1
Introduction

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1.1
Development of Chromatography

Adsorptive separations have been in use well before the twentieth century. Tswett (1905, 1906), however, was the first who coined the term "Chromatography" in 1903 for the isolation of chlorophyll constituents. Kuhn and Brockmann, in the course of their research recognized the need for more reproducible and also more selective adsorbents, specially tuned for specific separation problems. This recognized demand for reproducible stationary phases led to the development of first materials standardized for adsorption strength and describes the first attempt toward reproducible separations (Unger *et al.*, 2010).

Liquid Chromatography (LC) was first applied as a purification tool and has thereby 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 also allows the purification and isolation of synthetic industrial products in the ton range. The development of modern LC methodology and the corresponding technologies are based on three main pillars, which have developed over different time scales (Figure 1.1).

In the field of preparative and process chromatography the "restart" after the dormant period between the 1930s and the 1960s was not induced by the parallel emergence of analytical HPLC, but from engineering in search of more effective purification technologies. High selectivity of HPLC in combination with the principle to enhance mass transfer by counter current flow 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. The development of new processes was accompanied by theoretical modeling and process simulation which are a prerequisite for better understanding of transport phenomena and process optimization.

In the 1980s, highly selective adsorbents were developed for the resolution of racemates into enantiomers. These adsorbents were mainly employed in analytical

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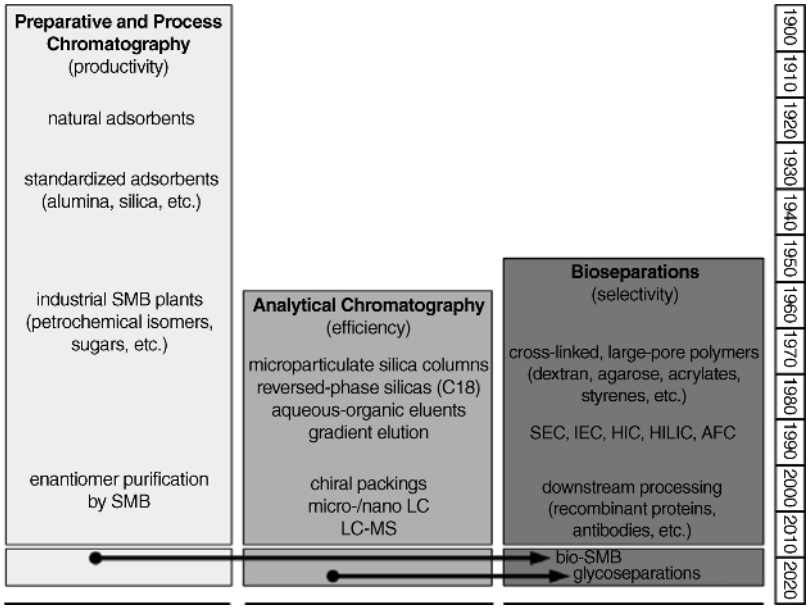

Figure 1.1 Development of chromatography (reproduced from Unger *et al.*, 2010).

HPLC (Allenmark, 1992). However, the availability of enantioselective packings in bulk quantities also enabled the production of pure enantiomers 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 concept was adapted and down-sized for the production of pharmaceuticals.

While preparative as well as analytical liquid chromatography were heavily relying on equipment and engineering and on the physical aspects of their tools for advancement in their fields, the domain Bioseparation 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 molecules from synthetic routes or from natural sources. Biopolymers have a molecular weight (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 bio-activity in 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 bio-chromatography differ substantially from the separation of low-weight molecules. For instance, process pressure which is in many cases much lower for bio-processes than for HPLC requires a different plant design. Selectivity makes another difference; due to the very different retention times of bio-solutes an effective separation is only possible with solvent gradients.

Taking a peek into the future reveals a technology trend toward the use of continuous process operations and also downstream processing. Costs and production capacities will have to be addressed, asking for more integrated and efficient approaches. Adapting counter current solvent gradient concepts for the isolation of antibodies from complex fermentation broths will probably allow for more cost effective downstream processing of biopharmaceuticals within the next couple of years. A similar path might be useful to consider for dealing with the "glyco"-issue. Knowing that glycosylation plays a significant role in therapeutic drug efficacy, the analytical approaches developed around mixed-mode separation methods might be transferred to the process scale within a short time.

Validation of methods and assays will become a key issue. This fits directly with the Process Analytical Technology (PAT) initiative launched already years ago by the Food and Drug Administration (FDA), calling for a better process understanding. Among other things, this requires a much deeper insight into the underlying interactions using model-based approaches, which should finally allow "predictable" process design and monitoring strategies in the future to enhance process robustness and safety.

1.2 Focus of the Book

The general objective of preparative chromatography is to isolate and purify products independent of the amount of material to be separated. During this process, the products have to be recovered in the exact condition that they were in before undergoing the separation. In contrast to this analytical chromatography, which is beyond the content of this book, focuses on the qualitative and quantitative determination of a compound, that is, the sample can be processed, handled, and modified in any way suitable to generate the required information, including degradation, labeling, or otherwise changing the nature of the compounds.

The book describes 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 matter and functionalities and on the other side mathematical modeling, simulation and plant design, as well as joined intersections in characterizing matter, process design, and plant operation. Such a joint view is necessary as the earliest possible interaction and cooperation between chemists and engineers is important to achieve time and cost-effective solutions and develop consistent methods that can be scaled up to a process environment.

With the second edition of this book the focus on fine chemicals and small pharmaceutical molecules is expanded to ion-exchange chromatography and the separation of biopolymers such as proteins. In accordance with the first edition these topics are restricted to those applications that can be modeled and simulated by current methods and procedures.

1.3 Recommendation to Read this Book

For most readers it is not necessary to read all chapters 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 different chapters are complementary to each other; therefore, it is recommended to be familiar with basic definitions explained in Chapter 2. The book may not provide answers to all questions. In which case, the reader can obtain further information from the cited literature.

Chapter 2 presents the basic principles of chromatography and defines the most important parameters such as retention, retention factor, selectivity, and resolution. It also explains the main model parameters as well as different kinds of isotherm equations including the IAS theory, and the determination of pressure drop. Other passages are devoted to plate numbers, HETP values as well as their determination based on first and second moments. The experienced reader may pass quickly through this chapter to become familiar with 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 focuses on stationary phases and the selection of chromatographic systems. The first part 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 part may be used as reference for special questions and will help those looking for an overview of attributes of different stationary phases. The second part 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 NP-, RP-, and 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. On one hand, the selection of the chromatographic system offers the biggest potential for optimization but, on the other hand, it is a potential source of severe errors in developing separation processes.

Chapter 4 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 bio-molecules. It starts with the market of chromatographic columns followed by chromatography systems, that is, all equipment required for production. This includes high performance as well as low-pressure batch systems and SFC plants as well as continuous SMB systems, supplemented by remarks on auxiliary equipment. Further topics are standard process control and detailed procedures for different methods of column packing. The section on trouble shooting might be an interesting source for practitioners. Especially for the manufacturing of bio-therapeutics special disposable technologies such as prepacked columns and single-use membrane chromatography are exemplified.

Chapter 5 gives an overview of process concepts available for preparative chromatography. Depending on the operating mode, several features distinguish chromatographic process concepts: batch-wise or continuous feed introduction, operation in single- or multicolumn mode, integration of reaction and separation in one process step, elution under isocratic or gradient conditions, recycling of process streams, withdrawal of two or a multitude of fractions, and SMB processes under variable conditions. 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. Besides modeling, a major part of this chapter 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. Chapter 6 ends with a selection of different examples showing that an appropriate model combined with consistent parameters can simulate experimental data within high accuracy.

After general criteria and parameters for process optimization are defined, Chapter 7 focuses first on 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 case of isocratic and gradient SMB processes, rigorous process simulations combined with short-cut calculations based on the TMB-model are useful tools for process optimization, which is illustrated by different example cases. Further sections discuss the improvements of SMB chromatography by variable operating conditions as given by Varicol, PowerFeed, or ModiCon processes. Finally, the latest scientific results on model-based advanced control of SMB processes are presented which are thought to be of increasing importance for practical applications.

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