PROTEIN PURIFICATION PROCESS ENGINEERING

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edited by
Roger G. Harrison

University of Oklahoma
Norman, Oklahoma

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Series Introduction

Bioprocess technology encompasses all the basic and applied sciences as well as the engineering required to fully exploit living systems and bring their products to the marketplace. The technology that develops is eventually expressed in various methodologies and types of equipment and instruments built up along a bioprocess stream. Typically in commercial production, the stream begins at the bioreactor, which can be a classical fermentor, a cell culture perfusion system, or an enzyme bioreactor. Then comes separation of the product from the living systems and/or their components followed by an appropriate number of purification steps. The stream ends with bioproduct finishing, formulation, and packaging. A given bioprocess stream may have some tributaries or outlets and may be overlaid with a variety of monitoring devices and control systems. As with any stream, it will both shape and be shaped with time. Documenting the evolutionary shaping of bioprocess technology is the purpose of this series.

Now that several products from recombinant DNA and cell fusion techniques are on the market, the new era of bioprocess technology is well established and validated. Books of this series represent developments in various segments of bioprocessing that have paralleled progress in the life sciences. For obvious proprietary reasons, some developments in industry, although validated, may be published only later, if at all. Therefore, our continuing series will follow the growth of this field as it is available from both academia and industry.

W. Courtney McGregor

Foreword

In the 20 years since the invention of genetic engineering, manufacture of recombinant proteins has become a mature industry impinging on almost every important aspect of our daily lives. Products range from extremely expensive diagnostic and therapeutic proteins to low-cost substances such as chymosin used in the manufacture of cheese. These latter substances compete successfully with traditional products in the commercial marketplace, on the basis of both quality and price. The number of commercial recombinant proteins continues to grow and both naturally occurring and genetically engineered molecules are now produced on a routine basis.

In order to maintain this remarkable momentum it is essential to solidify the technological base upon which this new industry is built. Almost obscured by the glamor surrounding these products is the simultaneous development of separation techniques needed for their production. Some of these techniques, such as large-scale cell disruption, exist only in biotechnology, and others have evolved into highly effective and specialized forms unique to protein purification. Rapid development of these processing techniques has given us little chance to consolidate and organize our understanding of them.

Although much information concerning protein purification is available, it has not been easy for the engineer or chemist to get the kind of detailed and explicit descriptions needed to develop effective expertise in either manufacturing or process development. It is particularly unfortunate that these newly important unit operations are being largely ignored in our educa-

tional institutions at all levels. A primary reason for this is the lack of reliable, readable, and well-organized texts.

Downstream processing, or recovery and purification, is particularly important because it typically accounts for nearly three-fourths of manufacturing costs in this new industry and because reliable and effective purification can be of the utmost importance to the user. Moreover, the developers of commercial purification processes must operate quickly and under heavy regulatory constraints. It is increasingly recognized that there must be effective communication among those engaged in research, process development, and manufacturing, from early stages of basic research through commercial production. At present, this is seldom the case.

Protein Purification Process Engineering is directed toward meeting these unfulfilled needs. The book begins with a basic overview of the facilities needed to work in this new area and the ways in which process development should be organized and implemented. The remainder of the text is devoted to individual separation and analytical techniques that are important to protein processing but are covered inadequately in other existing texts.

Analytical techniques are given strong emphasis. These techniques are essential in these systems, where the complexity of the product itself and the process streams can make it extremely difficult to close material balances and to characterize purity. Yields in the multistage purification chains characteristic of protein manufacture are usually low, and it is important to know where and why the losses occur, as well as to characterize the nature of the impurities. Moreover, analysis and quality control are expensive, often about one-third of total manufacturing costs in both upstream and downstream processing.

The most commonly used processing steps, described in the remainder of the text, all present unresolved engineering problems, and their effective use requires a great deal of judgment and experience. This is true for the humble but important mechanical rupture of cell walls as well as for more sophisticated techniques such as bioaffinity chromatography. Membrane filtration is an excellent example, as the potential economy and simplicity of these processes is clouded by poor understanding of boundary-layer behavior, fouling, and irreversible degradation. Liquid extraction is a potentially attractive process that suffers from both lack of suitable processing equipment and inadequate experience with solvent systems. Experience in the more conventional process industry cannot be simply translated into the complex chemistry and small flow rates of modern biotechnology.

The authoritative discussion of precipitation is also welcome. Selective precipitation is among the most powerful and potentially cheapest of all separation techniques, and it is widely used in selected protein purification processes. Extension to other applications is desirable, and the chief barrier is the complexity of this process for large unstable molecules such as proteins. The warning in the introductory chapter to pay close attention to

biochemistry is highly appropriate, and success will require close cooperation between protein chemists and engineers.

The most difficult separation problems are almost always solved by some form of chromatography, and two chapters discuss these highly selective but complex and expensive processes. Chapter 7 is devoted primarily to the details of column design and operation, which tend to be neglected in similar monographs but are of great importance in day-to-day operations. This discussion provides both a useful qualitative introduction as well as an appreciation for orders of magnitude, and it should prove especially helpful for those new to protein processing technology. Chapter 8 deals with the highly selective and somewhat mysterious processes of biospecific adsorption. This chapter reinforces the introductory chapter's advice that system biochemistry must be understood as thoroughly as possible.

This ambitious and comprehensive text concludes with an eminently practical discussion of freeze drying, an important process but often neglected in discussions of downstream processing. Freeze drying is normally the last step in the manufacturing process, and the care with which it is carried out can have a great impact on product stability and quality.

The authors are all recognized leaders in their areas of expertise; all have had a great deal of experience. They have produced a coherent product with its own "personality," one which should fill an important niche. They have provided the book with detailed information and given it a practical emphasis. Moreover, the bibliographies are extensive, permitting readers to delve deeper into topics of their own interest. The text will supplement more theoretically oriented monographs and should prove to be highly useful.

E. N. Lightfoot
University of Wisconsin
Madison, Wisconsin

Preface

The biotechnology industry, which originated in the late 1970s, is now well into the commercialization stage. A significant segment of this new industry is dedicated to bringing to market purified proteins. The economics of the processes to produce these proteins tend to be dominated by purification—typically, 80–90% of the manufacturing cost is for downstream recovery and purification. Thus, it is essential that a very efficient protein purification process be developed in order for the overall process to be cost competitive.

Protein Purification Process Engineering focuses on providing guidance for the substantial effort required in developing protein purification processes for large scale, commercial operation. It is written primarily for those engaged in this or related efforts in the industry. Readers doing research on protein purification in both industry and academia will find this book very useful as they work to improve existing processes or develop new ones.

Chapters cover various aspects related to protein purification: process development, scale-up, mathematical descriptions of processes and phenomena, technology, and applications. These topics fall within the general field of process engineering. Most of the technologies currently used at the commercial scale are covered in this book. Some of the chapters, particularly those discussing precipitation and affinity chromatography, have greater emphasis on the basic science involved. This is primarily because these technologies require a deeper science base to understand and utilize them. The chapters are also of varying length because some fields of protein purification are newer and less developed than others. Included is a chapter

on protein analytical methods by industrial practitioners, as these methods are essential to protein purification and should be in place before process development work begins.

The contributors were carefully selected, based on their substantial experience and expertise in their subject areas. The chapters are new, original treatments of the authors' respective subjects, thus constituting a new resource for those readers in the field.

I am grateful for the stimulating environment in purification process engineering at the Upjohn Company and Phillips Petroleum Company that helped lead to the idea for this book. Encouragement to pursue this project from colleagues in the School of Chemical Engineering and Materials Science at the University of Oklahoma is appreciated.

Roger G. Harrison

Contributors

Vincent Anicetti Quality Control, Genentech, Inc., South San Francisco, California

Clark K. Colton Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, Massachusetts

Cady R. Engler Department of Agricultural Engineering, Texas A&M University, College Station, Texas

Larry A. Gatlin Pharmaceuticals Department, Glaxo Research Institute, Research Triangle Park, North Carolina

William S. Hancock Department of Analytical Chemistry, Genentech, Inc., South San Francisco, California

Nikos K. Harakas Central Research Laboratory, Monsanto Company, St. Louis, Missouri

Roger G. Harrison School of Chemical Engineering and Materials Science, University of Oklahoma, Norman, Oklahoma

Dennis J. Kubek Biochemical Process R&D, Merck Sharp & Dohme Research Laboratories, West Point, Pennsylvania

Steven L. Nail Department of Industrial and Physical Pharmacy, Purdue University, West Lafayette, Indiana

Fred Rothstein Bio-Separations Consultants, Long Beach, California

John M. Simpson Medical Research Division, Department of Biochemical Engineering, Lederle Laboratories, American Cyanamid Company, Pearl River, New York

Zhi-Guo Su Department of Chemical Engineering, Dalian University of Technology, Dalian, People's Republic of China

Contents

1

Organization and Strategy

Roger G. Harrison
University of Oklahoma, Norman, Oklahoma

I. INTRODUCTION

During the development of a process to produce a protein, the initial emphasis of the work naturally is on the biological process. This focus on the biological process is often prolonged, because the time to develop this process can be lengthy. As a consequence, there can be a delay in shifting attention to the purification of the protein. However, it is important to realize that key organizational steps should be taken before experimental work on the purification process even begins, and that once the protein purification process development starts, the strategy to use in this development is crucial to the success of the project.

Several factors must be considered that relate to the organization of the work: The facilities and equipment must be appropriate for the job to be performed. The impact and applicability of the Current Good Manufacturing Practices (CGMP) regulations of the Food and Drug Administration (FDA) must be evaluated. The desirability of using a project team approach should be considered.

The complexity of most protein purification processes gives added importance to strategy considerations in the development of these processes. Purification processes for proteins nearly always involve more than one step and frequently involve a multitude of steps. Therefore decisions must be made about which individual unit operations to use and the order in which to use them. This effort is called process synthesis. The economics of the process should be evaluated at various times in the synthesis of the process in order to insure that the process is economically viable.

In addition to the strategy for the overall process synthesis, the strategy to apply in developing each individual process step is important. Four of these strategy considerations stand out, based on the author's practical experience.

In this chapter, elaboration of these organization and strategy considerations is given.

II. FACILITIES AND EQUIPMENT CONSIDERATIONS

Even before the actual process development work on purification begins, the issue of whether present facilities are adequate for the task needs to be addressed. Two situations need to be considered: laboratory scale work and pilot plant scale work.

For process development work at the laboratory scale, a good starting point is a typical protein chemistry laboratory. This would include a spectrophotometer with a UV lamp; a refrigerated centrifuge with centrifuging ability, expressed as relative *g* force times capacity in liters, on the order of 10,000–15,000; a wide variety of sizes of chromatography columns (glass or plastic) with adjustable plungers; a fraction collector with a UV monitor; a peristaltic chromatography pump; and a homogenizer for disrupting cells (1). Analytical equipment should include a system for analytical gel electrophoresis. In some cases, it may be highly desirable to have an analytical high-performance liquid chromatograph (HPLC) on hand for analyzing samples soon after they are taken.

Numerous operations for the purification of proteins need to be done at near 0°C to minimize proteolytic degradation and bacterial growth. The two options that arise for the lab scale are doing these operations in a refrigerated room and doing them in a chromatography refrigerator. The author has used only the latter option for lab process development work and found this to perfectly satisfactory. Chromatography refrigerators with glass doors, electrical outlets, and access portholes can be obtained with up to at least 75 cu. ft. of capacity.

The pilot plant is in essence a large-scale laboratory. Because of its larger scale, pilot plant equipment often must be constructed differently from laboratory equipment. Some equipment such as columns can be made of glass as in the lab. Other equipment such as vessels must be constructed of stainless steel or a plastic that has good chemical resistance, such as polypropylene. It is advantageous for each vessel to have its own pH probe for local and/or remote reading of pH. This is commonly done with an Ingold-type pH probe, which is made of glass and is capable of being sterilized. For applications involving food or pharmaceuticals, vessels should be of the "sanitary" design, which means that there are no threads on product contact surfaces and that surfaces must be smooth (150 grit or better finish). The sanitary equipment design standards usually employed are the "3A