CHALLENGES AND OPPORTUNITIES IN BIOPHARMACEUTICAL MANUFACTURING CONTROL

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Abstract

The total sales of biopharmaceuticals have been steadily increasing for decades with growing approval of new drug products, especially for monoclonal antibodies. This trend motivates the development of deeper understanding and advancement of biopharmaceutical manufacturing operations. This article describes opportunities, needs, and challenges in process control and operations for biopharmaceutical manufacturing associated with continuous operations, process data analytics, and novel bioseparations. Challenges and opportunities in continuous operations are described for (1) microscale technologies for high-speed continuous process development, (2) the design of plug-and-play modular unit operations with integrated process monitoring and control systems to facilitate straightforward deployment in the laboratory, (3) dynamic models for unit operations and entire biopharmaceutical manufacturing plants to support process development and plant-wide control, and (4) easy-to-use model-based control technologies for optimizing startup, changeover, and shutdown aided with real-time process analytical technology (PAT). A challenge is the derivation of process monitoring and control techniques that are able to simultaneously address the uncertainties, nonlinearities, time delays, nonminimum phase behavior, constraints, spatial distributions, and mixed continuous-discrete operations that arise in biopharmaceutical operations. New process data analytics and grey-box modeling methods are needed to deal with the heterogeneity and tensorial dimensionality of much of the most promising PAT for the measurement of biopharmaceutical data. Novel bioseparations as discussed as a potential cost-effective unit operation, with a detailed discussion of challenges for the widespread application of crystallization to therapeutic proteins.

Keywords

Biopharmaceuticals, Biomanufacturing, Biopharmaceutical manufacturing, Continuous manufacturing, Protein crystallization, Process data analytics.

Introduction

Biopharmaceuticals, which are also widely known as biologics or biologic drugs, are products derived from biological organisms for treating or preventing diseases. The global sales of biopharmaceuticals, which have continually increased for many years, was ~$300 billion in 2014, and is projected to reach ~$450 billion by 2019 (Deloitte, 2016). Over 30% of the drugs in the drug pipeline are biopharmaceuticals (Informa, 2016), with hundreds of approved products on the market and over 7000 medicines in development (PhRMA, 2016). The rate

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of biopharmaceuticals approval has remained relatively steady, with monoclonal antibodies (mAbs) accounting for an increasing proportion of the approvals (Walsh, 2014).

Monoclonal antibodies (mAbs) were the highest selling class of biopharmaceuticals with ~1,500 drugs in the product pipeline in 2016 (Informa, 2016). This class is of particular interest due to their specific action and reduced immunogenicity. With continued growth in sales of existing mAb products and a growing pipeline of mAb product candidates being developed, the total sales of mAb products and all biopharmaceuticals will continue to increase in the coming years (Ecker et al., 2015). The development of mAbs is expected to grow further as more diseases are understood at molecular and cellular levels.

Traditional biopharmaceutical manufacturing processes comprise a similar sequence of unit operations that are divided into two main parts: upstream and downstream. The upstream unit operations typically include cell culture and harvest steps, and the downstream consists of purification with multiple steps of chromatography, filtration, and diafiltration. For example, Figure 1 shows a process flow diagram for a typical platform used for the production of mAbs (see Kelley, 2009; Shukla and Thömmes, 2010; and citations therein for the figure and more details than the summary provided here). The upstream process starts with cell culture, which is fed inoculum prepared and expanded from a cell bank to a series of batch bioreactors of successfully larger volume for expansion of the cells and finally to the production bioreactor for protein expression. Then cells and cell debris are removed by centrifugation followed by depth and membrane filtration (for proteins that are secreted by the cells into the surrounding solution, that latter "harvest" steps are skipped).

The downstream process for mAbs begins its capture mAb by protein A affinity chromatography. Protein A affinity chromatography provides >98% purity in a single step with high binding affinity and specificity of protein A ligand to the Fc region of mAbs. The products bind to the stationary phase while impurities, such as host cell proteins and DNA, pass through at neutral pH. The products are then eluted from the adsorbents at low pH, which inactivates viruses. Next, two polishing chromatographic steps are typically used for further removal of impurities. Protein A affinity chromatography is unable to remove aggregates and product variants due to their chemical similarity with the derived protein, and introduces leached protein A as a new impurity. The most commonly used steps are cation exchange (CEX) chromatography and anion exchange (AEX) chromatography. CEX chromatography uses resin with negatively charged groups to bind the products during the loading step, then elutes the products by increasing pH or conductivity. AEX chromatography uses resin with positively charged groups and typically run in flow-through mode due to the high pI of mAbs, which is often >8. The operating conditions are chosen to allow the products to flow through while the impurities bind to the resin. A viral filtration step is then used to ensure viral safety by a sized-based virus removal. A subsequent step of ultrafiltration/diafiltration (UF/DF) formulates and concentrates the product for last step of the process.

Process control engineers have an important role to play in biopharmaceutical manufacturing, and this article describes its opportunities, needs, and challenges in process control and operations. The next section introduces three trends in biopharmaceutical manufacturing that are described in more detail in subsequent sections. A section on process data analytics discusses challenges in dealing with high-dimensional and heterogeneous data, and the potential for "grey-box" models that supplement first-principles models with data-based models. Another section discusses a recent trend towards continuous-flow operations, and how this development opens many opportunities for mathematical modeling and process simulation and model-based design, control, and optimization. A section on novel bioseparations primarily discusses crystallization as a non-chromatographic method for the purification of proteins from a large number of other components in solution. The challenges of crystallizing mAbs and other large-molecule therapeutic proteins are described along with potential control approaches for biopharmaceutical crystallization. The article ends with a summary and some closing comments.

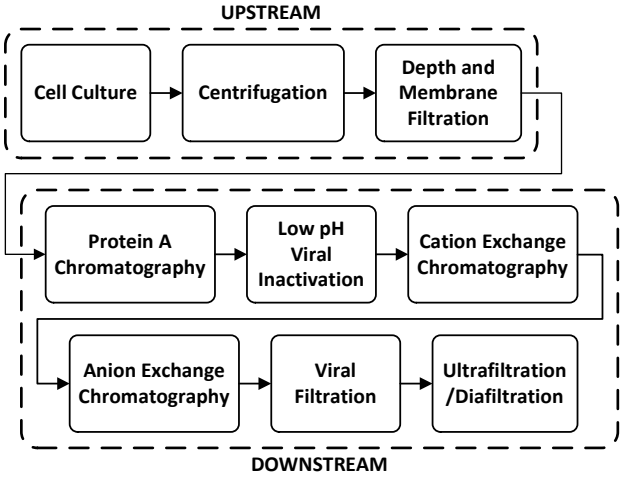

Figure 1. Process flow diagram for a typical production platform for mAbs.

Trends in Biopharmaceutical Manufacturing

The continued growth of biologics motivates developing a deeper understanding and advancement of biopharmaceutical manufacturing operations. This growth has increased interest in the application of process analytical technology (PAT), which is "a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality" (FDA, 2004). On-line measurements

of critical quality attributes (CQAs) provide much more data on the multivariable interactions and dynamics, with the potential towards increased process understanding. Depending on the degree of process understanding, the data have been used to construct first-principles and data-based models for each biopharmaceutical unit operation. The constructed models and real-time process monitoring enable the implementation of advanced control algorithms for producing products of higher quality. The consistent product quality achieved by the real-time measurements and advanced control strategy enable PAT to be viewed as mode for implementing quality by design (QbD) approach promoted by regulatory agencies (e.g., see FDA, 2009; Rathore, 2009; Rathore and Winkle, 2009; and citations therein).

The inherent complexity of biological molecules and processes creates challenges for the application of PAT to biopharmaceutical manufacturing, but the number and variety of high-tech instruments being developed ensure growing successful application (Read et al., 2009a). Due to their similar sequence of unit operations and shared CQAs of concern in this industry, single successful applications can be quickly moved to the manufacturing operations being developed for other biologic drug compounds throughout a company, and throughout the industry. This high degree of leveraging an advance in PAT is especially applicable for mAbs because, not only are a standard process flow diagram and equipment implemented for their production, this process platform enables applying data from one product to the other (Read et al., 2009b).

In addition to moving towards increasing online data collection and the construction of models, another trend in the biopharmaceutical manufacturing industry is to transition of many of processes from batch to continuous operation. This batch-to-continuous transition mirrors the recent trend for chemically derived pharmaceutical manufacturing (aka “small-molecule drugs”), driven by the goal of reducing manufacturing costs and increasing flexibility and quality (e.g., see Jiang and Braatz, 2016, and citations therein). Continuous perfusion bioreactors have been implemented in commercial biopharmaceutical manufacturing processes for decades (Konstantinov and Cooney, 2015). In contrast, although continuous downstream processes have been investigated in academia for decades, their application in the biopharmaceuticals industry has been limited. Industrial interest has increased in the last five years, and may lead to end-to-end continuous biopharmaceutical manufacturing (e.g., Lu et al., 2015; 2016). The increased data provided by PAT, and associated feedforward and feedback controls systems, will be key to providing reliable long-term continuous operations (Konstantinov and Cooney, 2015). The continuous manufacturing processes provide new process control problems to address, to handle the propagation of impurities and other disturbances caused by tight integration of continuous unit operations. Plant-wide control strategies that optimize the overall process operations, which have been demonstrated for small-molecule pharmaceuticals (Lakerveld et al., 2013; 2015), have promise for application to biopharmaceuticals.

Another area of interest in biopharmaceutical manufacturing is the invention of new designs for downstream processes, namely, the protein separations. Such a development, if successful, would be a major shift in the way that biopharmaceuticals are manufactured. Such developments would likely involve new processes to control, with crystallization being one of the more promising proposed technologies.

The next three sections describe each of these three topical areas in more detail: process data analytics, continuous operations, and novel bioseparations.

Process Data Analytics

Using High-Dimensional and Heterogeneous Data

Biopharmaceutical manufacturing data are often heterogeneous in both time scale and data type (Charaniya et al., 2008). For example, some measurements in a bioreactor can be performed continuously on-line such as dissolved oxygen, optical density, and pH. Other measurements are performed off-line, such as the distribution of oligosaccharides (glycans) attached to the proteins, usually at non-periodic, asynchronous intervals. Many process monitoring and control algorithms such as differential geometric methods are not well suited for heterogeneous data collected over varying sampling time intervals, and require revisions to be suitable for biopharmaceutical manufacturing applications.

Biopharmaceutical manufacturing studies that consider the handling of heterogeneous data have focused on the bioreactor. This focus is because, for most products and cell lines, bioreactors generate numerous compounds that are so closely related to the desired biologic drug compound that they are not removable by existing downstream separations technologies. Charaniya et al. (2010) and Le et al. (2012) have used kernel-based methods to integrate the various data types. These papers use a similarity kernel, which is an exponential transformation of the Euclidean distance, for the different bioreactor runs. Dynamic time warping (DTW) has also been investigated as a methodology for handling time heterogeneity (Ündey et al., 2002). DTW finds a nonlinear mapping to minimize the distance between two multivariate time series with certain event constraints, such as the beginning and end of a batch. González-Martínez et al. (2013) used information from the results of time-warping for process monitoring and fault classification.

A further complication with biopharmaceutical manufacturing data is the need for a higher degree of dimensionality reduction. This problem is most extreme when using state-of-the-art sensor technologies that employ spectral images, in which each sensor reading is a third- or fourth-order tensor. Because production runs can take many hours and are costly, the analyst may not have access to many replicates of the high-dimensional datasets, which complicates dimensionality reduction and subset learning.

Some approaches have been proposed to achieve this goal, employing such methods as hierarchical clustering (Charaniya et al., 2010) and greedy algorithms (Le et al., 2012).

A challenge in biopharmaceutical manufacturing is both high complexity of proteins and the close similarity of variants to the desired protein(s). These variants can include a very large number of combinations of different amino acid sequences, disulfide linkages, and glycosylation structures. Other subtle differences can include oxidation and/or deamidation of single sites on a complex protein. While some success has been seen in leveraging techniques from the machine learning community (e.g., as discussed by Severson et al., 2015), there are opportunities in the development of algorithms specially designed to handle the particularities of biopharmaceutical data.

Supplementing First-Principles with Data-based Models

First-principles models employ well-developed process understanding in the form of conservation equations, reaction mechanisms, and constitutive relations such as Fick's diffusion law. First-principles models and reliable monitoring of physical parameters, such as UV absorbance, pH, conductivity, and pressure, have enabled the control of individual process units (Konstantinov and Cooney, 2015). As one example, Karst et al. (2016) fed at-line high-performance liquid chromatography (HPLC) measurements of the harvest concentration subsequent to the bioreactor to a mechanistic model of a capture chromatography unit to optimize buffer consumption and productivity. Although not explicitly modeled in the study, stable production led to consistent measurements of aggregates, clipped forms, charge isoforms, and n-linked glycosylation for their system. More recently, it has been argued that significant value can be obtained by constructing first-principles models where possible—or black-box models where necessary—that integrate multiple unit operations, even for entire biopharmaceutical manufacturing (Lu et al., 2015; Severson et al., 2015). The value of such plant-wide models has been thoroughly demonstrated experimentally for both chemically and biologically derived drug manufacturing plants (Mascia et al., 2013; Severson et al., 2015; Lakerveld et al., 2015).

Statistical methods such as principal component analysis (PCA) and partial least squares (PLS) have been used to monitor process operations in biopharmaceutical manufacturing applications for years (Vaidyanathan et al., 2001; Wold et al., 2006; Kirdar et al., 2007). Certain attributes that may indicate product quality—such as aggregates, post-translational modifications, and n-glycosylation—cannot be measured directly, and are not well-characterized by PCA and PLS. Furthermore, some of the quality attributes are determined via biological processes that are not fully understood and first-principles models are not yet available (Read et al., 2009a). Advanced real-time sensor development could enable the large quantities of data needed to thoroughly evaluate potential hypothesized mechanisms and improve process understanding. Model-based experimental design methodologies and algorithms are available for carrying out such studies to generate process understanding and associated first-principles models, even for complex multiscale systems (e.g., see Braatz et al., 2006, and citations therein).

Until first-principles models become available for some of the more challenging biological reactions, there is an opportunity to build "grey-box" models using data to inform process monitoring and control decisions. Grey-box models are in between first-principles models (based on detailed process understanding) and black-box models, which are based on statistical correlations observed in experimental data (e.g., see Pearson and Pottmann, 2000; Togkalidou et al., 2004; Overgaard et al., 2005; and citations therein). Most grey-box modeling approaches combine first-principles models and black-box models to generate predictions that are more complete and/or accurate than either separate model type. One grey-box modeling strategy is to use a first-principles model to predict some states while using surrogate measurements to act as indicators of product quality for states that cannot be modeled using existing first-principles understanding (a "parallel" approach). Other grey-box strategies are to (1) define the nonlinear input-output behavior using first-principles equations or a basis function expansion fit to a simulation of first-principles equations and to use black-box models for describing the dynamics (e.g., Pearson and Pottmann, 2000), (2) use first-principles equations to define the model structure but replace unknown kinetic relationships by apparent relationships described by nonlinear basis function expansions (an "embedded" approach, e.g., van Can et al, 1997), or (3) replace the true system output in least-squares identification of a first-principles model with a surrogate measurement that closely tracks the true systems output (a "surrogate output" approach, e.g., Togkalidou et al., 2004).

Recent advances have been made in representing some phenomena that long seemed inaccessible to first-principles modeling. An example is the work of Villiger et al. (2016) on the first-principles modeling of n-linked glycosylation. In their work, the mechanistic glycosylation model of del Val et al. (2011) is combined with an unstructured cell culture model to predict the evolution of glycan profiles and used to make decisions about feeding policies. As first-principles models become available for more of the complex phenomena that arise in biopharmaceutical manufacturing processes, predictive accuracy will increase, enabling process monitoring and control with improved performance.

Continuous Operations

Classically, chemically and biologically derived pharmaceuticals were manufactured in a series of batch processes. Increased attention has been towards

transitioning to continuous operations, where "continuous" can refer to (1) a unit operation with the capability of operating under continuous flow and minimal holdup volume, or (2) a manufacturing plant with integrated continuous-flow unit operations with minimal hold volume in between (Konstantinov and Cooney, 2015; and citations therein). Operating under continuous flow enables: (1) higher process flexibility, consistency, and volumetric capacity; (2) lower equipment cost and operational complexity; and (3) tighter specifications on product quality (Jungbauer, 2013; Croughan et al., 2015; Jiang et al., 2015; and citations therein).

For small-molecule pharmaceuticals, an example of this trend is in purification. Purification processes are traditionally carried out in batch or semi-batch crystallizers, with a typical objective being to control the molecular purity, size, and shape of the product crystals. The degree of control that is needed depends on whether the crystallization is for an intermediate or for inclusion into the final pharmaceutical product, and the type of drug product. Many investigations have considered the effect of the process operations and seed crystal size distribution on the size distribution of product crystals generate in batch or semibatch processes; however, vastly improved control is obtainable in continuous crystallization, especially in terms of producing crystals of a narrower and more precisely controlled crystal size distribution (e.g., Myerson et al., 2015; Jiang et al., 2015abc; Jiang and Braatz, 2016; and citations therein).

With the rapid increase in market share and the drive to improve product quality with lower costs, industry and academia are increasingly investigating continuous-flow biopharmaceutical manufacturing processes (Konstantinov and Cooney, 2015; Jiang and Braatz, 2016; and citations therein) Progress has been made on unit operations for both upstream and downstream processes, including cell culture, centrifugation, filtration, and chromatography (Jungbauer, 2013; and citations therein). Ideas with prototypes for both hybrid systems (partially continuous) and fully integrated continuous processes (from media to drug substance) are available for further development (Konstantinov and Cooney, 2015). From existing publications (Konstantinov and Cooney, 2015; Jiang and Braatz, 2016; and citations therein) and the authors' perspectives, the challenges and future trends of continuous biopharmaceutical manufacturing operations include:

- Further understanding and optimization of each unit operation and component, such as cell culture media (Konstantinov and Cooney, 2015; and citations therein)
- Microscale technologies for high-speed continuous process development
- Availability of plug-and-play modular unit operations with integrated process control and monitoring systems to facilitate straightforward deployment in the laboratory (including single-use technology)
- Dynamic mathematical models for unit operations and entire biopharmaceutical manufacturing plants to support process development and plant-wide control
- Easy-to-use model-based control technologies for optimizing startup, changeover, and shutdown aided with real-time process analytical technology

To address these challenges, we propose several guidelines for constructing a virtual plant with modeling and control. First, first-principles models should be constructed wherever possible, with empirical or grey-box models used where necessary (see the Process Data Analytics section for a more detailed discussion of data-driven models and the associated challenges). Second, the highest complexity models should be used in the invention and optimization of process designs and process development. Such models should include computational fluid dynamics simulation of multiphase flow of bioreactors including momentum and multicomponent material balances of bubble and liquid phases including gas-liquid mass transfer relations, and first-principles models of chromatography columns and membrane filtration units. Third, lower complexity models of unit operations can be used to design most of the closed-loop control systems, and integrated to form a plant-wide model that is run in parallel with process operations, for process control and quality and equipment condition monitoring.

A continuous biopharmaceutical manufacturing platform (Integrated and Scalable Cyto-Technology, or InSCyT) (Lu et al., 2015; 2016) is being developed with these guidelines in mind. As an example of the specification of fidelity for such models, consider the continuous generation of liquid solutions needed for chromatography and polishing membrane unit operations. The unit operations require liquid solutions of tightly controlled pH and conductivity for optimal operation. The nonlinear phase equilibria can be complex, with high sensitivity to uncertainties in model parameters (Lu et al., 2016). The mixing in an initially designed tank was shown to be poor by running computational fluid dynamics, so the tank was replaced by two in-line static micromixers (Jiang et al., 2015ab) operating in series with continuous feed of inlet streams of multiple buffers and base in aqueous solution. This configuration is a fairly well-accepted approach to process intensification that creates fast mixing with simple dynamics. The mixing dynamics in each micromixer were described using a five tanks-in-series model. To improve the closed-loop performance and fast response time, the pH was modeled using the well-known reaction-invariant approach (Figure 2), which enables most of the nonlinearity to be represented algebraically. Model-based nonlinear control was designed to address the nonlinearities during the feedback controller design (Figure 3). An adaptive control approach, described by Lu et al. (2016), was developed to increase the robustness of

the closed-loop system to uncertainties in the pKa of the large number of ionizable groups for the molecules in the liquid feed solutions.

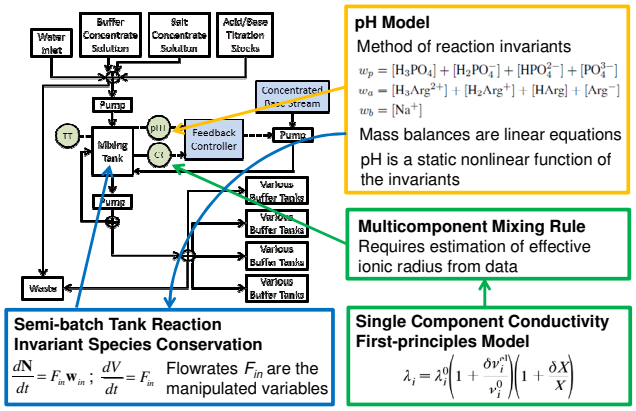

Figure 2. The pH and conductivity for inline mixing of liquid solutions in downstream biopharmaceutical manufacturing operations are nonlinear algebraic functions of the reaction invariants, which are computed from conservation equations. Adapted from Lu et al. (2015).

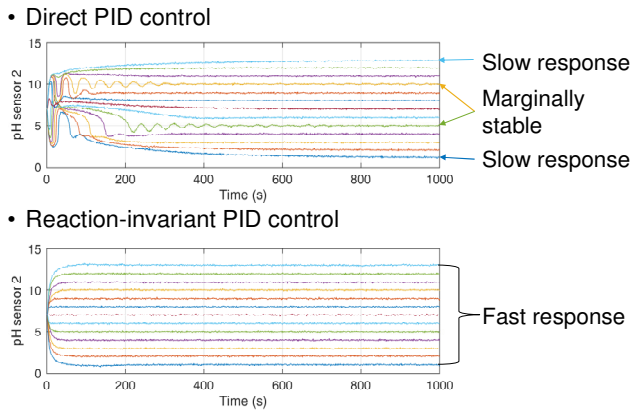

Figure 3. Performance comparison between PID controllers without and with the reaction-invariant method. Adapted from Lu et al. (2016).

Plant-wide simulation can guide the selection of the control strategy for each CQA, the design of startup, changeover, and shutdown operations, and the design of control systems that ensure that the CQAs of the drug product are insensitive to model uncertainties and disturbances while directly addressing all nonlinearities, time delays, non-minimum phase behavior, and constraints, as well as spatially distributed and mixed continuous-discrete character of the operations of some of the units. Most existing process monitoring and control theory cannot address much of these phenomena that occur in continuous biopharmaceutical manufacturing operations. Process models range in complexity from the aforementioned reaction-invariant multiple-tanks-in-series differential-algebraic models of base addition to static micromixers to control pH, to parabolic partial differential equations for the chromatography columns, to multiphase computational fluid dynamics for simulating bubble dynamics and shear stresses on cells in bioreactors to determine bioreactor internal geometries and mixing speeds. Better ways are still needed to design the real-time control systems that are implementable online. One awy to design such control systems is to exploit specific information on the underlying phenomena. For example, we have designed process control system designs for specific biopharmaceutical unit operations that include open-loop schedules, multi-level split-range control, and nonlinear model-based adaptive control by using a variety of techniques. A blending of distinct design strategies and theoretical approaches with a strong dose of nonconservative uncertainty analysis (e.g., Kim et al., 2013; Streif et al., 2016) has been effective for such nonlinear dynamical systems (Lu et al., 2015; 2016).

Novel Bioseparations

The currently dominant method for bioseparation is packed-bed chromatography, due to its high resolution. Chromatography for high-dose biopharmaceuticals is expensive, however, even when using operational improvements such as periodic countercurrent continuous chromatography (Bryntesson et al., 2011). When scaling manufacturing processes from bench-scale to production, it is generally desirable for the operating cost to scale sub-linearly with the material throughput. The method of purification via chromatography columns has operating costs that scale linearly with throughput, roughly the cost of resin divided by the number of times that the resin can be reused. As production demand will continue to increase, the operating costs of chromatography will rise proportionally. Also, the increased product titers that are being achieved with modern bioreactors also makes several non-chromatography separation methods more attractive (Zydney, 2016), such as countercurrent multi-stage aqueous two-phase extraction (Goja et al., 2013; Eggersgluess et al., 2014), precipitation (Hammerschmidt et al., 2014), and crystallization, which is discussed in more detail below. These latter three processes have been operated in continuous mode (Zydney, 2016).

Crystallization as a Non-Chromatographic Method for Bioseparations

Crystallization from liquid solution has proved to be an effective and inexpensive industrial operation for inorganic and organic molecules to achieve adequate purity and production in large quantities. This purification method has costs that scale sub-linearly with throughput because the costs scale with the volume of the solution. The improved cost-effectiveness is why crystallization is heavily used for the purification of small-molecule pharmaceutical compounds, that is, for amino acids, active pharmaceutical ingredients, and intermediates.

Crystallization is already used for the production-scale purification of some therapeutic proteins, the most

successful being insulin for the treatment of diabetes. Eli Lilly & Company first introduced the crystallization method for pancreatic insulin, and have been manufacturing insulin using this process for over thirty years (Jackson, 1973). After insulin is extracted from the pancreas by the usual aqueous phosphoric acid-alcohol process, the aqueous insulin-containing solution is added about 0.2 to 1.0 M alkali metal hydroxide at room temperature until a pH of 8.2 is attained. Crystallization takes about ¼ to 72 hours, depending upon quality and quantity of the starting material. The crystallized insulin is removed by decantation or filtration from mother liquor.

The insulin manufactured by Eli Lilly & Company today is called *insulin lispro*, which is a fast-acting insulin analogue also produced with a crystallization step (see Figure 4 for the flow diagram for the process) (Baker and Roberts, 1997). After proinsulin is produced from fermentation of *Escherichia Coli* and separated from the cells by centrifugation and filtration, insulin lispro is liberated from proinsulin by treating with trypsin and carboxypeptidase B. Then the mixture is purified using a number of chromatographic steps and lastly crystallized. For crystallization, the solution with 20 g/L protein, 37.5 mM NaCl, 0.75 M acetic acid, and 0.3% v/v phenol is raised to approximately pH 9 with 10% NaOH solution at 5°C. Well-defined crystals are obtained after 24 hours of gentle agitation.

Challenges for the Crystallization of Large-Molecule Therapeutic Proteins

While crystallization is already used for the production-scale purification of some therapeutic proteins as mentioned above, research and development are needed to develop a crystallization technology effective for mAbs and other large-molecule therapeutic proteins. One of the challenges in developing production-scale crystallization for the purification of large-molecule therapeutic proteins is that conditions must be found that produce reproducible crystallization using pharmaceutical-grade buffers and precipitants. Another challenge is that many of these proteins are easily denatured by pH variation, changes in temperature, addition of precipitants, and agitation. Characterizing the operating conditions and designing molecular additives that maximize process efficiency, protein stability, and protein yield while minimizing protein denaturation and aggregation is necessary to resolve these challenges.

The systematic approach to the solution would be constructing phase equilibrium model to characterize multidimensional solubility surfaces. The driving force for crystallization is supersaturation, which is rigorously defined in terms of chemical potential but is nearly always replaced by the ratio of the solution concentration to the solubility, this ratio minus one, or the solution concentration minus the solubility to avoid the time and expensive in constructing a non-ideal phase equilibrium model for computing the chemical potential. Regardless of the specific definition of supersaturation used, information on the solubility is needed to determine supersaturation. An example is the work of Ahamed et al. (2007) on applying first-principles models to calculate the phase diagram for mAb. Their work used the osmotic second virial coefficient (B_{22}), the parameter measuring solution non-ideality due to solute-solute interactions, to derive the generalized protein phase diagram.

A third challenge is that such proteins have orders-of-magnitude slower nucleation and growth rates, typically on the order of many hours to days, compared to small-molecule pharmaceuticals. Proteolytic degradation by proteases or denaturation may occur during this period, reducing the overall yield or quality of the product. This challenge may be addressed by using continuous-flow crystallizers designed to operate at high crystal surface area, to reduce the potential for protein denaturation and degradation by proteases. First-principles models based on population balances for crystal size distribution will be useful for the design of optimal crystallizer configurations.

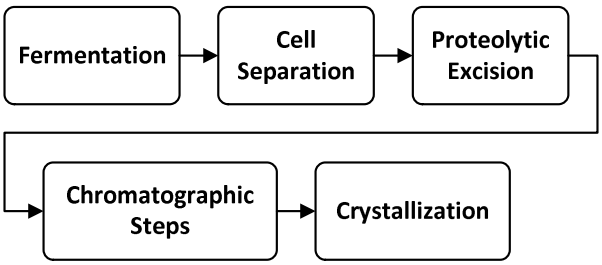

Figure 4. Flow diagram for the production of insulin lispro from fermentation broth using crystallization (Baker and Roberts, 1997).

Control of Biopharmaceutical Crystallization

Depending on how much the process is understood, the control of the biopharmaceutical crystallization can either take (1) first-principles approach or (2) the direct design approach (see Figure 5 for flowchart for both approaches) (Fujiwara et al., 2005; Nagy and Braatz, 2007; 2012; Simon et al., 2015; and citations therein). The first-principles approach controls the crystallization by optimizing objective function related to the crystal size distribution by using a first-principles model. With optimal experimental design and experimental data collection, the parameters for the models can be estimated and the best kinetic mechanism can be selected. The remaining uncertainties in the model parameters and structures can be assessed with modern robustness analysis methods (see Streif et al., 2016, and citations therein). Robust optimization can be handled with the worst-case objective (which is also known as min-max approach) as well as a weighted sum of the mean of the objectives for nominal performance and its variance for robustness. This first-principles approach was applied by Rawlings et al. (1993) and Miller and Rawlings (1994) to inorganic crystallization and Togkalidou et al. (2004) to pharmaceutical crystallization. In the latter work, experimental design and data collection were used for parameter estimation and model selection on expressions

for the supersaturation and the nucleation rate. Then, with the constructed model, batch operating procedures were designed to minimize nucleation and maximize the sharpness of the crystal size distribution.

For biopharmaceutical crystallization, especially for the case of large-molecule therapeutic proteins, constructing first-principles model may be time consuming due to the very slow nucleation and growth rates. When first-principles models and the crystallization kinetics are too expensive to constructed, the more efficient direct design approach can be taken (Fujiwara et al., 2005, and citations therein). This approach uses feedback control to follow a setpoint supersaturation curve in the experimentally determined metastable zone, which is region between the solubility curve and the metastable limit. Concentration control (C-control), also referred to as *supersaturation control*, implements direct design approach by manipulating the temperature (for cooling crystallization) and/or solvent addition (for antisolvent crystallization) to meet the setpoint supersaturation calculated by the solution concentration measurement and previously measured saturation concentration. This direct design approach has been recently applied by Simone et al. (2015) on a biopharmaceutical product. Their work showed that C-control gave narrow and larger crystals compared to simple linear cooling, which is a classical crystallization technique.

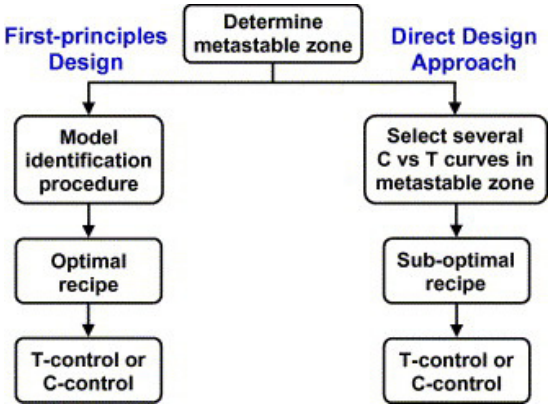

Figure 5. Flowchart for the first-principles and direct design approaches for crystallization control.

Conclusion

This article describes opportunities and challenges for the manufacturing of biopharmaceuticals from the perspective of chemical process control. One of the trends in the field is towards increased PAT, especially with the goal towards online sensor technologies. Characteristics of biopharmaceutical data such as heterogeneity in time scale and data type and tensorial dimensionality provide challenges for developing effective process data analytics methods. Existing proposed methods for heterogeneous data handling and dimensionality reduction were summarized. While some unit operations are well described by first-principles model, the lack of understanding required to construct first-principles models for some complex biological mechanisms motivates the construction of grey-box models, which combine process understanding and data analytics to improve the accuracy of predictions needed for process monitoring and control decisions. Various strategies in grey-box modeling for biomanufacturing processes were described.

Another major trend is the move in academia and industry towards continuous unit operations, which would greatly increase the application of more advanced process monitoring and control systems. Continuous manufacturing would be facilitated by (1) microscale technologies for high-speed continuous process development, (2) plug-and-play modular unit operations with integrated process control and monitoring systems, (3) dynamic models for unit operations and entire biopharmaceutical manufacturing plants to support process development and plant-wide control, and (4) industry-implementable model-based control technologies for optimizing startup, changeover, and shutdown. The article argues for more powerful control theories, which can explicitly address uncertainties, nonlinearities, time delays, nonminimum phase behavior, manipulated and output variable constraints, spatial distributions, and mixed continuous-discrete operations. Some successes have been achieved in the combination of multiple systems and control theories with robustness analyses.

Increases in demand for the production of biopharmaceuticals and in product titers in modern cultivations motivate the development of novel bioseparation methods to replace chromatography. Crystallization is a cost-efficient unit operation that is already extensively applied to chemically derived pharmaceuticals and to some biopharmaceuticals such as insulin. Some challenges and potential solutions for the application of crystallization to large-molecule therapeutics proteins such as mAbs were described. Furthermore, process control approaches applicable to biopharmaceutical crystallization were summarized that can be selected depending on the degree of the process understanding.

The field of biopharmaceutical manufacturing is in a state of flux right now as it further matures. Emerging technologies and market demands are driving companies to reconsider their manufacturing strategies and scales. As a result, there are new opportunities for chemical and process control engineers to make impactful contributions. Such contributions will only come from respecting the challenges associated with these biologically-derived complex processes, and working closely with experimental groups in academia or industry that are responsible for the unit operations, own the systems and control problems, and can implement proposed solutions.

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