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Process analytical technology (PAT) for biopharmaceuticals

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Process analytical technology (PAT), the regulatory initiative for building in quality to pharmaceutical manufacturing, has a great potential for improving biopharmaceutical production. The recommended analytical tools for building in quality, multivariate data analysis, mechanistic modeling, novel models for interpretation of systems biology data and new sensor technologies for cellular states, are instrumental in exploiting this potential. Industrial biopharmaceutical production has gradually become dependent on large-scale processes using sensitive mammalian cell cultures. This further emphasizes the need for improved PAT solutions. We summarize recent progress in this area based on an expert workshop held at the 8th European Symposium on Biochemical Engineering Sciences (Bologna, 2010), and highlight new opportunities for exploiting PAT when applied in biopharmaceutical production. We conclude with recommendations for advancing PAT applications in the biopharmaceutical industry.

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Abbreviations: CQA, critical quality attributes; **CPP**, critical process parameters; **MVDA**, multivariate data analysis; **PAT**, process analytical technology; **PCA**, principal component analysis; **PLS**, partial least squares; **QbD**, Quality by Design

1 Introduction

The term (and acronym) Process Analytical Technology (PAT) was introduced by the US FDA as an intiative to bring an improved understanding of pharmaceutical manufacturing processes to increase the quality of their products [1]. The FDA uses the expression "to build in quality into the pharmaceutical manufacturing process", thereby implying that high product quality should ideally be created already at the design stage of the manufacturing process [1–3], contrary to traditional processes that are often the result of empirical or rule-of-thumb design. In addition, they also emphasize the need for improved on-line monitoring and control methods to maintain high product quality during manufacturing operations. In the biopharmaceutical industry PAT principles are adopted with great care due to the fact that biopharmaceuticals and their production systems are very complex. Compared to the small molecule pharma industry the complexity of biopharmaceutical proteins multiplies the analytical quality issue.

Figure 1 illustrates a central concept of the PAT philosophy: to identify and control the critical quality attributes (CQA) of the process based on monitoring and adjusting the critical process parameters (CPP).

PAT forms a part of the Quality by Design (QbD) concept, also a regulatory-inspired methodology where PAT provides tools to enable the quality

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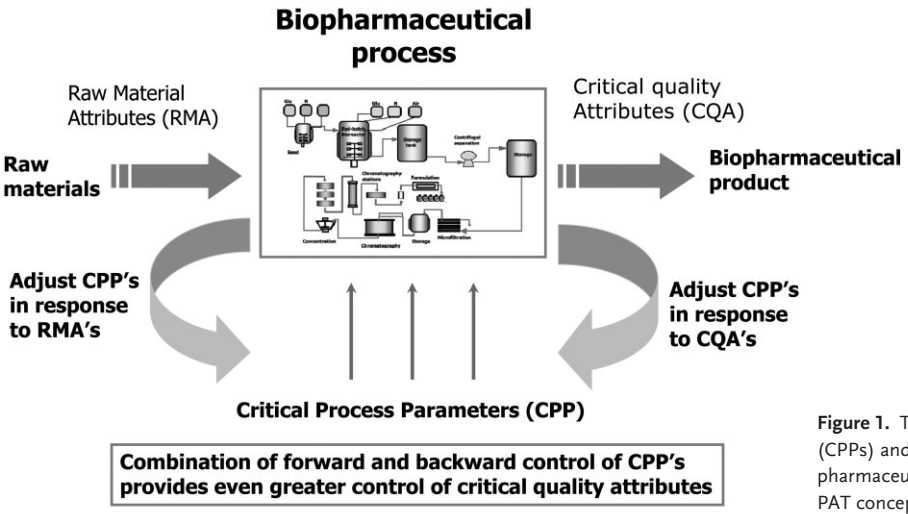

Figure 1. The relationship of critical process parameters (CPPs) and critical quality attributes (CQAs) in a biopharmaceutical manufacturing process according to the PAT concept.

goals [4, 5]. In QbD the acceptable ranges of the CQAs and the CPPs are defined for the manufacturing process (Fig. 2).

A large number of analytical methodologies and tools, here referred to PAT tools, are useful for realizing QbD. These PAT tools cover methodologies ranging from analytical chemistry, through control theory and mathematical and statistical modeling methods. In Figure 3 the most important of these methods and tools are compiled and related to their role in building quality into the pharmaceutical manufacturing process.

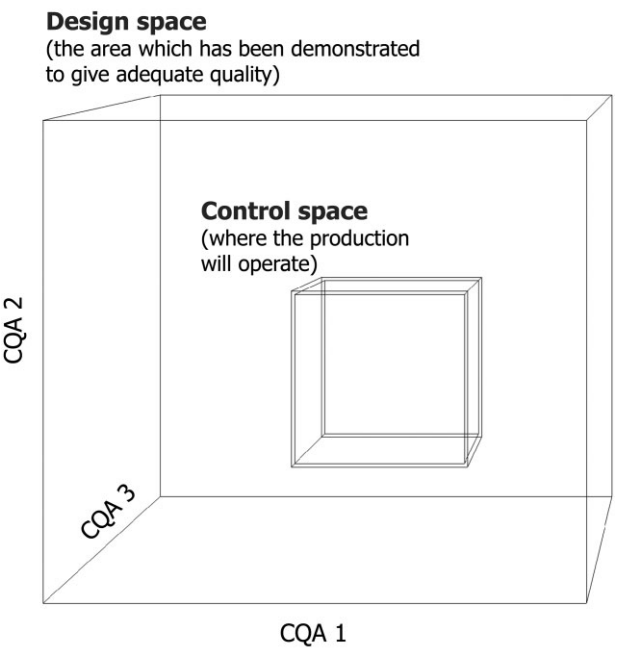

Figure 2. The design space and control space as defined in QbD. The axes represent the critical quality parameters.

The first tier in the figure shows three categories of methods for acquiring quality-related data from the pharmaceutical manufacturing process: methods for (1) on-line sensing, (2) 'omics'-based analysis, and (3) analysis of CQAs. These methods can show considerable overlaps based on deeper process understanding.

In the second tier, the measurement data obtained in the first tier are processed using models, and thereby converted to useful process-related information. Here multivariate data analysis models, mechanistic models or any other modeling approach (e.g., neural network models as a typical black-box model example) are highligthed.

The third tier in the figure shows alternative control methods that can be applied with any of the previous methods for data acquisition and analysis in the first and second tiers, or by combining these in appropriate ways.

In the most straightforward case it is an issue of feeding back the information to the process to correct it. In QbD, we define the control space from pre-knowledge created in separate experiments. This pre-knowledge is in the best case optimized, also using first tier methods.

The fourth tier represents a conceptually higher level of using the generated information. This may be a human decision maker, an operator on the plant, or a Quality Control (QC) officer deciding on the release of a batch based on a well-defined set of quality criteria. It may also be a more sophisticated decision making mechanism – implemented in an internet-based support system, e.g., using historical data and statistical procedures.

Figure 3 illustrates the rich collection of suitable alternatives for accomplishing the objectives of the PAT initative for biopharmaceuticals.

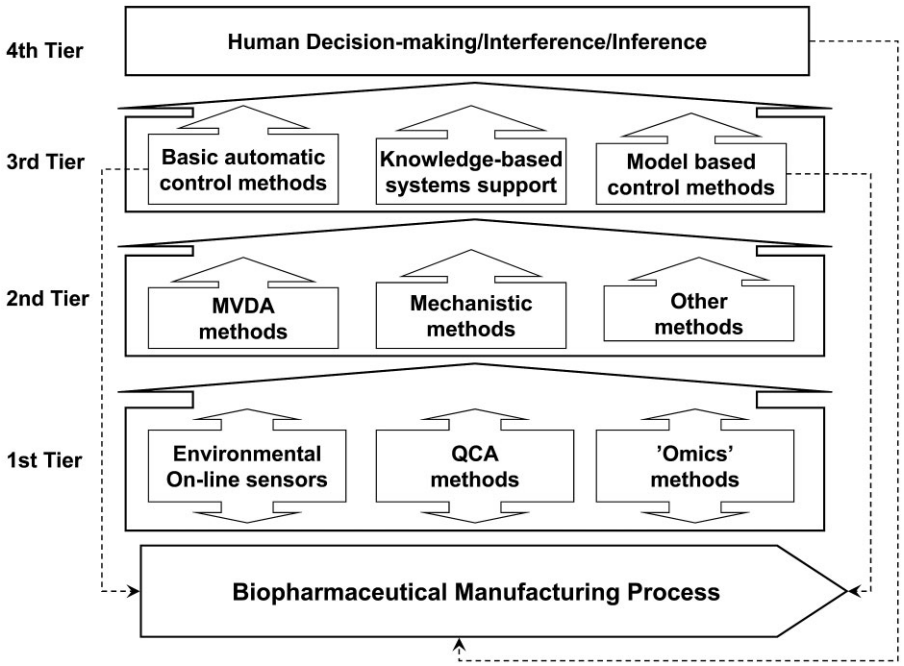

Figure 3. Conceptual interrelations of PAT tools and methods for biopharmaceutical processes structured on four consecutive levels (tiers). The first tier involves data acquisition tools, the second tier data processing of these acquired data. The methods of the third tier use the processed data for controlling the manufacturing process, and the fourth tier uses the data for advanced data analysis for further manufacturing control.

In this article we highlight a few areas of recent progress and opinions on application of PAT methodologies of relevance for biopharmaceutical production, based on a workshop held at the 8th European Symposium on Biochemical Engineering Sciences (Bologna, 5 September 2010). As typical biopharmaceutical products we include therapeutic antibodies, regulatory proteins such as insulin or growth hormone, enzymes with a pharmacological effect such as thrombin or tissue plasminogen activator, or vaccine components. But there can also be other biopolymers with therapeutic effect, for example, a gene therapy vector or a carbohydrate polymer.

The quality issues for all these biopharmaceuticals are similar, although not identical. The main concerns are the purity of the protein and the detection of impurities from the host organism, the culture media or formation of adverse product forms. The host organism and the biomolecular structure of the product have significant effects on the prerequisites of applying PAT. Progress in PAT and an improved understanding of its applications contribute to advanced manufacture of these complex biopharmaceutical products.

The article focuses on a few aspects of PAT: the furthering of multivariate data analysis (MVDA) for enhancing and building in quality into the biopharmaceutical process, the exploitaion of mechanistic models as an additional support tool, the use of systems biology data, the challenges of using mammalian cells in the processes, and examples of new opportunities with on-line sensing. We also relate these to industrial requirements and practice, and conclude with an identification of future needs to improve the impact of the PAT tools of benefit for biopharmaceutical production but also to biochemical engineering production in a more general perspective.

2 Potential of MVDA in implementing PAT on biopharmaceutical production

MVDA has the potential to play a central role in PAT and can interact with several other methods and techniques that analyze the biopharmaceutical manufacturing process, leading to better understanding of it and exerting control of its quality.

The use of a range of established analytical techniques alongside more advanced methods, such as near-infrared (NIR) spectroscopy, multi-wavelength fluorescence and electronic nose, within the PAT framework typically leads to the generation of large multivariate data sets. To extract useful information leading to deeper process understanding, it is essential to employ appropriate data analysis techniques capable of dealing with these multidimensional data. These data sets are highly heterogeneous, with varying frequency of various measurements, typically with significant delays in the off-line measurements. They are also often highly correlated, non-linear in nature and with high levels of redundancy and noise. Tech-

niques such as principal component analysis (PCA), partial least squares (PLS), parallel factor analysis (PARAFAC), unsupervised clustering methods and others, have long been used successfully by chemometricians for interpretation of multidimensional data sets in various subject areas, and their usefulness has also been proven in the area of biopharmaceuticals. A detailed description of these techniques is beyond the scope of this contribution and can be found in a variety of textbooks and manuscripts (e.g. [6–8]). Their ability to reduce dimensionality by removing the redundancy and noise leads to the identificaton of salient features in the data. These features can subsequently be used in bioprocess monitoring, fault detection and process optimization, as has been described extensively in the literature over the years. Successful applications cover a range of production systems from various microbial to cell culture systems and a wide range of industrially relevant products. Identification of deviations from nominal batch behaviour either in the seed or production cultivations (e.g. [9]), early detection of contamination or detection of faulty sensors are all critical process decisions that have been improved using MVDA techniques [2]. However, most importantly, the PAT methodology, as defined by the FDA [1], requires effective control of CPPs affecting the CQAs. This task is aided by the MVDA techniques enabling on-line estimation of such critical parameters as biomass, glucose and various other metabolites [10–12].

Biopharmaceutical processes are predominantly operated in a batch mode, which leads to further increase in the complexity of the resulting data arrays. The analysis of such data structures typically requires a modification of the traditional MVDA techniques or an application of non-linear alternative methods. Multiway PCA and PLS have been introduced in mid 1990 by Nomikos and MacGregor [13] and used sucessfully to account for the major non-linearity of the batch processes [9]. Alternative non-linear data analysis techniques, such as non-linear variants of PCA, PLS or various forms of artificial neural networks, have also been shown to be effective in bioprocess monitoring and control (e.g. [14, 15]). Whereas issues of regulatory approval of such 'black-box' data analysis techniques still represent a significant challenge in their application in a manufacturing environment, techniques such as autoassociative neural networks (AANN), self-organising maps (SOM) or support vector machines (SVM) can provide a more accurate representation of the fundamental features contained in the available measurements [16, 17].

3 Using mechanistic models as PAT tools for predicting CQA in the biopharmaceutical process

An alternative modeling approach to MVDA is to apply a mechanistic model description of the biopharmaceutical manufacturing system.

PAT, and especially its application to QbD, can only be realized in practice if sufficient process knowledge is available to explain the effect of CPPs on CQAs. In this respect, mechanistic modeling has gained renewed attention because a mechanistic model can be considered as a structured representation of the available process knowledge. Indeed, during the model-building procedure, the process knowledge is coded in a mechanistic model using appropriate mathematical expressions [18–21]. Such a model therefore incorporates process-relevant input (critical process variables) – output (product concentration and product quality attributes) relationships, which can be used to establish a proper design space. The mechanistic model therefore has great value in planning experiments, or in determining which critical process variables necessitate tighter control [21, 22].

Recent applications of mechanistic models for bio-based processes, such as biocatalysis [23] and fermentation [24] witnessed an interesting development. It should be emphasized indeed that the application of mechanistic modeling is not only relevant for the pharmaceutical industry, but also for fermentation and biocatalytic processes. In fact, many of the tools and techniques that are now adopted by the pharmaceutical industry for the implementation of PAT were developed much earlier by, or in close collaboration with, the fermentation industry [25].

One of the most frequently cited disadvantages of using mechanistic models is excessive time and resource requirements during the development phase. A systematic model development methodology addresses this drawback [26] and is beneficial for a number of reasons: (i) model development is more efficient (i.e., less time and resources required), and (ii) communication and knowledge transfer among the various members of the multidisciplinary team (i.e., researchers, engineers, operators, managers) typically involved in the development and implementation of a PAT system are facilitated. To this end, the matrix notation is commonly used because it allows a compact and visually appealing description of a complex mathematical model [20, 27].

The relatively large cost of developing a mechanistic model is increasingly justified if model analysis techniques are applied to the mechanistic

model as part of good modeling practice (GMoP) [28]. Moreover, mechanistic models also have great potential in Design of Experiment (DoE) applications. GMoP comprises the application of a set of mathematical and statistical techniques that allow improvements in the use and the reliability of the model. Examples include the calculation of correlation coefficients and confidence intervals for estimated parameters to verify sufficient data quality and quantity to allow reliable parameter estimation [20]. Additional examples include uncertainty analysis and sensitivity analysis [28–30]. Model analysis indeed provides answers to important questions such as: When is the time optimal for the collection of experimental data? Which variables should be measured? Which parameters can be estimated given an experimental data set and the mechanistic model?

4 Adapting the PAT tools to biopharmaceutical production with mammalian cells

The actual application of the MVDA and mechanistic models depends to an extent on the particular host organism used in the manufacturing process. Here we compare the two alternatives that are so far dominant, *E. coli* and mammalian cells, from a PAT perspective. Without a doubt, monitoring of living cells used as hosts, in an environment involving a multitude of parallel reactions and thousands of components as solid fraction in a mixture of gas and liquid, is a very ambitious and challenging task regardless of which organism is used. As a consequence, on-line data acquisition has to be individually designed for each organism used in bioprocessing. In contrast to bacterial cells, where synthesis of recombinant proteins is strictly associated with cellular growth, product formation in mammalian systems is more or less separated from growth. This fact leads to different process control strategies and specific monitoring requirements for each type of cell factory. Optimal exploitation of bacterial systems can only be obtained by setting conditions that allow for recombinant gene expression at high yet tolerable rates and simultaneous maintenance of cell viability and growth [31]. Growth rate, gene dosage and product titer are the central quality-related process variables. Real-time access to each of these variables is enabled by the application of comprehensive on- and off-line monitoring tools in combination with MVDA-based predictions [32, 33]. For example, based on these results an improved transcription tuning concept, utilizing the real-time predicted cell dry weight (CDW) for setting of a constant inducer to CDW ratio, has been established (to be published). Integration of comprehensive process data also yields an increased process understanding and new approaches for cell design [34]. In this work it was also demonstrated that the reduction of the induction level shows a significant increase of the soluble recombinant protein fraction. Real-time prediction of soluble and insoluble recombinant protein provides access to a CQA with transcription rate control as the CPP. This is just one example demonstrating a PAT solution with focus on product quality aspects specific for bacterial-based production processes.

Microbial production systems, especially *E. coli*, were the host of choice in the early biopharmaceutical processes. However, today mammalian cells have become the dominant system for the production of recombinant proteins mainly due to their capacity of required protein folding and post-translational modification. Thus, the resulting quality and efficiency of glycosylated proteins, e.g., monoclonal antibodies, produced by recombinant expression systems in mammalian cells can be considered to be superior to other expression systems such as bacteria, plants and yeasts [35]. Mammalian expression systems that are well known since the licensure of tissue plasminogen activator in 1987 include Chinese hamster ovary (CHO) or murine lypmphoid cell lines (e.g., NS0, Sp2/0). They offer the potential of increased productivities, which is one of the major driving forces for industrial manufacturing. Wurm [35] reported that 60–70% of all recombinant protein pharmaceuticals are produced in mammalian cells, usually following a well-established production scheme. Moreover, Farid [36] stated that antibodies played an important role in several of the important advances in pharmacotherapy that contributed to the treatment of infectious diseases, cancer and autoimmune diseases.

This clinical and commercial success of mammalian products, e.g., monoclonal antibodies, has led to the need for large scale production with mammalian cell cultures. Furthermore, these increased demands for therapeutic antibodies also resulted in a rapid expansion of global manufacturing capacity, e.g., increasing size of reactor capacity up to 10 m^3. At the same time process efficiency was improved to reduce manufacturing costs [37]. Clearly, besides the biological and quality-related advantages of the mammalian expression systems currently in use, economical aspects also play an important role in the industrial production of monoclonal antibodies. Werner [38] illustrated that high therapeutic dosages of monoclonal antibodies usually demand high capacity

needs, which in turn require significant capital investments, but also stimulate innovation for process improvements to decrease cost of goods (COG).

5 Integration of systems biology data from the host organisms of the bioprocess into the PAT methodology

This preference for mammalian systems in biopharmaceutical manufacture has placed particular quality aspects, such as protein glycosylation heterogeneity, charge distribution and polymorphism, at the forefront of research and development.

Such CQAs are linked to molecular details produced by highly regulated intracellular processes that are not part of the mainstream carbon and nitrogen fluxes. Although the dependency of, for example, protein glycosylation patterns on process operation parameters such as pH, temperature, medium composition and carbon source feeding rate has been demonstrated in several studies (e.g. [39, 40]), systematization of knowledge and demonstration of controllability become impossible without the consideration of intracellular processes. This is where systems biology can play an important role in PAT, specifically in: (1) the generation of knowledge, (2) the elucidation of mechanisms, and (3) the modeling of the biological functions at the basis of molecular level CQA.

Substantial efforts are made in academic and industrial research in collecting 'omics' data sets at various cultivation time to discover the fundamental regulation mechanisms of certain product CQAs, but also to find new ways to increase yield and productivity. In a PAT approach, it would be of particular use to concentrate attention on the exometabolomics in the process. The exometabolome, which consists of the total quantitative collection of small molecular weight compounds (metabolites) present in the extracellular medium, provides a very informative "footprint" of cellular activity, from which it is possible to infer its intracellular state up to the proteomic and genomic levels [41]. Today cheap, fast and high-throughput techniques with little or even no sample preparation exist that enable the measurement of the exometabolome. This opens an array of opportunities for advanced monitoring techniques that can support real-time control of critical intracellular processes at the basis of the CQA. Mass spectrometry, NMR spectroscopy and vibrational spectroscopy are the main techniques currently used for metabolomics. The need for high-throughput, short analysis time compatible with at-line or even on-line measurement restricts the choice to direct injection mass spectroscopy (DI-MS), Fourier transform infrared spectroscopy (FT-IR) and (1H) NMR [42]. Among these, DI-MS is considered the technique with the highest potential since it provides a fingerprint of the complete exometabolome, is highly sensitive, requires no sample treatment and is cheap, fast and high-throughput [41].

Chemometrics plays a very important role in PAT in MVDA, extraction of knowledge from large datasets, modeling and statistical process control. MVDA modeling is also widely applied in the analysis of 'omics' datasets to deduce the mechanisms that support a given (observed) biological function (the top-down approach) [43]. Systems biology is concerned with the interpretation of data to infer mechanisms (top-down) and to infer function from known mechanisms (bottom-up). In fact these two approaches can also be found in PAT modeling. The top-down approach is based on chemometric modeling, while the bottom-up approach is based on mathematical (mechanistic) modeling. In PAT, extensive challenges remain to be addressed in the integration of macroscopic (process monitoring and control) models with systems biology models [44, 45]. One important aspect is the efficient integration of mechanisms and MVDA. As new mechanisms are disclosed they represent constraints to top-down MVDA (top-down and bottom-up meet halfway). Hybrid semi-parametric modeling may provide an adequate answer to some of these challenges. It represents a compromise between rigorous mathematical modeling and empirical (chemometric) modeling, providing a flexible framework to merge *a priori* mechanisms with heterogeneous datasets of the different layers of information about the cell and the process [45].

6 Challenges limiting routine industrial application of PAT

The development of efficient mammalian bioprocesses in the biopharmaceutical industry has led to increased product yields above 5 g/L monoclonal antibody. These achievements can be attributed to the use of high-producer cell clones with enhanced cell productivity and to the optimization of key process characteristics, such as advanced process control and well-designed nutrient compositions, that are integrated into a platform technology for biopharmaceutical manufacturing processes. However, to identify potential process parameters that have an impact on CQAs, the process developer first need to identify and understand a set of CPPs.

When addressing QbD as described in the ICH guidelines (ICH Q8: pharmaceutical development, Q9: quality risk management and Q10: pharmaceutical quality system), typical questions in the field of bioprocess development arise: Can we identify CPPs and to what extent do these parameters have an impact on an industrial bioprocess? Can we adjust these process parameters? Thus, as a first step in addressing those questions from an industrial point of view, a 'proposed workflow' is presented with focus on new sensor techologies and advanced data analysis for process data based on multivariate projection techniques.

6.1 Industrial workflow to implement the QbD/PAT concept

The implementation of advanced PAT technologies into an already existing GMP manufacturing facility can be complex and faces many challenges. For example, new innovative process sensors for mammalian suspension cultures need to be easy-to-handle in a daily working routine, e.g., a simple calibration procedure is required. Moreover, PAT sensors can be applied in a robust and applicable manner for a broad range of process variants. This allows the amount of valuable process data to be increased and the corresponding know-how translated into a monitoring or control scheme to handle the sensitive process parameters. In other words, the collected data are translated into process information and subsequently further transformed into real process knowledge using advanced analysis techiques, e.g., PCA, PLS or orthogonal PLS (OPLS) (Fig. 4). The generated process knowledge can be subsequently used to monitor or control the critical sources of variability to ensure a consistent quality for an advanced manufacturing process.

The quality issues for all the biopharmaceuticals referred to in this contribution are similar, although not identical. The main concerns are the purity of the protein and the detection of impurities from the host organism, the culture media or formation of adverse product forms. The host organism and the biomolecular structure of the product have significant effects on the prerequisites of applying PAT.

Progress in PAT and an improved understanding of its applications contribute to advancing manufacture of these complex biopharmaceutical products.

7 Recommendations

Based on the workshop, the following recommendations for further developing PAT methodology for biopharmaceuticals are suggested:

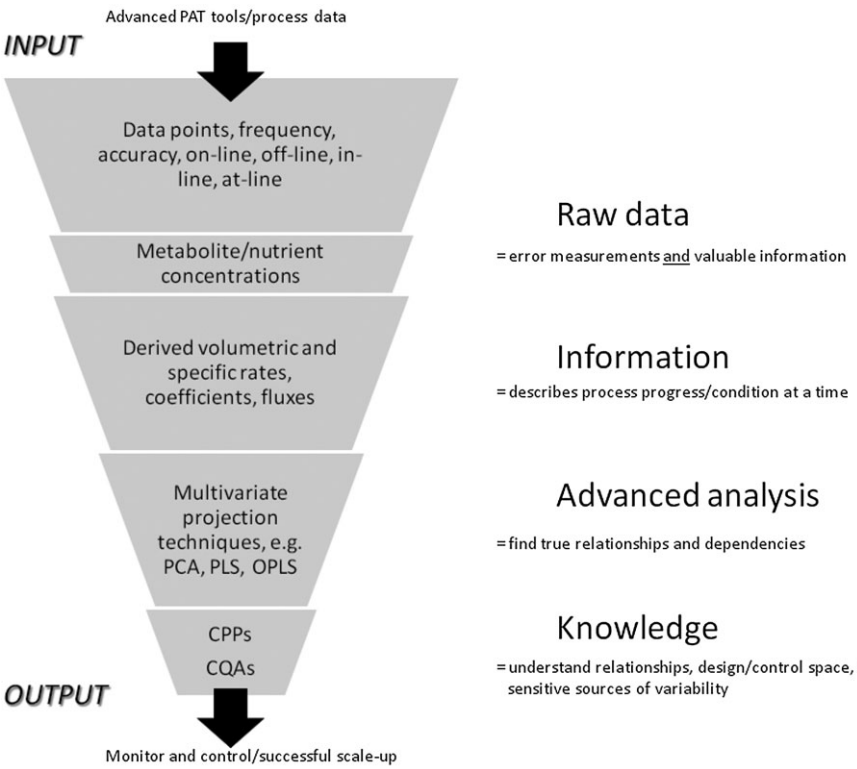

Figure 4. Proposed industrial workflow to address the QbD/PAT concept using multivariate projection techniques to identify CPPs and their relationship to CQAs.

- There is still a need to develop a more robust platform providing a structured framework for industrial acceptance of the PAT methodology. The methodology approaches presented in this article shoulde be integrated in such a framework.
- Systematic evaluation of such a platform should be carried out using real industrial production systems
- The success of such a platform will, to a large extent, also depend on the level of documentation and standardization that can be provided. Industry needs standard operating procedures and well-documented methods and tools, to be able to adopt new technology in a GMP environment. Good modeling practice (GMoP) forms a natural part of this.
- CQAs should be related to the molecular structures of the product. This information requires analytics that detects specific characteristics of the product media (broth, culture media, or released cellular components). Methods should preferably be based on immunogenic reactions or, possibly, rapid genomic analysis.
- Systems biology can provide critical information about the mechanisms that control CQAs. This information can be integrated in mathematical models to support PAT. On-line or at-line exometabolomics needs to be further developed as a process analytical technique with the capability to infer information about the endometablome, proteome and transcriptome.
- Merging mechanisms and MVDA into a common (hybrid) modeling framework still presents a major challenge to the integration of different layers of information about cells and macroscopic processes.
- The number of experiments required to establish a robust correlation model should be further reduced. At the same time it is necessary to differentiate between causal and non-causal correlation. Process knowledge should be used to overcome these issues. However, if little knowledge is available error prone interpretation of correlation structures might occur.

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