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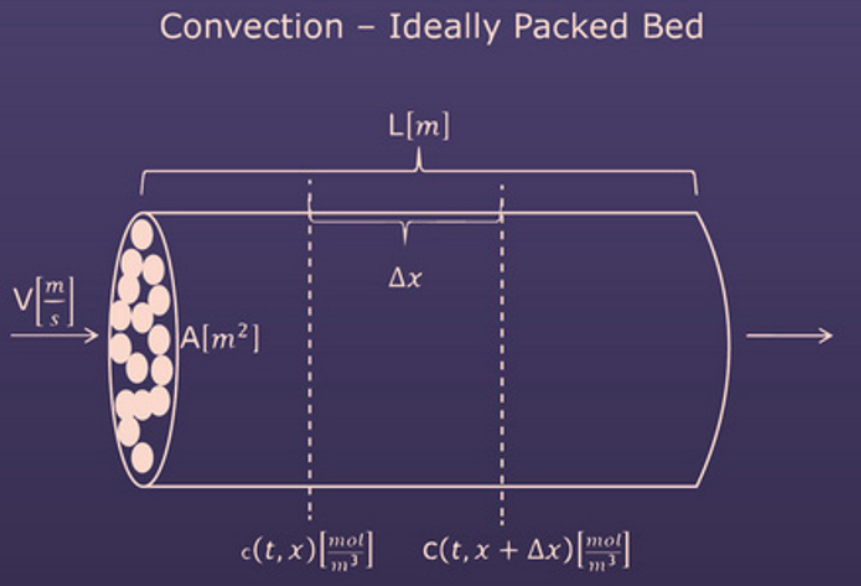
Preparative Chromatography for Separation of Proteins

EDITED BY
Arne Staby • Anurag S. Rathore • Satinder Ahuja



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Preparative Chromatography for Separation of Proteins

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Series Preface

The upcoming volumes will attest to the importance and quality of books in this series. I would like to acknowledge the fellow coeditors and authors of these books for their agreement to participate in this endeavor. Lastly, I would like to thank Ms. Anita Lekhwani, Senior Acquisitions Editor at John Wiley and Sons, Inc., for approaching me to develop such a series. Together, we are confident that these books will be useful additions to the literature that will not only serve the biotechnology community with sound scientific knowledge but also inspire as they further chart the course of this exciting field.

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Preface

This book covers various aspects of preparative chromatography, with a unique combination of academic research and industrial applications. We expect it to appeal to those in academia and industry who are involved in process development and the production of peptides and proteins, an area where the industry is typically reluctant to publicly share their knowledge because of trade secret considerations. Most of these major developments have either not been disclosed at all or exist only as oral conference contributions. This book aims to alleviate some of these gaps as we aim to supplement the academic contributions with industrial contributions. This aspect makes the treatment quite novel and unique when compared with other texts on the topic.

The book is divided into two parts: basic modeling and reviews and industrial separations/case studies. The basic modeling section aims to describe the recent developments in chromatographic theory and general approaches to research to provide increased understanding of the fundamentals behind chromatographic separation and behavior of proteins in these environments. The aim of this section is to provide a solid background in the theory of chromatography to the readers and to better prepare them for industrial case studies. Topics covered comprise the application of various approaches of modeling including computer simulations and mechanistic modeling. Chapter 1, by the editors, is designated to the general background for use of the various modeling tools and approaches.

The first section of the book contains fundamental contributions, general overviews, and reviews. Chapter 2, by Mollerup, provides a general and thorough overview of the thermodynamic tools and isotherm description necessary to model process chromatography in a double chapter. The author proposes approaches for acquiring accurate experimental data from which the model parameters in the adsorption isotherms can be estimated, in order to facilitate the use of simulation tools to the design and optimization of a chromatographic separation process.

Simulation of the performance of chromatographic separation of proteins is a powerful tool, and Chapter 3, by Nilsson and Andersson, presents a summary of the many methodologies applied to various chromatographic techniques

including ion exchange, affinity, and multimodal chromatography. Predictions of chromatographic behavior have been presented for a set of different separation problems, illustrating that a large number of common protein separation problems can be simulated quite easily with today's technology.

Chapter 4, by Yoshimoto and Yamamoto, describes simplified methods for understanding and designing chromatography processes for proteins and other biological products, with a focus on modeling of gradient elution chromatography. Simplified models based on the mechanistic model for linear gradient elution chromatography of proteins and other large molecule biological products are presented, together with several applications of the models to process design and process understanding and for bio-recognition.

Continuous processing, including chromatography, has gained much attention the last decade, and Chapter 5, by Riske and Ransohoff, presents industrial application of such multicolumn chromatography (MCC) systems for general capture. The authors suggest that the appropriateness and use of MCC in capture steps and in other parts of the downstream process depend on a number of factors, including the molecular characteristics and stability of the target molecule, the feed titer and product amount required, and the facility design and intention (multipurpose or dedicated). As industry gains more experience with MCC and other forms of continuous processing, the authors foresee that MCC is likely to be more commonly used throughout industry.

Molecular dynamics (MD) is another area that is getting much attention in recent years, and this approach will undoubtedly be key to better understanding of interactions on the molecular level and will ultimately result in better mechanistic models. This topic is described with case studies in Chapter 6, by Insaidoo, Banerjee, Roush, and Cramer. The authors summarize the current state of computational biophysics for determination of individual contributions of key interactions at an atomistic level. They conclude that there remains a significant gap in the linkage of experimental techniques (typically macroscopic) to biophysical modeling and that it is essential that these gaps be closed in order to realize the potential for rational process design.

Chapter 7, by Hansen, teaches the upscaling technique based on volumetric flow rate, which is founded in well-known chromatographic theory and equations, and the approach provides high process design flexibility. The chapter presents an overview of the underlying theory and also provides several examples of successful scale-ups on ion exchange and reversed-phase chromatography. A couple of industrial case studies related to these scale-ups are also presented. Finally, a step-by-step guide for scale-up is presented together with recommendations and a discussion of the challenges that a practitioner is likely to face.

The industrial separations section presents new and existing chromatographic unit operations and discusses how mechanistic and empirical modeling approaches are used to optimize equipment and methodologies. Equipment includes column

hardware, scale-down equipment, continuous operation mode, etc., as well as tools for monitoring and control; for example, on-, in-, and at-line equipment for improved process development and manufacturing methods. Improved methodologies comprise scaling approaches, the use of models for validation, uncertainty and robustness evaluations, and process design. A mix of industrial, equipment vendor, and academic authors contributed to this section. Chapter 8, by Antoniou, McCue, Natarajan, Thömmes, and Yuan, provides a number of examples where modeling may help in scale-up of chromatography in industry and how computational fluid dynamics (CFD) has been applied. The authors explore why column packing is such an important criterion that has to be consistent across scales, and they discuss how models can be utilized to predict column packing across scales and to perform packing consistently in an industrial environment.

Chapters 9, 10, and 11 (by Pirrung and Ottens; Diederich and Hubbuch; and Li, Pollard, and Tugcu, respectively) present industrial applications of process development, optimization, and small-scale practice. Chapter 9, among others, demonstrates the use of the high-throughput process development (HTPD) setup to generate mechanistic model parameters for process development, optimization, and design. The authors have discussed the pros and cons of the various experimental approaches, including the one-factor-at-a-time (OFAT), design of experiments (DOE), mechanistic modeling, and hybrid approaches. Chapter 10 provides guidance to process development using robot systems, including modeling/simulation of peak shapes for mechanistic modeling and validation. Factors that have been examined include the influence of pipetting precision, absorption measurements in microtiter plates, peak fractionation, flow patterns, and salt step heights in gradient elution experiments. Separate and combined effects have been qualitatively and quantitatively investigated using both experiments and simulations based on a mechanistic model. The authors demonstrate that with a sufficient number of fractions collected per peak, a significant improvement in precision can be obtained despite low analytical precision. Finally, Chapter 11, focuses on DOE and OFAT in an HTPD setup and presents the state-of-the-art experimental process development approach. A methodology for lab-scale chromatography process development utilizing high-throughput tools in conjunction with traditional column-based methodologies has been presented. The proposed experimental plan for process development relies heavily on a DOE approach supplemented with OFAT experiments. It fully utilizes HTPD and transitions into lab-scale column experiments where additional confirmation is required for defining parameter ranges and scale-up.

Chapters 12, 13, and 14 (by Breil, Frederiksen, Kidal, and Hansen; Hunt, Larsen, and Todd; and Sejergaard, Ahmadian, T.B. Hansen, Staby, and E.B. Hansen, respectively,) present three industrial case studies of mechanistic modeling for use in-process development, optimization, challenge, and

identification of critical process parameters, troubleshooting, deviation handling, control strategy setup, and establishing a design space for chromatographic purification. Also included are equation systems and computer coding that may help new applicants in setting up models. Chapter 13 presents an example where the general rate model has been used to describe transport behavior in the column and in the beads and the steric mass action binding model to describe protein binding to the resin matrix. This approach has been used successfully to describe the primary mechanisms involved in cation exchange chromatography of proteins. An open-source chromatography solver was used to estimate model parameters and evaluate the impact of operating parameters on process performance. Model parameters were estimated by performing a set of specific model calibration experiments. Pulse injection experiments were used to estimate the general rate model transport parameters, while steric mass action binding parameters were estimated by backfitting the model to a set of fractionated gradient elution runs. Chapter 14 discusses a specific application involving the use of a size-exclusion chromatography step for reducing aggregated product forms for the commercial production of turoctocog alfa. It has been illustrated how the different quality by design (QbD) elements of risk assessment and process knowledge can be linked through identification of key critical quality attributes (CQAs), which may be affected by the step and the different process parameters responsible for such influence on the CQAs.

Continuous processing including chromatography has gained much attention in the last decade, and Chapter 15, by Bisschops and Brower, presents industrial applications of such MCC systems for dynamic simulations as predictive models for MMC separation. This chapter describes a numerical simulation approach for predicting the performance of continuous chromatographic separations of biopharmaceutical proteins. The numerical simulations are based on the linear driving force model for mass transfer kinetics and a Langmuir isotherm for equilibrium behavior. The numerical simulations have been compared with the experimental capture efficiency of monoclonal antibodies on Protein A media in a continuous MCC system for two different monoclonal antibodies and two different (agarose based) Protein A media. The authors demonstrate the possibility of using simulation models for process characterization, thereby enabling the knowledge space with limited experimentation significantly speeding up the development program.

Chapter 16, by Rathore and Singh, presents the general state of the art of multivariate data analysis and review of current process analytical technology (PAT) methods available to facilitate process chromatography. This chapter presents a review of chemometrics applications in process chromatography. The various data preprocessing methods and modeling approaches have been discussed along with two case studies illustrating the utility of chemometrics in analyzing process chromatographic data.

Process control and PAT are topics of great interest in the industry, and new tools that may move the analytical release test burden to the unit operation process control are highly desirable. A recent tool exploiting UV spectra for this application is shown in Chapter 17, by Hansen, Brestrich, Staby, and Hubbuch. The proposed tool has a response time of <1 s and allows real-time pooling decisions. Both the screening and the PAT tool have been based on partial least squares (PLS) regression models, correlating mid-UV protein absorption spectra with selective protein concentrations. The fundamentals of intrinsic protein absorption and PLS as well as their application for selective protein quantification have been also addressed.

Finally, Chapter 18, by Hearn, presents the more sustainable and green approach to chromatographic separation and to many practical considerations needed in future manufacturing. This chapter examines recent progress toward the incorporation of the concepts underlying sustainable manufacturing of protein-based products, with emphasis of the downstream aspects of the recovery and purification of value-added protein products derived from biotechnological procedures. Lessons gained from the use of similar approaches developed within the chemical, traditional pharmaceutical, and food ingredient industries have been examined in terms of their applicability to the downstream processing of protein products derived from genetic engineering, cell culture, and associated biotechnology strategies.

The book may be read for individual contributions; however, all of the book chapters complement each other with state-of-the-art implementation of modeling in the biopharmaceutical industry and academic research within the field. All chapters of the book have been peer reviewed. We would like to thank all authors for their valuable contributions and hope the academic, industrial, and regulatory scientists will benefit from this book.

Arne Staby
20 December 2016 *Anurag S. Rathore*
Satinder Ahuja

1

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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Edited by Arne Staby, Anurag S. Rathore, and Satinder Ahuja.

> 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).

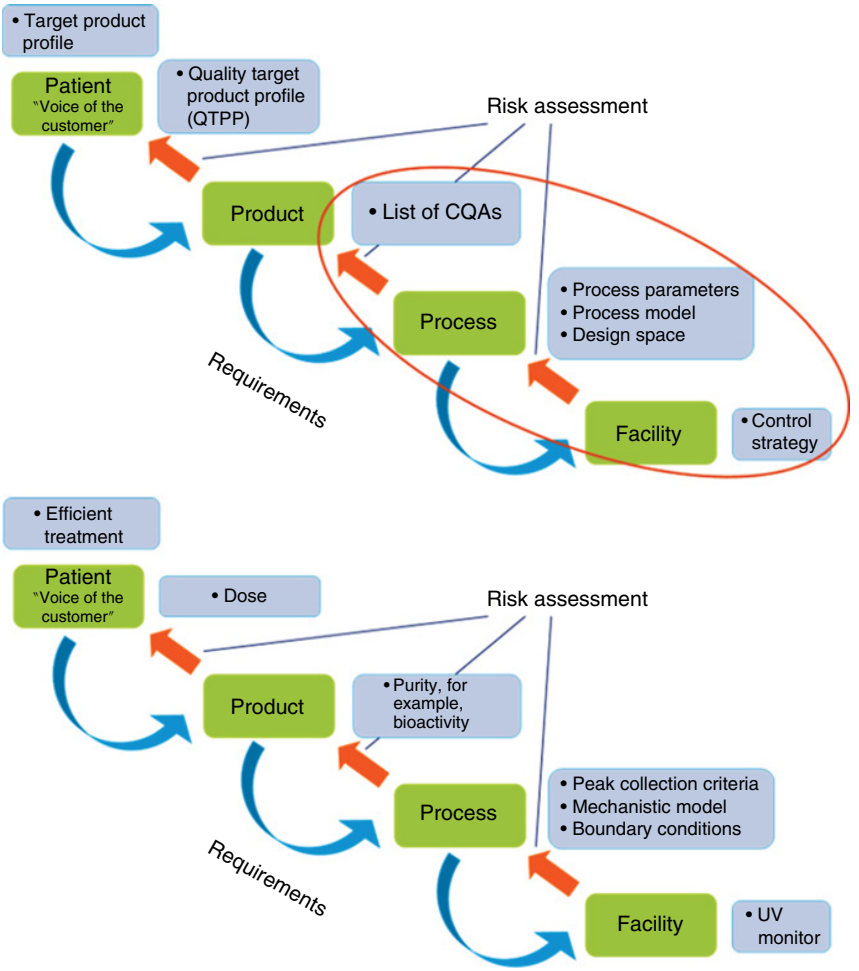

Figure 1.1 (Top) The framework of QbD. (Bottom) Example of QbD elements contained in the QbD framework for a preparative chromatography step. (*See insert for color representation of the figure.*)

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

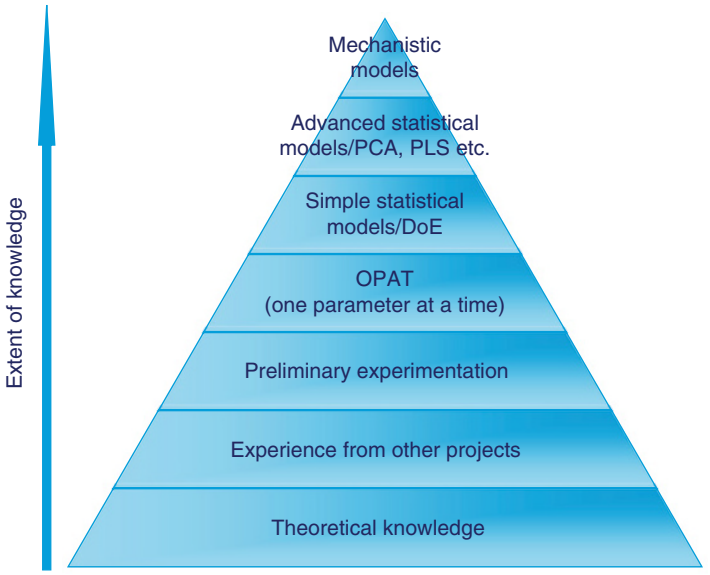

Figure 1.2 General extent of knowledge and process understanding obtained employing various methodologies and approaches.

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

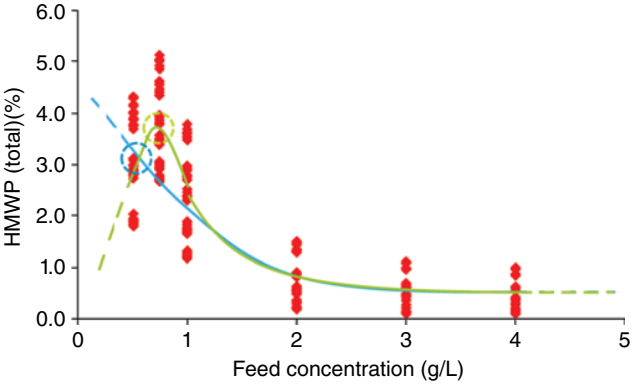

Figure 1.3 HMWP content after purification on SEC for a biopharmaceutical as a function of feed concentration. ◊, experimental results; —, model prediction by mechanistic model; and —, model prediction by statistical model based on DoE. (*See insert for color representation of the figure.*)

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