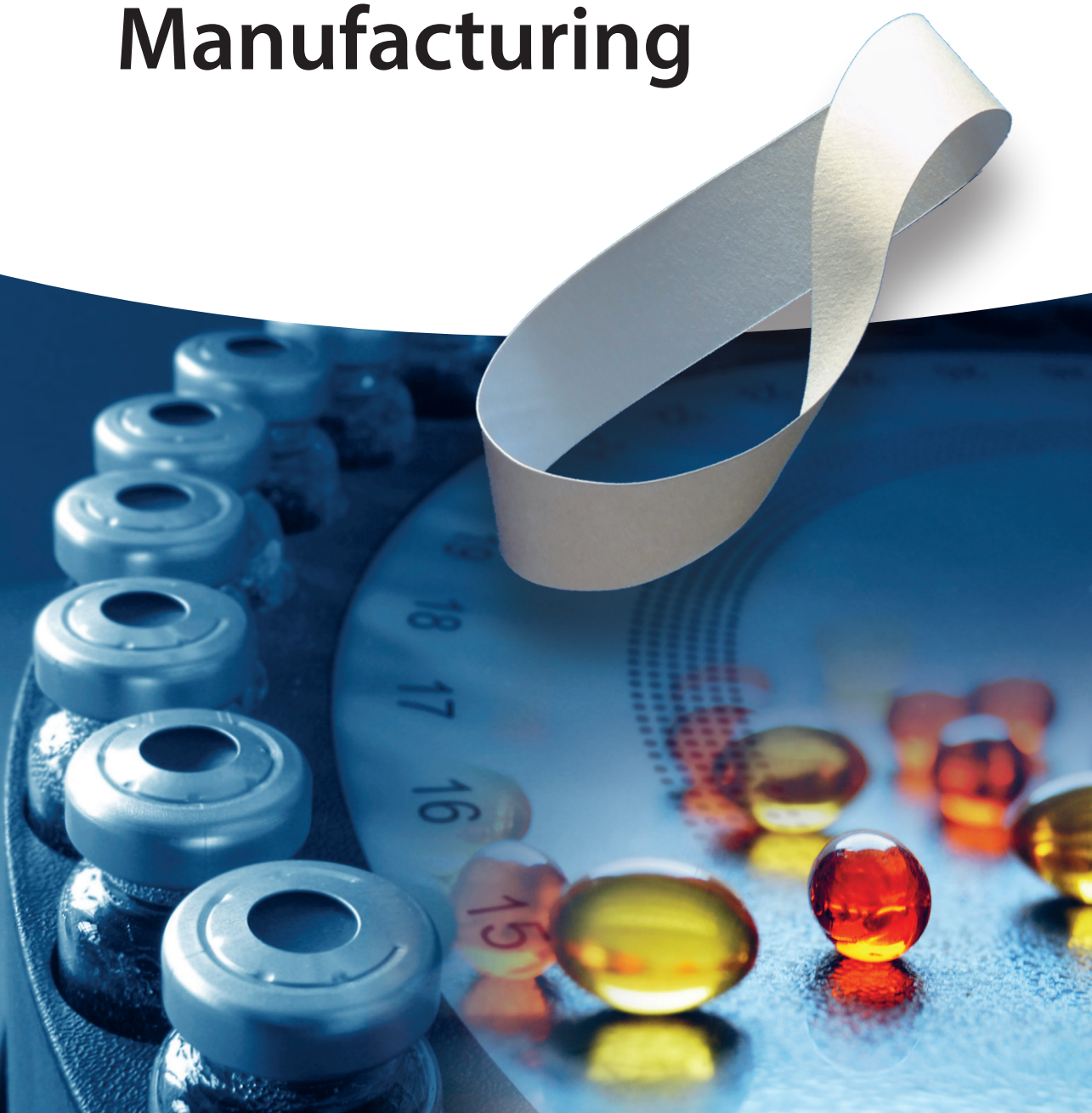
WILEY-VCH

Edited by
Ganapathy Subramanian

Continuous Processing in Pharmaceutical Manufacturing



Edited by
Ganapathy Subramanian

Continuous Processing in Pharmaceutical Manufacturing

Related Titles

Subramanian, G. (ed.)

Biopharmaceutical Production Technology

2012
Print ISBN: 978-3-527-33029-4 (Also available in a variety of electronic formats)

Flickinger, M.C. (ed.)

Downstream Industrial Biotechnology

Recovery and Purification

2013
Print ISBN: 978-1-118-13124-4 (Also available in a variety of electronic formats)

Eibl, R., Eibl, D. (eds.)

Single-Use Technology in Biopharmaceutical Manufacture

2011
Print ISBN: 978-0-470-43351-5 (Also available in a variety of electronic formats)

Flickinger, M.C. (ed.)

Encyclopedia of Industrial Biotechnology

Bioprocess, Bioseparation, and Cell Technology, 7V

2010
Print ISBN: 978-0-471-79930-6 (Also available in a variety of electronic formats)

Carta, G., Jungbauer, A.

Protein Chromatography

Process Development and Scale-Up

2010
Print ISBN: 978-3-527-31819-3 (Also available in a variety of electronic formats)

Edited by Ganapathy Subramanian

Continuous Processing in Pharmaceutical Manufacturing

WILEY-VCH
Verlag GmbH & Co. KGaA

The Editor

Dr. Ganapathy Subramanian
44 Oaken Grove
Maidenhead, Berkshire
SL6 6HH
United Kingdom

Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available on the Internet at http://dnb.d-nb.de.

Print ISBN: 978-3-527-33595-4
ePDF ISBN: 978-3-527-67371-1
ePub ISBN: 978-3-527-67370-4
Mobi ISBN: 978-3-527-67369-8
oBook ISBN: 978-3-527-67368-1

Cover Design Bluesea Design, McLeese Lake, Canada
Typesetting Thomson Digital, Noida, India
Printing and Binding Markono Print Media Pte Ltd

Printed on acid-free paper

Contents

List of Contributors

Marc Bisschops
Tarpon Biosystems, Inc.
Worcester, MA
USA

and

Tarpon Biosystems Europe B.V.
BioScience Park
Archimedesweg 17
2333 CM Leiden
The Netherlands

Mark Brower
Merck & Co., Inc.
Merck Research Labs
BioProcess Development
2000 Galloping Hill Road
Kenilworth, NJ 07033
USA

Leda R. Castilho
Federal University of Rio de Janeiro (UFRJ)
Cell Culture Engineering Laboratory
COPPE, Chemical Engineering Program
21941-972 Rio de Janeiro
Brazil

William Cataldo
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Véronique Chotteau
KTH (Royal Institute of Technology)
School of Biotechnology
Cell Technology Group
Roslagstullsbacken 21
106 91 Stockholm
Sweden

Marie-Francoise Clincke
KTH (Royal Institute of Technology)
School of Biotechnology
Cell Technology Group
Roslagstullsbacken 21
106 91 Stockholm
Sweden

and

UCB Pharma S.A.
Allée de la Recherche, 60
1070 Brussels
Belgium

Aloke Das
Supply chain Management
Senior Analyst at Dell Inc.
Ireland

Willem A. de Jongh
ExpreS2ion Biotechnologies
DTU Science Park
Agern Allé 1
2970 Horsholm
Denmark

Alison Dupont
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Suzanne S. Farid
University College London
Department of Biochemical Engineering
The Advanced Centre for Biochemical Engineering
Torrington Place
London WC1E 7JE
UK

Pedro Ferreira Gomes
University of Porto
Faculty of Engineering
Laboratory of Separation and Reaction Engineering (LSRE)
Associate Laboratory LSRE/LCM
Department of Chemical Engineering
Rua Dr. Roberto Frias, s/n
4200-465 Porto
Portugal

Christopher Gillespie
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Sanjeev K. Gupta
Ipca Laboratories Ltd.
Department of Biotechnology (R&D)
Plot #125, Kandivali Industrial Estate, Kandivali (W)
Mumbai 460007
Maharashtra
India

Sa V. Ho
Pfizer
Biotherapeutics Pharmaceutical Sciences
1 Burtt Road
Andover, MA 01810
USA

Ying Hou
Merck & Co., Inc.
Merck Research Labs
BioProcess Development
2000 Galloping Hill Road
Kenilworth, NJ 07033
USA

Jad Jaber
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Nika Janež
The Centre of Excellence for Biosensors, Instrumentation and Process Control – COBIK
Center for Biotechnology
Tovarniška 26
5270 Ajdovščina
Slovenia

Maik W. Jornitz
G-CON Manufacturing Inc.
6161 Imperial Loop
College Station, TX 77845
USA

Mark-Henry Kamga
University of Massachusetts Lowell
Department of Chemical Engineering
1 University Avenue
Lowell, MA 01854
USA

Namjoon Kim
University of Massachusetts Lowell
Department of Chemical Engineering
1 University Avenue
Lowell, MA 01854
USA

Mikhail Kozlov
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Hae Woo Lee
Clinical Manufacturing Center
Daegu-Gyeongbuk Medical Innovation Foundation
Cheombok-ro 80
Dong-gu, Daegu
South Korea

Ping Li
East China University of Science and Technology
State Key Laboratory of Chemical Engineering
College of Chemical Engineering
130 Meilong Road, Xuhui
Shanghai 200237
China

José M. Loureiro
University of Porto
Faculty of Engineering
Laboratory of Separation and Reaction Engineering (LSRE)
Associate Laboratory LSRE/LCM
Department of Chemical Engineering
Rua Dr. Roberto Frias, s/n
4200-465 Porto
Portugal

Sunil Mehta
kSep Systems
1101 Hamlin Road
Durham, NC 27704
USA

Massimo Morbidelli
ETH Zurich
Institute for Chemical and Bioengineering
Vladimir-Prelog-Weg 1
HCI F 129
8093 Zurich
Switzerland

and

Chairman Dept. of Chemistry &
Applied Biosciences
Institute for Chemical and
Bioengineering
ETH Zurich
Vladimir-Prelog-Weg 1/HCI F129
CH-8093 Zurich-Hoenggerberg

Thomas Müller-Späth
ETH Zurich
Institute for Chemical and
Bioengineering
Vladimir-Prelog-Weg 1
HCI F 137
8093 Zurich
Switzerland

Nico M.G. Oosterhuis
Easthouse Biotech Solutions BV
Landschrijverlaan 35
9451KT Rolde
The Netherlands

Sadettin S. Ozturk
MassBiologics of the University of
Massachusetts Medical School
Process and Analytical
Development
460 Walk Hill Street
Mattapan, MA 02126
USA

Matjaž Peterka
The Centre of Excellence for
Biosensors, Instrumentation and
Process Control – COBIK
Center for Biotechnology
Tovarniška 26
5270 Ajdovščina
Slovenia

Michael Phillips
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Aleš Podgornik
The Centre of Excellence for
Biosensors, Instrumentation and
Process Control – COBIK
Center for Biotechnology
Tovarniška 26
5270 Ajdovščina
Slovenia

and

Faculty of Chemistry and Chemical
Technology
Ljubljana University
Večna pot 113
1000 Ljubljana
Slovenia

David Pollard
Merck & Co., Inc.
Merck Research Labs
BioProcess Development
2000 Galloping Hill Road
Kenilworth, NJ 07033
USA

James Pollock
University College London
Department of Biochemical
Engineering
The Advanced Centre for
Biochemical Engineering
Torrington Place
London WC1E 7JE
UK

Ajish Potty
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Lars Poulsen
ExpreS2ion Biotechnologies
DTU Science Park
Agern Allé 1
2970 Horsholm
Denmark

Thomas C. Ransohoff
BioProcess Technology
Consultants, Inc.
12 Gill Street
Woburn, MA 01801-1728
USA

Alirio E. Rodrigues
University of Porto
Faculty of Engineering
Laboratory of Separation and
Reaction Engineering (LSRE)
Associate Laboratory LSRE/LCM
Department of Chemical
Engineering
Rua Dr. Roberto Frias, s/n
4200-465 Porto
Portugal

Romas Skudas
Merck Millipore
Frankfurter Str. 250
64293 Darmstadt
Germany

Franc Smrekar
Jafral d.o.o.
Koprska ulica 94
1000 Ljubljana
Slovenia

L. Richard Stock
BioProcess Technology
Consultants, Inc.
12 Gill Street
Woburn, MA 01801-1728
USA

Matthew Stone
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

William G. Whitford
GE Healthcare
HyClone Cell Culture
925 West 1800 South
Logan, UT 84321
USA

Alex Xenopoulos
EMD Millipore
80 Ashby Rd.
Bedford, MA 01730
USA

Seongkyu Yoon
University of Massachusetts Lowell
Department of Chemical
Engineering
1 University Avenue
Lowell, MA 01854
USA

Ye Zhang
KTH (Royal Institute of
Technology)
School of Biotechnology
Cell Technology Group
Roslagstullsbacken 21
106 91 Stockholm
Sweden

Preface

A continuous process requires the ability to think laterally and have a proactive mindset across the entire team from lab development through to production. Continuous manufacturing process is not new. It has been in use by the chemical, food, and beverage industries successfully. The biopharmaceutical industries are reluctant to engage in applying advanced technology on continuous processes, and are still using the batch process, which has been is use since the nineteenth century. The batch process is an archaic process that progresses sequentially step by step, creating a specified and fixed amount of therapeutic product, which in modern times is not state-of-the art. Several reviews and articles have shown that considerable advances have been made by technologist in offering systems for continuous processes. It has been established that continuous processing promises efficiency because it is a well controlled and flexible process, and there is less waste and produces higher quality products. There is considerable economic benefit in applying the continuous process in manufacturing.

Momentum is gathering pace behind the implementation of continuous manufacturing in the pharmaceutical industry. The regulatory bodies are now encouraging companies to move toward continuous manufacturing. Consequently, leading biopharma industries seem to be in the mend of thinking that the time is right for a major effort in the development of continuous processes in their organizations. As more companies look at the practical evidence from pilot and demonstration units, the adoption and commercialization of the new technology is picking up speed and currently several leading global biopharmaceutical industries are moving to implement continuous manufacturing processes in collaboration with technologist and suppliers. It will not be far away that industries will apply the continuous manufacturing process and thus we are setting up a Gold standard for the future, maybe in 10 years or more.

This book presents the most recent scientific and technological advances of continuous processing, as well as methods and applications in the field of biomanufacturing. Each chapter provides introductory material with an overview of the topic of interest; a description of the technology and methods, protocols, instrumentation, and application, and a collection of published data with an extensive list of references for further details.

It is our hope that this book will stimulate a greater appreciation of the usefulness, efficiency, and the potential of single-use systems in continuous processing of biopharmaceuticals, and that it will stimulate further progress and advances in the field of continuous processing to meet the ever-increasing demands and challenges in the manufacturing of therapeutic products.

The completion of this book has been made possible with the help and encouragements of many friends and colleagues. It is a great pleasure for me to acknowledge, with deep gratitude, the contribution of 19 authors of the chapters in this book. Their outstanding work and thoughtful advice throughout the project have been important in achieving the breadth and depth of this book.

I would be most grateful for any suggestions that could serve to improve future editions of this volume.

Finally, my deep appreciation to Dr Frank Weinreich of Wiley-VCH for inviting me to edit the volume and also to Lesley Fenske and her colleagues for their sustained encouragement and help.

Maidenhead, UK
June 2014

G. Subramanian

1
Proteins Separation and Purification by Expanded Bed Adsorption and Simulated Moving Bed Technology

Ping Li, Pedro Ferreira Gomes, José M. Loureiro, and Alirio E. Rodrigues

1.1
Introduction

Proteins not only play an important role in biology, but also have large potential applications in pharmaceuticals and therapeutics, food processing, textiles and leather goods, detergents, and paper manufacturing. With the development of molecular biology technologies, various kinds of proteins can be prepared from upstream processes and from biological raw materials. However, there exist various proteins and contaminants in these source feedstocks, and the key issue is that proteins can be separated and purified efficiently from the source materials, in order to reduce the production cost of the high-purity protein. The development of techniques and methods for proteins separation and purification has been an essential prerequisite for many of the advancements made in biotechnology.

Most separation and purification protocols require more than one step to achieve the desired level of protein purity. Usually, a three-step separation and purification strategy is presented, which includes capture, intermediate separation and purification, and final polishing during a downstream protein separation and purification process. In the capture step the objectives are to isolate, concentrate, and stabilize the target proteins. During the intermediate separation and purification step the objectives are to remove most of the bulk impurities, such as other proteins and nucleic acids, endotoxins, and viruses. In the polishing step most impurities have already been removed except for trace amounts or closely related substances. The objective is to achieve final purity of protein.

In the capture step, as the primary recovery of proteins, the expanded bed adsorption (EBA) technology has been widely applied to capture proteins directly from crude unclarified source materials, such as, *Escherichia coli* homogenate, yeast, fermentation, mammalian cell culture, milk, and animal tissue extracts [1,2]. The expanded bed is designed in a way that the suspended adsorbent particles capture target protein molecules, while cells, cell debris,

Continuous Processing in Pharmaceutical Manufacturing, First Edition. Edited by Ganapathy Subramanian.

particulate matter, and contaminants pass through the column unhindered. After loading and washing, the bound proteins can be eluted by elution buffer and be concentrated in a small amount of elution solution, apart from the bulk impurities and contaminants in source materials. With specially designed adsorbents and columns, the adsorption behavior in expanded beds is comparable to that in fixed beds. Various applications of EBA technology have been reported from laboratory-scale to pilot-plant and large-scale production [1–9].

During the intermediate purification and final polishing steps, the techniques of the conventional elution chromatography have been applied successfully. A new challenge should be the application of simulated moving bed (SMB) to the separation and purification of proteins. SMB chromatography is a continuous process, which for preparative purposes can replace the discontinuous regime of elution chromatography. Furthermore, the countercurrent contact between fluid and solid phases used in SMB chromatography maximizes the mass transfer driving force, leading to a significant reduction in mobile and stationary phase consumption when compared with elution chromatography [10–14]. Examples of products that are considered for SMB separation and purification are therapeutic proteins, antibodies, nucleosides, and plasmid DNA [15–23].

When the binding capacities of proteins on adsorbent are close to each other, an isocratic SMB mode may be used to separate and purify the proteins, where the adsorbents have the same affinity capacity to proteins in all sections in SMB chromatography. However, usually the binding capacities of proteins are so different that we cannot separate them by the isocratic mode with a reasonable retention time. In conventional elution chromatography, a gradient mode should be used for the separation of proteins. It is most commonly applied in reversed-phase and ion exchange chromatography (IEC), by changing the concentration of the organic solvent and salt in a stepwise gradient or with a linear gradient, respectively. For SMB chromatography, only a stepwise gradient can be formed by introducing a solvent mixture with a lower strength at the feed inlet port compared with the solvent mixture introduced at the desorbent port; then the adsorbents have a lower binding capacity to proteins in sections I and II to improve the desorption, and have a stronger binding capacity in sections III and IV to increase adsorption in SMB chromatography. Some authors state that the solvent consumption by gradient mode can be decreased significantly when compared with isocratic SMB chromatography [17–19,24–29]. Moreover, when a given feed is applied to gradient SMB chromatography, the protein obtained from the extract stream can be enriched if protein has a medium or high solubility in the solution with the stronger solvent strength, while the raffinate protein is not diluted at all [24].

In this chapter, we shall describe the developments made at the Laboratory of Separation and Reaction Engineering (LSRE) for proteins separation and purification by expanded bed chromatography and salt gradient ion exchange simulated moving bed technology.

1.2
Protein Capture by Expanded Bed Technology

1.2.1
Adsorbent Materials

The design of a special adsorbent is a key factor to enhance the efficiency of expanded bed adsorption. The EBA process will be more effective for those adsorbents that have both high-density base matrix and salt-tolerant ligand. The high-density matrix means minimizing dilution arising from biomass or viscosity in feedstock and reducing dilution buffer consumption; the lack of sensitivity of the ligand to ionic strength and salt concentration means there is no need for dilution of feedstock [30–32].

"Homemade" adsorbents are commonly used for research purposes. Agarose and cellulose are the major components utilized on the tailoring of the adsorbents. Table 1.1 shows a list of such adsorbents.

Table 1.1 "Homemade" adsorbents.

Year	Core	Adsorbent	Reference
1994	Crystalline quartz	6% Agarose	[33]
1994	Perfluorocarbon	Polyvinyl alcohol – perfluorodecalin	[34]
1995	Crystalline quartz - Red H-E7B	6% Agarose	[35]
1995	Perfluorocarbon	Polyvinyl alcohol – perfluoropolymer	[36]
1996	Crystalline quartz - Cibacron blue (3GA)	6% Agarose	[37]
1997	Fluoride-modified porous zirconium oxide		[38]
1999	Polyacrylamide gel	Silica	[39]
1999	Glass	Agarose	[40]
2000	Celbeads[a)]	Cellulose	[41]
2000	Stainless steel	Agarose	[30]
2001	Celbeads[a)]	Cellulose	[42]
2001	Nd–Fe–B alloy powder	Agarose	[43]
2002	Stainless steel	6% Agarose	[44]
2002	Stainless steel	6% Agarose	[45]
2002	Crystalline quartz	6% Agarose (Streamline DEAE) modified with a layer of polyacrylic acid (PAA)	[46]
2002	Nd–Fe–B with Cibacron Blue 3GA (CB)	4% Agarose	[47]
2002	Zirconia-silica (ZSA) ZSA - Cibracron Blue (CB)	4% Agarose 4% Agarose	[9]
2003	Zirconia-silica (ZSA)	Agarose	[48]
2003	CB-6AS	Cellulose	[49]
2003	Titanium oxide	Cellulose	[50]

(*continued*)

Table 1.1 (*Continued*)

Year	Core	Adsorbent	Reference
2004	Glass	4% Agarose	[51]
2005	Titanium oxide	Cellulose	[52]
2005	Stainless steel powder	Cellulose	[53]
2006	Stainless steel powder	Cellulose	[54]
2007	Nickel powder	Cellulose	[55]
2007	Tungsten carbide	Cellulose	[56]
2008	Tungsten carbide	Cellulose	[57]
2008	Stainless steel powder with benzylamine (mixed mode)	Cellulose	[58]
2008	Zirconia-silica	Agarose	[59]
2009	Zirconium dioxide	Polyglycidyl methacrylate β-cyclodextrin	[60]
2009	Tungsten carbide	β-Cyclodextrin polymer	[61]
2010	Tungsten carbide	β-Cyclodextrin polymer	[62]
2010	Tungsten carbide	Agarose	[63]
2011	Tungsten carbide	Cellulose	[64]
2012	Nickel (nanoporous)	Agarose	[31]
2012	Zinc (nanoporous)	Agarose	[32]
2013	Tungsten carbide	3% Agarose	[65]
2013	Titanium dioxide	Polyacrylamide-based Cryogel	[66]

a) Celbeads: Rigid spherical macroporous adsorbent beads with surface hydroxyl groups.

The drawback of agarose/cellulose-based adsorbents is their low density. Therefore, EBA adsorbents were developed by incorporating a dense solid material in the beads. Table 1.2 shows a list of commercial adsorbents.

Adsorbents used in EBA have been developed by some major companies as shown in Table 1.3. The name of the adsorbents are influenced by the ligand used, for example, diethylaminoethyl (DEAE), sulphopropyl (SP), quaternary amine (Q), recombinant protein A (r-Protein A), imino diacetic acid (Chelating), multimodal function (Direct CST I), carboxymethyl (CM), sulfopropyl (S) and polyethyleneimine (PEI) [3,30,71].

The trend is to use a dense solid core material to allow processing of higher flow rates and therefore reach a better productivity [30–32].

Streamline DEAE and Streamline SP (specially designed for an expanded bed), are classical ion exchangers, in which binding proteins are primarily based on interactions between charged amino acids on the protein surface and oppositely charged immobilized ligands. Protein retention on an ionic surface of adsorbent can be simply explained by the pI-value (isoelectric point) of a protein. But in practical applications, it is found that these ion exchangers have a lower binding capacity to proteins in high ionic strength and salt concentration feedstock. Streamline Direct CST I is a cation exchanger with multimodal functional groups, which not only takes advantage of electrostatic interaction, but also takes advantage of hydrogen bond interaction and hydrophobic interaction to tightly bind proteins. In other words, the new type of