

Preface

Chromatography has become an essential unit operation in the production of biopharmaceuticals. This method facilitates the processing of the complex mixtures encountered in this industry using readily available stationary phases and equipment suitable for large-scale sanitary operation. Moreover, its practice as a process purification tool is recognized by regulatory agencies so that chromatography is an integral part of essentially all licensed biopharmaceutical processes. An in-depth understanding of the process is desirable and is increasingly being sought by regulatory agencies. As a result, chemists, engineers, and life scientists working in this field need to become familiar with the theory and practice of process chromatography.

While, in general, the theory of chromatography is well established for small molecule separations, the design and scale-up of chromatography units for biopharmaceutical purification remain largely empirical. Thus, optimum designs often remain elusive. On one hand, engineers, while possessing a strong foundation in transport phenomena and unit operations, often have a limited understanding of biomolecular properties. On the other, biochemists and biologists often have a limited understanding of the key scale-up relationships and models needed for optimum design. In an effort to address this dichotomy, in 2000 we started a new short course at BOKU in Vienna, Austria, with the principal aim of merging the theory and practice of biochromatography to achieve optimum design and scale-up of process units. Our goal was to help educate engineers who understand the biophysical properties of proteins and other bio-macromolecules and can implement this understanding in the bioprocess setting; and life scientists who understand transport phenomena and engineering models and who can apply these tools to the design of process units. Since 2000, the course, which has been open to both industrial and academic participants, has been held annually both in Vienna and at the University of Virginia, in Charlottesville, Virginia, USA. The course has both theoretical and practical, hands-on components. The participants learn the fundamentals of protein production, their structural and biophysical properties, and the varied nature of their contaminants. In the lectures, they learn the theory of chromatography, the properties of stationary phases, how to describe the equilibrium and kinetic factors that govern process performance, and how to achieve a proper balance of separation efficiency and productivity. In the labora-

tory, they learn to pack columns which are useful as scale-down models, plan experiments to identify critical parameters, and use advanced chromatography workstations to measure the critical physiochemical properties needed to model retention and band broadening in different types of chromatographic operations. Ultimately, the participants complete a design exercise, in which they are asked to design an optimized column on the basis of the laboratory measurements and theories learned during the course.

This book is based on the same framework. After teaching the course for more than ten times and after discussions with several hundred participants with very broad ranges of educational backgrounds and job functions, we now have a better understanding of the main difficulties that are encountered in understanding protein chromatography from both theoretical and practical viewpoints. Therefore, following the spirit of the course, we begin with a chapter on the biochemical and biophysical properties of proteins and their contaminants. We focus on the properties that are relevant for chromatography such as size, surface charge and hydrophobicity, solution viscosity, and diffusivity and on how to preserve biological activity. In Chapter 2, we provide a succinct, general introduction to chromatography identifying the key factors that are important for design and scale-up. This allows the reader who is not familiar with chromatography to put the various issues discussed in Chapters 3 to 10 into proper context. Chapter 3 addresses the chemistry and structure of many different stationary phases while Chapter 4 discusses laboratory and process columns and equipment. Both of these chapters are limited in scope to familiarizing the reader with examples of commercially available materials and equipment. No attempt has been made to provide comprehensive coverage, in large part because the field is rapidly expanding and new media and equipment are constantly being introduced. The mechanical design of equipment has also been omitted, since separation scientists and engineers in the biopharmaceutical production setting are rarely required to undertake this task. Chapters 5 to 9 are structured to acquaint the reader with theory and models to design and scale-up chromatography units. Emphasis has been placed on phenomenological models whose parameters can be determined using suitable experimental studies. Many specific numerical examples are provided to illustrate the application of these models to the analysis of laboratory data and to the prediction of column performance. A great deal of emphasis has been placed on describing transport in the stationary phase, since adsorption kinetics is often limiting in industrial applications of biochromatography. Thus, Chapter 6 provides a detailed coverage of mass transfer effects and their relationship to the structure of the stationary phase. Chapter 7 explores the dynamic behaviour of chromatography columns to establish a link between equilibrium properties, which are described in Chapter 5, and column behaviour. Chapter 8 discusses how equilibrium and rate factors combine to determine column performance and how to model band broadening for practical conditions. Chapter 9 focuses on gradient elution chromatography. We chose to devote a separate chapter to this mode of operation, since, in our experience, it is frequently less well understood despite its major importance in the practice of biochromatography. Finally, Chapter 10 is designed

in hopes of bringing everything together and providing guidance for the optimum design of process units. Although most of the emphasis is on conventional, batch chromatography processes, we conclude with an overview of continuous or semi-continuous multicolumn systems that are attracting increasing interest for biopharmaceutical applications. It should be noted that the main intent of this book is not to address *de novo* process development—rather, the main focus is on the optimal design and scale-up of columns for a process whose steps have already been defined. Nevertheless, understanding these concepts will also aid the scientist who is involved in early process development to identify process steps that are scalable and can be efficiently translated from the laboratory to the manufacturing suite.

We are convinced that proper application of theory combined with adequate experiments is instrumental to the successful application of biochromatography on a large scale. We would be happy, of course, if the book encouraged some of the readers to attend our course and learn about the practical, laboratory aspects that accompany the theory. The book also provides extensive references to original literature, textbooks, and books on chromatography, for those seeking greater detail. We have endeavored to make the notation consistent throughout the book and to check the correctness of the mathematical equations. Notwithstanding these efforts, we strongly suspect that there may still be some inconsistencies. We would be very grateful to readers who inform us of any such issues so that they can be remedied.

Finally, we would like to thank our students who, over the years, have helped us to develop and teach the laboratory and discussion sessions used in our short courses, which could not have been held without their input and enthusiastic support. We would particularly like to thank our students Timothy Pabst, Emily Schirmer, Jamie Harrington, Melani Stone, Jeremy Siebenmann-Lucas, Theresa Bankston, Yinying Tao, Robert Deitcher, and Ernie Perez-Almodovar at the University of Virginia and Tina Paril, Kerstin Buhler, Rene Überbacher, Anne Tschelesnig, Alfred Zochling, and Christine Machold at BOKU and our colleague Rainer Hahn for their support. We also thank all the participants who have attended our courses and who have provided very valuable feedback and have shared with us much of their practical experience.

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