during elution phase. Dashed red lines indicate acceptance criteria. Each data point represents a simulation at 12000 L manufacturing-scale. Measurement data of 20 clinical manufacturing runs was used to simulate the variance of load material composition, loading density, pH, and salt concentrations of the different chromatographic phases

consisting of 400 simulations. As a result, Figure 5A shows step yield as a function of mobile phase conditions during the wash phase. The edge-of-failure was defined as the cutting curve of the surface function calculated based on in silico results and the AC for step yield. Set-point conditions for wash salt and pH conditions are located in the center of x- and y-axis, respectively. Therefore, the contour plot reveals that step yield cannot fall below the AC when varying only one factor at a time. When increasing both, salt and pH during wash above set-point conditions the step yield drops from >98% to a minimum of 77% within the investigated parameter space. Elution of protein during the wash phase resulted in non-linear correlations between process parameters and step yield, which would be difficult to cover using an experimentally limited DoE approach coupled with empirical response surface modeling. The selection of wet-lab experiments at conditions close to the edge-of-failure (scatter plot in Figure 5) validated that a

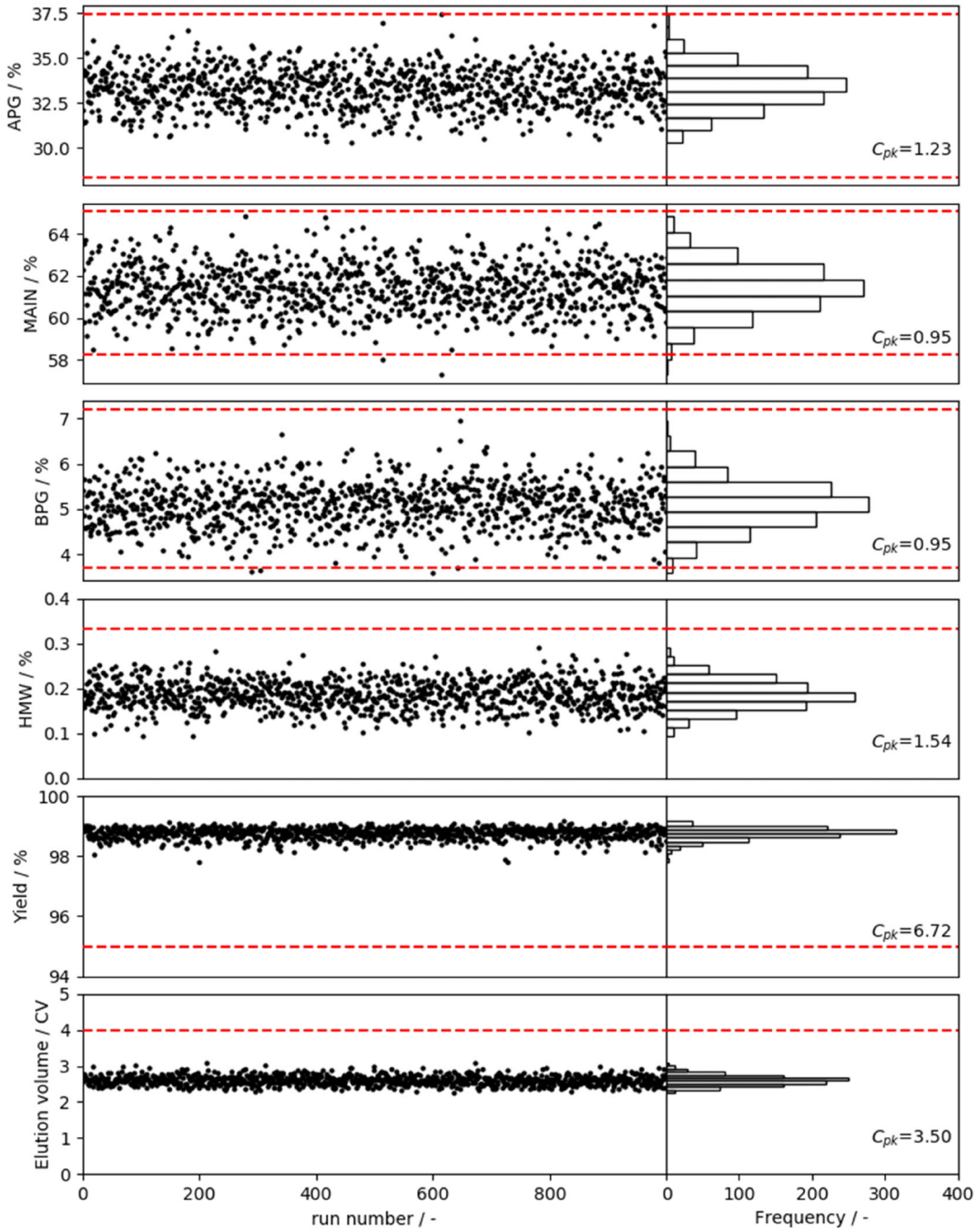

FIGURE 7 Monte-Carlo simulation of the CEX unit operation at pH 5.7 during elution phase. Dashed red lines indicate acceptance criteria. Each data point represents one simulation at 12000 L manufacturing-scale. Measurement data of 20 clinical manufacturing runs was used to simulate the variance of load material composition, loading density, pH, and salt concentrations of the different chromatographic phases

simultaneous increase of salt concentration and pH during the wash phase would result in a violation of the AC for step yield. Instead of conducting wet-lab experiments in the entire parameter space, in silico identification of the edge-of-failure enabled a reduction of the experimental design to process conditions relevant for proofing process robustness.

The identical methodology was applied to mobile phase conditions during the elution phase and their effect on HMW concentration in the elution pool. Figure 5B depicts HMW concentration as a function of elution salt concentration and elution pH. Compared to elution salt concentration, the elution pH had a strong impact on the HMW levels in the product. Again, wet-lab experiments around the edge-of-failure could validate the correlations obtained using in silico data. With targeted experiments close to the in silico determined edge-of-failure, the total number of wet-lab experiments was reduced from 35 to 9 compared to the traditional DoE-based PCS. The contour plot in Figure 5B supports the finding of the previous OFAT analysis, that elution pH 5.7

could be a more robust set point, showing an increased distance to the edge-of-failure compared to pH 5.8. The true capability of the process to deplete HMW species in the desired quantity demands further in silico analysis considering material and process parameter variability.

4.3 | Process capability and continual improvement

Following the QbD elements described by Yu et al., process capability and continual improvement represents the final building block of the in silico PCS.[5] Process capability c_{pk} describes the ability of the purification process to achieve CQAs and KPIs located within the AC under consideration of the intrinsic process variability. As depicted in Figure 3, Monte-Carlo simulation enabled calculation of process capabilities. Feed stream and process parameter variances were used as model input. The input distributions were obtained from 20 CEX chromatography cycles at clinical manufacturing-scale. Variance in load composition and mobile phase properties were approximated with Gaussian functions. The input variance of the loading density was described by a Gaussian function limited to a maximum of 45 g/L_{resin}. The 1000 samples were taken from the distributions calculated based on manufacturing-scale data. The intended mobile phase pH value of the unit-operation was pH 5.8 for all chromatographic phases. Although a pH range of pH 5.8 ± 0.1 is well controllable, simulations in Figure 4 suggest that pH 5.7 is a more robust set point for HMW removal. Therefore, both elution pH scenarios were evaluated using the Monte-Carlo method. Figure 6 and Figure 7 show the resulting distribution of CQAs and KPIs for pH 5.8 and 5.7, respectively.

The comparison between Figure 6 and Figure 7 reveals that a reduction of the elution pH from pH 5.8 to pH 5.7 increases process capability for HMW removal when considering the intrinsic variance of the CEX unit operation. The capability of the process to achieve an HMW concentration below the AC increased from 0.43 to 1.54. Assuming normal distribution of model outputs, the probability for an HMW concentration be located outside the AC reduced to 0.0004% from 19.4%. The adaption of the elution set point pH had no negative effect on process capabilities of other CQAs and KPIs. Consequently, Monte-Carlo simulation could support the decision to shift the set point pH from pH 5.8 to pH 5.7.

Despite the simplification of assuming normal distribution for the majority of CPPs, KPIs, CMAs, and KMAs as model input, step yield and elution volume showed an asymmetric distribution at pH 5.7. These trends underline the importance of considering non-linear correlations in preparative chromatography. Similar to the loading material compositions, charge variant concentrations in the elution pool were found to be normally distributed. Process capabilities for charge variants ranged between 0.92 and 1.23. A $c_{pk} = 1$ corresponds to a distance of 3 sigma between the mean output value and the AC, resulting in a 0.27% probability for a CQA or KPI to be located outside the AC.

Probabilistic simulation using mechanistic modeling is a simple and effective way to estimate process capabilities before a sufficient amount of real data from commercial manufacturing campaigns is available. Here, adaption of the set point pH based on Monte-Carlo simulation improved process robustness with regards to aggregate removal and reduced the risk of an out of specification (OOS) event. During the product lifecycle, input distributions for CMAs, KMAs, CPPs. and KPPs can be continuously updated and fed-back into the mechanistic model. This procedure would allow an early identification of root-causes for process variability enabling an adjustment of the control strategy if needed.

5 | CONCLUSION

In the present study, a mechanistic chromatography model was applied to the PCS of mAb polishing step. The in silico methodology fulfilled the essential elements of the QbD concept. OFAT sampling allowed classification of process parameters and establishment of PARs. Wet-lab studies derived from in silico screening can lead to a significantly reduced experimental effort compared to purely DoE driven PCS studies. Calculation of process capability considering a posteriori variabilities of feed stream materials and process parameters at manufacturing-scale enabled the identification of a robust set point condition. In this study, in silico PCS results were complemented with experimental data, which reduced the overall impact of mechanistic modeling on the control strategy. When relying exclusively on in silico predictions, consideration of the effects of model parameter uncertainty on model predictions will further increase the trust in the final control strategy.

Considering the complexity of polishing chromatography steps compared to other unit operations in mAb purification processes and the related experimental efforts that must be invested for their characterization, the here presented in silico techniques have the potential to debottleneck process development timelines. While accelerating development and disrupting experimental constraints, mechanistic modeling generated a deep process understanding ensuring consistent product quality in the light of QbD. This work represents a possible concept for the application of digital process twins to QbD related tasks in biopharmaceutical process development, with the focus on preparative chromatography. The proposed methods could further enable the in silico PCS of other unit operations when validated mechanistic models are available.

ACKNOWLEDGMENT

The authors would like to acknowledge the experimental support and thorough review of their colleagues, including Roland Pfänder, Stephanie Combe, Jessica Stolzenberger, Heike Krischollek, and Jens Smiatek. Open Access funding enabled and organized by Projekt DEAL.

AUTHOR CONTRIBUTIONS

Gang Wang: Conceptualization; formal analysis; investigation; methodology; project administration; writing - original draft; writing-review & editing. **Federico Rischawy:** Conceptualization; data curation; formal analysis; investigation; methodology; writing - original draft; writing-review & editing. **Simon Kluters:** Conceptualization; data curation; methodology; project administration; supervision; writing - original draft; writing-review & editing. **Joey Studts:** Conceptualization; formal analysis; investigation; project administration; supervision; writing - original draft; writing-review & editing.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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How to cite this article: Saleh D, Wang G, Rischawy F, Kluters S, Studts J, Hubbuch J. *In silico* process characterization for biopharmaceutical development following the quality by design concept. *Biotechnol Progress*. 2021; e3196. https://doi.org/10.1002/btpr.3196