Polymer Applications for Biotechnology

MACROMOLECULAR SEPARATION AND IDENTIFICATION

David S. Soane, Editor

PRENTICE HALL POLYMER SCIENCE AND ENGINEERING SERIES

POLYMER APPLICATIONS FOR BIOTECHNOLOGY
Macromolecular Separation and Identification

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Editor

Prentice Hall, Englewood Cliffs, New Jersey 07632

Library of Congress Cataloging-in-Publication Data

Polymer applications for biotechnology: macromolecular separation and identification / David Soane, (editor).

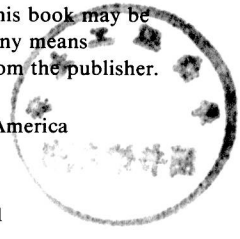
p. cm.
Includes bibliographical references and index.
ISBN 0-13-683251-2
I. Soane, David S.
TP248.25.S47P65 1992 91-20225
660'.6—dc20 CIP

Editorial/production supervision
and interior design: *Barbara Marttine/Jean Lapidus*
Cover designer: *Lundgren Graphics, LTD.*
Prepress buyer: *Mary Elizabeth McCartney*
Manufacturing buyer: *Susan Brunke*
Acquisitions editor: *Betty Sun*

A Simon & Schuster Company
Englewood Cliffs, New Jersey 07632

The publisher offers discounts on this book when ordered in bulk quantities. For more information, write:

Special Sales/Professional Marketing
Prentice-Hall, Inc.
Professional and Technical Reference Division
Englewood Cliffs, New Jersey 07632



Printed in the United States of America

10 9 8 7 6 5 4 3 2 1

ISBN 0-13-683251-2

PRENTICE-HALL INTERNATIONAL (UK) LIMITED, *London*
PRENTICE-HALL OF AUSTRALIA PTY. LIMITED, *Sydney*
PRENTICE-HALL CANADA INC., *Toronto*
PRENTICE-HALL HISPANOAMERICANA, S.A., *Mexico*
PRENTICE-HALL OF INDIA PRIVATE LIMITED, *New Delhi*
PRENTICE-HALL OF JAPAN, INC., *Tokyo*
SIMON & SCHUSTER ASIA PTE. LTD., *Singapore*
EDITORA PRENTICE-HALL DO BRASIL, LTDA., *Rio de Janeiro*

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To Nicholas

PREFACE

Synthetic polymers for biotechnology applications abound, chiefly in areas of biomacromolecular separation and analysis. Examples include membrane processes, chromatography (normal and reverse phase, affinity, and gel filtration), electrophoresis, gel swelling and collapse, and two-phase partition. Established materials (such as polyacrylamide and agarose for gel electrophoresis) and procedures (such as gradient versus isocratic elution for liquid chromatography) have been employed extensively in the biotechnology industry. However, the fundamental principles governing the optimal selection of process material and condition have not been clearly elucidated, partly because the practitioners are not necessarily experts in the science and technology of synthetic polymers. This situation presents a great research opportunity to the polymer community.

In this book, we offer an introductory chapter devoted to the fundamental physics and chemistry of synthetic polymers. It is intended to be a brief capsule, reviewing only the major topics for polymers. With this knowledge, users of polymers for bioseparation can better appreciate the major as well as the subtle issues in their material selection and process optimization. Throughout the book, each separation methodology is also presented with this desire in mind.

The reverse situation is also true. Traditional polymer chemists and engineers can definitely benefit from understanding the many practical problems daily confounding biologists, biochemists, molecular geneticists, microbiologists, pharmacokineticists, genetic engineers, biochemical engineers, and even physicians and clinicians. Many of these problems are related to the separation and identification of biomacromolecules. Only after

full comprehension of the users' perspectives can polymer designers effectively develop new and improved materials for bioprocessing. Hence, this book will serve both groups of readers: those using polymers as tools and those refining the tools for the advancement of biotechnology. In this spirit, what we are trying to achieve here is similar to that stated for a recent book by Zoya Martynenko and me, *Polymers in Microelectronics; Fundamentals and Applications* (Elsevier 1989), where the state of the art polymer technology for integrated circuit applications and the remaining critical issues are both addressed, bridging the gap of users and researchers.

A profound distinction between the two books nevertheless exists. Whereas *Polymers in Microelectronics* was painstakingly written by Zoya Martynenko and me, *Polymer Applications for Biotechnology* could not be accomplished without all the contributing authors. I have the good fortune of having professional and sometimes personal association with most of these individuals. Collectively, they impart to this book a sound balance of industrial and academic viewpoints. I am grateful to them: David Gray (Cetus), Bob King (Molecular Devices), Don Rose (Hewlett-Packard), Sue Behrens (Merck), and Young Bae, Harvey Blanch, John Dorgan, Paul Grossman, John Prausnitz, and Alex Sassi (University of California, Berkeley).

Finally, I gratefully acknowledge the skilled typing assistance of David Seligman and drafting services of Nancy Monroe, without whom this book would not have been possible.

David Soane

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David Gray is a biochemical engineer in the process development of Cetus Corp., Emerville, California, USA. He is currently involved in bacterial fermentation and product recovery process development and scale-up.

Mr. Gray obtained a B.Sc. in Biochemistry in 1978 at the University of Liverpool, U.K., his M.Sc. in Biochemical Engineering in 1979 from University College, London. He previously worked at Biogen, both in the U.S. and in Switzerland and began his industrial career with Dista Products in Liverpool, U.K., a subsidiary of Eli Lilly in the U.K.

David S. Soane has been a professor in the Department of Chemical Engineering at the University of California at Berkeley for the past twelve years where he also earned his Ph.D. (1978). He received his B.S. degree in chemistry at the National Taiwan University (1973). Actively involved in research dealing with polymers, he includes among his special interests the processing and properties of advanced composites and structural materials, polymer applications in microelectronics and optoelectronics, microcapillary and thin-film gel electrophoresis, and membrane science and technology.

John Robert Dorgan received his B.Sc. degree in Chemical Engineering from the University of Massachusetts at Amherst in 1986. In the same year, he entered the Chemical Engineering doctoral degree program at the University of California at Berkeley where his thesis research has been in the area of critical phenomena, with an emphasis on the morphological consequences of spinodal decomposition in polymer blends containing liquid crystalline

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Susan N. Behrens is a 1990 graduate of the University of California at Berkeley, where she completed her doctoral degree with the guidance of David Soane and Harvey Blanch. She received her B.S. Chemical Engineering in 1983 from University of Michigan. Dr. Behrens is currently employed as a Senior Staff Engineer at Merck and Co. in Elkton, VA. Her research interests include the applications of polymeric materials in biotechnological processes, with an emphasis on downstream processing.

Paul D. Grossman has just finished his Ph.D. degree in Chemical Engineering at the University of California at Berkeley under the direction of Professor David Soane. Dr. Grossman's interests are in the area of electrophoretic separations of biologically relevant macromolecules for applications in life science research. He received the B.Sc. degree in Chemical Engineering from the University of California at Berkeley (1983) and the M.S. degree in Chemical Engineering from the University of Virginia at Charlottesville (1987). Dr. Grossman is currently employed by Applied Biosystems, Inc.

Don Rose received his B.S. (Honors) in nutrition in 1982 and his Ph.D. in analytical chemistry in 1988, both from the University of North Carolina at Chapel Hill. Since then, he has been a member of the technical staff at Hewlett-Packard Laboratories (Palo Alto, CA) and is currently with Glaxo. His research interests include microscale separation techniques for bio-analytical chemistry, specifically focusing on capillary electrophoresis and capillary liquid chromatography. His most recent work involves the preparation, characterization, and application of polyacrylamide gel columns for capillary electrophoresis.

Robert Siang-shu King graduated with a Bachelor of Science degree in Chemical Engineering from the University of Washington, Seattle, WA in 1983. He obtained his Ph.D. under the supervision of Professors Harvey Blanch and John Prausnitz in the Department of Chemical Engineering at the University of California, Berkeley, CA in 1988. He was formerly an Applications Scientist at Molecular Devices Corporation, Menlo Park, CA, where his activities encompassed business development, biochemical research and development, and instrument engineering and marketing. He is currently employed by California Biotechnology.

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Harvey W. Blanch is Professor of Chemical Engineering at the University of California at Berkeley, where he has taught since 1978. He is also a senior faculty scientist with Lawrence Berkeley Laboratory and has taught previously at the University of Delaware (1974–78). His research interests include kinetics of hybridoma and mammalian cell growth, enzyme activity in non-aqueous environments (supercritical fluids, organic solvents and reverse micelles), enzymatic synthesis of novel polymers and bioseparations using aqueous two-phase systems and hydrogels. He is editor for Bioprocess Engineering and Applied Biocatalysis and serves on the editorial board of Biotechnology and Bioengineering. Dr. Blanch received the B.Sc. degree (First Class Honors) from the University of Sydney, Australia, (1968) and the Ph.D. degree in chemical engineering from the University of New South Wales (1971).

John M. Prausnitz is Professor of Chemical Engineering at the University of California at Berkeley, where he has taught since 1955. He is a senior scientist with Lawrence Berkeley Laboratory and has been elected to the National Academy of Engineers (1979), the National Academy of Sciences (1973) and the American Academy of Arts and Sciences (1987). He is a fellow of the AAAS (1982), AIChE (1985) and the Institute for Advanced Studies, Berlin (1985). His research centers on the physico-chemical interpretation and molecular thermodynamic correlation of experimental data to provide reliable estimates of thermodynamic properties for process design. Dr. Prausnitz's recent interest have been directed at the molecular thermodynamics of aqueous 2-phase systems, reverse micelles, polymer solutions, hydrogels, heavy fossil fuels, supercritical fluids and electrolyte solutions. He is editor of the Princeton Series in Physical Chemistry and Engineering. Dr. Prausnitz received the B.Ch.E. degree from Cornell University (1950), the M.S. degree from the University of Rochester (1951) and the Ph.D. degree in chemical engineering from Princeton University (1955). He has also received honorary doctor of engineering degrees from the University of L'Aquila in Italy (1983) and the Technical University of Berlin (1989).

Young Chan Bae received his B.S. degree in Polymer Engineering at the Hanyang University in Seoul, Korea in 1981. He obtained his Ph.D. degree in Chemical Engineering at Wayne State University in Detroit, Michigan in 1989. He has been a postdoctoral fellow in Chemical Engineering at the University of California, Berkeley, where his research interests focused on phase equilibria of polymer solutions and rheological properties of polymer solutions (or melts) using dynamic light scattering. Currently Dr. Bae is employed by Soane Technologies, Inc.

POLYMER APPLICATIONS FOR BIOTECHNOLOGY

CONTENTS

Chapter 1

OVERVIEW OF RECOMBINANT DNA PROTEIN BIOPROCESSING

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INTRODUCTION

During the 1980s, industrial biotechnology grew with the emergence of genetic engineering as a means of producing medically important proteins. By the end of the decade, several such proteins had been granted product license status by the FDA and are now being sold as medicines for the treatment of previously untreatable diseases (Table 1-1), or as vaccines, which have eliminated the dependence on human serum and improved the efficacy of antibody induction in recipients. In the next few years, many more products will gain approval and will be available to clinicians for the treatment of specific cancers, to combat septic shock, and as vaccines against microbial and viral diseases.

Prior to genetic engineering, valuable biologically active proteins were available only in limited amounts. Interferon-α (leukocyte interferon) was initially extracted from the buffy coat fraction of centrifuged whole blood (1) and only a few micrograms of active protein could be obtained from large volumes of blood. Human growth hormone (HGH), used to treat pituitary dwarfism, was previously extracted from cadaver pituitaries. This yielded only small quantities of HGH-containing pituitary product, with contaminants capable of producing unwanted side effects, such as temporary feminization of males undergoing treatment. Today such proteins are made through genetic-engineering-based processes and are now available in sufficient quantity and devoid of the clinical side effects of the tissue-derived products. The HGH produced by recombinant DNA (rDNA) methods is more homogeneous than the tissue-derived material, lacking aggregates and antigenic properties (2).

TABLE 1-1 Approved Biotechnology Drugs/Vaccines

Product Name	Company	Indication	Date of U.S. Approval
Humulin: human insulin	Eli Lilly	Diabetes	10/82
Protropin: human growth hormone	Genentech	Human growth hormone deficiency in children	10/85
Intron A: interferon-alpha-2b	Schering-Plough	Hairy cell leukemia Genital warts AIDS-related Kaposi's sarcoma	6/86 6/88
Orthoclone OKT3: monoclonal antibody CD3	Ortho pharmaceutical Corporation	Kidney transplant rejection	6/86
Recombivax HB: hepatitis B vaccine	Merck	Hepatitis B prevention	7/86
Roferon-A: interferon alpha-2a	Hoffan-LaRoche	Hairy cell leukemia AIDS-related Kaposi's sarcoma	6/86
Humatrope: human growth hormone	Eli Lilly	Human growth hormone deficiency in children	3/87
Activase: tissue plasminogen activator	Genentech	Acute myocardial infarction	11/87
HibTiter: haemophilus B conjugage vaccine	Praxis Biologics	Haemophilus influenza type B prevention	12/88
Epogen: erythropoietin	Amgen	Dialysis anemia	6/89
Engerix-B: hepatitis B vaccine	SmithKline Beecham	Hepatitis B prevention	9/89

Bioprocessing, the subject of this chapter, is a crucial part of recombinant DNA technology. Proteins produced or expressed in bacterial cells require extensive removal of host-produced contaminants before the end product is suitable for clinical administration. Bioactivity of the recombinant protein is essential: process techniques must be chosen so as to maintain the correct protein structure, yielding a product of high specific activity. Finally, process scale-up involves a number of stages and interactions (Figure 1-1). These interactions are important in order to ensure that products can be rapidly moved into clinical testing and, eventually, commercial manufacturing processes.

GENETIC ENGINEERING AND PROTEIN PRODUCTION

The ability to transfer genetic information (DNA) between prokaryotic

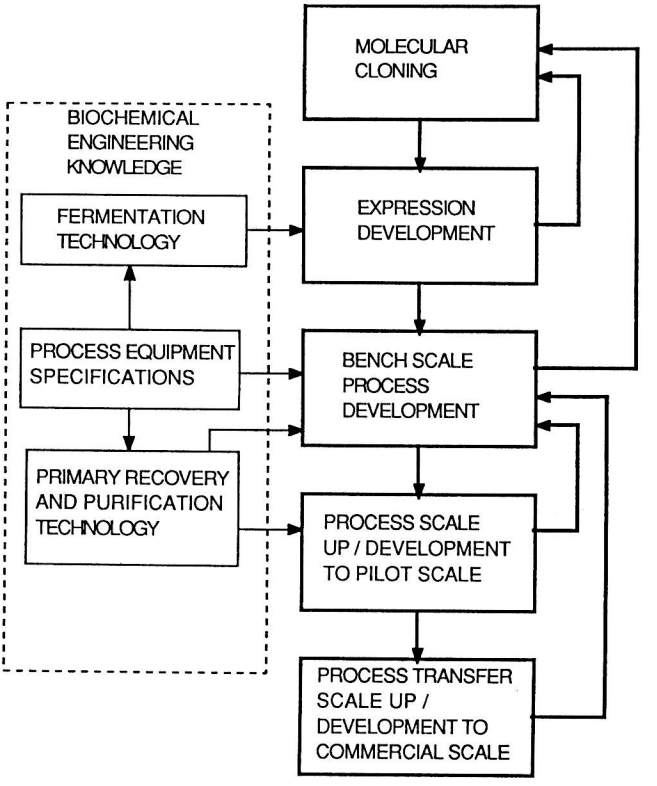

Figure 1-1 Typical flow of activities in process development.

organisms was demonstrated in the early 1970s (3). Expression of eukaryotic genes in prokaryotes, particularly in the gram-negative enterobacterium *Escherichia coli* (*E. coli*), followed. Plasmids, circular double-stranded DNA present in antibiotic resistant bacteria, were used as the vehicle for insertion of the foreign genes into the host cell. Soon useful clinical and industrial polypeptides were being expressed in *E. coli* (4).

Today proteins may be expressed in a variety of hosts, but the earliest system employed was *E. coli.* Recently, mammalian cells have been used for glycoprotein production. Glycosylated proteins are an important family of biologically active molecules in which the protein is responsible for biological activity and the oligosaccharide appears to have a role in targeting the site of action. Variation of the glycosyl composition may alter the biological properties of the glycoprotein, and so it is important to obtain consistency in its carbohydrate structure through control of bioreactor environmental parameters during cell culture (5, 6).