cytiva

Quality by design in biotherapeutics purification: understanding and addressing sources of process variability

The principles and methodologies of biopharmaceutical manufacturing are well established today. However, the increased molecular diversity brings new challenges for biomanufacturers.

One of these challenges is how process developers might gain a deeper understanding for sources of variability and set mitigation strategies.

This white paper summarizes information around chromatography resin variability, serving as a reference and providing support for developing robust purification processes for biopharmaceuticals.

Post-launch challenges for manufacturing of biopharmaceuticals

The biopharmaceutical industry has had a tremendous decade of success with now more than 1600 commercially available drugs approved for therapeutic use. As of 2022, over 300 antibodies and recombinant proteins are in late stage development and a great number are expected to enter late stage development phases in the coming years (1). After such a successful decade, it is worthwhile to reflect on how this experience can be leveraged to increase operation efficiency.

It becomes even more relevant to reflect on this experience when considering novel molecular formats in the drug development pipeline with properties that differ from conventional biomolecules like monoclonal antibodies (mAbs), where a substantial amount of prior knowledge can be utilized to guide process development activities.

Although the principles and methododologies for biopharmaceutical development and manufacturing are well established today, there are still a few blind spots worth discussion, one of which is raw material variability.

The chromatography blind spot

Quality by design (QbD) is described as a systematic approach that incorporates prior knowledge, results of studies using design of experiments (DoE), use of quality risk management, and use of knowledge management throughout a product's lifecycle to build quality into the product, rather than only testing the final product (2). QbD incorporates the identification of critical quality attributes (CQAs) that ensure patient safety and drug efficacy, and links to critical process parameters (CPPs) and critical material attributes (CMAs).

Chromatography resin properties are sometimes suggested as potentially critical raw material attributes that could impact CQAs. They are also considered important parameters for consistent process performance (e.g., step yield or productivity). These thoughts are reflected in statements from our discussions with process developers:

- "Several observations have been made regarding impact of resin variability on product quality."
- "Developing an understanding of resin variability impacting product quality is required for Biologic License Application (BLA)."
- "Resin variability is a blind spot to us."

In 2017 at BPI West, Dave Kolwyck stated three common post launch challenges (3):

- Raw material consistency
- Process robustness to variation
- Supply chain continuity

Of these, raw material consistency and supply chain continuity are both closely linked to the capabilities of the raw material producer and so relate to supplier qualification and assessments that determine success in this area (4). Process robustness to variation on the other hand is dependant on activities and knowledge generated during process development. A supplier's manufacturing process capability determines the degree of resin variability and does not constitute a problem in itself. All resins are produced against a specification interval. Investigating whether the inherent variability within a manufacturer's given specification range has an impact on the process outcome is a task for process development.

Voice of process developers

To understand the challenges facing process developers, we conducted a series of interviews with nine biomanufacturing or contract development and manufacturing organization (CDMO) companies. The intention was to investigate the industry's current approach to assessing the impact of chromatography resin variability and the appetite to improve this in the future.

The general findings of these interviews are that:

- Seven out of nine interviewed process developers had seen an impact from resin properties on purification performance during process development or in manufacturing, but most processes show none.
- Roughly half of those interviewed claim their organization complies with the principles of QbD as part of their development methodology.
- Two respondents have implemented adaptive process controls to mitigate the impact of resin variability (*see* "Using an adaptive control strategy for resin variability"), but the majority rely on definition of acceptable ranges for process parameters and "fixed" or less flexible process designs.
- Resin variability is mainly addressed during late-stage process characterization with some front-loading of effort at early stage development.
- Some companies see resin variability as a potential early-stage resin selection criterion, especially for early process development for novel molecular formats.
- Process characterization of resin variability is not required for all processes, but a risk assessment can identify when resin variability should be characterized.
- Some chromatography techniques or modes are more relevant for study:
  - Hydrophobic interaction chromatography (HIC) and multimodal chromatography are more relevant to study than ion exchange chromatography (IEX) and affinity capture.
  - Bind/elute mode (B/E) is a higher priority for study than flow-through mode (FT).
- Risk assessment points to ligand density as the resin attribute most likely to have an impact on product quality and process performance, but it can be useful to evaluate the impact of base matrix properties (like pore size and particle size) for delicate separations.
- The amount of effort directed towards resin variability depends on company strategy and experience.

Process robustness collaborations

There is an increased focus on and drive for process understanding, evidenced by an increase in the number of collaborations we have with biopharmaceutical companies. Typically, these requests are driven by risk assessments that indicate a need to understand the potential impact of resin variability on CQAs or process performance indicators to help ensure robust process outcomes.

Figure 1 shows the number of process robustness collaborations we at Cytiva have engaged in each year. The collaborations include both process characterization studies that take place prior to registration, as well as platform studies that are intended to build additional process understanding by retrospectively looking at the impact of raw material variability in an existing process or platform. The number of collaborations has been almost constant the last five years. Furthermore, the studies are evenly distributed between IEX, HIC, and multimodal chromatography (data not shown), where the somewhat surprisingly high relative number of IEX studies likely reflects the higher frequency of ion exchange chromatography steps in typical downstream processes.

Process and platform characterization:
Number of collaborations with Cytiva

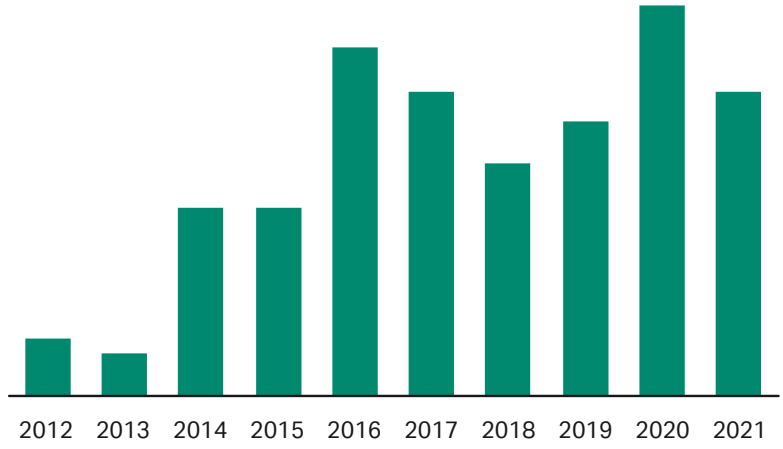

Fig 1. Trends in process robustness collaborations concerning resin variability between Cytiva and biopharmaceutical developers and manufacturers.

How can process developers approach the challenge of resin variability?

Resin variability needs to be viewed in the context of all other parameters that could influence quality attributes or process performance. Although process parameters typically have a higher risk of impact than resin attributes, understanding the interplay between the two, as illustrated in Figure 2, is the key to maximizing robustness and predictability at full manufacturing scale.

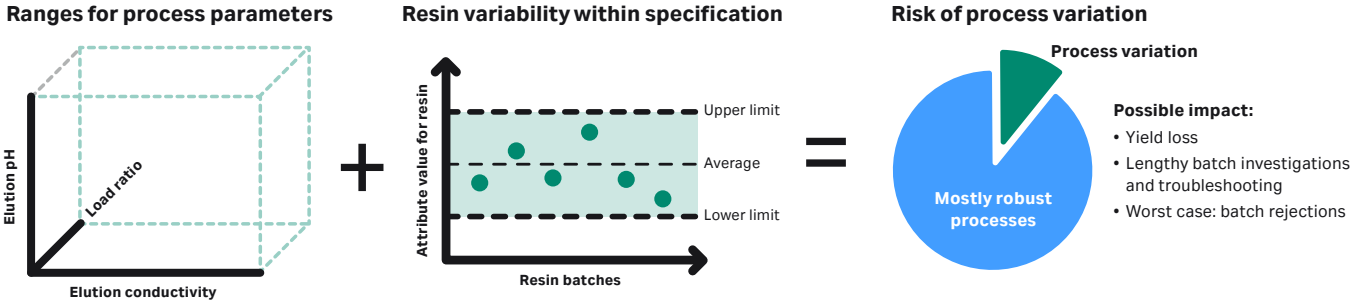

Fig 2. Illustration of the interplay between process parameters and resin attributes.

Suggestions to consider when approaching chromatography process development and characterization include:

Utilize platform knowledge

The key focus in early phase development is to reduce time to clinic, and resin variability tends not to be the most important parameter to consider. Utilizing platform knowledge is an efficient way to reduce risk while still facilitating speed.

Adopt a risk-based approach

For most chromatography steps, resin variability has marginal to no impact on CQAs or process performance attributes such as yield and productivity. A risk-based approach and using indications of process sensitivity can help identify when it is beneficial to include resin variability in process characterization.

Stay close with your resin supplier

As outlined in the BPOG whitepaper: Patient-Centric Requirements for the Supply of Raw Materials into Biopharmaceutical Manufacturing (5), "*The best way to understand, manage, and minimize sources of variation is via mutually-beneficial partnerships between Suppliers and Biopharmaceutical Manufacturers.*"

Impact of chromatography resin attributes on process performance

Review of literature covering impact of chromatography resin attributes

The effect of resin attributes on chromatographic performance is well known from the basic theory and is thoroughly discussed in published literature (6). However, most of the examples relate to ranges of attributes that are typically broader than the specification ranges for commercially available resins designed for bioprocessing applications.

From a practical process robustness perspective, it is more relevant to examine the effect of variations within a tighter range corresponding to the actual variation of the resin manufacturing process, that is, the manufacturing envelope or resin specifications. Published examples of such studies are less common, partially because of a lack of relevant resin samples covering the specification range, but more importantly because most purification processes are robust enough to accommodate these small variations in resin attributes. It is possible for us to speculate whether this also holds true for the new generation of molecular formats that have increasing challenges regarding product-related impurities. Table 1 shows a selection of examples that indicate that resin attributes can impact the outcome of real-world biopharmaceutical processes.

However, it is also clear that the potential impact often is process-specific, where the sensitivity is influenced by the overall process design, as well as the amount of process development effort spent on characterization of raw material variability (*see* "Risk assessment support for quality by design").

Table 1. Literature examples of robustness studies of resin variability (FT = Flow-through mode)

Chromatography technique	Type of separation	Resin attribute	Impact of variability	Reference
AIEX (anion exchange chromatography)	Product related impurities	Unknown	Elution peak appearance	Biogen, 2011 (7)
	Product related impurities	Ligand density	Product loss during wash	Biogen, 2009 (7, 8)
	Aggregate removal Product related impurities	Ligand density	Aggregate removal Product yield Chromatogram appearance	Biogen, 2013 (9)
CIEX (cation exchange chromatography)	Aggregate removal	Lysozyme capacity (particle size showed no impact)	Product yield Aggregate clearance	AstraZeneca, 2017 (10)
	Product variants	Multiple	Product form distribution	Roche, 2016 (11)
	Aggregate and HCP removal	Ligand density	No impact	Genentech, 2012 (12, 13)
	Aggregate and HCP removal	Particle size	Elution volume	Genzyme, 2008 (14)
	Aggregate removal	Ligand density and porosity (particle size showed no impact)	Aggregate removal Product yield	Cytiva, 2014 (15)
HIC (hydrophobic interaction chromatography)	Aggregate removal	Ligand density	Aggregate removal Product yield	Biogen, 2009 (16)
	Product variants	Binding strength	Product form distribution	Pfizer, 2014 (17)
	Product related impurities	Multiple	Impurity removal	Genzyme, 2017 (18)
	HCP removal Glycoform profile	Lysozyme retention*	Product yield Glycoform profile	Genzyme, 2009 (19)
HIC FT	Aggregate and HCP removal	Lysozyme retention	No impact within specification	BMS, 2010 (20)
HIC FT Low salt	Aggregate removal	Lysozyme retention	No impact	Biogen, 2017 (21)
	Aggregate removal	Three lots	No impact	Biogen, 2013 (22)
Multimodal AIEX	Aggregate removal	Ligand density	Product yield Aggregate clearance	Cytiva, 2013 (23)
Protein A affinity chromatography	mAb capture	Ligand density Particle size Accessible pore fraction	No impact	Cytiva, 2015 (24)

* Lysozyme retention is sometimes used to characterize the resin hydrophobicity for HIC resins instead of ligand density.

Insights from our resin development experience

The strategy for development of Cytiva's BioProcess™ chromatography resins has changed since the late 1950s when Sephadex™ resin was launched. In the early days of chromatography resins, the acceptable material specifications were mainly based on the supplier's manufacturing process capability rather than the needs of the user. That is, the specifications would be set as wide as possible. In the last 20 years, the industry has moved to a more user-focused approach, and we at Cytiva are using Design for Six Sigma (25), which is very similar to the quality by design paradigm. The specifications are now based on the intended use of the resin and structure-function relationships determined during the development (Fig 3).

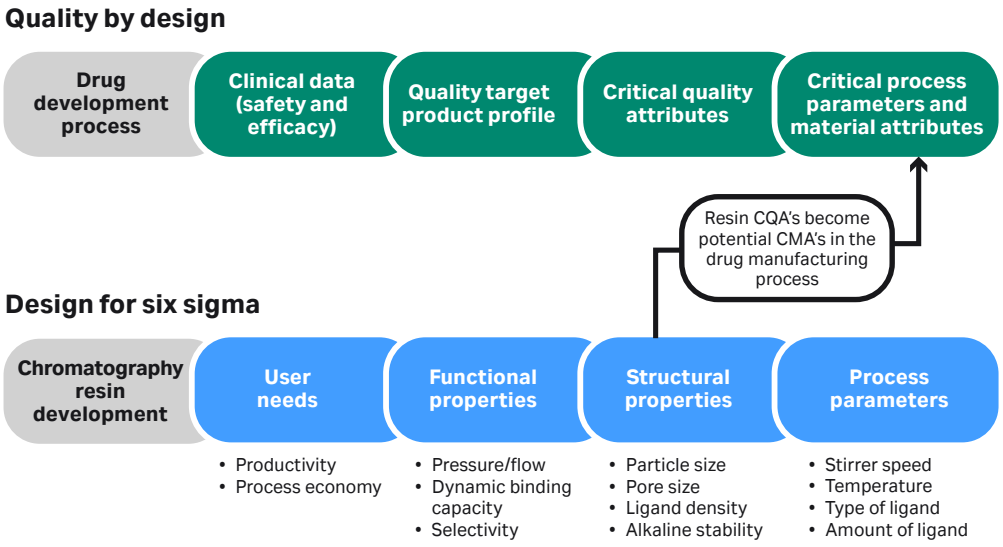

Fig 3. Schematic of resin development based on Design for Six Sigma.

In the Design for Six Sigma approach, the resin specifications are set based on application examples where the manufacturer studies the effect of resin structural properties on functional properties (e.g., dynamic binding capacity [DBC], host cell protein [HCP] clearance, and resolution/selectivity). This typically includes studies of variation far outside the final specification for resin properties, indicating that the effects seen during resin development are larger than expected for the final product. The final specifications of the product are set with the aim of minimizing variation in the studied functional properties while maintaining sufficient manufacturability. At Cytiva, we have gained a lot of experience regarding how structural (i.e. physical and chemical) properties of the resin affect functional properties. Table 2 summarizes the findings from several of our resin development projects.

Note, these findings are only relevant for specific samples under specific process conditions with a specific studied range in resin properties, which in these cases are often larger than the final product specification. The trends for other molecules, conditions, and resin attribute ranges might be different, non-existent, or even reversed. One example seen in Table 2 is the different effects on DBC when evaluated for several proteins that differ in size and charge. This example clearly points to the need to study these aspects under process-specific conditions as described in "Risk assessment support for quality by design".

Table 2. Summary of effects identified in Cytiva resin development projects

Chromatography technique	Resin	Resin attribute (Structural property)	Functional property	Impact on functional property at high attribute level
CIEX	Capto™ S ImpAct	Ligand density	DBC	Increased for IgG
			Aggregate clearance	Decreased
		Particle size	Aggregate clearance	Decreased
			DBC	Decreased for IgG
		Pore size	Aggregate clearance	No effect
			DBC	Increased for IgG
	Capto™ SP ImpRes	Ligand density	DBC	Increased for bovine serum albumin (BSA)
				No effect for IgG
				Increased for lysozyme
			Model protein resolution	Increased
		Particle size	DBC	Decreased for BSA
				Decreased for IgG
				Decreased for lysozyme
			Model protein resolution	Decreased
		Pore size	DBC	Increased for BSA
				Increased for IgG
				No effect for lysozyme
			Model protein resolution	Increased
AIEX multimodal	Capto™ adhere ImpRes	Ligand density	Aggregate clearance	Decreased
			DBC	Increased for IgG
		Particle size	Aggregate clearance	Decreased
			DBC	Decreased for IgG
		Pore size	Aggregate clearance	No effect
			DBC	Increased for IgG
CIEX multimodal	Capto™ MMC ImpRes	Ligand density	Aggregate clearance	Decreased
			DBC	No effect IgG
				Increased for lysozyme
			HCP clearance	No effect
			Pool volume	Increased
		Particle size	Aggregate clearance	No effect
			DBC	Decreased IgG
				Decreased for lysozyme
			HCP clearance	No effect
		Pore size	Aggregate clearance	No effect
			DBC	Increased for IgG
				No effect for lysozyme
			HCP clearance	No effect

Risk assessment support for quality by design

One of the central concepts of QbD, as well as the ICH Q8 guideline (2), is the focus on a risk-based approach. The efforts to reduce variability should be based on an assessment of the potential impact on the quality of the drug substance, which essentially boils down to safety and efficacy. There is a lot of focus placed on establishing best practices and tools for such risk assessment procedures. Roche/Genentech describe one example of this approach (26). The output from such risk assessments should be used to set the extent of process characterization studies, potentially excluding factors from the DoE studies or suggesting the interactions that are most important to investigate. Figure 4 outlines the general steps for this approach.

Fig 4. General steps in a QbD aligned risk assessment procedure.

Process description: gathering existing knowledge

The first step of a risk assessment procedure is focused on gathering existing knowledge of the unit operation, including:

- **Purpose:** what is the intention of the unit operation (e.g., aggregate removal)?
- **Mode:** bind/elute or flow-through?
- **Target column size:** is there a need for resin lot mixing to reach the column volume?
- **Protein properties:** isoelectric point (and charge variant distribution) of target protein vs binding buffer pH.
- **Pooling criteria:** based on UV-cutoff or column volumes, and so on.
- **CPPs:** known critical process parameters and ranges, including load ratio range.
- **Known variability from previous experience:** platform experience from similar unit operations or variability seen from resin lots previously used during development and clinical manufacturing.
- **In-process controls associated with unit operation:** with respect to feed material and elution pool.

Identifying factors that may have an impact on process outcome

There are many types of factors that could potentially affect product quality and process performance, as illustrated in Figure 5 and detailed in the fishbone diagram in Figure 6. Although this white paper is focused on the potential impact of resin attributes on process robustness, we can make the general observation that process parameters like load ratio, pH, and conductivity are more likely to have an impact than resin attributes in any given process. This is reflected in the current practice of focusing on the process parameters first during process characterization.

Fig 5. Input/output diagram showing factors that could affect product quality and process performance.

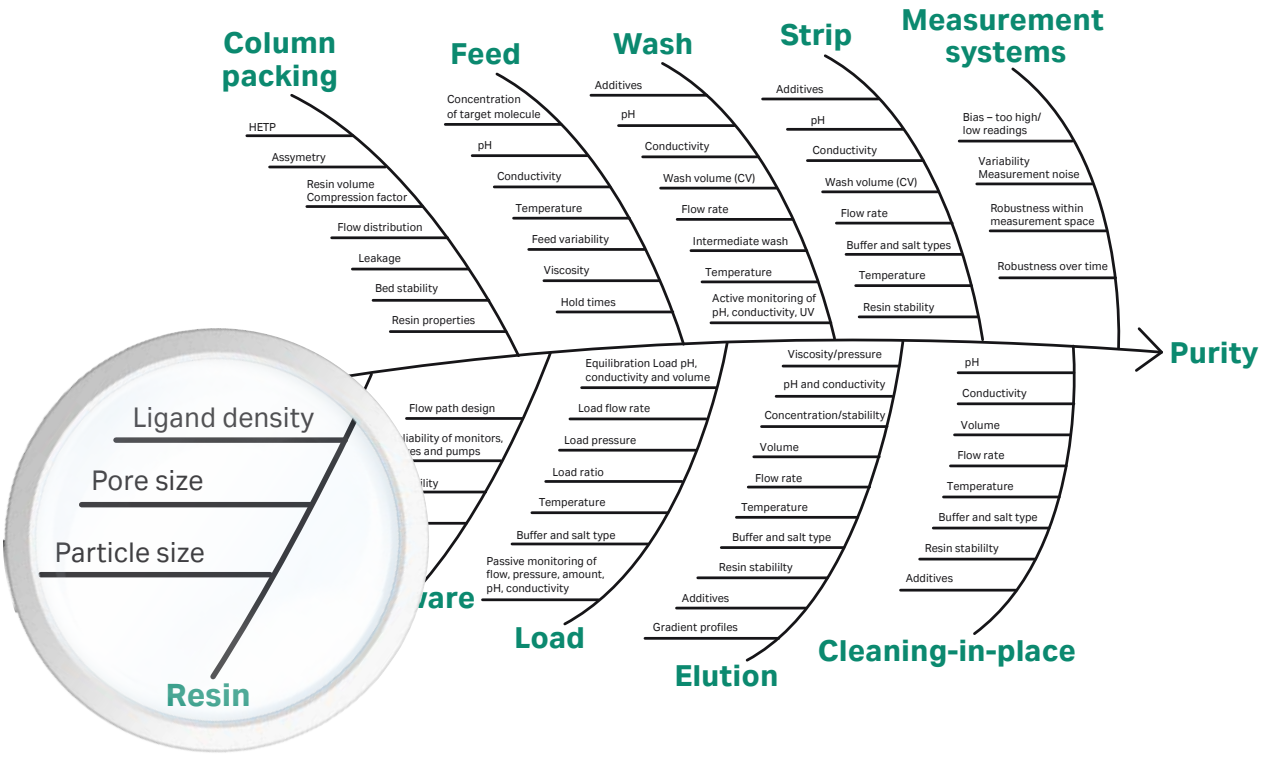

Fig 6. Fishbone diagram (Ishikawa) showing resin as one of the multiple factors that might affect purity.

Factors that can impact robustness towards resin variability

The final risk assessment is process- and potentially product-specific, and so must be performed by the process development team. However, at Cytiva, we believe that there is merit to using our resin experience as input to such risk assessments. Table 3 lists some general aspects for consideration.

Table 3. Factors that can impact robustness towards resin variability

Topic	Considerations
Purification platform	Variation in process parameters and resin attributes are more likely to affect delicate separations (e.g., those of closely-related product variants) than, for instance, an affinity capture step. A downstream process with only two chromatography steps increases the need for thorough characterization of the polishing step.
Process parameters	Narrow control ranges for pH and conductivity increase the risk that resin attributes (e.g., ligand density) could impact process outcome. If the safety margin for the load ratio towards the dynamic binding capacity is small, there is an increased risk that resin variability could impact process performance (e.g., by early breakthrough of product). Developing pooling criteria based on volume or column volumes. Impurities might be collected if the peak becomes narrower (e.g., as an effect of smaller particle size).
Chromatography resin	Using a resin outside its intended use (e.g., a capture resin for polishing) increases the risk of unexpected effects of resin variability since the specifications typically reflect the intended use. Optimizing the process using a resin lot on the outskirts of the specification range increases the risk of suboptimal performance from other future resin lots.
Column packing	Column packing quality (HETP and asymmetry) can impact process outcome and, in some cases, be impacted by resin variability. The column packing compression factor is an indirect measure of the amount of resin in the column and variation could affect binding capacity. Resin attributes could also affect the resin compressibility and require different compression factors to achieve good packing.

Risk assessment: when should you study resin variability during process characterization?

Our risk assessment support is based on the collected experience of our resins, both from the resin development DoE studies and interactions with biopharmaceutical companies. This support provides background material for the final process-specific risk assessment and covers the typical use of the listed resins, mainly for mAb applications. In other cases, for example, bind/elute of large entities that only bind to the outer surface of the beads, the impact of particle size distribution can be greater than assessed and described in this white paper.

Table 4 outlines a risk assessment support matrix where we have assessed the different chromatography techniques on a graded scale from "Considered robust" to "Characterization recommended". The typical process parameters studied in process characterization (e.g., load ratio, pH, and conductivity) are graded for comparison. It is important to acknowledge that interactions between process parameters and resin attributes are possible, for example, the impact of elution conductivity may depend on the ligand density. The variability in base matrix properties is less likely to have an impact than ligand density and has not been divided between particle size and pore size since their impact might be hard to generalize. The primary effect of base matrix variability for bind/elute separations is related to the dynamic binding capacity, which can be mitigated by a proper safety margin for loading. Setting a conservative load ratio can also be a good way of reducing the risk of impact from resin variability.

Table 4. Risk assessment support matrix

Chromatography technique	Mode	Process parameters	Resin ligand density[1]	Resin base matrix properties
AIEX	B/E	●	●	●
	FT	●	●	●
CIEX	B/E	●	●	●
	FT	Not applicable/not assessed		
HIC	B/E	●	●	●
	FT	●	●	●
Multimodal CIEX	B/E	●	●	●
	FT	Not applicable/not assessed		
Multimodal AIEX	B/E	●	●	●
	FT	●	●	●
Protein A affinity	B/E	●	●	●
	FT	Not applicable/not assessed		

[1] Ligand density is typically represented by model protein retention for HIC resins

● Considered robust
● Characterization to be considered
● Characterization recommended

More specific risk assessment considerations (when using selected resins from Cytiva) are given in the appendix.

Considerations for studying the impact of resin variability

Resin variability and its impact on the process can be studied at various stages. This could be using resin samples that already express variability in the first resin screening, where robustness could be used as one of the selection criteria. Another opportunity would be to include such samples in the development activities for the unit operation and so potentially design a process that is inherently robust towards resin variability. A third approach (and most common) is to include resin attributes as potential critical raw material attributes based on risk assessment during the process characterization phase. The final opportunity is to retrospectively look back at an existing process or platform (perhaps developed before the introduction of the QbD paradigm) and perform resin variability studies to increase the process understanding.

Resin variability in process characterization

There are multiple ways of incorporating resin variability in process characterization studies. The most comprehensive approach would be to include resin attributes as factors in the first process characterization studies. However, you might find this impractical because of the resulting increased number of experiments. Currently, the approach used most frequently is to perform the study in two stages, first with focus on process parameters only, and then adding resin variability together with the identified critical process parameters or by selecting "worst case" conditions from the first stage (10). The advantage of performing a DoE study with resin attributes together with process parameters (either all or a selected subset) is that it enables estimation of interaction effects and a comparison of the relative effects of variability. This also enables identification of potential adaptive process control strategies (*see* "Using an adaptive control strategy for resin variability").

Use of Process Characterization Kits

Any study of the potential impact of variability in raw material attributes like resin ligand density on the process outcome requires access to representative resin samples. A Process Characterization Kit consists of three bottles with 25 mL of the same resin where each of the bottles has a different ligand density, representing a low, average, and high value in the manufacturing envelope (Fig 7). Such kits are available for multiple resins from the Capto™ resin family, supporting the following techniques: ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), and multimodal chromatography (MMC) (*See* "Ordering information").

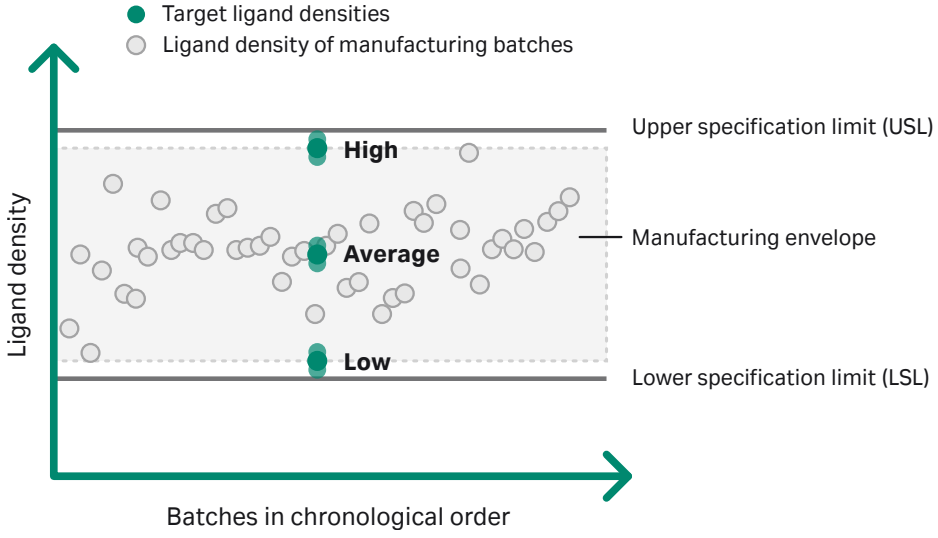

Fig 7. The target ligand densities in a Process Characterization Kit superimposed on the manufacturing envelope.

Design of experiments

DoE is the quantitative tool that drives QbD and comes in numerous shapes and sizes, depending on the aim of the investigation. A DoE study is preferably based on a thorough risk assessment, an evaluation of prior experience, good control of measurement systems, cost and time considerations, and so on. This type of information increases the probability of a successful DoE study and helps in choosing which and how many factors to include in the study and the responses to measure and model. This influences the choice of the experimental design for the study at hand, bearing in mind the degree of complexity that needs to be quantitated by the model.

The purpose of the study (screening, optimization, or robustness) also has a profound impact on the choice of design.

The screening designs are in general designs where you would try to fit the maximum number of useful factors into the minimum number of experiments, identifying the most vital factors for the process. This will inevitably lead to designs that will have little or no support for higher order effects, such as interactions and quadratic effects.

Once you have identified the vital factors, either via a screening DoE or from experience and pre-work, you can perform an optimization DoE in fewer factors to identify higher order effects. This optimization DoE will quantitate possible interaction effects as well as quadratic effects of the input factors.

A robustness DoE is a good next step once a process has been developed and there is a need to test the process on small variations in the vital factors. For robustness testing, the DoE studies will not resolve higher order effects since the aim is only to show that "nothing" happens with small variations in the input factors. This is most applicable when resin variability is tested under "worst-case" conditions.

Once performed, the results can be evaluated statistically and significant effects identified. However, it is important to bear in mind the difference between statistical significance and practical significance. There are many cases where the experimental data is of such good quality that even very small effects become statistically significant, despite being negligible in practice. The opposite is, of course, true if the experimental data is of too low quality to draw any conclusions.

Use of mechanistic modeling

It is also possible to use mechanistic modeling (*see* cytiva.com/modeling), e.g., with GoSilico™ Chromatography Modeling Software, to estimate the influence of raw material variability. Both ligand density and particle size have been studied by variation of model parameters (30, 31).

Control strategy options to prevent process variation due to resin variability

A control strategy is a planned set of controls, derived from current product and process understanding, that assures process performance and product quality (ICH Q10) (27). The outcome of the process development and process characterization efforts is a robust process control strategy that includes approved ranges for process parameters, designated in-process controls, and potentially strategies to mitigate for raw material variability (ICH Q10 & Q11) (26, 28).

In most cases, you would not expect to need further actions with respect to variability in resin attributes since there is a lot of (at least circumstantial) evidence that most purification processes are robust to such variability.

Even when you identify a statistically significant effect of resin variability, the impact on product quality will also commonly depend on the settings for the critical process parameters. In some cases, it is possible to use Monte Carlo simulations to demonstrate that the risk of having the process parameters and resin attributes coincide towards the worst-case scenario is minimal. If this is the case, with an ability to detect impact, you might be able to exclude resin variability from the control strategy. However, in the event you find a practically significant effect of resin variability, there are several different potential mitigation strategies that can be explored. Most of these strategies rely on a high degree of process understanding and so are facilitated by the inclusion of process parameter variation together with the resin variability in the characterization study.

Shifting process parameter target values to account for variability

It might be possible to shift the settings for one or more process parameters to a process operating point less sensitive to raw material variability, as described in Figure 8. Examples of potential adjustments include: pH, conductivity set points, or a reduced load ratio. However, if sample displacement effects are involved, the reduction of load ratio could lead to lower purity. Another opportunity is to adjust the pooling criteria to increase the robustness. From a classical "purity vs yield or throughput" optimization perspective, this could be thought of as designing the process for the worst-case conditions, adjusting the operating point so that product quality is always ensured at the potential expense of process economy. This is not the most attractive solution, but it has the advantage of simplicity and robustness.

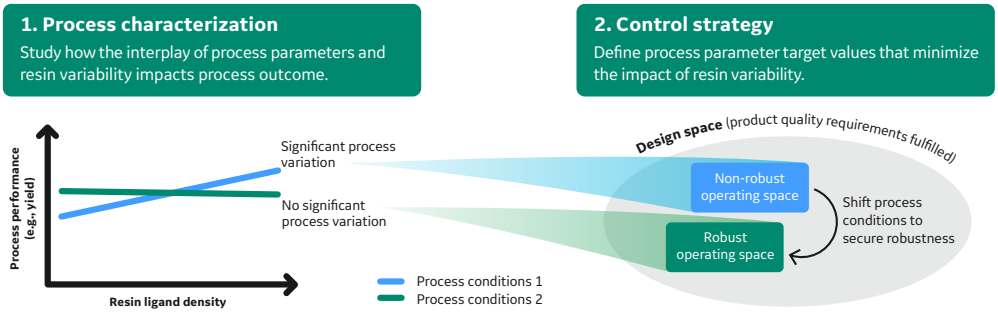

Fig 8. Shifting process parameter settings to improve robustness to resin variability.

Using an adaptive control strategy for resin variability.

To avoid having to always perform under worst-case scenario conditions, an adaptive process control strategy allows the settings for process parameters to be adjusted to the properties of a given resin lot (or lot mix, if several lots are blended in the column). This enables maximum process performance for the entire resin variability range while still ensuring consistent product quality. Development of such an adaptive control strategy requires a higher level of process understanding compared to shifting target values since you need to consider dynamic effects of process parameter changes, including higher order effects and interactions with resin attributes. This approach might be seen as more complex by the manufacturing operations team but provides an improved overall process economy and robustness.

The most common approach for an adaptive control strategy is to evaluate each resin lot in a "use test" that provides process-specific information about the impact of the resin attributes. This is a much-preferred approach compared to using the supplier's certificate of analysis data since they are not specifically connected to the particular unit operation performance. The outcome of the use test is transferred to process performance and product quality through a process model, empirical or mechanistic, enabling prediction of favorable process parameter settings for the given resin lot (or mix). This procedure is only necessary once per new resin lifetime, so the increased effort is not overwhelming. There are several published examples of this approach. Biogen (9) described how the effects of variation in ligand density for an AIEX resin could be counteracted by adjustment of the mobile phase conditions, estimated from the retention time of the therapeutic protein determined in a use test.

Lot mixing

One option sometimes discussed is the ability to mix resin lots already present in the biopharmaceutical company inventory to reduce the overall variability. The average value of any resin attribute for the mixture will, of course, tend towards the middle of the specification but that does not necessarily guarantee that the performance of the mixture is the same as what the average indicates. McCue *et al*. presented one example at the ACS meeting in 2008 (29) showing that the average ligand density of an HIC resin could not predict the behavior of the mixture. Another aspect to consider is the possible effects when removing trace impurities where even a very small amount of a lot with different properties to the other resins lots could play a significant role in the overall mixture's properties.

A final note is that this approach might not be a long-term sustainable supply situation as it relies on always having a lot to blend in to compensate for any "undesired" value for a resin attribute.

Custom specification

You might want to consider a custom company-specific specification if the process performance or product quality cannot otherwise be secured across the entire resin specification. This approach needs to be in collaboration with the resin manufacturer and results in a custom product (with a new article number) at a potentially higher price. The ability to produce such custom products depends on the overall resin process capability and will be easier if the entire specification range is shifted up or down, rather than moving to a narrower specification range. It might only be possible to address the latter situation by lot mixing, as there would be a risk to supply reliability if resin lots are produced against a specification window that is too narrow for the manufacturer's process capability.

Lot selection

The least desirable control strategy, based on our experience, relies on lot selection, that is, the biopharmaceutical company only accepting a subset of all resin lots that fulfil user specific criteria. Such criteria could be based on a company-specific narrower specification range or the outcome of a process-specific use test. The latter approach does not allow any predictability of supply reliability since the outcome of the use test for a given resin lot is unknown to both parties. However, even if the acceptance criteria are based on a user-defined specification range, the supply chain continuity is at severe risk since the supplier's process capability will not necessarily match that specification window. Essentially, the standard specifications for a resin (or any other raw material) are set to ensure uninterrupted supply to the biopharmaceutical industry, and any attempt to reduce the specification range will put the supply in jeopardy and be unfavorable for both parties.

Table 5. Summary of control strategy options

Control strategy option	+	–
Shifting process parameter values	Simple to implement	Extended process development work Not always possible to implement
Adaptive control strategy	Maximizes process performance and product quality	Might be seen as complex by manufacturing operation teams Extended process development work required
Custom specifications	Maximizes process performance and product quality	Need close collaboration with supplier Higher cost Not always possible to implement
Lot mixing	Simple to implement	No guarantee that the performance of the mixture is the same as the average indicates Driving inventory cost and complexity
Lot selection		Supply chain disruption and uncertainty Requires development of use test

Summary

Chromatography unit operations are usually robust to variability in resin attributes like ligand density. However, there are cases where unexpected variability in resin attributes, coupled with process parameters, have shown an impact on product quality and/or process performance.

A risk based approach can be used to assess when to include resin attributes as part of process characterization.

This deeper process understanding enables the development of control strategies based around quality by design principles that help mitigate any impact of variation within the limits of a resin manufacturer's specification. Ultimately this will ensure that the chromatography process remains robust and predictable at full manufacturing scale.

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Ordering information

Process Characterization Kits

Product	Pack size	Product code
Process Characterization Kit Capto™ adhere	3 × 25 mL	17544470
Process Characterization Kit Capto™ MMC ImpRes	3 × 25 mL	17371670
Process Characterization Kit Capto™ MMC	3 × 25 mL	17531770
Process Characterization Kit Capto™ adhere ImpRes	3 × 25 mL	17371570
Process Characterization Kit Capto™ S ImpAct	3 × 25 mL	17371770
Process Characterization Kit Capto™ SP ImpRes	3 × 25 mL	17546870
Process Characterization Kit Capto™ Phenyl (high sub)	3 × 25 mL	17545170
Process Characterization Kit Capto™ Phenyl ImpRes	3 × 25 mL	17548470
Process Characterization Kit Capto™ Butyl ImpRes	3 × 25 mL	17371970

Process Characterization Kits supplied in prepacked columns[1]

Product	Pack size	Product code
Tricorn™ 10/200, 20–21 cm Process Characterization Kit, Capto™ Phenyl ImpRes	3 columns	29496552
Tricorn™ 10/200, 20–21 cm Process Characterization Kit, Capto™ Butyl ImpRes	3 columns	29496553

[1] This list is not exhaustive. Our Custom Product Team can pack the column of your choice on-demand

Appendix: additional considerations for selected Cytiva resins

AIEX resins

Resin	Design for six sigma (DFSS) studies = "Developed for"	Current use	Additional points to consider
Capto™ Q	B/E capture	FT DNA removal	When designing a flow-through step for mAbs, it is important to consider the mAb charge variant profile to avoid binding of the most acidic species. If the separation relies on displacement effects, the load ratio becomes more important to optimize.
Q Sepharose™ FF	General chromatography (prior to DFSS)	FT DNA removal	
Capto™ Q ImpRes	B/E mAb polishing	B/E polishing	

CIEX resins

Resin	DFSS studies = "Developed for"	Current use	Additional points to consider
Capto™ SP ImpRes	B/E polishing	B/E polishing	Some studies show very little impact of ligand density.
Capto™ S ImpAct	B/E mAb polishing	B/E polishing	Potential for "non-traditional" IEX behavior where DBC increases with salt concentration. Split peaks have been observed but can be mitigated by optimization of salt and pH.

Flow-through applications have not been graded since both resins were designed for polishing in bind/elute mode.

HIC resins

We suggest that the retention time of a model protein, rather than the ligand density, should represent the resin hydrophobicity since it is a more representative measure of the chromatographically relevant hydrophobicity. The recommendation to study the potential impact of resin hydrophobicity becomes even stronger in the case of using HIC in capture mode since the complex feed material contains many hydrophobic species. HIC is also the most common modality for publications of adaptive process control strategies based on a "use test", for example, a scale down mimic of the unit operation as described by Biogen (9). See also "Using an adaptive control strategy for resin variability".

Resin	DFSS studies = "Developed for"	Current use	Additional points to consider
Capto™ Phenyl	FT mAb polishing (20)	FT polishing	Sometimes used for binding at low salt.
Capto™ Phenyl ImpRes	B/E polishing	B/E polishing	
Capto™ Butyl ImpRes	B/E polishing	B/E polishing	

Multimodal AIEX resins

Resin	DFSS studies = "Developed for"	Current use	Additional points to consider
Capto™ adhere	FT mAb polishing	FT polishing	The recommendation to study the impact of ligand density is stronger if the purpose of Capto™ adhere resin in flow-through mode includes aggregate removal, while DNA removal and viral clearance are considered robust. If the separation relies on displacement effects, the load ratio becomes more important to optimize. The antibody concentration might also influence the outcome in such cases.
Capto™ adhere ImpRes	B/E mAb polishing	B/E polishing	

Multimodal CIEX resins

Resin	DFSS studies = "Developed for"	Current use	Points to consider
Capto™ MMC	High salt binding capture	B/E polishing	
Capto™ MMC ImpRes	B/E polishing	B/E polishing	The lower ligand density range of Capto™ MMC ImpRes resin can imply a stronger case for a study of the potential process impact of variability compared to Capto™ MMC resin.

Protein A resins

The performance of affinity capture resins is considered robust with respect to resin variability. This has been shown in several cases, for example by Åberg at PREP conference 2015 (23).

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