QbD AND PAT IN BIOPHARMACEUTICAL DEVELOPMENT

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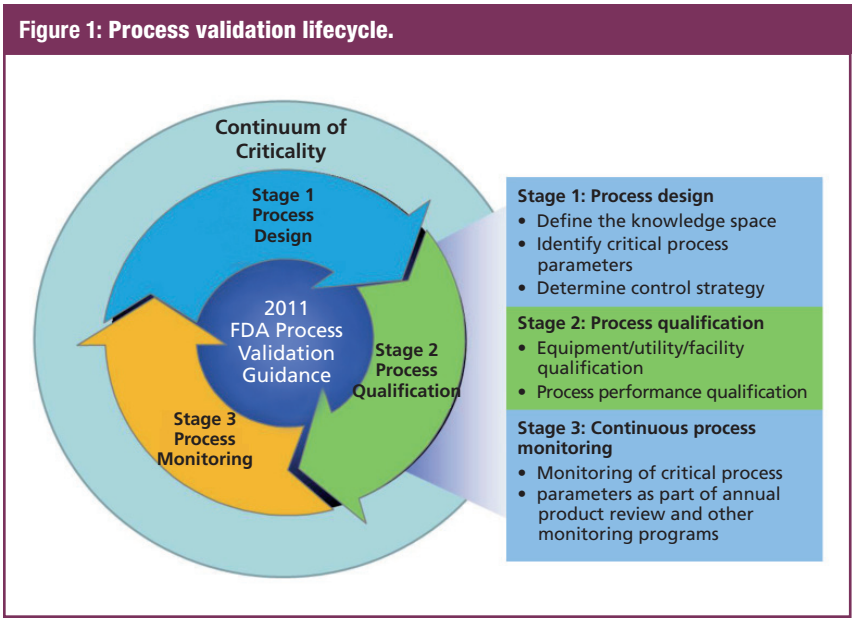
DETERMINING CRITICALITY-PROCESS PARAMETERS AND QUALITY ATTRIBUTES: CRITICALITY AS A CONTINUUM

Mark Mitchell

This article presents a practical roadmap that applies scientific knowledge, risk analysis, experimental data, and process monitoring throughout the three phases of the process validation lifecycle to first determine and then refine criticality. In this approach, criticality is used as a risk-based tool to drive control strategies (Stage 1), qualification protocols (Stage 2), and continued process verification (Stage 3).

As the pharmaceutical industry tries to embrace the methodologies of quality by design (QbD) provided by the FDA's process validation (PV) guidance (1) and International Conference on Harmonization (ICH) Q8/Q9/Q10 (2–4), many companies are challenged by the evolving concept of criticality as applied to quality attributes and process parameters. Historically, in biopharmaceutical development, criticality has been a frequently arbitrary categorization between important high-risk attributes or

Figure 1: Process validation lifecycle.



parameters and those that carry little or no risk. This binary designation was usually determined during early development for the purposes of regulatory filings, relying heavily on scientific judgment and limited laboratory studies.

With the most recent ICH and FDA guidances endorsing a new paradigm of process validation based more on process understanding and control of parameters and less on product testing, the means of determining criticality has come under greater scrutiny. The FDA guidance points to a lifecycle approach to process validation (see **Figure 1**). "With a lifecycle approach to process validation that employs risk-based decision making throughout that lifecycle, the perception of criticality as a continuum rather than a binary state is more useful." The problem is that a practical approach of determining this criticality "continuum" using risk analysis has been left to each company to develop.

This article presents the first part of a practical roadmap that applies scientific knowledge, risk analysis, experimental data, and process monitoring throughout the three phases of the process validation lifecycle to first determine and then refine criticality. In this approach, criticality is used as a risk-based tool to drive control strategies (Stage 1), qualification protocols (Stage 2), and continued process verification (Stage 3). Overall, a clear roadmap for defining, supporting and evolving the criticality of parameters and attributes throughout the process-validation lifecycle will allow pharmaceutical companies to easily embrace the new process-validation paradigm. Furthermore, processes will be more robust and continuous improvement opportunities more easily identified.

In Part I of this series presented here, the author used risk analysis and applied the continuum of criticality to quality attributes during the process-design stage of process validation. After using process knowledge to relate the

attributes to each process unit operation, the inputs and outputs of each unit operation were defined to determine process parameters and in-process controls. An initial risk assessment was then completed to determine a preliminary continuum of criticality for process parameters.

In Part II, the preliminary risk levels of process parameters provided the basis of characterization studies based on design of experiments (DOE). Data from these studies were used to confirm the continuum of criticality for process parameters.

In Part III, the control strategy for the process was developed from a design space established from characterization studies. As the process-qualification stage proceeds, the continuum of criticality was used to develop equipment qualification criteria and strategies for process performance qualification. Finally, in the continued-process-verification stage of process validation, criticality was used to determine the frequency of monitoring and analysis.

From binary to continuum

On the surface, deciding whether an attribute or parameter is critical or not may seem clear and simple. After all, data are compared to acceptance criteria in countless decisions regarding clinical trials, experimental studies, qualifications, and product release. Either the acceptance criteria are met, or they are not. Companies that take this familiar path have tried to draw a definitive line between the "critical" and "not critical" sides. Once a decision has been made about criticality, there is no need to look again. It doesn't help that the guidance documents for industry have been vague on where this criticality threshold lies. The FDA's PV guidance avoids the issue: "attribute(s) … and parameter(s) … are not categorized with respect to criticality in this guidance" (1).

ICH Q8(R2) provides the following definitions using the term critical:

- **Critical process parameter (CPP).** A process parameter whose variability has an impact on a critical quality attribute and, therefore, should be monitored or controlled to ensure the process produces the desired quality.
- **Critical quality attribute (CQA).** A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.

This interpretation of CQA is most applicable to in-process and finished-product specification limits, which suggests that these limits must be critical given that they were designed to ensure product quality. During the early stages of

process development and design, other quality attributes may be measured that, over the course of development, do not end up as either in-process or finished-product tests in the commercial process. These test results may show little variation and present little to no risk to product quality. In other cases, while process duration or yield is measured, they are not related to the product quality and are, therefore, not CQAs. However, even when defined as critical, not all CQAs have equal impact on safety and effectiveness (3, 5).

The definition for CPP states that a parameter is considered critical when its variability has an impact on a CQA. The amount of impact is not defined, which leads to the question, does even a small impact to a CQA mean that the parameter is critical? It is not difficult to imagine the example of an extreme shift of a process parameter having a minor impact on a CQA, whether measurable or not. Extreme temperatures can destroy many pharmaceutical products; however, if a process inherently cannot produce such temperatures, is temperature still considered to be critical and, therefore, required to be monitored and controlled?

When these definitions are strictly interpreted, some companies find themselves in one of two extremes:

- Every quality attribute is critical (they all ensure product quality); every parameter is critical (product cannot be made without controlling them)
- No parameter is critical because if they are controlled, all quality attributes will pass specifications.

Reality lies somewhere between these extremes. Logic and common sense dictate that additional criteria must be necessary to aid in determining criticality. There is great value in understanding not only if a parameter/attribute is critical (i.e., has an impact), but also how much impact the parameter/attribute has. All companies have limited time and resources; therefore, the focus must be on that which provides the greatest benefit for the effort.

By using risk analysis as a means to determine criticality, an opportunity arises to help resolve these potential conflicts. CQAs should be classified based on the potential risks to the patient. CPPs should be separated into those that have substantial impact on the CQAs and those with minor or no impact. The binary yes/no decision transforms into a continuum of criticality ranging from high impact to low impact critical to not critical. As knowledge increases or as improvements are made to a process throughout the lifecycle, risks may be reduced and the level of impact for a CPP can be modified and control strategies adjusted accordingly.

The number of levels in the continuum is a matter of choice and the risk analysis method used. Each company must

procedurally define what risk tools and risk levels it will use and consistently apply them across similar products. In the following examples, three levels of impact are used for simplicity's sake. The different levels drive decision-making and action plans throughout the lifecycle.

For CQAs, a continuum of criticality provides a tool to designate particular attributes as the most important to the protection of the patient. For CPPs, a continuum of criticality allows for process control and monitoring strategies to focus where the greatest impact on product quality is achieved.

Quality risk management

Risk is the combination of the probability of occurrence of harm and the severity of that harm (5, 6). The value of risk assessment models is the formalized evaluation criteria that comes from agreed-upon ranking tables. Even though some may argue that the assessment is not quantitative, the benefit derived from framing the evaluation to an agreed upon risk criteria dramatically improves the ability to objectively evaluate the process risk profile. Per ICH Q9, there are two primary principles of quality risk management (3):

- The evaluation of risk to quality should be based on scientific knowledge and ultimately link to the protection of the patient
- The level of effort, formality, and documentation of the quality risk management should be commensurate with the level of the risk.

Formal risk management tools such as failure mode effects analysis (FMEA) or failure mode effects and criticality analysis (FMECA) (7) can be used to provide a structured semi-quantitative summary of risk. For Stage 1, however, often a qualitative risk assessment evaluating low, medium, and high risk is sufficient to distinguish relative differences in risk.

Continuum of CQAs

Prior to the development of a new drug, companies frequently decide and document a therapeutic need in the marketplace for a new pharmaceutical. It is through this effort that the quality and regulatory aspects of the new drug are defined such as the type of dosage form, the target dose, the *in-vivo* drug availability, and limit of impurities. Current guidance identifies this documentation as the quality target product profile (QTPP). The QTPP provides the basis of the desired quality characteristics of the drug product, taking into account safety and efficacy (i.e., purity, identity, strength, and quality). The QTPP should not be confused with the drug product specification, which created later, is generally a list of specific test methods to perform and their acceptance criteria

designed to ensure drug efficacy and safety. The QTPP is an input to these activities whereas the quality attributes and specifications are outputs.

The initial list of quality attributes from the QTPP should be created as early as possible in the development process so that data can be collected from experimental runs. To assign the continuum of criticality to that initial list of quality attributes, knowledge of the severity of the risk of harm to the patient is paramount. This comes from prior knowledge such as early safety trials and scientific principles.

Quality attributes are rated as the highest criticality level because they have a high severity of risk of harm. Severity is the primary criteria for assessing quality attribute criticality because it is unlikely to change as understanding increases over the life-cycle. For example, an impurity may be determined to severely harm the patient (high severity score) if beyond its limit. If its level does not increase in the process or on stability testing, the occurrence score is low and its overall risk to the patient may be low. However, it is still rated as high risk due to its high severity. That severity will not change and as a high-risk CQA, it has to be tested and monitored.

Examples of risk levels for CQAs:

- High: assay, immunoreactivity, sterility, impurities, closure integrity
- Medium: appearance, friability, particulates
- Low: container scratches, non-functional visual defects.

For a quality attribute to be designated as "not critical," it has to have no risk to the patient (e.g., yield, process duration). Attributes that are not critical to quality are sometimes named process performance attributes to distinguish from quality attributes.

Not all CQAs are tested as part of finished-product testing. Some are tested in-process to define limits such as pH and conductivity. Although frequently designated as "in-process controls," they are still quality attributes that should be assessed for their criticality. Consideration should be given to the relationship between in-process controls and finished-product CQAs when making this decision. While this is one example of how to assign a continuum of criticality to quality attributes, other examples are also available (8–10).

Cause-and-effect matrix

Once risk levels have been assigned to the CQAs, the next activity is to begin to relate which parts of the process have impact on these attributes. This cause-and-effect analysis breaks the process into its unit operations and conveys its impact on the CQAs. An example of the cause-and-effect matrix for a biologic is given in **Table I.**

Table I: Example of cause-and-effect matrix for a biologic. H = high, M = medium, L = low.

Critical quality attributes (risk level):	Unit operations							
	Preculture & expansion	Fermentation and harvest	Centri-fugation	Cation exchange chromato-graphy	Anion exchange chromato-graphy	Viral filtration	Concen-tration & diafiltration	Vial filling
Appearance (M)	Low	Low	Medium	No	No	Low	Low	Low
Impurities (H)	Medium	High	Medium	High	High	Medium	Low	Low
Protein content (H)	Low	High	Medium	Medium	Medium	Low	Medium	No
Immunoreactivity (H)	Low	Medium	Low	Low	Low	Low	Low	No
Purity (H)	Low	Low	Low	Medium	Medium	Medium	Low	No
pH and ionic strength (M)	Low	Low	No	Low	Low	Low	Medium	No
Amino-acid content/ ratio (H)	Medium	Low	No	Low	Low	No	No	No
Bioburden (H)	Low	Low	No	High	High	Medium	Medium	Low
In-process controls								
Fill-weight check (M)	No	No	No	No	No	No	No	High
Visual inspection (M, L)	No	No	Medium	No	No	Low	Low	Medium

In addition to the matrix, it is important to document the justification for these decisions as part of this analysis. For example, the parameters of the cation- and anion-exchange chromatography processes are expected to have a high impact on impurities because they are designed to remove impurities of different ionic charge than the desired product. With the knowledge of which unit operations have impact on particular CQAs, it is now possible to analyze each process' inputs and outputs to determine how process parameters affect the CQAs.

Input and output process variables

Each unit operation has both input and output process variables. Process parameters signify process inputs that are directly controllable and can theoretically impact CQAs. Process outputs that are not directly controllable are attributes. When the attribute ensures product quality, it is a CQA. The output of one unit operation can also be the input of the next unit operation. These parameters and attribute designations and their justification should be documented in either a formal process description document, or process flow diagram/drawing. This documentation should also include the scale of each unit operation, equipment and materials required, sampling/monitoring points, test methods, and relevant processing times and storage times/conditions.

The intent of assessing process parameters is to determine how they affect the process variation of CQAs along with their control strategy. Each company should clearly document their

methodology for defining and assessing parameters. The following may be considered in making that assessment:

- Raw material attributes are outputs of the release of materials. Critical material attributes (CMA) should be considered along with CPPs as impacting process variability.
- Fixed parameters such as equipment scale, equipment setup, pre-programmed recipes should be documented but are assessed as either low or non-critical.
- Parameters for sterilization processes and cleaning process and the preparation of process intermediates can be included in the primary process assessment. Alternatively, they can be treated as independent processes with their own process parameters, quality attributes, criticality assessments, and process validation.
- Calibration and standardization setting for equipment and instruments are usually not included as process parameters.
- Formulation recipes can be considered fixed parameters (low or not critical); these parameters generally have relatively tight limits, which are justified during formulation development. Such a parameter, which does not vary, cannot impact process variability. An exception to this rule is the case where operators must calculate a quantity based on a variable input such as biological activity; this variable process parameter may lead to process variation.
- Holding/storage times and conditions where no processing occurs should be qualified to show little to no impact on the product. These should be documented and, if these factors are included as process parameters, they are considered low or non-critical.
- Environmental conditions during process (room temperature, humidity), such as holding times, are to have set limits so that they have little to no impact to the process. Process-specific environmental conditions such as cleanrooms, cold rooms, and dry rooms are included as process parameters because they are monitored to ensure product quality.

When a process parameter is determined to be non-critical either by process knowledge or by process study, companies may choose to further designate the parameter as a key performance parameter if that parameter impacts a process performance attribute.

From knowledge to risks

Once each unit operation is related to CQAs through a cause-and-effect matrix

Table II: Example of initial risk assessment of process parameters.

Process parameters	Initial risk assessment	Justification
Inoculum *in-vitro* cell age	Low	• Separate end of production studies have justified limit of cell age.
Osmolality	Medium	• Can affect impurities and cell viability. • Keep constant in scale-up.
Antifoam concentration	No	• Knowledge from previous studies have defined acceptable range to have no impact to quality. • Keep constant in scale-up.
Nutrient concentration	Medium	• Must be sufficient to maintain cell viability. • Keep constant in scale-up.
Medium storage time and temperature	No	• Knowledge of medium storage from previous studies. • No effect when kept within pre-established limits.
Medium expiration (age)	No	• No effect when kept within pre-established limits.
Volume of feed addition	Medium	• Related to component concentration. • Scale by fermentor volume.
Component concentration in feed	Medium	• Yield impact and impacts cell viability • Related to volume of feed addition. • Keep constant in scale-up.
Amount of glucose	Low	• Glucose fed as needed to maintain cell viability leading to different cell concentrations. • Scale by fermentor volume.
Dissolved oxygen	High	• Must be sufficient to maintain cell viability. • Impacts yield by low cell growth. • Controlled by rate of aeration. • Scale to large scale by pre-defined model.
Temperature	High	• Impacts cell growth and viability. • Keep constant on scale up.
pH	High	• Impacts cell growth and viability. • Keep constant on scale up.
Agitation rate	Low	• Speed set by previous process experience. • Scale to large scale by pre-defined models.
Culture duration (days)	High	• Related to nutrient concentration for cell viability.

and the process parameters and attributes are documented, an initial risk assessment to determine the potential impact of each process parameter is performed. Prior to process characterization experiments, this risk assessment may be more high level using primarily prior knowledge and scientific principles. However, a more formal FEMCA may also be considered.

Table II is an example of an initial risk assessment for a single unit operation. Included in the justification is the expected relationship with CQAs and how the parameter may be influenced during scale-up. Fixed parameters are set to non-critical as they do not impact process variability. For the initial process characterization experiments, process parameters with medium to high impact will be included.

In Part I of this series, the author looked at criticality as a continuum to apply risk analysis during process design, and to

relate process unit operations to quality attributes using a cause-and-effect matrix.

In Part II, the continuum of criticality for parameter and attributes will be used to design process characterization studies using DOE. From the initial risk assessment of critical parameters, experimental data from formal studies will confirm the criticality assignment—critical or not—and help to assess the level of impact to CQAs.

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Additional Resources: DoE and Process Control Strategies

This eBook presents Part I of a *BioPharm International* three-part series on "Determining Criticality—Process Parameters and Quality Attributes." Starting on Page 3, Mark Mitchell, principal engineer at Pharmatech Associates, introduces the concept of a continuum of criticality, and applies it to the concepts of critical quality attributes and critical process parameters.

Building on this piece in Part II, Mitchell uses the preliminary risk levels of process parameters as the foundation of characterization studies based on Design of Experiments (DoE). These studies confirmed the continuum of criticality for process parameters.

In Part III, Mitchell develops the process control strategy from a design space identified by data collected in the characterization studies. The continuum of criticality was used to develop equipment qualification criteria and strategies for process performance qualification. Criticality also helped establish the frequency of monitoring and analysis.

Read more here:

Determining Criticality—Process Parameters and Quality Attributes, Part II: Design of Experiments and Data-Driven Criticality

Determining Criticality–Process Parameters and Quality Attributes, Part III: Process Control Strategies—Criticality throughout the Lifecycle

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QbD AND PAT IN UPSTREAM AND DOWNSTREAM PROCESSING

Susan Haigney

BioPharm International talks to industry experts about the implementation of QbD and PAT tools in biopharmaceutical manufacturing.

To gain perspective on the implementation of quality by design (QbD) and process analytical technology (PAT) in biopharmaceutical processing, *BioPharm International* spoke with Clinton Weber, associate director of bioprocess sciences, CMC Biologics Organization; Henrik Johanning, director QAtor; Nathan L. McKnight, PhD, principal engineer, late stage cell culture, BioProcess Development, Genentech; Anurag Rathore, professor, Indian Institute of Technology (IIT) Delhi; Frederic Girard, CEO, Spinnovation Biologics; and Thomas J. Vanden Boom, PhD, vice-president, global biologics research, development and manufacturing operations, Hospira.

Upstream Processing

BioPharm: In implementing QbD, what would you identify as the critical quality attributes (CQAs) in

a typical upstream bioprocess using cell-culture?

Vanden Boom (Hospira): Generally, upstream critical quality attributes for a cell-culture manufacturing process are limited to the adventitious agent and bioburden testing of the cell-culture harvest material. This also holds for biosimilar products.

The variability and complexities associated with the upstream biological process makes QbD a complex process. —Girard

McKnight (Genentech): CQAs are defined for the product, not identified as part of upstream or downstream portions of the manufacturing process. There are key performance indicators (KPIs) that are defined for upstream steps, including culture productivity (i.e., titer), cell growth, and viability. While KPIs may be correlated with CQA results, KPIs are not themselves product quality attributes. However, particular CQAs may be generated or modified during specific upstream or downstream steps. Protein glycosylation, for example, is generally determined during cell culture and minimally altered in downstream unit operations (at least for uncharged glycosylation species). Using this definition of CQAs, those CQAs observed to be potentially impacted during cell-culture steps include product attributes contained within the protein glycan distribution (e.g., afucosylated glycan), charge-variant distribution (e.g., glycated, deamidated forms), and to a lesser extent, molecular-size distribution (e.g., dimer or aggregate forms).

It should be noted that knowledge of an association between cell-culture steps with certain product quality attributes is not a result of implementing a QbD approach but, rather, a result of knowledge gained through the basic scientific and engineering endeavors that should be elements of developing a bioprocess.

Rathore (IIT Delhi): In the past, most CQAs could not be measured directly in the fermenter broth due to the interference from the numerous components present in the broth. As a result of major advancements in analytical science, direct measurements of CQAs are performed in bioprocessing today. These would be product-quality related parameters, including host-cell impurities (e.g., host-cell proteins, DNA), process-related (e.g., Protein A leachate), and product related (e.g., aggregate, basic variants, acidic variants, and glycoxylation pattern). Some of these CQAs, such as glycoxylation pattern for monoclonal antibody (mAb) products, are primarily impacted by the upstream process and

are particularly important to monitor during process development.

Weber (CMC Biologics): The goal of a QbD approach is to develop additional knowledge of the impact of upstream process unit operation performance on the final purified product quality. The most likely or desired outcome is to develop a quantifiable correlation between upstream process outputs, such as cell viability or viable cell density (VCD), and product attributes, such as glycosylation. This approach can lead in the determination of CQAs for the cell production bioreactor unit operation. Upstream outputs classified as CQAs have proven to be controversial, because the bioreactor is so far upstream from the final product. However, if a strong correlation can be established between VCD/viability and other CQAs or final product specifications, this can be an appropriate approach.

Girard (Spinnovation): The underlying concept for QbD of an upstream bioprocess is that the desired quality of the biological or biopharmaceutical product is assured every time. CQAs vary for each cell line depending on the nature of the bioprocess, with typical critical qualities such as metabolites and contaminants. CQAs usually include properties that affect product quality and eventually overall performance of the bioprocess. CQAs are typically also release tests, although they don't have to be as there are no real release tests as such in upstream processing.

Typical CPPs for a fermentation step would be pH, sparge rate, agitation rate, and temperature. —Rathore

The variability and complexities associated with the upstream biological process make QbD a complex process, one that relies on defining operation specific critical process parameters (CPPs). CPPs are those likely to impact on the quality of a product or intermediate. For biological products, process control can be difficult to define and implement. O_2 pressure, catalyst concentration, and pH are examples of critical parameters. It is important to note that mAbs are currently the leading area of biopharmaceutical research. One of the key parameters to monitor in the implementation of QbD in mAb production is the glycosylation process during formulation. Glycosylation is one of the overriding contributors to mAb heterogeneity and has significant implications for the function of the antibody *in vivo* and immunogenicity. This means that glycosylation has been isolated as a critical parameter to follow during mAb manufacture. QbD for mAb development with specific glycosylation

patterns enables researchers to optimize manufacturing and clinical efficiency.

Johanning (QAtor): the QbD concept works its way back so to speak from the patient to product to process and ultimately to the facility. A risk assessment will outline the risk profile in and between each area.

The bioanalytical characterization of originator products over the approved shelf life provides a powerful input for use of a QbD paradigm. —Vanden Boom

The starting point for the risk assessment is R&D, which upstream has determined the CQAs on the product. CQAs are often product specifications, including eventual GMP requirements (if GMP is used as part of the biopharmaceutical process, which is often the case in multinational pharma companies in order to ensure a fast-track initiative from R&D to license to operate and market). The risk assessment includes a review and assessment of the products CQAs when manufactured on specific equipment.

BioPharm: In implementing QbD, what would you identify as the critical process parameters (CPPs) in a typical upstream bioprocess using cell culture?

McKnight (Genentech): Among cell-culture parameters, culture pH is typically the most difficult to control relative to its impact on cell-culture performance and product quality. This is generally the case for both mAbs and other products.

Johanning (QAtor): Common CQAs in an upstream bioprocess (i.e., bio formulation process) using cell-culture include sterility and bioactivity.

Rathore (IIT Delhi): Typical CPPs for a fermentation step would be pH, sparge rate, agitation rate, and temperature. These are typically easy to control. Issues arise when one goes to volumes greater than 1,000 L and when it increasingly becomes more difficult to strip off the CO_2 generated by the cells and in ensuring uniform supply of O_2 and other critical nutrients to the cells. Another set of challenges comes from raw materials that are complex and not well characterized (such as yeastolates) as these can result in significant variation in the CQAs from lot to lot. For mAbs, besides the afore-mentioned factors, concentration of critical nutrients has been known to affect the glycoxylation pattern of the product, thereby impacting product efficacy.

Vanden Boom (Hospira): Parameters such as temperature, pH, osmolality, and dissolved oxygen have the potential to impact the CQAs of mAbs and other mammalian cell-culture derived products. However, with current bioreactor engineering controls, these parameters

may be tightly and confidently controlled within the design space of the manufacturing process permitting these parameters to be downgraded from a CPP to a key process parameter (or other lower parameter designation used by different drug sponsors).

Weber (CMC Biologics): For an upstream process, the process of expanding the cells is the primary purpose until the culture goes into the production bioreactor. Because the majority of product produced is in the production bioreactor, the expansion process is considered to have minimal impact on the final product. Additionally, most products have shown the ability to recover from suboptimal conditions during expansion without serious product quality impact. Therefore, for a typical upstream process, CPPs are identified at the production bioreactor stage. CPPs for the production bioreactor may include seed density, temperature, and process duration. Initial seed density can impact the overall growth profile and viability. Temperature is critical in maintaining viability and may impact the quality of product being produced by the cells. Process duration will impact the viability of the culture at harvest, which can be tied to product quality.

Among the most difficult parameters to control in an upstream process is CO_2 concentration. The difficulty of controlling this parameter depends on the complexity of the aeration control strategy and availability of dissolved CO_2 probes. Though there can be typically broad ranges of acceptable CO_2 during production, very high CO_2 concentrations can impact the product quality. A balance needs to be maintained between lowering CO_2 and maintaining pH at a set point. Furthermore, these conclusions seem to be supportive of most cell-culture processes, not just mAb production.

BioPharm: What measurement tools are typically used to measure CPPs and the resultant process inputs and outputs, including PAT tools, in an upstream process?

McKnight (Genentech): CPPs are a subset of the environmental and batch-recipe settings (e.g., timing of feeds, culture duration) used to perform the process. Temperature and pH, which are commonly CPPs, are measured using on-line probes (a technology that is officially PAT, but far preceding the "PAT initiative"). Timing of events and generation of basal and feed media are controlled with traditional process controls and standard operating procedures. Online cell-density measurement is an area of active development as is online nutrient measurement technology to enable advanced feeding or timing strategies.

Weber (CMC Biologics): Assuming this question is referring to the monitoring and control of CPPs and not the tools used to actually establish CPPs, the majority of upstream CPPs are monitored online and offline. Online instruments such as CO_2 probes are verified against offline instruments to ensure that the conditions within the bioreactor have not caused the probes to "drift." Adjustments are made to the online instruments if drift is detected. Other outputs such as cell count and viability are measured strictly offline on a routine basis. PAT tools can be effective, though not necessary, to monitor parameters such as temperature and pH, but are not necessarily value-added for culture health outputs such as viable cell density, viability, or doubling time.

Rathore (IIT Delhi): For upstream process, tools that researchers have used include:

- Surface plasmon resonance (to assess product concentration and affinity)
- High-performance liquid chromatography (HPLC) (to assess product concentration and structure)
- Capillary electrophoresis (to assess product concentration and structure)
- Dielectric spectroscopy (to determine biomass)
- *In-situ* microscopy (to characterize cell population)
- Flow cytometry (to characterize cell population)
- Metal oxide field effect transistor (to sense biological contaminants)
- Infrared spectroscopy (to detect media components)
- *In-situ* 2D fluorometry (to detect media components and metabolic end products)
- Raman spectroscopy (to detect media components and metabolic end products)
- UV spectroscopy (to measure homogenate components)
- Mass spectroscopy (to detect metabolic end products)
- HPLC (to detect media components and metabolic end products).

Not all of these are amenable for online applications, but together they capture various attributes of upstream processing.

BioPharm: Given the inherent variability in biologics manufacturing, how does QbD improve process understanding and control? What are the limitations of QbD in upstream bioprocessing?

McKnight (Genentech): The inherent variability in biologics manufacturing, and the general inability to define mechanistic equations (i.e., mechanistic process models versus empirical process models), limits the ability to precisely

predict the outcome of specific runs at manufacturing scale. Application of multivariate, statistically designed experiments, however, is still valuable for identifying CPPs, defining parameter acceptable ranges, and understanding the variability that may be expected from the manufacturing-scale process. Biological variability likely limits the ability to control even the best understood process solely through control of process parameters—the need for some degree of product testing will be necessary to control for inherent variability.

Rathore (IIT Delhi): Implementation of QbD necessitates creation of information relating the process to the product and the product to the clinic. It is this understanding that lays the foundation for appropriate process control. A major limitation that I see with respect to implementation of QbD in upstream processing is the complexity of the sample medium due to the presence of the large variety of process related, host-cell related, and product-related impurities. Another limitation is the fact that the fermentation process is so complex. With the aforementioned, tools it is easy to monitor different process and product attributes. However, many different alterations in operating conditions and raw-material attributes can lead to similar changes in the process outputs and hence monitoring is merely the first and simpler step. The difficulty comes in diagnosing the root cause of variability and effectively dealing with it in real time.

Girard (Spinnovation): Precisely because QbD is a scientific, risk-based, and proactive approach to biologics development, one can use it to define the ideal characteristics of a product to achieve CQAs relating directly to its clinical performance. On the basis of this information, product formulation and processes are designed within a specific framework to ensure the product meets these attributes. Variability within this framework can be monitored allowing scrutiny of the process to assure consistent product quality. However, it is important to consider the CQAs in a matrix since one knows that a biological system has the capability to compensate or adjust its metabolic pathway.

Vanden Boom (Hospira): The enhanced design-space knowledge derived from the systematic risk assessments and design of experiment (DOE) work completed in association with a QbD approach offers the potential to significantly improve the level of process control for mammalian cell culture-derived products. A key factor to realize the full benefit of QbD is the establishment of robust small-scale model(s) of the upstream manufacturing process. This may be more challenging

for certain products resulting in limitations to fully using QbD for upstream bioprocessing steps for these products. In the case of biosimilar products, the bioanalytical characterization of the originator product provides another useful input to determine the significance of product and process variability.

Weber (CMC Biologics): A QbD approach can lead to an early focus and understanding of what influences the CQAs. While scale-down models combined with screening and design space DOEs can be used to understand cell expansion and the cell-production bioreactor processes, full purification can be time consuming and costly for full characterization of the upstream process. In addition, as the downstream process is characterized, optimizations/changes in the downstream process can lead to the need to repeat upstream characterization efforts. Hence, the sooner a correlation between the main upstream outputs (such as viability) and primary product quality attributes (such as glycosylation) can be established, bench-scale work can be minimized.

Downstream Processing

BioPharm: In implementing QbD, what would you identify as the CQAs in a typical downstream bioprocess in which the product was produced using cell culture?

Weber (CMC Biologics): This is probably the most difficult question to answer. We have found the CQAs for downstream are very product dependent. Glycosylation and sialylation are definitely two outputs that require product and product quality understanding and are the most common across different cell-culture processes. After that, variation in the product profile begins to manifest itself too much, preventing universal CQAs to be established.

McKnight (Genentech): Some CQAs are generated in, or substantially changed by, certain downstream unit operations. Size variants or charge variants, for example, may be generated during hold times, and product quality attributes such as host-cell proteins and size variants are typically reduced through the purification process and controlled to an acceptable level by consistent purification process performance.

Rathore (IIT Delhi): The downstream process attributes would be similar to those mentioned earlier for upstream processing. These include process-related impurities (e.g., antifoam, additives added during the processing, Protein A leachate), host-cell impurities (e.g., host-cell proteins), and product-related impurities (e.g., aggregate, basic variants, acidic variants, glycoxylation pattern). The big difference is that the ease of measurement of these attributes improves

significantly in downstream processing as the samples are relatively cleaner.

BioPharm: In implementing QbD, what would you identify as the CPPs in a typical downstream bioprocess in which the product was produced using cell culture? What are the most difficult parameters to control and why?

McKnight (Genentech): Chromatography unit operations are typically most sensitive to the pH and ionic strength of wash and elution conditions. Control of these critical buffers is managed through batch preparation and release prior to use based on acceptable pH and conductivity ranges. Process capability is sufficient to reproducibly prepare buffers within narrow pH and conductivity ranges, such that only a subset of the most sensitive buffers are typically determined to be CPPs.

Rathore (IIT Delhi): Typical CPPs in downstream processing would include parameters such as pH, conductivity, temperature, and gradient for chromatography steps; temperature, agitation rate, and sparge rate for refolding steps; and pH and hold time for the viral inactivation step. The challenge in downstream processing mainly comes from the fact that the steps are relatively short in duration. For example, a typical elution in chromatography column may be 30–60 minutes. Our ability to measure and then take action, therefore, is quite challenged if the assay is not a real-time assay (such as HPLC).

Johanning (QAtor): CPPs in a typical downstream process involving cell culture include holding time, pH control, temperature control, and UV control. UV control/cutting is the most difficult parameter to control because UV cutting techniques/equipment are still an area for further development. They relatively often challenge batch release (out of specification).

Vanden Boom (Hospira): Originator and biosimilar products have similar downstream CPPs. For chromatography steps, these may include protein load, pH (load or elution depending on step), temperature, flow rate, and conductivity for ion-exchange steps. Viral inactivation steps may have temperature, pH, or detergent concentration as a CPP depending on the modality of inactivation. Pressure and filter volume represent CPPs for viral filtration steps. Again, if engineering controls permit tight control of these parameters, some may be downgraded to a lower parameter designation.

Weber (CMC Biologics): Using a QbD approach, downstream CPPs would be proposed only through using risk assessments of the possible impact to pre-identified CQAs. Downstream CPPs will vary greatly depending on the nature of the molecule, the purification

strategy, and the order and timing of unit operations. Nevertheless, there are certain potential CPPs common to many downstream processes. The following list of operations/parameters is potentially responsible for affecting product quality/CQAs, particularly toward the end of a purification process. Not all of these would necessarily end up as CPPs:

- *Viral inactivation:* pH of inactivation, time of inactivation, and concentration of inactivation solution
- *Viral filtration:* filter load density (mg/mL membrane) and filtration pressure
- *Chromatography operations:* load density, pH and/or conductivity of buffers, residence time, volumes, and eluent concentration
- *Filtration operations:* load density, transmembrane pressure, crossflow rate, and diafiltration diavolumes.

The most difficult parameters to control are those with narrow allowed ranges. Using QbD, well-designed DOEs are intended to grant maximum flexibility in defining allowed ranges.

BioPharm: What tools are typically used to measure CPPs and the resultant process inputs and outputs in a downstream process, including PAT tools?

McKnight (Genentech): CPPs for chromatography operations frequently include pH and conductivity of the most sensitive wash or elution buffers. Control of these critical buffers is managed through batch preparation and release prior to use based on acceptable pH and conductivity ranges. This practice predates the PAT initiative, but serves the intended purpose of PAT.

Rathore (IIT Delhi): The same aforementioned tools would be applicable here as well as we are measuring the same attributes. As mentioned previously, the difference lies in the fact that the samples are much cleaner and hence easier to analyze. However, the time for decision is significantly less, thus making PAT implementation more of a challenge.

Weber (CMC Biologics): Put simply, the right downstream equipment for chromatography and filtration should have built-in monitoring of all potential CPP parameters and should cover a wide range of operational values. This can either transmit out for a PAT approach, or can allow for well-designed process monitoring.

QbD and PAT in Bioprocessing

BioPharm: What are some recent advances in PAT or other analytical tools that better enable product characterization and the increased process understanding desired in a QbD paradigm? How is QbD applied to equipment design and implementation in biologic API manufacturing?

Girard (Spinnovation): FDA identified the use of new analytical methods, such as nuclear magnetic resonance (NMR), to monitor and control processes as important in increasing manufacturing quality through QbD. NMR could change the face of bioprocess development and monitoring because it provides access to component identity, plus quantitative data, rapidly and easily from a single analysis. NMR profiling can provide full visibility of the presence and concentration of feed components, contaminants, and metabolites. The technique is capable of providing access to accurate concentration data for media components. NMR-based methods can provide rapid and accurate quantitative monitoring of more than 50 media components, contaminants, and metabolites within a culture at any stage in the process to meet QbD requirements.

The use of NMR, combined with statistical approaches, provides rapid solutions to performance inconsistencies in simple and complex raw materials within upstream processes of virtually any bioprocess, offering the ability to characterize chemically complex media. NMR techniques have the potential to contribute significantly to an understanding of process-critical parameters, helping to reduce performance variability and minimize the risk of process failure in large-scale biopharma production.

Johanning (QAtor): Before (traditional) installation, test and qualification of equipment a QbD approach involves a design qualification, where the user requirement specification (URS), which includes the basic CQAs from the master and manufacturing production files, is reviewed and qualified against the proposed equipment design. This, of course, involves specialists from R&D, manufacturing, and quality assurance. The design qualification and review is challenging the equipment design with special attention on CQAs such as contamination with germ and fibrillation.

Vanden Boom (Hospira): In the case of biosimilar products, the bioanalytical characterization of originator products over the approved shelf life provides a powerful input for use of a QbD paradigm in process development. Biosimilar developers also have the opportunity to employ modern manufacturing technologies, including PAT tools, to enhance process control. The enhanced monitoring of potential CPPs on current buffer dilution and chromatography skids represent one example of improved equipment design contributing to the improved process control of biopharmaceutical manufacturing processes.

Weber (CMC Biologics): PAT implies an online monitoring system that can determine if a process is trending negatively in 'real time.' The essentials of PAT have been in the industry nearly from the beginning: data historian. Although PAT allows for maximal design space control and understanding, whether one has real-time information and automated response capability is not essential to the QbD paradigm. However, having a well-understood and robust historian is. As long as processing trends and capabilities are assessed on a per-run basis, there is an opportunity to monitor and adjust to ensure that the process performs optimally within the design space. Many unit operations within cell-culture manufacturing processes have control capabilities built into the equipment; as long as the assessment is performed, the equipment can repeatedly perform within the required space. Couple that with strong operator training and having real-time process adjustments is already inherent in a process without PAT. Generating dynamic and static control charts on a per-run basis of the process allows for trend analysis, and adjustments can be made should a negative trend be detected. This can all be accomplished without a formal PAT system.

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Susan Haigney is the managing editor of *Pharmaceutical Technology* and *BioPharm International.*

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RECONCILING SENSOR COMMUNICATION GAPS

Angelo DePalma

Process controls get some upgrades to better reflect real-time conditions.

Process analytical technology (PAT), quality by design, and individual company- or process-centric quality initiatives are driving the development of bioprocess sensors and probes. Single-use systems generally suffer from a "sensor gap" related to the need to balance measurement accuracy against the well-known benefits of single-use processing, including the avoidance of cleaning, cleaning validation, and calibration.

For most of the history of the biomanufacture of therapeutic proteins, in-process measurement and control focused on upstream operations. Historically, cell culture and fermentation persist longest among unit operations. Expression is "where the action is"—where throughput and volumetric productivity exert the greatest influence on cost of goods.

The desirability of platform purification, best exemplified in monoclonal antibody (mAb) downstream processing, simultaneously creates and reduces the need for extensive downstream

monitoring. Platforming, whether it occurs upstream or downstream, relies on controls to maintain operations within specification. Yet the imperative to improve process understanding—even for exquisitely controlled operations—increases the need for sensing and monitoring. Fulfilling this need has only been possible relatively recently with the ability to monitor product directly, more or less in real time, as opposed to managing surrogate parameters such as pH, dissolved oxygen, amino acids, and gases.

At the interface

Cell viability does not fall within downstream operations, but it can determine when upstream processing ends and purification begins. For Kelsey McNeel, market segment manager, process analytics at Hamilton (Reno, NV), the key issue for bioprocess monitoring and controls is reconciling information obtained at bench- or process-development scale with what may be expected during manufacturing. "The setup is different, the sensors are different," explains McNeel. "There's a definite disconnect."

Because of the tight dependence of cell density on reactor conditions, viable cell count is one of those measurements where the "disconnect" becomes pronounced. Dead cells, cellular debris, and adventitious particles confound conventional optical cell counting, which often over-report cells that are no longer doing their job.

Manual counting is accurate but involves sampling—avoided whenever possible—and considerable time, effort, and reagents for plating, staining, and counting cells. "By the time you're done you're measuring something that existed up to three hours ago—you're looking into the past and potentially missing an event that caused stress on the cell," McNeel says. Hence, process scientists desire real-time measurements. Moreover, total cell measurements are most reliable only during the cells' log phase, when they are expanding.

Hamilton's online measurement system, Incyte, uses permittivity instead of optical proxies for viable cell density. Think of permittivity as the capacity of cells to hold an electrical charge, analogously to the operation of an electronic capacitor. Living cells hold charges while dead cells or debris do not. Where anchorage-dependent cells confound optical methods, permittivity easily quantifies them because the microcarriers are coulombically inert.

"You can also get information during the cell death phase, which could be relevant for some processes," McNeel adds. "With total cell counts you get a plateau—you know cells have stopped multiplying but not their viability status."

Single-use sensors

As a general strategy for keeping up with sensing and control technologies, contract manufacturer Lonza (Slough, UK) looks to innovator companies that are clients or potential clients. "We need to be in line with their thinking to increase their comfort in outsourcing with us," says Atul Mohindra, PhD, head of mammalian process research and technology at Lonza.

Further on the single-use trend, the biggest gap in downstream monitoring and control is the lack of appropriate single-use sensors beyond the standard devices for pH, conductivity, and product concentration. "We need to get past those parameters and start developing sensors that quantify product quality and impurity profiles in a way that is actionable," Mohindra explains.

One complicating factor in the development of such tools is the necessity that they operate more or less in real time, as downstream operations occur on compressed timelines and in rapid succession. To that extent, downstream operations cause a re-thinking of which properties to measure (e.g., specific quality attributes or impurity concentrations), how to quantify them (particularly in single-use systems), and how to react meaningfully.

For example, during chromatographic purification in gradient mode, a product's charge characteristics may change. Are such changes meaningful? Do they affect quality? Can they be measured during purification? And if so, what can be done about deviations?

Downstream sensing gaps highlight the need to provide the same level of sophistication downstream as upstream, particularly for single-use processes. Techniques that quantify some over-arching characteristic come to mind—such as refractive index in the chemical-pharmaceutical world—but the quality attributes for biopharmaceutical molecules are so much more complex that a one-measure-fits-all approach will almost surely fail.

Whatever techniques are ultimately adopted, their ultimate goal must be to eliminate manual sampling and lengthy analyses, or at least, to streamline these operations so they become relevant within the timeframe of purification.

Higher-level controls

Single-use operations are inherently more space-conserving than stainless-steel processes because they eliminate clean-in-place and sterilization-in-place operations and associated piping, instruments, valves, and resource configurations. "However, single-use processing means that there are more manual and complicated set-up steps required that include tubing, bags, sensors, devices, and connectors," says Michalle Adkins, director of life sciences consulting at Emerson Automation Solutions (Pittsburgh, PA).

To manage these complexities, Emerson is betting on augmented reality—a technology under evaluation in the oil and gas industry—which the company believes could help the design and validation of biomanufacturing processes. Augmented reality is the superimposition of an idealized or theoretical computer-generated image onto a user's view of the real world. Unlike virtual reality, which is completely artificial, augmented reality expands perception by adding new information to it.

For now, biomanufacturers must settle for less sophisticated distributed control systems such as Emerson's DeltaV coupled with an operations management system like Syncade, also from Emerson, to integrate processes and procedures within the increasingly complex single-use processing environment.

"Pulling together materials, set-up, sequences, and process control data along with asset data is essential to provide the necessary analytics to support reliable operations," Adkins adds.

Continuous processing has been available in some form, but not completely implemented, for the manufacture of therapeutic proteins. Widespread familiarity with single-use equipment has greatly improved prospects for continuous unit operations and elevated "continuous" to official buzzword status. With continuous processing comes new issues related to process dynamics, the management of lots, batches, and materials. With those changes, process modeling to ensure product quality and smooth operation becomes crucial. Quality by design and its enablers—process analytics and advanced control strategies—move to the front of the line of priorities.

"As the industry moves to continuous manufacturing, it is certain that more data and data analytics tools will be needed to ensure product quality and prevent deviations," Adkins observes. "It is also interesting to see how some companies are moving in the direction of both continuous and single use for some of their processes, adding new layers of complexity and opportunity."

In an environment of increasingly complex processes and associated monitoring and controls that generate unprecedented quantities of information, data integrity becomes crucial. PAT data, models, traditional process sensor data, material traceability, and tracking of single-use consumables all funnel into this data stream. Ensuring operational success requires integrated control system and manufacturing execution platforms that work across process units, the entire process, and even through various stages of product development.

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Angelo DePalma is a contributing writer to *BioPharm International.*

MULTIVARIATE DATA ANALYSIS IN BIOPHARMACEUTICAL DEVELOPMENT

Dimitris Alexandrakis, Stephen Craven, and Stephan Zelle

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Multivariate Data Analysis in Biopharma Development

Multivariate data analysis and Design-of-Experiment techniques enable biopharmaceutical companies to improve their efficiency and ultimately speed data analysis and development, reduce process-related costs, and reduce time to market.

Overview

Bioprocesses—such as for the production of vaccines, antibiotics, cell-based therapies, and more—are quite complex. Bioprocessing within fermenters or bioreactors involves controllable process parameters (e.g., pH and temperature) as well as a series of output measures (e.g., biomass and impurities). In addition, several uncontrollable factors can affect the quality and reproducibility of the process and the product (see **Figure 1**).

The development of a robust biopharmaceutical process requires numerous experiments, measurements, and data analyses. To deal with the great complexity, three main strategies are useful for biopharmaceutical process development: Design of Experiment (DoE), multivariate analysis

(MVA), and process analytical technologies (PAT) in combination with MVA.

DoE

With respect to biopharmaceutical process development, DoE is a series of tests in which purposeful changes are made to controllable input factors so developers can pinpoint the root cause(s) of changes in output responses. It is a useful tool to gain process knowledge by understanding the main interactions and the impact of possible sources of variability (like raw materials and process parameters). This allows individuals to identify critical material attributes and critical process variables and therefore defining design and control spaces.

Moreover, DoE is helpful for generating large amounts of information about a process with the minimum number of experiments, which saves time, resources, and money.

DoE can be implemented using a step-by-step workflow summarized in **Figure 2**.

- The first screening step is screening out trivial factors.
- The next step characterizes the various effects and interactions that exist within the process.
- If you have curvature, or non-linear relationship between inputs, optimization experiments help developers identify the control space.
- Last are verification tests, which confirm the findings.

MVA

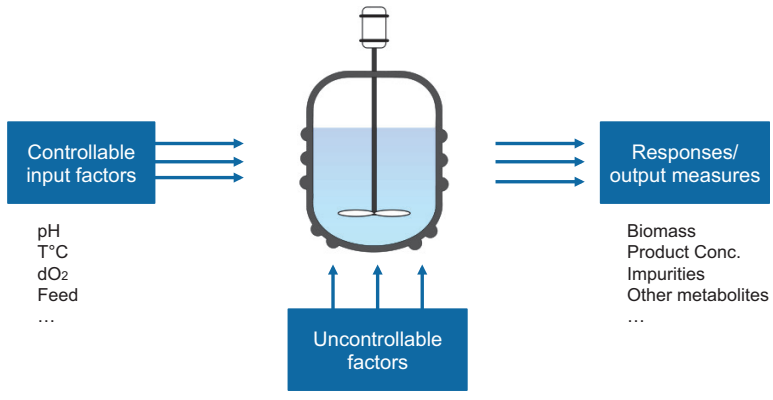
A second tool for dealing with the large amounts of data created during biopharmaceutical process development is MVA. MVA can be used to understand the variability of the process and inputs/outputs and help design a process monitoring technique as part of the overall process control strategy. Design process monitoring strategies include:

- Multivariate statistical process control (MSPC), which identifies if the product is of the right quality and meets critical quality attributes (CQAs).
- Batch statistical process control (BSPC) provides information about whether the batch process is behaving as expected.
- Process analytical technologies (PAT), which, combined with MVA, allow for CQA predictions in real time.

All these techniques can be used for real-time process monitoring, and they detect any faults or deviations from normal process behaviors. These methods have outputs, whether they are diagnostics or the predictions, which can be communicated through a control system and help developers move toward powerful model predictive control.

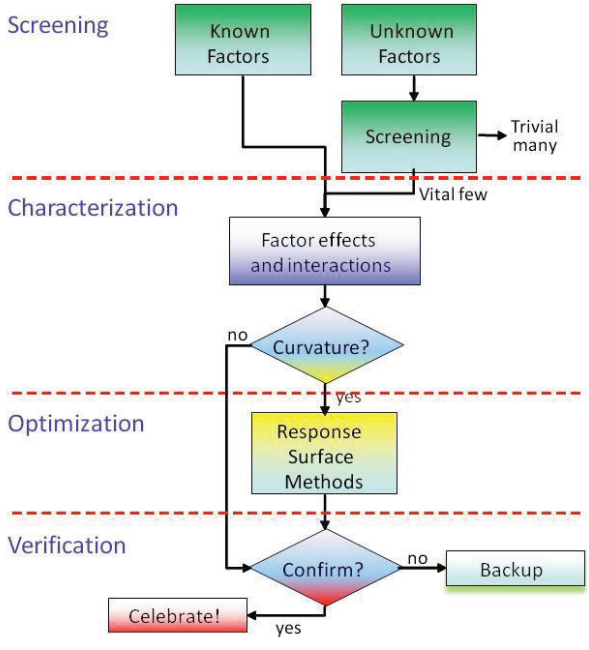
Why conduct MVA? Figure 3 illustrates the difference that MVA versus single variable analysis can make. In this

Figure 1: Bioprocessing within bioreactors.



Large amount of experiments, lot's of measurements and in general large amout of data to be analysed

Figure 2: Sample DOE workflow.

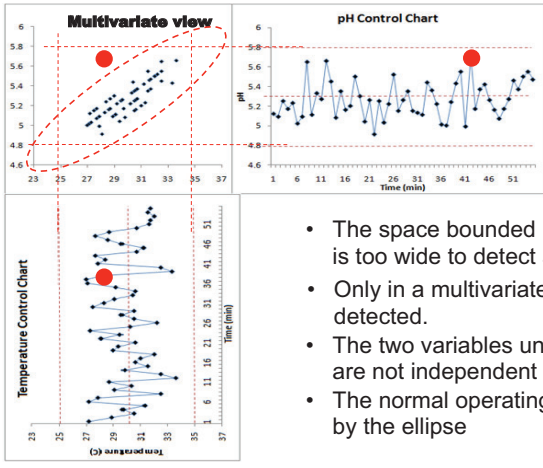


example, temperature and pH appear to be within control limits; however, a trend is observed when plotting the two charts against each other in the multivariate view. One data point is separate from the main cluster, which was not apparent in the univariate control charts. The reason why is that most process parameters are not independent of one another. Because they are correlated, MVA provides a powerful look at the complete picture. Through MVA, data can be summarized for an entire batch in a single trend.

Moreover, each data point contains information about each process variable, which can be used for deeper, more-focused analysis of and troubleshooting about a specific variable. One can also create multivariate charts that contain limits and can be used to follow the trajectory of a batch over time.

MVA case study. To investigate how MVA can be used, scientists looked at publically available process data generated during the fermentation process of penicillin production. Data from 13 batches were available. Ten batches

Figure 3: Multivariate analysis provides a more complete view of the data than single variate analysis.



- The space bounded by the univariate limits is too wide to detect a multivariate fault.
- Only in a multivariate context can the fault be detected.
- The two variables under consideration are not independent
- The normal operating range is defined by the ellipse

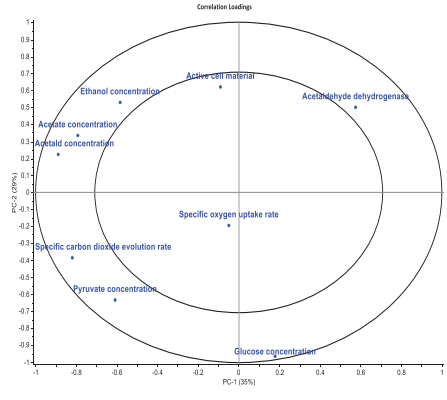
Figure 4: Through multivariate analysis, correlations of the data were revealed in a loadings plot.

Fermentation data for production of penicillin. 10 batches for modelling, 3 for testing

Process variables collected on an hourly basis:

- Glucose
- Pyruvate
- Acetaldehyde
- Acetate
- Ethanol
- Biomass
- Active cell material
- Acetaldehyde dehydrogenase
- Specific oxygen uptake rate
- Specific carbon dioxide evolution rate

End product quality - **Biomass**



yielded a good product and were used as the basis for the calibration model. Three batches failed, and these data were used for testing and verification of the process model.

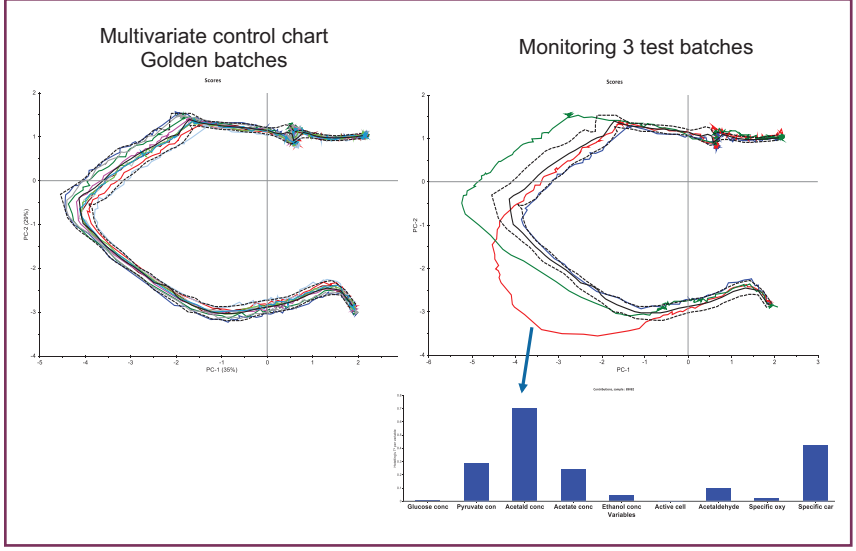
The process variables in this analysis were extensive and the end-product quality indicator was biomass.

Through MVA, the structure and various correlations of the data were revealed in a loadings plot, which shows how the process variables are correlated (see **Figure 4**).

The glucose concentration at the bottom of the chart and the active material at the top of the chart (together with the ethanol and acetate data points) indicate that when the cells are reproducing, the glucose concentration starts to get depleted. The more the cells grow, the more metabolites occur; therefore, concentrations of ethanol, acetate, and other variables increase. The plot gives a map that indicates the correlations among the variables.

Using the 10 batches, an initial model was made and a multivariate control chart displayed. The three monitoring test batches were used to see whether deviations could be detected versus the

Figure 5: Multivariate analysis provide rich information about deviations.



model. It was found that the deviations were quite easy to detect. The red and green lines in **Figure 5** represent two batches that had large deviations at different stages of the batch process, and the blue line represents a batch that was borderline.

Importantly, one can track the evolution of the deviation. At the top of the chart, batches are within the limits, and then they deviate for most of the process. At the end the process, they are back within the limits. Therefore, with these methods, there is early detection of a deviation, with which one could deal during the process. Conversely, without this data, the only information one would have is that the yield was lower at the end of the process.

The bar graph details which variables are responsible for the deviations in the red batch.

PAT with MVA

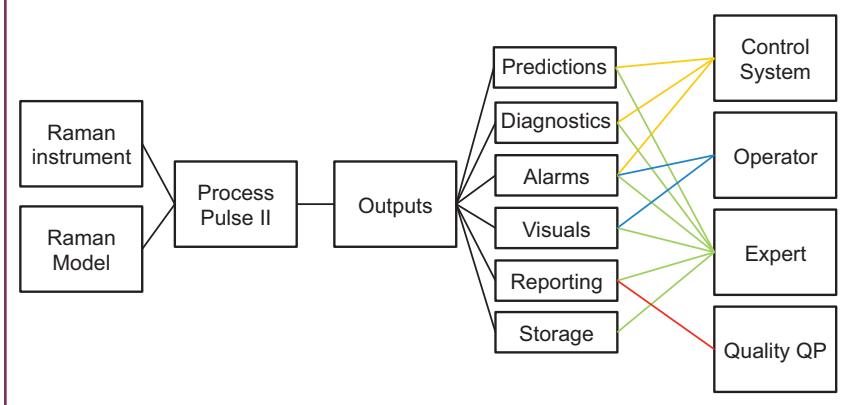
A third tool is PAT combined with MVA. With sensor technology and hardware, PAT allows for indirect measurements of various CQA and allows for real-time predictions as the process is evolving. In turn, that means that one can replace the offline testing and save resources and time.

The various signals from the PAT sensors provide a large amount of information. If MVA is layered onto this, then the multivariate nature of the signal can provide even richer process information with a correlation model. The PAT sensors can be used for a wide range of applications, like for end point measurements, measure nutrient levels, and identify impurities.

A sample workflow of PAT and MVA analysis is presented in **Figure 6**. Spectral measurements and offline reference measurements are taken and a calibration model is created that is basically a regression or a prediction model. For real-time analysis, the model is loaded into a multivariate platform. This method can replace expensive and destructive testing.

In the sample workflow, a Raman instrument connects directly to the data

Figure 6: Sample of real-time workflow with PAT and MVA.



management solution. The model is run in real time and the calculations can be used as an output. Some examples for outputs are predictions, diagnostics, alarms, visuals, and reporting. Dependent on the intended use, one must decide how the information is further processed. For example, alarms are set for process control deviations so operators can take appropriate actions, or predictions go into the control system for model predictive control.

Advanced Process Technology

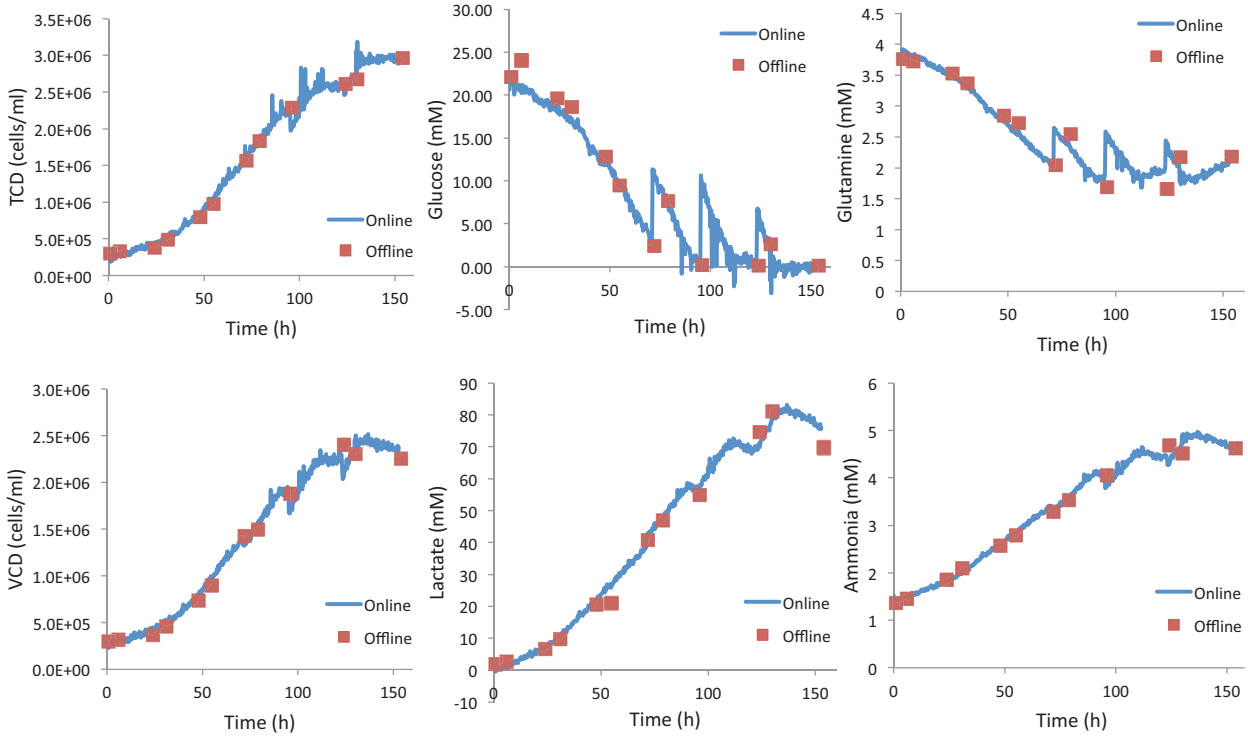
The following case studies involve APC's use of the CAMO Multivariate Data Analysis (MVDA) Unscrambler® X software package to support biopharma partners and bioprocess development and optimization. The CAMO Unscrambler® X software package offers several tools that allow the user to extract the potential correlative structure between the garnered spectral data.

Raman spectral PLS modelling. The first case study demonstrates the pivotal role that MVA plays in supporting a PAT-enabled bioreactor process where chemometric partial least squares (PLS) models are used to translate a Raman spectral dataset into mammalian cell growth and metabolite data streams.

In mammalian bioprocesses, process parameters measured traditionally online are those for which robust process sensors exist, such as temperature, pH, and dissolved oxygen. However, to determine the critical quality attributes of the typical bioprocess, we require information of factors like the substrate and byproducts, which often affect the product quality and product titer. Identification of metabolites in a cell culture process is not trivial; because cell culture media are very complex, the concentration of different chemical species is low and many are structurally similar.

APC used a Kaiser Raman spectroscopic system to monitor in real time a typical fed batch mammalian cell bioreactor process using a DASGIP® Parallel Bioreactor System from Eppendorf. Because Raman

Figure 7: Output of chemometric partial-least-squares modelling.



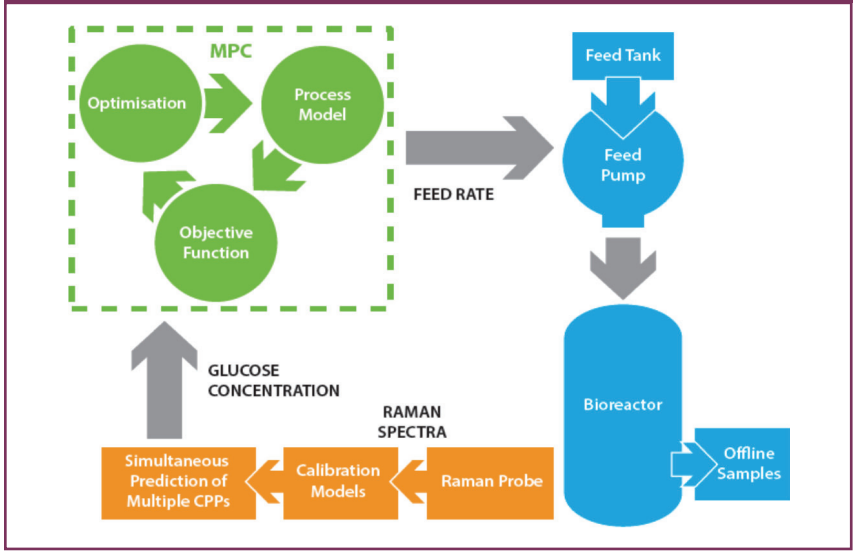
spectroscopy requires the use of MVA, the Unscrambler® X package was helpful.

Applying PAT complemented by MVA was key in transitioning from a traditional bioreactor approach, which lacks online measurements of the key critical bioprocess parameters.

Figure 7 illustrates the output of the chemometric PLS model built by APC for a standard bolus Chinese hamster ovary cell fed batch bioprocess. There is good agreement between the online Raman determined readings and the offline reference method determined readings for biomass, the main nutrients (i.e., glucose and glutamine), and the main byproducts (i.e., lactate and ammonia) for this bioprocess.

Overall, with the ability to generate a validated chemometric model for biomass and the main nutrients and byproducts for this bioprocess, the researchers used the dual action of the PAT instrumentation (i.e., Raman) and the MVA package (i.e., CAMO Unscrambler® X) to close the loop on a critical process parameter (i.e., glucose). With the introduction of a control algorithm (i.e., model predictive control), they could deliver a continuous feed to support cell growth and productivity (see **Figure 8**).

Figure 8: Delivering continuous feed with the introduction of a control algorithm.



The end-result was increased cell longevity and productivity, compared to a traditional bolus fed batch approach.

Bioprocess batch level modelling. The second case study used MVA to identify the root causes of batch-to-batch variability in a typical monoclonal antibody (mAb) production bioprocess.

In this example, a large amount of data was involved from two manufacturing sites encompassing all unit operations from initial vial thaw through to drug product filling. Therefore, the CAMO Unscrambler® X package was used to get an understanding of what unit operations, process variables, and process parameters contributed to variability in product quality between the sites.

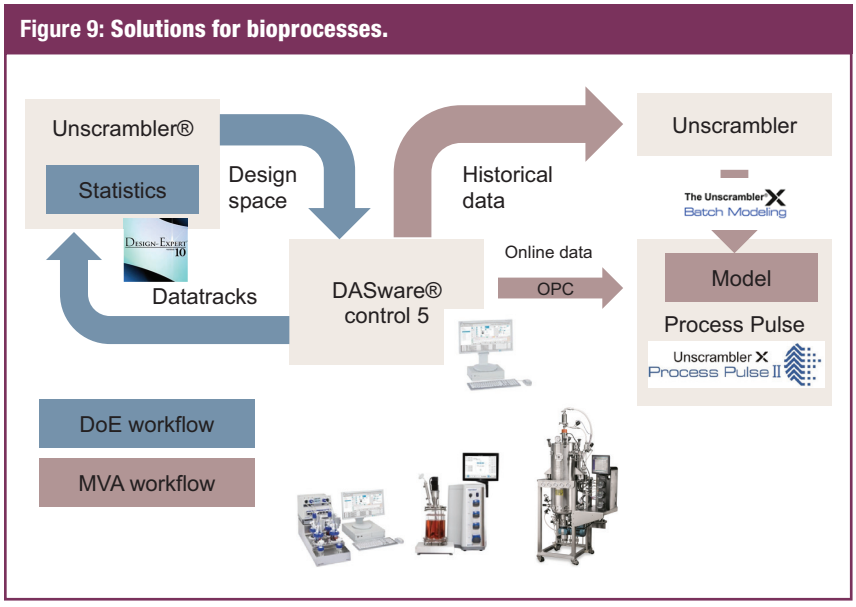
MVA indicated the bioreactor was the key unit of operation causing the variability in product quality attributes. Bioreactor processes are very complex, nonlinear, and multivariate, as stated. Bioprocess performance depends on the cell quality, the media composition (i.e., raw material lots), and bioreactor environmental parameters (pH, dissolved oxygen, temperature).

The CAMO Unscrambler® X package was used to drill down and understand what parameters were contributing to the variability. MVA score plots increased process understanding of the batch data, and combined with evolution plots, it was determined that the two sites were using different pH deadbands.

Bioprocess Solutions

Several solutions are available to support DoE workflows and MVA. Regarding DoE, a small-scale system may be most efficient for helping to run the numerous experiments required in a DoE workflow, which are often done in parallel. The Eppendorf DASbox® Mini Bioreactor System, for example, is well-suited for this purpose. With an integrated DoE plugin, one can easily map different set points coming from a design space or statistical tool.

Figure 9: Solutions for bioprocesses.



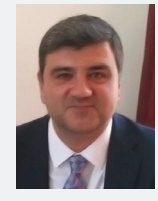
For MVA, one can generate a "golden" batch model based on historical data collected from bench-scale systems (e.g., Eppendorf BioFlo® 120 or 320). Those bench models are easily generated in the CAMO Unscrambler® X software with the bench model plugin and then can be transferred and applied in the CAMO Process Pulse II software for real-time MVA.

Seamless integration between all tools is essential for both DoE and MVA workflows, and control software (e.g., DASware® control 5) is helpful for this purpose.

Figure 9 illustrates the interplay of different components. The DoE tool is seamlessly integrated into the bioprocess control software (blue). Online data from the bioprocess system is used to monitor and control the bioprocess based on the golden batch model (brown).

Summary

MVA and DoE techniques enable biopharmaceutical companies to improve their data analysis and ultimately speed development, reduce process-related costs and reduce time to market. MVA has significant advantages over traditional statistical methods and can be an especially powerful tool for when combined with PAT.



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Q&A on MVA

The following Q&A was excerpted from a recent *BioPharm International* webcast on Multivariate Data Analysis in Biopharmaceutical Development, sponsored by Eppendorf AG. Reponses were provided by Dimitris Alexandrakis, PhD, business development director, Europe at CAMO Software; Stephen Craven, PhD, life sciences team leader at APC Ltd; and Stephan Zelle, PhD, product manager bioprocess at Eppendorf AG.

Which parameters can be used with an MVA? Everything or only a limited set?

Alexandrakis: It depends on what you're trying to achieve with the MVA. In principle, parameters should hold useful information, but also capture changes in the product's chemical or physical state, and evolve over time. So, things like set points or stepped cross-parameters really should not be used. When using PAT, we have a multivariate signal but don't need to use the entire signal because it covers a large wavelength range. After cleaning up the chemisaturated areas at the beginning and the end, there are also areas where you would find either specific peaks or absorbance ranges that are not necessarily relevant to what you're trying to model. So, some sort of wavelength selection can be used to make the method more specific.

If you're using golden batches to determine the MVA model for a process, how do you determine if those results are representative of the process and don't contain any outliers or failures that could make a faulty model?

Alexandrakis: When you are building those models, usually it is done with historical data. Depending on the type of process and the development stage, you would also have some developed experiments and knowledge. And if it's an established process, you will have a large amount of historical batches and know which ones gave you good product. You would probably also have some critical quality attributes for them. So, they can be used to filter out batches that didn't yield a good product. When you're building those models, make sure that you are capturing the entire range of parameters that yields an acceptable product. These parameters can be used to create the calibration model.

You should also have some batches in the historical data that were kind of intermediate. This information is very useful to test whether your model is not only accurate in predicting deviations, but also not giving false positives.

How was the communication between the predictive model and the bioreactor system established and at which rate did the systems exchange data?

Craven: We integrated the predicitive model developed by APC into the DASGIP® Parallel Bioreactor System. We executed the model predictive control algorithm in MATLAB® (in .exe file format) and the control algorithm was designed to execute every 12 hours. Exchange of information between the Raman spectroscopic instrument, the Unscrambler® X, and the DASGIP® Control (DASGIP® Control is now DASware® control 5.) software occurred every 12 hours. During this execution, the APC controller, via OPC communication, would take readings from the Raman spectroscopic instrument and from the chemometric model and look to determine the control action, that is the glucose feed rate to maintain the glucose concentration at a defined set point. The calculated control action would then be sent to the DASGIP® Control software via OPC to actuate the DASGIP® MP8 pumps. The overall OPC communication between the Kaiser Raman system, chemometric software package, Unscrambler® X, APC controller, and DASGIP® Control software is essential.

Is the Unscrambler® X easily incorporated into the DASware® control 5 software?

Zelle: For that incorporation, we are currently using mostly OPC technology. Depending on the use, we also developed a dedicated optimized filter through which we can transfer the different data between software tools. In the future, we are planning to have a really seamless integration, meaning that the customer can easily copy or transfer the data between the different software tools and then get the results.