Received: 10 January 2019 | Revised: 13 August 2019 | Accepted: 9 September 2019

DOI: 10.1002/btpr.2908

RESEARCH ARTICLE

BIOTECHNOLOGY PROGRESS

Process characterization strategy for a precipitation step for host cell protein reduction

Jessica Prentice | Diemchi Vu | David Robbins | Gisela Ferreira

AstraZeneca, Gaithersburg, Maryland

Correspondence
Gisela Ferreira, AstraZeneca One MedImmune Way, Gaithersburg, MD 20878.
Email: ferreirag@medimmune.com

Peer Review
The peer review history for this article is available at https://publons.com/publon/10.1002/btpr.2908.

Abstract

Process characterization using QbD approaches has rarely been described for precipitation steps used for impurity removal in biopharmaceutical processes. We propose a two-step approach for process characterization in which the first step focuses on product quality and the second focuses on process performance. This approach provides an efficient, streamlined strategy for the characterization of precipitation steps under the Quality by Design paradigm. This strategy is demonstrated by a case study for the characterization of a precipitation using sodium caprylate to reduce host cell proteins (HCP) during a monoclonal antibody purification process. Process parameters were methodically selected through a risk assessment based on prior development data and scientific knowledge described in the literature. The characterization studies used two multivariate blocks to decouple and distinguish the impact of product quality (e.g., measured HCP of the recovered product from the precipitation) and process performance (e.g., step yield). Robustness of the precipitation step was further demonstrated through linkage studies across the overall purification process. HCP levels could be robustly reduced to ≤100 ppm in the drug substance when the precipitation step operated within an operation space of ≤1% (m/v) sodium caprylate, pH 5.0–6.0, and filter flux ≤300 L/m^2-hr for a load HCP concentration up to 19,000 ppm. This two-step approach for characterization of precipitation steps has several advantages, including tailoring of the experimental design and scale-down model to the intended purpose for each step, use of a manageable number of experiments without compromising scientific understanding, and limited time and material consumption.

KEYWORDS
host cell protein, precipitation, process characterization, QbD

1 | INTRODUCTION

The goal of biopharmaceutical development is to design a quality product and a manufacturing process to consistently deliver that product.[1] Per ICH Q8 (R2),[1] biopharmaceutical development should result in a manufacturing process with an appropriate control strategy to meet adequate critical quality attributes as defined in a predefined quality target product profile. While the focus is appropriately on product quality, ICH Q8 (R2) also shows consistency of process performance is an important element of the control strategy.

Strategies for product development vary from company to company and from product to product.[1] Quality by Design (QbD) is an enhanced approach to biopharmaceutical development based on systematic and risk-based process understanding, which can be used to

establish a design space (a multivariate operational space that yields the desired product quality). This approach seeks to link the manufacturing process design and operation to product quality.[1-3]

Development studies provide the scientific understanding to meet these goals.[1-3] In later phases of development (e.g., moving from clinical to commercial applications), the development studies are often referred to as process characterization. Process characterization studies demonstrate the impacts of individual or interacting process parameters over the intended operational space on product quality and process performance.[1-3] The choice of relevant process parameters is based on scientific assessments and identification of critical quality attributes (that need to be within specified ranges for appropriate safety and efficacy of a therapeutic).[1-3] Models based on mechanistic understanding and scientific first principles are preferred but they are less commonly used due to the complexity of the characterized unit operations (e.g., bioreactor systems, complex feeds into chromatography steps). Experimental execution yields empirical mathematical models obtained through univariate and multivariate experimental designs.[1-3] The results of these studies (unit operation centered or linking all production steps) are used to confirm the operational space, or on occasion, restrict or "refine" the operational space as a result of deeper process understanding.[1-3]

Much has been published on the application of the QbD approaches for biopharmaceutical purification process characterization, especially on its application to chromatography steps.[4-7] However, there is limited published information on the characterization of precipitation steps under the QbD paradigm. Precipitation is a method for impurity removal in biopharmaceutical processes[8-15] that can be advantageous, because it is often simple, gentle, inexpensive (relative to chromatography), and scalable.[9,12,14-18] The published literature for characterization of precipitation steps has focused mostly on product quality. While some may explore process performance aspects of precipitation (e.g., sizing of filters for precipitate removal), the studies are not necessarily tailored to characterize and establish an appropriate control strategy as part of a manufacturing process.[9,15,19-21]

Precipitation steps call for special considerations when it comes to the design of the scale-down models used for characterization studies that are unique to this mode of separation. While precipitation is seemingly simple (often a batch operation in simple tanks with impellers for mixing), it is actually complex to scale down/up.[22] The precipitation conditions in the scale-down model (e.g., pH, conductivity, final precipitant concentration, and temperature) need to be maintained at values representative of the full-scale process.[22-24] Vessel design (e.g., tank diameter to height ratio, impeller type and diameter, impeller placement, and baffle geometry) needs to mimic manufacturing scale tanks such that mixing (as defined by tip speed, power per volume ratio, shear, and/or residence time) results in the same precipitation endpoints (e.g., extent of precipitation and floc size distribution).[22-25] Finally, the removal of the precipitate, for example (often by depth filtration, carries its own challenges. Depth filters are scaled by area ratio, but limitations in the scale-down model for filters leads to the consumption of large volumes of product.[22-26] Occasionally, filtration scale-down models are abandoned altogether and non-representative methods (such as benchtop centrifugation) are used instead.[22]

Based on these considerations, characterization studies of precipitation steps are likely to require an extensive number of complex experiments and resources (personnel, time, material, data collection and analysis). We propose an alternative streamlined strategy for the characterization of precipitation steps using QbD principles. The strategy uses a two-step approach to multivariate experimental design, with the first step focused on product quality and the second focused on process performance. Additionally, this strategy allows the use different scaled-down models to reflect the relevant outputs being studied in each step, minimizing the complexity of the studies and use of resources.

This strategy is demonstrated by a case study for the characterization studies of a precipitation step using sodium caprylate to reduce host cell proteins (HCP) during a monoclonal antibody purification process. Sodium caprylate can interact with other molecules due to both hydrophobic and polar properties.[14,15,27-29] Under acidic conditions, hydrophobicity dominates causing sodium caprylate to become unstable and precipitate along with acidic proteins (e.g., some HCP), while basic molecules (such as antibodies with basic pI) remain in solution. Results from the characterization studies using this two-step approach demonstrated effective and robust clearance of HCP. This was further verified using linkage studies in which the characterized precipitation step was included in the entire downstream process.

2 | MATERIALS AND METHODS

2.1 | Materials

2.1.1 | Antibody-containing product intermediate

The antibody was a human IgG_1 monoclonal antibody with a pI of 9.0–9.1 (range due to varying levels of charge variants) produced from CHO cell culture. The antibody-containing product intermediate was the eluted product from a cation-exchange chromatography that served as the capture step for the antibody purification process (see *Methods* below for description of the purification process). Antibody concentration in the starting material ranged from 6 to 15 mg/mL (depending on the operation of the cation-exchange chromatography) in 75 sodium phosphate buffer pH 6.5 with approximately 25 mM sodium chloride. The antibody was >99% monomer by high-performance size-exclusion chromatography. The antibody-containing product used in the Step 1 study had 740 ppm HCP. The product used in the Step 2 study had HCP varying between 125 and 6,225 ppm (the varying HCP achieved through well understood method modifications in the cation-exchange chromatography operation). See *Characterization of the precipitation step using a QbD approach* below for description of Steps 1 and 2.

2.1.2 | Reagents

Sodium caprylate was obtained from EMD Millipore (Darmstadt, Germany). A 20% (mass/volume) stock solution was prepared by dissolving sodium caprylate in 10 mM sodium phosphate pH 6.5 solution. Sodium chloride, sodium phosphate monobasic monohydrate, sodium phosphate anhydrous, sodium phosphate dibasic heptahydrate, sodium

sulfate, Tris base, Tris hydrochloride, and glycine were obtained from Avantor Performance Materials (Center Valley, PA).

2.1.3 | Filters

Millistak+ pod grade X0HC (nominal retention <0.1 μm) and D0HC (nominal retention 0.6–9.0 μm) depth filters and Express SHC 0.5/0.2-μm sterilizing grade filters were purchased from EMD Millipore (Darmstadt, Germany).

3 | METHODS

3.1 | General description of the purification process

The antibody was produced and secreted by CHO cells cultivated in a fed-batch cell culture using proprietary in-house media, feeds, and bioreactor set points. Cells were separated from the cell culture fluid using a combination of continuous disc-stack centrifugation ($Q/\sum$ 4.4–9.1 $\times 10^{-9}$ m/s, discharge interval determined from packed cell volume measured immediately before harvest) fed directly to a filter train comprised of a depth filter (Millistak+ pod grade X0HC, nominal retention <0.1 μm, loaded to ≤200 L/m^2 at 30–60 L/m^2-hr and ≤30 psi) and a 0.2-μm filter (Express SHC loaded to ≤3,000 L/m^2 at ≤30 psi). The antibody was captured using cation-exchange chromatography (SO_3-based resin operated in bind-and-elute mode with loading at pH 5.3, two washes designed to clear HCP and charge variants, and elution at pH 6.5 through increased conductivity). The cation-exchange chromatography eluted product was acidified to pH 3.5 and held for a minimum of 60 min for viral inactivation. The product was then adjusted to pH 5.2, subjected to the precipitation treatment, and followed by neutralization to pH 7.5. Subsequently, the antibody was purified by anion-exchange chromatography (quaternary amine resin operated in flow-through mode at pH 7.5) and mixed-mode chromatography (anion exchange/hydrophobic interaction resin operated in bind-and-elute mode with loading at pH 7.5 and elution at pH 5.2) polishing steps, filtered through a virus filter (20-nm nominal pore size), and formulated to the Drug Substance composition.

3.2 | Detailed description of the precipitation

The cation-exchange capture chromatography eluted product (starting material) was adjusted to pH 3.5 by addition of 0.5 M glycine pH 2.35, held for a minimum of 60 min, and subsequently adjusted with 1.0 M Tris pH 9.0 to pH 5.2 (observed conductivity 8.5–9.5 mS/cm). Sodium caprylate stock solution was added to a final caprylate concentration of 1% (mass/volume) to initiate precipitation (observed conductivity 13–14 mS/cm). The precipitated material was allowed to mix for 1 hr at an agitation rate of 4 W/m^3. The precipitate was then removed using a filter train including Millistak+ pod grade D0HC (nominal retention 0.6–9.0 μm) pod depth filters loaded up to 925 L/m^2 capacity at 100 L/m^2-hr constant flux, followed in series by an Express SHC 0.5/0.2-μm filtration (differential pressure across filter train <30 psi). Following filtration, the product was further neutralized with 1.0 M Tris pH 9.0 to pH 7.5 to halt the precipitation process and prepare the product for the subsequent anion-exchange chromatography. Any departures from the target experimental values used during process characterization are described in the *Results and Discussion* section and Table 1.

3.3 | Characterization of the precipitation step using a QbD approach

1. *Critical quality attributes, process performance, and corresponding acceptable ranges*: The relevant critical quality attributes (characteristics of the product that should be within an appropriate limit, range or distribution to ensure the safety and efficacy of the drug[1-3]) were HCP in the precipitated product and Drug Substance, monomer purity, and charge variants. The acceptable ranges for HCP in the antibody-containing product after the caprylate precipitation step and Drug Substance were ≤650 ppm and ≤100 ppm, respectively. The acceptable ranges for monomer purity and charge variants were ≥98% by high-performance size-exclusion chromatography and ≤10% by high-performance ion-exchange chromatography, respectively. Process performance measurements (step yield and filter capacity) were also studied as an added layer of control to ensure step consistency and manufacturing feasibility. The acceptable ranges for the process performance measurements were step yield >90% step yield and filter capacity >260 L/m^2.
2. *Risk assessment* –The potential impact of process parameters on product quality and process performance outputs involved in the precipitation step were scored as high, low, or no impact. Process parameters identified in the risk assessment as having potential impact on product quality and process performance were included in multivariate studies (see *Characterization Study*). Process parameter ranges studied can be found in Table 1.
3. *Characterization Study* –A two-step approach was used to further refine the multivariate study design and is illustrated in Figure 1.
    - *Step 1 – product quality*. The impacts of the amount of sodium caprylate added, pH for precipitation, hold time, and temperature on product quality (HCP, monomer purity, and charge variants) were determined using a multivariate full-factorial design of experiments (DOE) over the process parameter ranges as shown in Table 1. This two-level, full-factorial study design comprised 18 experiments (including two center points using target values as described in the "*Detailed description of the precipitation*" for estimation of process variability). It determined all main effects and two-way interactions between process parameters. The design had 100% power for detecting differences in response of 2 SDs (see Table 2). Based on the chemistry of precipitation by sodium caprylate where precipitation of HCP is based on solution pH, amount of sodium caprylate, and molecule pI, the assessment of product quality required only that precipitation occurred. Thus, scaling of vessel design, mixing and filtration systems was not necessary,[22-26] and centrifuge tubes or small beakers with magnetic stir bars could be used.

TABLE 1 Risk assessment of the sodium caprylate precipitation step

Process outputs	Process parameter range evaluated	Product quality		Process performance			
Process parameters		Step yield	Filter capacity	HCP	Monomer purity	Charge variants	Rationale
Temperature	15-25°C	No	No	High	Low	Low	Literature[9,14,15] and prior development data suggest potential impact on the kinetics of precipitation and therefore potentially impact HCP in the product. Prior data showed no impact to filterability, though. Temperature may also impact antibody stability.
Antibody concentration in capture product	6-15 mg/mL	No	No	No	No	No	No impact of antibody concentration is anticipated because it is not precipitated in this process.
HCP in capture product	<6,225 ppm	No	High	No	No	No	Prior development data showed that HCP in the starting product impacts the amount of precipitate formation and therefore filter capacity. HCP in the product was not observed to depend on the HCP in the starting product.
pH for precipitation	pH 4.5-6.5	No	High	High	Low	Low	Literature[9,14,15] and prior development showed pH impacts formation of precipitate, and thus HCP in the product and filterability. pH may also impact antibody stability.
Conductivity for precipitation	6.5-10.0 mS/cm	No	No	No	No	No	Literature and prior development data showed that precipitation was pH driven, and conductivity had minimal impact.
Amount of caprylate added	0.75-2.0% (mass/volume)	No	High	High	No	No	Literature[9,14,15] and prior development data showed impact of the amount of sodium caprylate on the formation of precipitate, and thus HCP in the product and filterability.
Rate of caprylate addition	0-120 min	No	No	No	No	No	Prior development data showed no impact of rate of caprylate addition on product quality or process performance.
Agitation rate	1-22 W/m^3	No	High	No	No	No	Prior development data showed impact on floc size distribution and thus could impact filterability. Precipitate is fully retained by the filters, so HCP in the product is not impacted. Shear within this agitation range is not expected to impact antibody stability.
Hold time	30-300 min	No	High	High	Low	Low	Literature[9,14,15] and prior development data showed impact of hold time on floc size distribution and therefore filter capacity. Hold time could induce further precipitation and thus impact HCP in the product. Hold time may impact antibody stability.
Filter flux	100-300 L/m^2-hr	No	High	No	No	No	Literature[9,14,15,35] and prior development data showed flux impacts filter capacity. Precipitate is fully retained by the filters, so HCP in the product is not impacted.
Neutralization pH after precipitation step	pH 7.3-pH 7.7	No	No	No	No	No	The intent of this step is to quench precipitation and prepare to product for the next purification step.

Note: Severity of potential impact scoring and recommended study strategy: No impact = no study recommended; low impact and high impact = multivariate study recommended.

- *Step 2–process performance*. The impacts of filter flux, agitation rate, hold time, HCP in the starting material, amount of sodium caprylate added, and pH for precipitation on process performance on step yield and filter capacity were determined using a multivariate fractional-factorial DOE over the process parameter ranges given in Table 1. This two-level, resolution IV study design had 14 experiments (including two center points for estimation of process variability) and determined all main effects and some two-

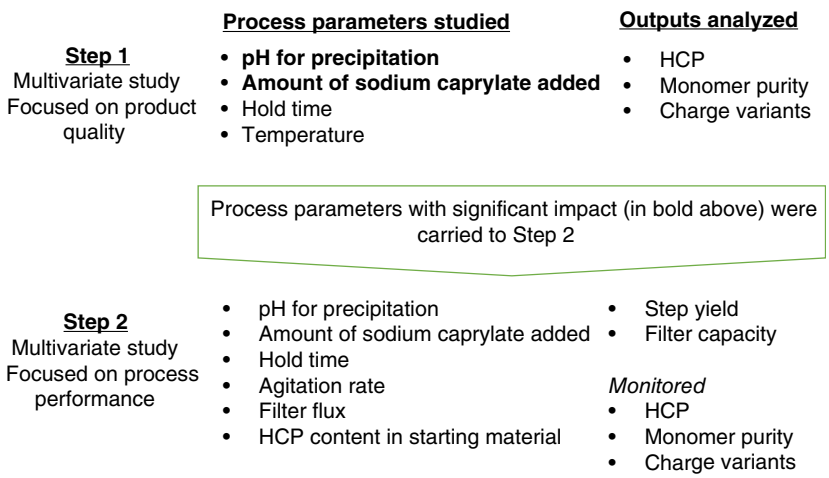


FIGURE 1 Schematic representation of the two-step characterization of the precipitation with sodium caprylate

TABLE 2 Multivariate study design summary

Response	Desired difference in response to detect	Expected SD for the response	Power to detect response for step 1	Power to detect response for step 2
HCP	100 ppm	50 ppm	100%	99%
Monomer purity	0.2%	0.1%	100%	99%
Charge variants	1%	0.5%	100%	99%
Yield	10%	5%	N/A	99%
Filter capacity	10 L/m^2	5 L/m^2	N/A	99%

way interactions between process parameters. While 2-way interactions were aliased, should interactions be found, the design could be augmented to include additional experiments to de-alias interactions. The design had 99% power for detecting differences in response of 2 SDs (Table 2). In addition to impact to process performance, the impact to the product quality attributes from Step 1 continued to be monitored. The assessment of process performance required scale-down models of vessel design, mixing, and filtration systems.[22-26] Therefore, 3-L bioreactors fitted with 1 cm diameter impellers were used as precipitation vessels (Applikon Biotechnology, Delft, the Netherlands), with mixing based on similar power per volume (4 W/m^3) to that of the clinical manufacturing facility. Filtration for removal of precipitate was scaled based on constant filter grade, volume per area ratio, and flux.[22-26,30]

4. *Definition of the operational space / linkage studies*: Process parameter ranges were tested to confirm the robustness and multivariate nature of the operational space using linkage studies. In this case, the entire downstream process (capture through formulation) including the characterized precipitation step was used. A cation-exchange chromatography eluted product with atypically high HCP (19,000 ppm) was produced and subjected to the precipitation step conditions determined in the multivariate studies to be least effective at HCP clearance. The precipitated product was then purified through the remainder of the purification process as described in the *General Description of the Purification Process*, using target/typical running conditions. The HCP of the Drug Substance produced using this approach was compared to the Drug Substance using a typical HCP input into the precipitation step (1,300 ppm) and run through target precipitation conditions. Figure 2 illustrates the linkage study design.

3.4 | Analytical assays

Total protein concentration was measured by UV absorbance at 280 nm (Agilent, Santa Clara, CA). Monomer purity was measured by high-performance size-exclusion chromatography using a TSKgel G3000SWxl 30 cm × 7.8 mm column (Tosoh Bioscience LLC, King of Prussia, PA) operated at 1 mL/min in a sodium phosphate/sodium sulfate mobile phase at pH 6.8. Charge variants were measured by high-performance ion-exchange chromatography using a Propac WCX-10 25 cm × 4 mm column (Thermo Fisher Scientific) operated at 1 mL/min in sodium phosphate mobile phase with a sodium chloride gradient. HCP was measured using Gyrolab xP technology (Gyros AB, Uppsala, Sweden) and proprietary in-house-derived immunogenic reagents.

3.5 | Statistical analysis

Multivariate design of experiments and statistical analysis was performed using JMP version 10 software (SAS, Cary, NC).[31]

4 | RESULTS AND DISCUSSION

4.1 | Identification of relevant critical quality attributes to be part of the characterization studies

While severity risk assessments on the criticality of product attributes for a therapeutic antibody identify multiple critical quality attributes

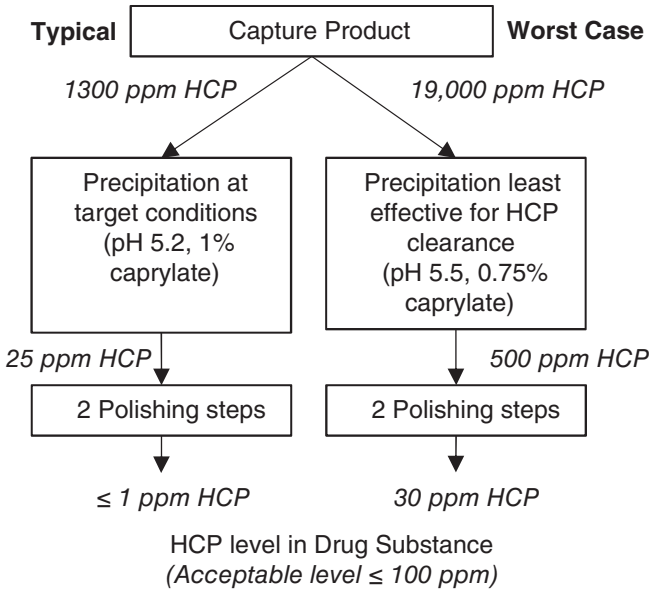


FIGURE 2 Linkage study design and results

affecting safety and efficacy, the attributes identified based on the chemistry of precipitation by sodium caprylate and degradation pathways for this particular antibody as potentially being impacted were HCP, monomer purity, and charge variants. HCP were measured with an ELISA-based assay developed with in-house-derived immunoreagents. Acceptable levels for HCP were related to the amount identified by the assay and not related to HCP identity. The wide coverage of these reagents for HCP populations (approximately 80%, as verified by in-house assay characterization) imparted a direct comparison of HCP results and relieved the data analysis from the characterization from specific cleared HCP populations. Monomer purity could be impacted through the formation of aggregates and/or fragments during precipitation conditions. Charge variants were monitored due to the propensity of this antibody to deamidate upon bioreactor production and/or product storage.

4.2 | Risk assessment

Following a QbD-based approach, a risk assessment was performed to determine which process parameters made scientific sense to be further studied during characterization studies. Table 1 shows the process parameters associated with the precipitation step, the corresponding ranges that were assessed, the risk score associated with the extent of impact from each process parameter on the relevant outputs, and justification for the final scoring (highest identified for each process parameter). The parameter ranges were informed by prior knowledge from early process development and by manufacturing control capabilities, but some were also widened to better understand the process. Seven process parameters were identified as having potential impact on the product quality and process performance of the precipitation step and were included in the characterization studies: pH for precipitation, amount of caprylate added, temperature, hold time, agitation rate, filter flux, and HCP in the starting material.

4.3 | Characterization studies

If all seven of the parameters identified in the risk assessment were to be studied in a single multivariate design of experiments using a full-factorial design, 128 experiments would be needed.[34] Numerous fractional-factorial designs exist with reduced number of experiments, but at least 64 experiments (resolution V to identify main effects and 2-way interactions) would still be required.[34] Coupled with the 3-L volume of product required for scale-down of mixing and filtration, the amount of product needed for the characterization study would be substantial.

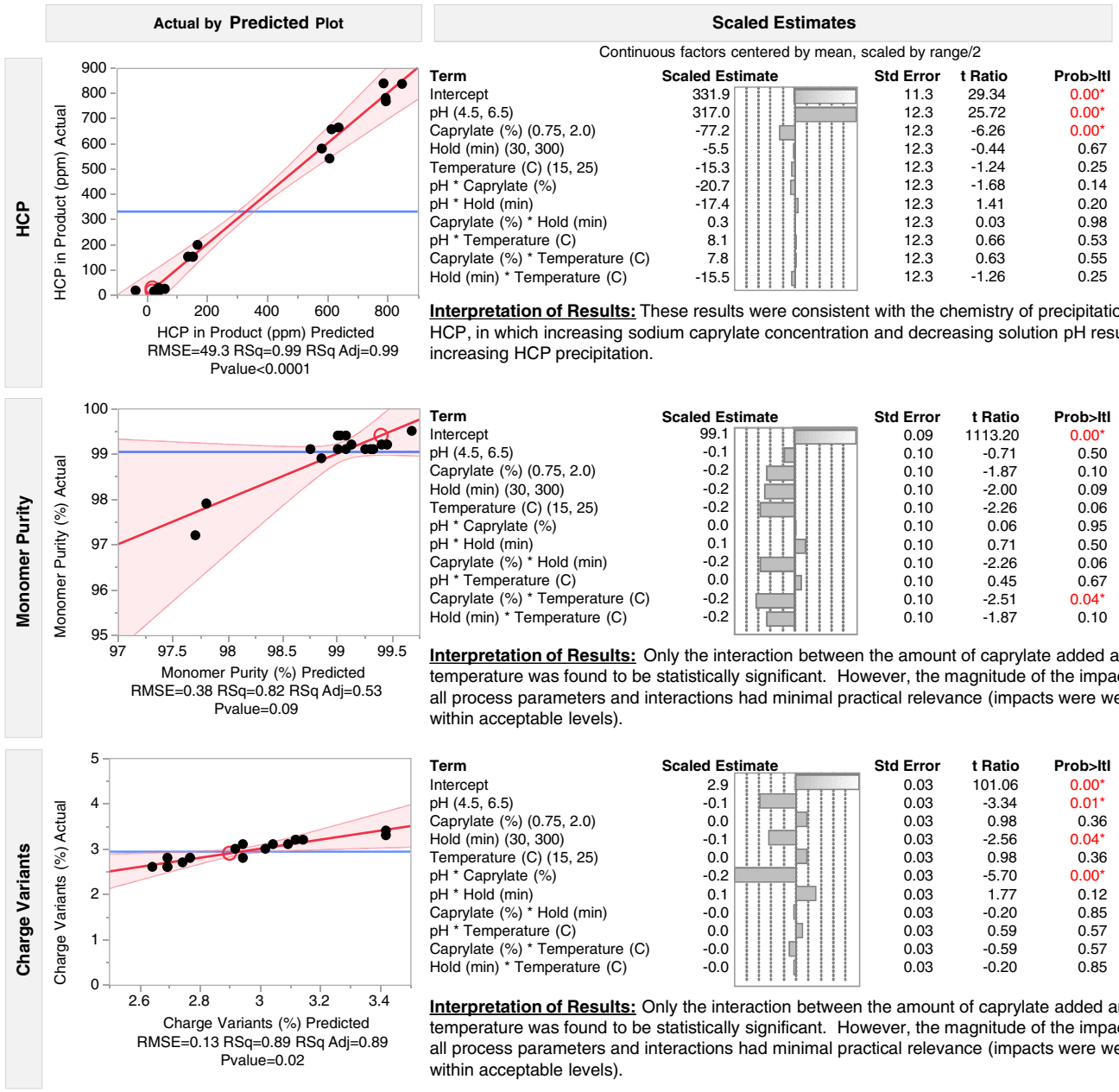
Our proposed two-step characterization approach, focusing first on product quality and second on process performance, reduced the overall number of experiments to 32 without compromising process understanding. Step 1 used a full-factorial design. This study design was chosen because it provides the greatest resolution since product quality is of greatest importance under the QbD paradigm. Step 2 used fractional-factorial design. This design had some aliasing between two-way interactions and had lower resolution than a full-factorial design. The fractional-factorial design balanced the material requirement (which was larger for this study due to the scale-down model design for this study) with the ability to detect responses and augment the design to de-alias interactions, should interactions be found. This is a typical approach used in industry and thus illustrated a representative characterization study design. Therefore, this design was deemed satisfactory for characterization studies for process performance during Step 2.

The two-step approach also allowed for selection of separate suitable scale-down models based on the intended purpose of each step. Step 1 did not require the use of scale-down vessels or filters, so tubes and small beakers were used. This reduced the volume of product per experiment from 3-L (scaled-down mixing vessel) to <50 mL per condition studied. Additionally, experiments in Step 1 could be run in parallel, allowing for significant time savings. Meanwhile, Step 2 used scaled-down vessels and filters to mimic the actual clinical manufacturing facility.

4.3.1 | Step 1—product quality

Figure 3 shows the statistical analysis and interpretation of results from the characterization study, including the selected factors and their impacts, significance of main effects and two-way interactions, measurements of model fit, and determination coefficients (R^2 adjusted R^2).

The pH for precipitation and amount of caprylate added had the most significant and substantial impacts on product quality of the four parameters studied in Step 1. Therefore, these parameters were carried to Step 2 for further evaluation in the process performance characterization, in addition to parameters that could impact process performance. Temperature was excluded from Step 2 characterization study as its influence on product quality was minimal. Although hold time also had minimal impact on product quality, it was included in



Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	331.9	11.3	29.34	0.00*
pH (4.5, 6.5)	317.0	12.3	25.72	0.00*
Caprylate (%) (0.75, 2.0)	-77.2	12.3	-6.26	0.00*
Hold (min) (30, 300)	-5.5	12.3	-0.44	0.67
Temperature (C) (15, 25)	-15.3	12.3	-1.24	0.25
pH * Caprylate (%)	-20.7	12.3	-1.68	0.14
pH * Hold (min)	-17.4	12.3	1.41	0.20
Caprylate (%) * Hold (min)	0.3	12.3	0.03	0.98
pH * Temperature (C)	8.1	12.3	0.66	0.53
Caprylate (%) * Temperature (C)	7.8	12.3	0.63	0.55
Hold (min) * Temperature (C)	-15.5	12.3	-1.26	0.25

Interpretation of Results: These results were consistent with the chemistry of precipitation of HCP, in which increasing sodium caprylate concentration and decreasing solution pH result in increasing HCP precipitation.

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	99.1	0.09	1113.20	0.00*
pH (4.5, 6.5)	-0.1	0.10	-0.71	0.50
Caprylate (%) (0.75, 2.0)	-0.2	0.10	-1.87	0.10
Hold (min) (30, 300)	-0.2	0.10	-2.00	0.09
Temperature (C) (15, 25)	-0.2	0.10	-2.26	0.06
pH * Caprylate (%)	0.0	0.10	0.06	0.95
pH * Hold (min)	0.1	0.10	0.71	0.50
Caprylate (%) * Hold (min)	-0.2	0.10	-2.26	0.06
pH * Temperature (C)	0.0	0.10	0.45	0.67
Caprylate (%) * Temperature (C)	-0.2	0.10	-2.51	0.04*
Hold (min) * Temperature (C)	-0.2	0.10	-1.87	0.10

Interpretation of Results: Only the interaction between the amount of caprylate added and temperature was found to be statistically significant. However, the magnitude of the impact for all process parameters and interactions had minimal practical relevance (impacts were well within acceptable levels).

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	2.9	0.03	101.06	0.00*
pH (4.5, 6.5)	-0.1	0.03	-3.34	0.01*
Caprylate (%) (0.75, 2.0)	0.0	0.03	0.98	0.36
Hold (min) (30, 300)	-0.1	0.03	-2.56	0.04*
Temperature (C) (15, 25)	0.0	0.03	0.98	0.36
pH * Caprylate (%)	-0.2	0.03	-5.70	0.00*
pH * Hold (min)	0.1	0.03	1.77	0.12
Caprylate (%) * Hold (min)	-0.0	0.03	-0.20	0.85
pH * Temperature (C)	0.0	0.03	0.59	0.57
Caprylate (%) * Temperature (C)	-0.0	0.03	-0.59	0.57
Hold (min) * Temperature (C)	-0.0	0.03	-0.20	0.85

Interpretation of Results: Only the interaction between the amount of caprylate added and temperature was found to be statistically significant. However, the magnitude of the impact for all process parameters and interactions had minimal practical relevance (impacts were well within acceptable levels).

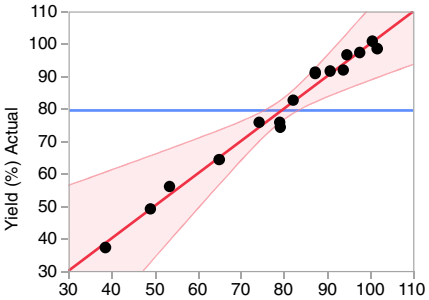
FIGURE 3 JMP statistical analysis and discussion of results from step 1—product quality characterization. The "Actual by Predicted" plots show how well the statistical model fits the experimental data. Residuals are depicted by the experimental data points along the solid red line representing the model. The shaded red region around the red line corresponds to the 95% confidence interval for the model obtained. The blue line represents the model predicted response at center point. The determination coefficients and assessment of model fit (RMSE, R2, adjusted R2, model *p*-value) are found below the plot. The "Scaled Estimates" reflect the magnitude of the impact of process parameters and their interactions. Values in parenthesis next to the process parameter names correspond to the ranges that were tested. Statistically significant process parameters are indicated by *p*-values <.05 in red/orange text with asterisk. The Pareto-like plot illustrates the relative magnitude of impacts. The "Interpretation of Results" discusses the statistical analysis

Step 2 due to the identified potential impact to process performance, as described in the risk assessment (Table 1).

4.3.2 | Step 2—process performance

Similarly to the results from Step 1 (Figure 3), Figure 4 shows the statistical analysis and interpretation of results from the process performance characterization study (Step 2), including the selected factors and their impacts, significance of main effects and two-way interactions, measurements of model fit, and determination coefficients. The interactions shown for each output in the figure are those which impact the outputs based on the understanding of the chemistry of precipitation of HCP and principles of depth filtration. Two interactions impacting the HCP in the product after the precipitation step (starting HCP and pH with amount of caprylate added) were found to be statistically significant. While these interactions

Yield



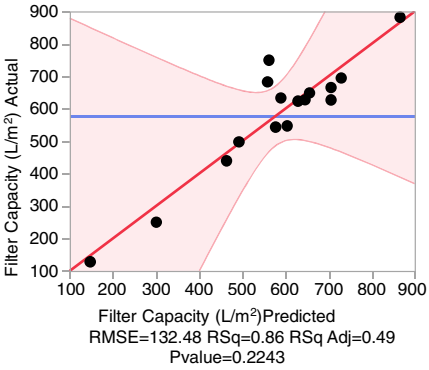
Scaled Estimates

Continuous factors centered by mean, scaled by range/2

Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	78.4	1.3	58.34	0.00*
Starting HCP (ppm) (128, 6214)	2.0	1.4	1.42	0.23
pH (4.5, 6.0)	8.7	1.5	5.92	0.00*
Caprylate (%) (0.75, 2.0)	-19.0	1.5	-12.67	0.00*
Hold (hr) (0.5, 5.0)	-0.7	1.4	-0.50	0.64
Agitation (W/m³) (1, 25)	2.1	1.5	1.41	0.23
Flux (LMH) (100, 300)	1.2	1.5	0.83	0.45
Starting HCP (ppm) * pH	-0.8	1.6	-0.53	0.62
Starting HCP (ppm) * Caprylate (%)	3.2	1.6	1.97	0.12
pH * Caprylate (%)	5.8	1.6	3.57	0.02*
pH * Hold (hr)	-1.3	1.7	-0.75	0.50
Caprylate (%) * Hold	0.7	1.6	0.43	0.69

Interpretation of Results: Consistent with the chemistry of precipitation of HCP, as the caprylate concentration increased and pH decreased, precipitation increased, including non-specific precipitation of the antibody.

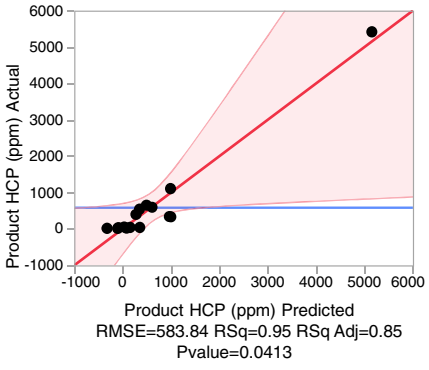
Filter Capacity



Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	582.9	38.6	15.11	0.00*
Starting HCP (ppm) (128, 6214)	-67.0	38.9	-1.72	0.16
pH (4.5, 6.0)	21.3	40.7	0.52	0.63
Caprylate (%) (0.75, 2.0)	-141.1	41.2	-3.43	0.03*
Hold (hr) (0.5, 5.0)	-11.8	39.5	-0.30	0.78
Agitation (W/m³) (1, 25)	49.3	40.7	1.21	0.29
Flux (LMH) (100, 300)	19.3	41.5	0.47	0.67
Starting HCP (ppm) * pH	85.3	66.9	1.28	0.27
Starting HCP (ppm) * Caprylate (%)	-13.9	43.9	-0.32	0.77
pH * Caprylate (%)	101.2	42.4	2.39	0.08
Caprylate (%) * Hold (hr)	9.4	46.8	0.20	0.85
Caprylate (%) * Flux (LMH)	25.2	64.9	0.39	0.72

Interpretation of Results: Consistent with the chemistry of precipitation of HCP, as the caprylate concentration increased, the particulate load for the filters increased and filter capacity decreased. Similarly, increasing starting HCP and the interaction of decreasing increasing caprylate concentration resulted in increased particulate load to the filters and pH with substantial decrease in filter capacity, though these parameters/interactions were not statistically significant.

HCP



Term	Scaled Estimate	Std Error	t Ratio	Prob>|t|
Intercept	776.1	163.7	4.74	0.01*
Starting HCP (ppm) (128, 6214)	622.8	175.9	3.54	0.02*
pH (4.5, 6.0)	567.6	178.6	3.18	0.03*
Caprylate (%) (0.75, 2.0)	-287.0	183.1	-1.52	0.20
Hold (hr) (0.5, 5.0)	95.0	177.6	0.53	0.62
Agitation (W/m³) (1, 25)	-396.9	181.0	-2.19	0.09
Flux (LMH) (100, 300)	457.0	181.7	2.51	0.07
Starting HCP (ppm) * pH	439.4	192.5	2.28	0.08
Starting HCP (ppm) * Caprylate (%)	-572.2	205.1	-2.79	0.05*
Starting HCP (ppm) * Hold (hr)	7.6	213.1	0.04	0.97
pH * Caprylate (%)	-611.0	202.5	-3.02	0.04*
Caprylate (%) * Hold (hr)	-94.4	190.6	-0.50	0.65

Interpretation of Results: As the starting level of HCP increased, the amount of HCP in the product also increased, suggesting a threshold of HCP in the feed for effective HCP clearance. The influence of this parameter dominates the analysis. Consistent with the analysis of product quality (Step 1) and the chemistry of precipitation of HCP, pH was significant. Unlike Step 1, the main effect of caprylate concentration was not significant, though its magnitude of impact was in alignment with Step 1 and its interactions with pH and starting HCP are significant.

FIGURE 4 JMP statistical analysis and discussion of the results from Step 2—process performance characterization. The "Actual by Predicted" plots show how well the statistical model fits the experimental data. Residuals are depicted by the experimental data points along the solid red line representing the model. The shaded red region around the red line corresponds to the 95% confidence interval for the model obtained. The blue line represents the model predicted response at center point. The determination coefficients and assessment of model fit (RMSE, R2, adjusted R2, model *p*-value) are found below the plot. The "Scaled Estimates" reflect the magnitude of the impact of process parameters and their interactions. Values in parenthesis next to the process parameter names correspond to the ranges that were tested. Statistically significant process parameters are indicated by *p*-values <.05 in red/orange text with asterisk. The Pareto-like plot illustrates the relative magnitude of impacts. The "Interpretation of Results" discusses the statistical analysis

were aliased with other two-way interactions, the study design was not augmented to de-alias these interactions due to the deemed limited value added. Step 1 already showed pH and the amount of sodium caprylate added to have a statistically significant impact on HCP, while step 2 showed the additional statistically significant main effect of starting HCP level in the starting material impacting the HCP level in the product after precipitation. Other process parameters (flux, agitation rate, and hold time) were not statistically

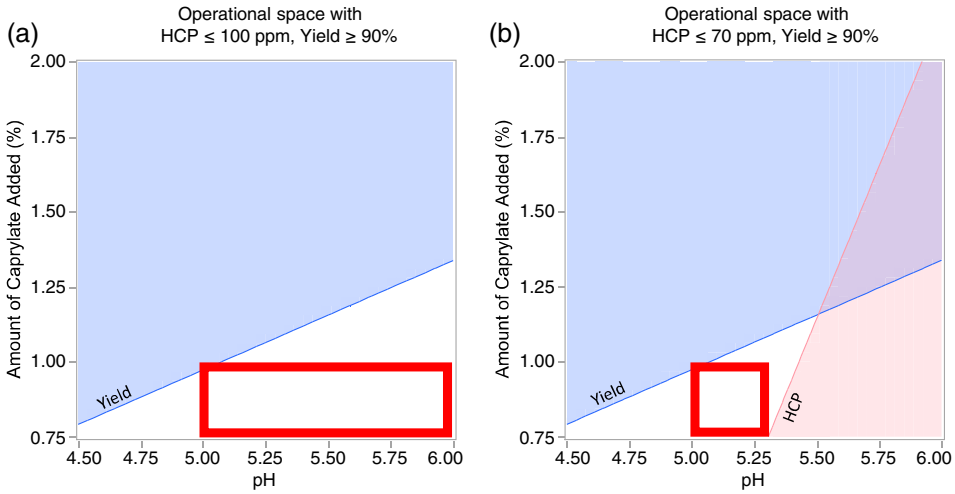


FIGURE 5 Contour plots illustrating potential operational spaces for the precipitation unit operation. The operational space is influenced by the impacts of pH for precipitation and the amount of caprylate added on the precipitation step yield (blue) and HCP in the drug substance (pink). The red boxes denote example operational spaces where step yield is ≥90% and drug substance HCP is ≤100 ppm (a) or more conservatively ≤70 ppm (b)

impactful on the HCP level in the product after precipitation, suggesting that the interactions that are shown (i.e., pH, amount of sodium caprylate added and HCP in the starting material) were descriptive of the process characterization. These findings were consistent with the scope of the study for each step and the chemistry of the precipitation step.

The HCP level in the starting material, pH for precipitation, and the amount of sodium caprylate added had the most significant and substantial impacts on process performance, as well as on product quality. At some limits of these characterized ranges, HCP and step yield exceeded predetermined acceptable ranges (≤650 ppm and ≥90%, respectively) in the product. The HCP in the starting material was dependent on the operation of the cation-exchange chromatography step, and thus was controlled during the capture cation-exchange chromatography. The ranges for the other process parameters were controlled during the precipitation and thus these ranges could be limited to where impacts were found to be within the acceptable ranges (pH for precipitation pH 5.0–6.0 and amount of caprylate added).

Monomer purity and charge variant were monitored during Step 2. However, the techniques used to generate high HCP starting material used in Step 2 also resulted in high levels of aggregate (reduced monomer purity) and charge variants in the starting material. The monomer purity and charge variant levels in the product after the precipitation step were the same as the levels in the starting material (results not shown), showing these attributes were not impacted by the precipitation step process parameters within the ranges studied. Statistical analysis of monomer purity and charge variants in the product would be confounded by the HCP level in the starting material and therefore were excluded from analysis during Step 2.

4.4 | Linkage study to determine process robustness

A linkage study was performed to verify the results from the characterization studies, to ensure that the precipitation was robust enough to handle typical variability of HCP in the starting material (125–6,225 ppm), and also result in HCP ≤100 ppm for the Drug Substance. The linkage study design and results are illustrated in Figure 2. The results showed that even when the precipitation step was challenged with atypically high HCP (19,000 ppm) in the starting material and the precipitation was carried out at the least favorable conditions for HCP clearance, after two polishing steps the HCP was reduced to similar values to those obtained from typical starting material and well under the acceptable range of ≤100 ppm for Drug Substance.

4.5 | Operational space analysis

The HCP level in the starting material, pH for precipitation, and the amount of sodium caprylate added had the most significant and substantial impacts on process performance and product quality during the characterization studies. Therefore, the operational space for the precipitation unit operation would be defined based on these process parameters. As discussed above, the HCP in the starting material was dependent on the operation of the cation-exchange chromatography step, but the precipitation step was able to accommodate HCP in the starting material approximately three-fold higher than typical high HCP levels during normal process operation. The ranges for pH and the amount of sodium caprylate added could be controlled during the precipitation and thus could be limited to where impacts were found to be within the acceptable ranges for product quality and process performance. Figure 5 shows contour plots of the combined impacts of pH and amount of sodium caprylate added on HCP in the Drug Substance and step yield. The areas marked by the red borders denote a proposed example of an operational space in which the precipitation step yield is ≥90% and Drug Substance HCP levels are ≤100 ppm (Figure 5a). This operational space is limited to sodium caprylate concentration ≤1% (mass/volume) and pH 5.0–6.0. If (for illustrative purposes) a more conservative HCP limit of ≤70 ppm is used (Figure 5b), the operational space is reduced to a pH range of 5.0–5.3. Since this was a multivariate study, one could arguably claim a Design Space (operational space within which any combination of the defined parameter ranges results in appropriate quality). The knowledge

accumulated from this characterization exercise allowed for and successfully achieved the needed purity, even for HCP in the Drug Substance lower than 100 ppm.

5 | CONCLUSIONS

A two-step QbD-based approach to the characterization of precipitation steps was proposed and successfully demonstrated with a case study using sodium caprylate-induced precipitation of HCP. The two-step approach sequentially addressed the impacts of process parameters on product quality (HCP, monomer, and charge variants) and then process performance (step yield, and filterability). Additionally, linkage studies verified the robustness of the characterized operational space to achieve Drug Substance with HCP < 100 ppm and proving the successful application of the two-step QbD strategy to the characterization of this precipitation step.

This characterization strategy had several advantages, including tailoring the experiment design and scale-down model to the intended purpose of each step and reducing the total number of experiments, material consumption, and time by approximately 75, 90, and 90%, respectively (relative to process characterization of product quality and process performance in a single step using a representative scaled-down mixing and filtration model for all experiments).

The two-step strategy described here to characterize precipitation in a downstream process has already been adopted in-house, for precipitation that is forced through addition of components that induce floc formation (as presented here) as well as precipitation, which may manifest during biopharmaceutical manufacturing like the turbidity from a chromatography column effluent.

ACKNOWLEDGMENTS

The authors would like to acknowledge William Wang, Guillermo Miro-Quesada, and Tolulope Ogunsola of AstraZeneca for helpful insights, support with the experimental execution and statistical analysis.

CONFLICT OF INTEREST

All authors have no conflict of interest to declare and approved the manuscript and this submission.

DATA AVAILABILITY STATEMENT

Research data are not shared.

ORCID

Jessica Prentice https://orcid.org/0000-0002-1719-103X
Gisela Ferreira https://orcid.org/0000-0001-8357-3004

REFERENCES

1. ICH Harmonised Tripartite Guideline. Pharmaceutical Development Q8 (R2). International Conference on Hamonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf. Accessed August 29, 2016.
2. Rathore AS, Winkle H. Quality by design for biopharmaceuticals. *Nat Biotechnol*. 2009;27(1):26-34.
3. Yu LX, Amidon G, Khan MA, et al. Understanding pharmaceutical quality by design. *AAPS J*. 2014;16(4):771-783.
4. DPQR. Peer-reviewed publications on QBD topics (2007-2011). FDA https://www.fda.gov/downloads/AboutFDA/CentersOffices/OfficeofMedicalProductsandTobacco/CDER/UCM288858.pdf. Accessed April 7, 2019.
5. Cook S, Patton KA, Bazemore LR. Quality by design in the CMO environment. *BioPharm Int*. 2007;20(12):28-35.
6. Thakor NS, Amrutkar SV. Implementing quality by design (QbD) in chromatography. *J Anal Pharm Chem*. 2017;4(1):1078.
7. Hanna Brown M, Barnette K, Harrington B, et al. Using quality by design to develop robust chromatographic methods. *Pharm Tech*. 2014;38(8):48-64.
8. Arunakumari A, Wang J, Ferreira G. Advances in non-protein a purification processes for human monoclonal antibodies. *BioPharm Int*. 2009;2009(2):22-26.
9. Brodsky Y, Zhang C, Yigzaw Y, Vedantham G. Caprylaic acid precipitation method for impurity reduction: an alternative to conventional chromatography for monoclonal antibody purification. *Biotechnol Bioeng*. 2012;190(2):2589-2598.
10. Chollangi S, Parker R, Singh N, Li Y, Borys M, Li Z. Development of robust antibody purification by optimizing protein-a chromatography in combination with precipitation methodologies. *Biotechnol Bioeng*. 2015;112(11):2292-1304.
11. Giese G, Myrold A, Gorrell J, Persson J. Purification of antibodies by precipitating impurities using polyethylene glycol to enable a two chromatography step process. *J Chromatogratogr B Analyt Technol Biomed Life Sci*. 2013;938:14-21.
12. Glynn J. Process-scale precipitation of impurities in mammalian cell culture broth. *BioPharm Int*. 2008;2008(3)12-18.
13. McDonald P, Victa C, Carter-Franklin JN, Farmer R. Selective antibody precipitation using polyelectrolytes: a novel approach to the purification of monoclonal antibodies. *Biotechnol Bioeng*. 2009;102(4):1141-1151.
14. Wang J, Diehl T, Aguiar D, Dai XP, Arunakumari A. Precipitation of process-derived impurities in non-protein a purification schemes for antibodies. *Biopharm Int*. 2009;2009(7):4-10.
15. Zheng J, Wang L, Twarowska B, et al. Caprylic acid-induced impurity precipitation from protein a capture column elution pool to enable a two-chromatography-step process for monoclonal antibody purification. *Biotechnol Prog*. 2015;31(6):1515-1524.
16. Gottschalk U. The renaissance of protein purification. *Biopharm Int*. 2006;2006(3):8-9.
17. Low D, O'Leary R, Pujar NS. Future of antibody purification. *J Chromatogr B*. 2007;848:48-63.
18. Thommes J, Etzel M. Alternatives to chromatographic separations. *Biotechnol Prog*. 2007;23(1):42-45.
19. Gong X, Chen H, Chen T, Qu H. Unit operation optimization of the manufacturing of botanical injections using a design space approach: a case study of water precipitation. *PLoS One*. 2014;9(8):e104493.
20. Xu ZL, Huang WH, Gong XC, et al. Design space approach to optimize first ethanol precipitation process of Dangshen. *Zhongquo Zhong Yao Za Zi*. 2015;40(22):4411-4416.
21. Buyel JF, Opdensteinen P, Fischer R. Cellulose-based filter aids increase the capacity of depth filters during the downstream

processing of plant-derived biopharmaceutical proteins. *Biotechnol J*. 2015;10(4):584-591.
22. Godavarti R, Petrone J, Robinson J, Wright R, Kelley BD, Boklon GR. Scaled-down models for purification processes: approaches and applications. In: Rathore AS, Sofer G, eds. *Process Validation in Manufacturing of Biopharmaceuticals*. Boca Raton, FL: CRC Press; 2012:106-109.
23. Rathore AS, Joshi VS. Scaledown of biopharmaceutical purification operations. In: Flickinger MC, ed. *Downstream Industrial Biotechnology: Recovery and Purification*. Hoboken, NJ: John Wiley and Sons, Inc; 2013:129-146.
24. Moscariello J. Scale-down models: an indispensable tool to biopharmaceutical process development. *Am Pharmaceut Rev*. https://www.americanpharmaceuticalreview.com/Featured-Articles/187246-Scale-Down-Models-An-Indispensable-Tool-to-Biopharmaceutical-Process-Development/. Published April 30, 2016. Accessed October 8, 2019.
25. Buyel JF, Hubbuch J, Fischer R. Comparison of tobacco host cell protein removal methods by blanching intact plants or by heat treatment extracts. *J Vis Exp*. 2016;114:1-9.
26. Buyel JF, Fischer R. Scale-down models to optimize a filter train for the downstream purification of recombinant pharmaceutical proteins produced in tobacco leaves. *Biotechnol J*. 2014;9:415-425.
27. Bull HB, Breese K. Denaturation of proteins by fatty acids. *Arch Biochem Biophys*. 1967;120(2):303-308.
28. Bull HB, Breese K. Binding of fatty acids by proteins. *Arch Biochem Biophys*. 1967;120:309-315.
29. Chanutin A, Curnish RR. The precipitation of plasma proteins by short-chain fatty acids. *Arch Biochem Biophys*. 1960;89(2):218-220.
30. Millistak+ Pod disposable depth filter system. Merck KGaA. Available for download at: http://www.emdmillipore.com/US/en/product/Millistak+-Pod-Disposable-Depth-Filter-SystemsMM_NF-C9485#documentation. Accessed April 7, 2019.
31. *JMP version 13 Design of Experiments Guide*. 2nd ed. Cary, NC: SAS Institute; 2017.

How to cite this article: Prentice J, Vu D, Robbins D, Ferreira G. Process characterization strategy for a precipitation step for host cell protein reduction. *Biotechnol Progress*. 2020;36:e2908. https://doi.org/10.1002/btpr.2908