1

Introduction

Henner Schmidt-Traub[1] *and Reinhard Ditz*[2]

[1] *TU Dortmund Fakultät für Bio- und Chemieingenieurwesen, Lehrstuhl für Anlagen- und Prozesstechnik, Emil-Figge-Str. 70, 44227 Dortmund, Germany*
[2] *Bristenstrasse 16, CH 8048 Zürich, Switzerland*

1.1 Chromatography, Development, and Future Trends

Ink dripping on a blotting paper thrills children when they realize the rainbow of colors spreading out. It is chromatography, an effect first coined by Tswett (1906) in 1903 for the isolation of chlorophyll constituents. Now, more than a hundred years later, children still enjoy chromatographic effects. Chromatography has developed into an important method for chemical analysis and production of high purity product in micro- and macroscale, and today pharmaceuticals are unthinkable without chromatography.

Liquid chromatography (LC) was first applied as a purification tool and has therefore been used as a preparative method. It is the only technique that enables to separate and identify both femtomoles of compounds out of complex matrices in life sciences and allows the purification and isolation of synthetic industrial products in the ton range. Figure 1.1 characterizes the development of chromatography and its future trends.

In the 1960s, analytical high-performance liquid chromatography (HPLC) emerged when stationary phases of high selectivity became available. At the same time, an essential technology push for preparative chromatography was created by the search of engineers for more effective purification technologies. The principle to enhance mass transfer by countercurrent flow in combination with high selectivity of HPLC significantly increased the performance of preparative chromatography in terms of productivity, eluent consumption, yield, and concentration. The first process of this kind was the simulated moving bed (SMB) chromatography for large-scale separation in the petrochemical area and in food processing (Broughton and Gerhold 1961).

These improvements were closely coupled to the development of adsorbents of high selectivity. In the 1980s, highly selective adsorbents were developed for the resolution of racemates into their enantiomers. The availability of enantioselective packing in bulk quantities enabled the production of pure enantiomers

Preparative Chromatography, Third Edition.
Edited by Henner Schmidt-Traub, Michael Schulte, and Andreas Seidel-Morgenstern.

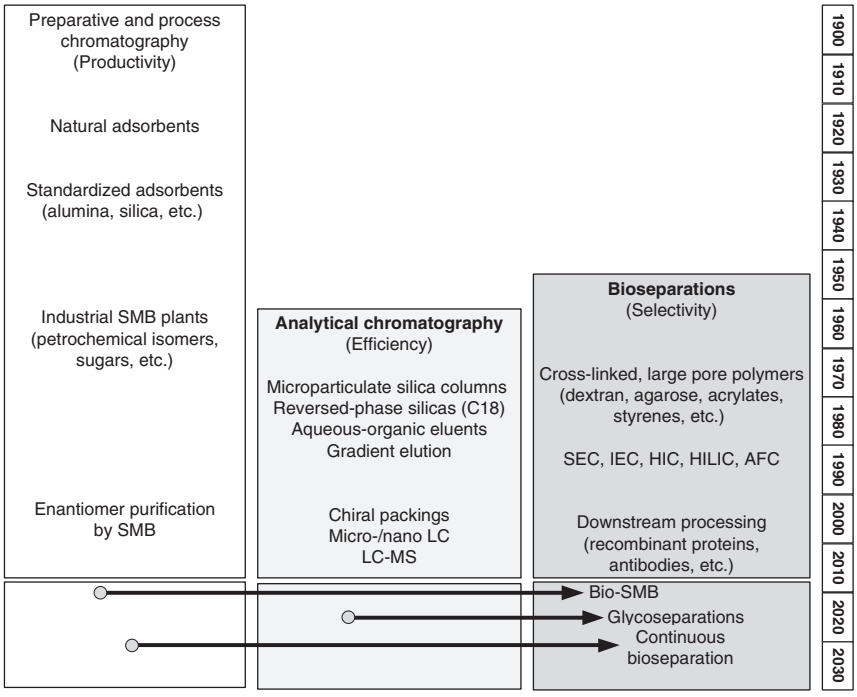

Figure 1.1 Development of chromatography. Source: Unger et al. (2010). Reproduced and modified with permission of John Wiley and Sons.

by the SMB technology in the multi-ton range. Productivities larger than 10 kg of pure product per kilogram of packing per day were achieved in the following years.

In the 1990s, the SMB process concept was adapted and downsized for the production of pharmaceuticals. The development of new processes was necessarily accompanied by theoretical modeling and process simulation, which are a prerequisite for better understanding of transport phenomena and process optimization.

While preparative as well as analytical LC were heavily relying on equipment and engineering and on the physical aspects of their tools for advancement in their fields, the bioseparation domain was built around a different key aspect, namely, selective materials that allowed the processing of biopolymers, for example, recombinant proteins under nondegrading conditions, thus maintaining bioactivity. Much less focus in this area was on process engineering aspects, leading to the interesting phenomenon, that large-scale production concepts for proteins were designed around the mechanical instability of soft gels (Janson and Jönsson 2010).

The separation of proteins and other biopolymers has some distinctly different features compared with the separation of low molecular weight (MW) molecules from synthetic routes or from natural sources. Biopolymers have an MW ranging from several thousand to several million. They are charged and characterized

by their isoelectric point. More importantly, they have a dynamic tertiary structure that can undergo conformational changes. These changes can influence or even destroy the bioactivity in the case of a protein denaturation. Biopolymers are separated in aqueous buffered eluents under conditions that maintain their bioactivity. Moreover, these large molecules exhibit approximately 100 times lower diffusion coefficients and consequently slower mass transfer than small molecules (Unger et al. 2010). Due to these conditions, processes for biochromatography differ substantially from the separation of low molecular weight molecules. For instance, process pressure, which is in many cases much lower for bioprocesses than for HPLC, requires a different plant design. Selectivity makes another difference; due to the very different retention times of biosolutes, an effective separation is only possible with solvent gradients.

Since the 1990s modeling and simulation tools for chromatographic separations of fine chemicals have developed considerably and are meanwhile well established, stimulating the efficiency of practical processes, while bioseparations were mainly based on empirical knowledge because of the complex nature of biomolecules. In the past regulation policies of FDA and other authorities focused on certified process schemes and process conditions as well as quality control by measuring the composition of intermediates and final products in order to guarantee drug safety. This resulted in overregulation and threatened to lead drug production into a dead end. Therefore, FDA started the process analysis technology (PAT) initiative in the 2000s and stated, "The goal of PAT is to enhance understanding and control of the manufacturing process, which is consistent with our current drug quality system: quality cannot be tested into products; it should be built-in or should be by design" (FDA 2004). PAT implies a paradigm change in pharmaceutical industries and created a momentum for better understanding of processes and products. Meanwhile it is extended by quality by design (QbD), which in summary aims at: "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and control, based on sound science and quality risk management" (Yu et al. 2014).

In the recent past countercurrent SMB processes have been highlights of chromatographic separations. With a focus on increased productivity and to make chromatographic separations more economical especially for bioproducts, the SMB principles are becoming a source to create new and more flexible processes with a reduced number of columns. Here the extreme case is a proposal for a SMB-like process with only one column (Zobel et al. 2014). Process control is another example for improvements. Robust processes are essential for high-quality products. Prerequisites for all these developments are stable and highly selective stationary phases and reliable equipment and on the other side rigorous models and increased computer power, which enables fast and reliable process simulations.

Peeking into the future reveals a technology trend toward the use of continuous process operations and downstream processing for fine chemicals and especially biopharmaceuticals. Costs and production capacities will have to be addressed, asking for better integrated and efficient approaches. Adapting countercurrent

solvent gradient concepts for the isolation of antibodies from complex fermentation broths will allow for more cost-effective downstream processing of biopharmaceuticals within the next couple of years.

Preparative and large production chromatography in their current major fields of application and scale have reached a level of maturity, which turns it from a breakthrough technology into a commodity. Major future opportunities will be in the field of continuous operation in the form of new SMB variants and especially in combination with other unit operations like extraction, crystallization, precipitation, etc. Such combinations will provide new and viable opportunities in fields like natural and renewable plant-based products, for example, in healthcare applications and other regulated industries. Work in a variety of applications and combinations is in progress with a focus on regulated products. This, however, is outside and beyond the scope of this book.

1.2 Focus of the Book

The general objective of preparative chromatography is to isolate and purify products in high quality. During this process, the products have to be recovered in the same condition that they were in before undergoing the separation. As preparative chromatographic processes have to produce the target with a desired purity and as economical as possible, they are usually operated under overloaded (nonlinear) conditions.

In contrast to this, analytical chromatography, which is not in the core of this book, focuses on the qualitative and quantitative determination of a compound. Thus, the sample can be processed, handled, and modified in any way suitable to generate the required information, including degradation, labeling, or changing the nature of the compounds under investigation. Such processes operate generally under diluted (linear) conditions.

The book provides and develops access to chromatographic purification concepts through the eyes of both engineers and chemists. This includes on one side the fundamentals of natural science and the design of materials and functionalities. On the other side mathematical modeling, simulation, and plant design, as well as joint efforts in characterizing materials, designing processes, and operating plants, are exemplified. Such a joint approach is necessary at the earliest possible moment as interaction and cooperation between chemists and engineers is important to achieve time and cost-effective solutions and to develop consistent methods that can be scaled up to a process environment.

In comparison with the second edition published in 2012, this book is completely restructured, revised, and updated.

1.3 Suggestions on How to Read this Book

For most readers it is probably not necessary to read all chapters of this book in sequence. For some readers the book may be a reference to answer specific

questions depending on actual tasks; for others it may be a guide to acquire new fields of work in research or industrial applications. The book may not provide answers to all questions. In such situations, the reader can obtain further information from the cited literature.

The different chapters are complementary to each other. It is recommended to be familiar first with basic definitions explained in Chapter 2. This chapter presents the basic principles of chromatography and defines the most important parameters such as retention factor, selectivity, and resolution. It also explains the main model parameters as well as different kinds of isotherm equations including the IAS theory, the determination of pressure drop, and the effect of mass transfer. Other passages are devoted to plate numbers, height of an equivalent theoretical plate (HETP) values as well as their determination based on first and second moments and column overloading. The experienced reader may pass quickly through this chapter to recall the definitions used. For beginners this chapter is recommended in order to learn the general terminology and acquire a basic understanding. A further goal of this chapter is the harmonization of general chromatographic terms between engineers and chemists.

Chapter 3 gives a survey of packings and stationary phases. It explains the structure and specifies the properties of stationary phases such as generic and designed phases, reversed phase silicas, cross-linked organic polymers, and chiral phases and gives instructions for their maintenance and regeneration. This chapter may be used as reference for special questions and will help those looking for an overview of attributes of different stationary phases.

Chapter 4 deals with the selection of chromatographic systems, that is, the optimal combination of stationary phases and eluent or mobile phases for a given separation task. Criteria for choosing normal phase (NP), reversed phase (RP), and chiral stationary phase (CSP) systems are explained and are completed by practical recommendations. Other topics discussed are the processing of monoclonal antibodies and size exclusion. Finally, practical aspects of the overall optimization of chromatographic systems are discussed.

The selection of chromatographic systems is the most critical for process productivity and thus process economy. This step offers the biggest potential for optimization, but, on the other hand, it is also a potential source of severe errors in developing separation processes.

Chapter 5 gives an overview of process concepts available for preparative chromatography. Depending on the operating mode, several features distinguish chromatographic process concepts: batchwise or continuous feed introduction, operation in single- or multicolumn mode, elution under isocratic or gradient conditions, recycling of process streams, withdrawal of two or a multitude of fractions, integration of reaction, and separation in one process step. It finishes with guidelines for the choice of a process concept.

In Chapter 6, modeling and determination of model parameters are key aspects. "Virtual experiments" by numerical simulations can considerably reduce the time and amount of sample needed for process analysis and optimization. To reach this aim, accurate models and precise model parameters for chromatographic columns are needed. Validated models can be used predictively for optimal plant design. Other possible fields of application for

process simulation include process understanding for research purposes as well as training of personnel. This includes the discussion of different models for the column and plant peripherals.

Chapter 7 is devoted to the consistent determination of the model parameters, especially those for equilibrium isotherms. Methods of different complexity and experimental effort are presented, which allow a variation of the desired accuracy on the one hand and the time needed on the other hand. The chapter ends with a selection of different examples showing that an appropriate model combined with consistent parameters can simulate experimental data within high accuracy.

Chapter 8 focuses on process design and optimization and starts with basic principles and continues with single-column processes. Design and scaling procedures for batch as well as recycle processes are described, and a step-by-step optimization procedure is exemplified. In the case of isocratic and gradient SMB processes, rigorous process simulations combined with shortcut calculations based on the true moving bed process (TMB) model are useful tools for process optimization, which is illustrated by different examples. Further sections discuss the improvements of SMB chromatography by variable operating conditions as given by Varicol, PowerFeed, or Modicon processes and gradient operation. Comments on multicolumn systems for bioseparation are completing this chapter.

Chapter 9 starts with an introduction to standard process control and presents scientific results on theoretical online optimization of batch chromatography and model-based advanced control of SMB processes, which are compared with experimental results. Further aspects are adaptive parameter estimation and cycle control as well as control of coupled SMB processes.

Chapter 10 focuses on practical aspects concerning equipment and operation of chromatographic plants for the production and purification of fine chemicals and small pharmaceutical molecules as well as proteins and comparable biomolecules. It starts with chromatographic columns followed by chromatography systems, that is, all equipment required for production. This includes high-performance as well as low-pressure batch systems as well as continuous SMB systems, supplemented by remarks on auxiliary equipment. Further topics are detailed procedures for different methods of column packing. The section on troubleshooting might be an interesting source for practitioners. Especially for the manufacturing of biotherapeutics, special disposable technologies such as prepacked columns and single-use membrane chromatography are exemplified.

References

Broughton, D.B. and Gerhold, C.G. (1961) Continous sorption process employing fixed bed of sorbent and moving inlets and outlets, *US Patent* No. 2.985.589

FDA (2004). Guidance for industry PAT-A framework for innovative pharmaceutical development, manufacturing and quality assessment, http://www.fda.gov (accessed 03 October 2019).

Janson, J.C. and Jönsson, J.-A. (2010). *Protein Purification* (ed. J.C. Janson). Weinheim: VCH.

Tswett, M.S. (1906). Physical chemical studies on chlorophyll adsorption. *Berichte der Deutschen botanischen Gesellschaft*, 24, 316–323 (as translated and excerpted in H.M. Leicester, Source Book in Chemistry 1900–1950). Cambridge, MA: Harvard, 1968.

Unger, K., Ditz, R., Machtejevas, E., and Skudas, R. (2010). Liquid chromatography – its development and key role in life sciences applications. *Angew. Chem.* 49: 2300–2312.

Yu, L.X., Amidon, G., Khan, M.A. et al. (2014). Understanding pharmaceutical quality by design. *AAPS J.* 16 (4): 771–778.

Zobel, S., Helling, C., Ditz, R., and Strube, J. (2014). Design and operation of continuous countercurrent chromatography in biotechnological production. *Ind. Eng. Chem. Res.* 53: 9169–9185.