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Model-Based Preparative Chromatography Process Development in the QbD Paradigm

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1.1 Motivation

Preparative chromatography for separation of proteins and peptides continues to be the primary workhorse in purification of biopharmaceuticals. Numerous papers and books exist describing theory and implementation of preparative chromatography; however, this is the first book that combines academic progress in modeling with industrial implementation. Although theory and models have been available for many years, industrial usage of these tools has been scarce due to labor- and material-intensive requirements. However, with the biotech industry moving to implement the expectations underlined in the recent regulatory initiative of quality by design (QbD), interesting and outspread applications of modeling tools for commercial process development and manufacture have emerged.

1.2 Regulatory Context of Preparative Chromatography and Process Understanding

QbD expectations to biopharmaceutical production including preparative chromatography are described in the ICH quality guidelines Q8, Q9, Q10, and Q11 [1–4]. Further, ICH Q8-R2 [1] provides the overall definition of QbD in a regulatory context.

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> A systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control based on sound science and quality risk management.

The focus of this book is on the underlined parts of this definition, and the framework of QbD may be outlined as presented in Figure 1.1. In the top part of the figure, the primary focus of biopharmaceuticals is the patient, and the patient needs are defined through the quality target product profile (QTPP), which in turn is affected by chemistry, manufacturing, and controls (CMC) activities. Fulfilling patients' needs places some requirements on the product, and these elements are obtained through linkage of the QTPP to the list of critical quality attributes (CQAs). The CQAs will have acceptable ranges for the manufacturer to comply with, and to obtain product of the desired quality, the process needs to be run within acceptable ranges of process parameters. Proper knowledge of how process parameters affect the product quality may be obtained through process models that may end up in a regulatory, enhanced application for approval of a design space. To control process parameters within defined ranges, process models and/or even a design space will provide some requirements to the GMP facility and linkage to the control strategy, which will include various process monitors, process analytical technology (PAT) tools, process validation, and release tests and specifications. All elements are linked through risk assessment exercises to address the risk-based approach of QbD in a regulatory setting.

Figure 1.1 (bottom) displays an example of QbD elements contained in the QbD framework for a preparative chromatography step. A key patient need is of course to get efficient treatment, and one element affecting this is to get a proper dose of the biopharmaceutical. To obtain proper dosing, the purity and among others the bioactivity of the biopharmaceutical needs to be correct. Purity is significantly affected by the peak collection criteria used in preparative chromatography, and a well-known methodology for peak collection is by UV monitoring as part of the control strategy (e.g., see Chapters 12 and 17). A proper understanding and control of the preparative chromatography process may be obtained by a mechanistic or statistical model and their boundary conditions that may define an operational design space. Thus, the idea of this linkage exercise is to obtain a complete overview of the process in a way that will elucidate, for example, how a defect in or removal of a UV monitor in a preparative chromatographic purification step will affect the patient through cascading back in the figure through a series of risk assessments. The focus of this book is to obtain "process understanding and process control based on sound science" as described earlier, and it can be visualized by observing the elements within the red circle in Figure 1.1 (top).

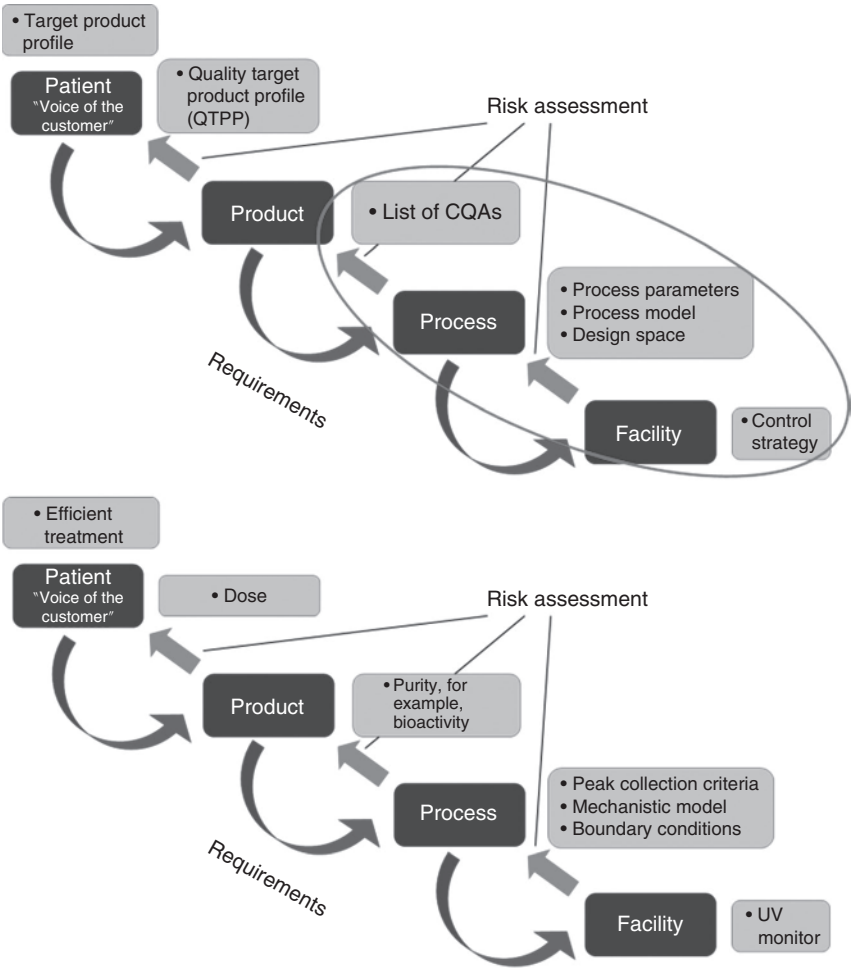

Figure 1.1 (Top) The framework of QbD. (Bottom) Example of QbD elements contained in the QbD framework for a preparative chromatography step. (*See insert for color representation of the figure.*)

A proper control strategy is achieved through sufficient process understanding. Traditionally, process understanding in the biopharmaceutical industry was obtained through a combination of theoretical knowledge based on the following: (i) education; (ii) experience from other projects and proteins optionally of similar nature, for example, mAbs; (iii) preliminary experimentation of less systematic nature; and (iv) "one parameter at a time" (OPAT) experimentation

where all variables are kept constant while systematically altering one variable. This concept has worked well for many years, and most legacy products have been developed using this approach. Figure 1.2 presents the general level of knowledge obtained by the different methodologies including more recent concepts. Although some companies have also used multivariate methods for development and documentation of legacy products, the extensive use of more advanced methods for process understanding has been affected by implementation of QbD concepts. The general methodology used in the industry today is based on multivariate statistical analysis such as design of experiments (DoE) often combined with various high-throughput process development (HTPD) techniques (see e.g., Chapter 11). DoE is a very broad and important tool that does not require mechanistic understanding prior to implementation, and it works quite efficiently if the user has prior knowledge of which parameters are significant and if the number of parameters is limited. Today, the most comprehensive application of statistical methods to support QbD and a true enhanced approach filing has been accomplished by Genentech/Roche with its recent regulatory approval of Gazyva. Disadvantages of DoE include less optimal identification of assumptions and the general lack of opportunities for extrapolation outside the experimental area used to set up the statistical models. DoE is used extensively for validation of parameter ranges in preparative chromatography; however for other unit operations

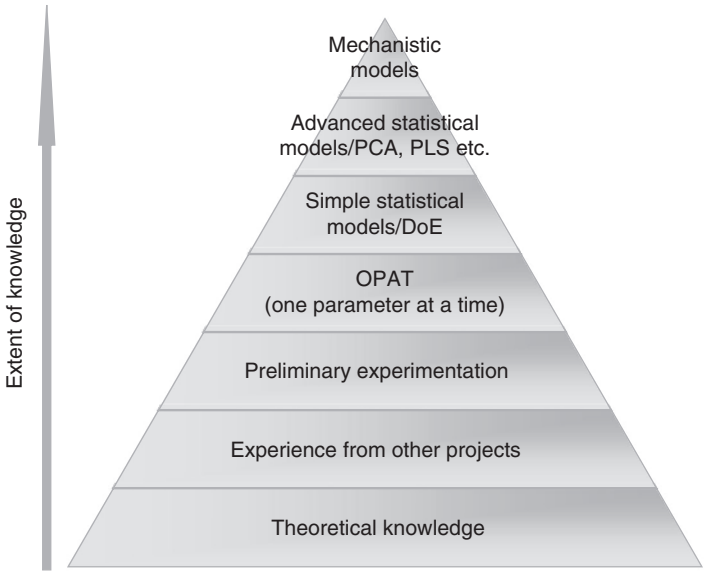

Figure 1.2 General extent of knowledge and process understanding obtained employing various methodologies and approaches.

such as fermentation, more advanced statistical methods like principal component analysis (PCA), partial least squares (PLS) methods, etc. are used due to their capability to handle very high number of variables (see also Chapter 16). At the top of the pyramid in Figure 1.2 and at the highest extent of knowledge obtainable are models based on mechanistic principles because full mechanistic process understanding is typically achieved. Depending on assumptions, these mechanistic models are also referred to as first-principle models, and they provide optimal evaluation of assumptions as well as opportunities for extrapolation outside the experimental area of parameter estimation.

An example of the difference in process understanding achieved from application of mechanistic modeling and a DoE approach for a preparative SEC step is presented in Figure 1.3 [5] (see also Chapter 14). The figure shows the effect of the feed concentration of a biopharmaceutical on the content of high molecular weight proteins (HMWP)—a typical CQA in the drug substance addressed by purification. The different experimental values for a given feed concentration (red diamonds) are due to controlled variation of other variables. Predictions based on a mechanistic model and on a statistical model by DoE are shown with full green and light blue colors, respectively. It is noticed that the model based on DoE cannot predict the worst-case conditions at a feed concentration of 0.75 g/L (indicated by the green, dashed circle) and instead the DoE-based model predicts the lowest concentration of 0.5 g/L as the worst-case conditions (indicated by the light blue, dashed circle). Further, the prediction error increases if extrapolation is performed outside the experimental area. The problem is partly caused by the general setup of

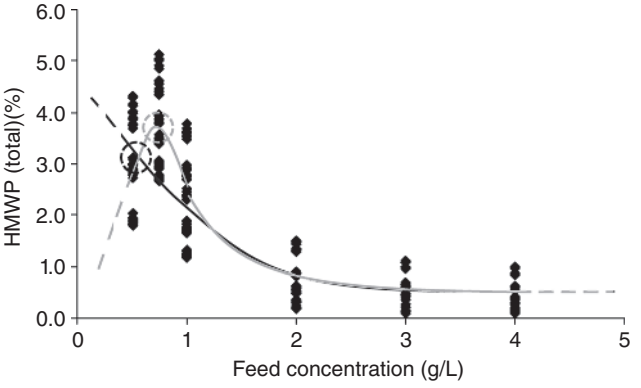

Figure 1.3 HMWP content after purification on SEC for a biopharmaceutical as a function of feed concentration. ◊, experimental results; —, model prediction by mechanistic model; and —, model prediction by statistical model based on DoE. (*See insert for color representation of the figure.*)

experiments supporting DoE where center points and parameter range limits are often applied (in the current case ~2 g/L and 0.5 and 4 g/L, respectively). DoE-based models are good in capturing monotonous functions, but they have problems capturing functions containing inflection points, and it would require a very comprehensive experimental setup for DoE-based models to capture functions with inflection points—far more than what is used in general in the industry. The experimental setup to obtain mechanistic models is typically not more comprehensive, but it is different. This example illustrates some of the pitfalls of applying DoE the way it is usually performed in the biopharmaceutical industry and how a mechanistic model may provide more process understanding.

1.3 Application of Mathematical Modeling to Preparative Chromatography

Mathematical models and modeling tools have been available for decades in academia, for example, Van Deemter [6], Giddings [7], Guiochon et al. [8], Melander and Horváth [9], Brooks and Cramer [10], Yamamoto et al. [11], Hearn et al. [12], Lenhoff [13], Carta and Jungbauer [14], Frech et al. [15], Łącki et al. [16], Hansen and Mollerup [17], Ottens et al. [18], Bracewell et al. [19] and many, many more, and the tools have been applied to academic problems such as separation of standard proteins like BSA, lysozyme, etc. and occasionally to more industry-relevant proteins. The experimental burden required and essential access to large amounts of pure experimental material made it very difficult and in fact too cumbersome for the biopharmaceutical industry to implement the methodology for many years. Motivation and requirements have, however, changed over the last years. The regulatory environment as described earlier [1–4] access to HTPD techniques [20, 21] facilitating fast experimentation and low demands of experimental material, and, in the specific case of polishing chromatography, proper assumptions and approaches to minimize the experimental task of generating preparative modeling parameters [22]. These aspects have aided the industry into initiating application of mechanistic modeling, and this book also presents numerous examples of such implementation for preparative chromatography.

Another aspect challenging the biopharmaceutical industry in implementation of mechanistic modeling tools is access to skilled personnel that can master modeling and computer coding at an expert level as well as to have comprehensive insight into preparative chromatography at manufacturing scales. Many implementation attempts in industry have failed due to lack of management support and critical mass of skilled personnel. In contrast, statistical modeling based on DoE or similar methods are much more easily implemented. An approach to initiation of implementation of mechanistic modeling is collaboration between

academics or specialized consultants and the biopharmaceutical industry, and numerous examples of such collaboration exist, for example, Borg et al. [23], Ghosh et al. [24], Rathore et al. [25], Nfor et al. [26], and many more. Another approach may be to look at trends in the small molecule pharmaceutical area, which are typically several years ahead of the biopharmaceutical industry in implementation of new tools and approaches.

New trends and hot topics in the industry include the utilization of semi- and continuous techniques (see also Chapters 5 and 15), PAT method implementation (see also Chapter 17), production of antibody–drug conjugates (ADCs) and other conjugates, and manufacturing of biosimilars, and many of these applications will benefit from the use of mechanistic modeling. As examples, the insulin purification method using MCSGP presented in Figure 1.4 was modified extensively from the original batch process by a mathematical model (L. Aumann et al., Chromacon AG, internal report to Novo Nordisk), and conjugate products that require reactions may benefit from reaction models as presented elsewhere [27]. Finally, the manufacture of biosimilars could significantly benefit from access to mechanistic modeling of preparative chromatography and other unit operations to demonstrate optimal process understanding, identification of critical process parameters, PAT-based process control, and demonstration of consistently achieving product profile that is similar to that of originator products.

Once a mechanistic model for a preparative chromatography step has been developed, the applications of the model are numerous depending on the approach and assumptions made. Figure 1.5 lists some common applications of mechanistic and statistical modeling in industry. Topics presented in black text in the figure represent themes that are covered by the subsequent book chapters, and a more thorough guidance to the individual chapters is given in the preface.

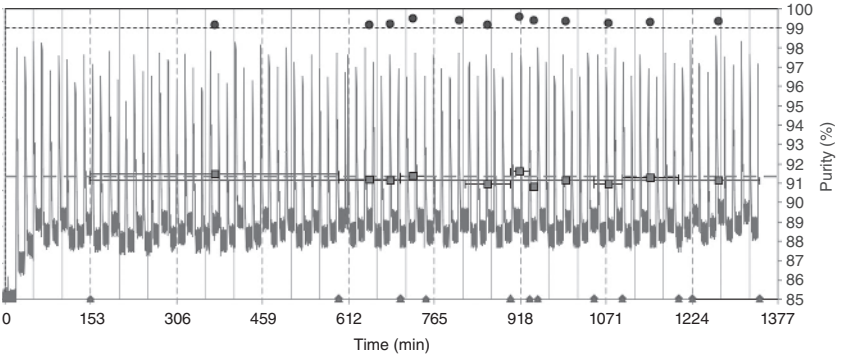

Figure 1.4 Chromatogram and purity of a three-column MCSGP unit as a function of time for a 23 h semi-continuous chromatographic purification of insulin (L. Aumann et al., Chromacon AG, internal report to Novo Nordisk).

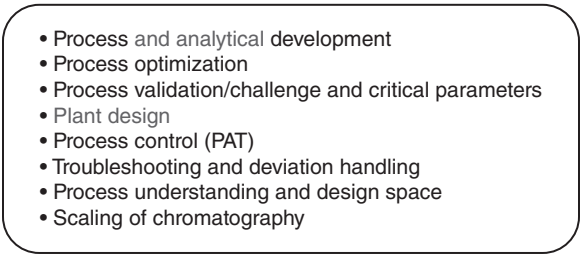

Figure 1.5 Application options of mechanistic modeling.

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