1
Introduction

R. Ditz

1.1
Liquid Chromatography – its History

For obvious reasons the 'official' birth date of chromatography is linked with the first use of the name 'Chromatography' (writing with colors) by M.S. Twsett in his article on chlorophyll substances published in 1903.

However, the existence and utilization of adsorptive methods for substance purification was practiced well before that time. Applications of liquid phase separations started as early as the turn of the 19th century, that is if one does not acknowledge wood as the first described adsorption medium (Moses 2, 15 (23–25))

Interesting or naturally enough the early history of liquid chromatography has been all preparative. Also, a significant amount of separation work was performed in an engineering environment long before the fundamental work of Michael Twsett, and this is a kind of parallelism, which has continued until today. For almost a century, separation methodology therefore has been developed and practiced in the two different fields without too much interaction and interfacing. In today's world, where as fast a transfer as possible from basic research to practical application has become mandatory, the integration of chemical research and process engineering is key to success. This is one of the major driving forces for producing this book, in order to describe the approaches to and the aspects of chromatography from the side of the chemist as well as from the side of the engineer.

Milestones in the development of Chromatography are linked to the work of many outstanding scientists, such as Ramsay, Langmuir, Berl and Schmidt, Kuhn, Martin and Synge, Cremer and others. It would expand the scope of this contribution to highlight the historical merits of these scientists and the reader is referred to special references (Wintermeier, s. Unger, 1990 and Ettre, 1996).

Also, as this book deals with preparative and process scale aspects and applications of chromatography, the vast efforts made in the analytical fields cannot be covered here.

After the first, and in many ways pioneering, work of Michael Twsett, further development of Column Liquid Chromatography (CLC) was hampered because his

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work had only limited public exposure as it was not published in English or, at least, German. Therefore, it took more than a decade, before Kuhn and Lederer in Heidelberg applied Twsett's approach to carotinoid separation in the late 1920s, which was followed by another dormant period until the late 1940s (Fig. 1.1).

Towards the end of the Second World War basic and directive studies on Gas Chromatography (GC) were performed. GC became a powerful separation method in the analysis of hydrocarbon mixtures obtained from petroleum fractions. It was followed by Thin Layer Chromatography (TLC) in the mid-1950s.

Between 1950 and 1960 Size Exclusion Chromatography (SEC) became a popular technique in two branches: the fractionation of synthetic polymers, described as gel permeation chromatography, and in the resolution of biopolymers, termed as gelfiltration. The former was performed on cross-linked porous synthetic polymers, the latter on cross-linked polysaccharides (Sephadex).

The real advancement of High-Performance Liquid Chromatography (HPLC) began during the late 1960s when small porous particles became available. It took several years before spherical porous particles were accepted over irregular chips. However, before porous particles took the floor porous layer beads with a solid core of 40 μm and a thin porous layer of 1–2 μm were the adsorbents of choice. The serious limitations of such packings were quickly recognized. The breakthrough of HPLC as a routine technique occurred in the mid-1970s with commercial availability of reversed phase silica packings that carried bonded n-octadecyl and n-octyl groups at the surface. One of the latest developments takes advantage of the thermodynamic properties of super-critical fluids and modified HPLC technology – so-called Supercritical Fluid Chromatography (SFC).

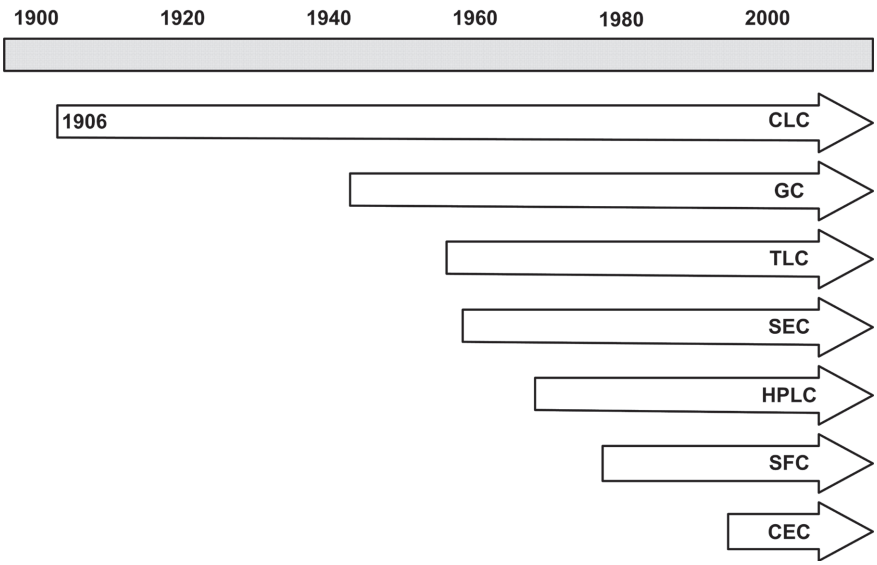

Figure 1.1 Development of chromatography over one century.

Finally, as a hybrid between pressure-driven chromatography and capillary electrophoresis, Capillary Electrochromatography (CEC) has emerged over the last decade.

In retrospect, the development of chromatography was not straightforward, following circles and dead ends before real breakthroughs.

The rapid development of HPLC columns for example was due to three major technical achievements: the ability to manufacture micro-particulate silicas, the invention of air elutriation technique as sizing technology and progress in the slurry technique for packing HPLC columns. However, it took more than ten years to build up sufficient know-how to produce stable, robust and reproducible HPLC columns that satisfied the continuously increasing demands of the chemical and pharmaceutical industry (Fig. 1.2).

The advancement of chromatographic separation techniques during the last century is closely linked to the synthesis of novel products and the isolation and purification of natural substances. The production needs for high-value compounds employed as pharmaceuticals, agrochemicals, food additives etc. put high demands on the purity of these products. The separation of racemates into pure enantiomers is one of the striking examples that highlight the importance and achievements of chromatography.

However, the 'Twsett' period of chromatography has also gone through a bifurcation during its maturization process. Originally conceived by Michael Twsett as a

Moses 2, 15 (23-25)	removal of bitter taste from waters of mara by addition of specific wood
turn of 19th century	adsorptive capacity of carbon for purification of beet juices
appr. 1850	*Runge's* capillary work with coloured chemicals on paper
1870's	ion exchange studies by *Eichhorn* and *Boecker*
1886	use of natural and synthetic ion exchangers in sugar production patented
1903	*Twsett* – chromatographic separation of plant pigments explained by adsorptive effects
1904	*Wislicenus* requests defined materials for adsorptive purposes
1930	*Lederer*, *Kuhn* – separation of carotin and zeaxanthin
1934	standardised aluminium oxides according to *Brockmann*
1941	*James* and *Martin* – gas liquid chromatography – trigger for development of chromatographic principles at analytical and preparative levels
since 1970's	liquid chromatography plays an ever increasing role
1981	1st process scale HPLC system (Kiloprep)
1986	1st preparative chromatography symposium in Paris
1993	1st scaled down SMB units for pharmaceutical applications
1996	1st example of large scale chiral purification process (*UCB*)
2000	1st 800 mm inner diameter SMB unit for contract purification (*Aerojet*)
2001	advanced SMB-applications (Multi-Component, VariCol, …)

Figure 1.2 Historic dates in preparative column liquid chromatography.

preparative method for the isolation and identification of plant pigments, the method still retained its preparative thrust during the reinvention in the 1930s by the Heidelberg research group of Kuhn, Lederle and Brockmann. Then, during the second rebirth in the 1960s as the extension of gas chromatography, it became reoriented for quite some time to serve as an analytical tool, driven not least by the instrument manufacturers. Preparative applications of liquid chromatography remained an exotic aspect for quite some time, served only by a minority of suppliers and estimated to be employed by a few hundred users throughout the world.

During the 'historical' chromatographic period, separations were developed mostly on a trial-and-error experimental basis due to lack of understanding of the underlying principles and the inability to address the complex interactions in fluid-phase systems by a computing approach.

Therefore, the 'chemical' scale-up approach was based on

1. Finding separation conditions for the mixture
2. 'Optimizing load' until elution profiles start to overlap (touching bands)
3. Go through a 'linear scale up' protocol by increasing the column geometry to deliver the required amount and purity of material while maintaining load and separation conditions.

While this protocol produced the 'predicted' product, its process economics were usually close to disastrous, allowing only extremely expensive materials to be processed. Therefore, preparative HPLC was long considered unsuitable for large-scale operation.

Both the 'understanding' as well as the 'computing' aspect have been significantly advanced during recent years by

- Applying more and more already well-known engineering concepts and approaches to chromatography and
- The availability of high-speed computing power at reasonable cost, allowing fast processing of complex data on-line to enable monitoring and control of chromatographic systems even under complex operating conditions.

1.2 Focus of the Book

The present book focuses on preparative chromatography, and is distinct from earlier approaches in that it will describe and develop access to chromatographic purification concepts through the eyes of both engineers and chemists. This is because, to develop a method that can be scaled up to a process environment, the earliest possible interaction and cooperation between chemist and engineer is required to achieve time and cost-effective solutions.

The differentiation between preparative and analytical chromatography has often been a point of heated discussion in the chromatographic community, especially

over the last 30 or so years. Quite often, this differentiation has been based on size (column size, particle size of the packing or sample size). Unfortunately, this has often led to less than appropriate separation strategies and modes of operation (Fig. 1.3).

The difference between „analytical“ and „preparative“ work is not defined by the size of either sample or equipment. It is exclusively determined by the „GOAL“ of the separation process. If „INFORMATION“ is the goal of the separation, it is „analytical“ chromatography. If the COLLECTION OF PRODUCTS is the intention, it is a „preparative“ separation. This implies that „Sample Preparation“ is always a „preparative“ method. In an „analytical“ mode, 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 under investigation, as long as a correct result can be documented. In a „preparative“ mode, the sample has to be recovered in the exact condition that it was in before undergoing the separation, i.e. no degrading elution conditions, etc. This determines the whole separation strategy far more than any consideration of the size of the process or dimensions of columns ever would.

By this definition, one of the smallest scale operations could, and maybe should, be included in this context, which is the isolation, and subsequent characterization or identification of so-called low-abundant peptides in modern proteomics research.

At first glance, this is considered as nanoscale analytics, challenging the separation and detection capabilities of the most sensitive equipments and columns available

'Classical' *(by evolution)*	**'Goal –oriented'** *(by design)*	
	Goal of separation	
size-based ▪ **column size** ▪ **particle size of packing** ▪ **sample size (amount)**	**information** *peak-chromatography* **for fastest information generation**	**material** *product-chromatography* **for best product recovery**
▪ unstructured ▪ separation based on column dimensions, equipment etc. ▪ often separation modes used, which are unsuitable for preparative operation, like buffers, ion-pair reagents etc. (which are difficult to remove from purified product) ▪ use of strongly diluting separation mechanisms ▪ insufficient characterisation of relevant or critical separation parameters, esp. solubility optimisation	▪ all separation modes allowed ▪ optimize for necessary separation sensitivity (elution between k`= 1 – 5 or in gradient mode) ▪ optimize for peak capacity ▪ optimize for speed ▪ optimize for selectivity	▪ only operations permitted, that allow intact recovery of sample submitted to the purification process ▪ optimize for purity ▪ optimize for speed ▪ optimize for productivity ▪ minimize eluent consumption

Figure 1.3 Development of a separation strategy.

today. At a second look, the boundary conditions for such isolations and characterizations under reproducible conditions put the same requirements on method development, sample handling and chromatographic packing materials as process-scale separations do.

1.3 How to Read this Book

It is not necessary to read all chapters of this book in sequence. For some readers the book may be a reference to answer specific questions. However, all readers should familiarize themselves with the content of Chapters 2–4 and 7 before they start solving chromatographic separations. Chapter 5 gives an overview of different chromatographic processes while Chapter 6 provides a detailed introduction to modeling and parameter estimation. These chapters, as well as Chapter 8 on chromatographic reactors and Chapter 9 on process control, should be read as the need arises. The book may not provide answers to all questions. In which case, the reader can obtain further information from the cited literature.

Brief contents of the individual chapters are: 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, such as dispersion, porosity or void fraction, as well as different kinds of isotherm equations. Other topics are plate number, HETP values as well as their determination based on the first and second moments. Finally, the effects of linear and nonlinear chromatography, and the different kinds of column loading, are discussed. The experienced reader may pass quickly through this chapter to become familiar with definitions used. For beginners this chapter is a must in order to learn the general terminology and acquire a basic understanding. A further goal of Chapter 2 is the harmonization of general chromatographic terms between engineers and chemists, avoiding „slang terms“, which are quite common in the literature.

Chapter 3 focuses on all aspects related to the chromatographic column. A major part explains and specifies the kinds of stationary phases as well as their chemical structure and properties. This part may be used as reference for special questions and will help those looking for an overview of attributes of different stationary phases. Packing procedures, as well as the design of the inlet and outlet heads, are very important for column efficiency. When deciding on the dimensions of a column the pressure drop also has to be taken into account.

Chapter 4 deals with the selection of a chromatographic system, i.e. the optimal combination of stationary phase, eluent or mobile phase for a given separation task. These key issues focus not only on solely scientific questions, but also take into account economy, speed, time pressure, hardware requirements, automation and legal aspects towards documentation, safety and others. Obviously, such rules of thumb may not cover all possible scenarios, but they may be useful in avoiding pitfalls.

The selection of chromatographic systems is 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 5 focuses on process concepts. The basis of every preparative chromatographic separation is the proper choice of the chromatographic system, as described in the previous chapters. Its implementation in a preparative process concept plays an important role in serving the different needs of substance production in terms of system flexibility and production scale. Depending on the operating mode, several features distinguish chromatographic process concepts:

- Batch-wise or continuous feed introduction
- Operation in single- or multi-column mode
- Elution under isocratic or gradient conditions
- Co-, cross- or counter-current flow of mobile and stationary phase
- Withdrawal of two or a multitude of fractions

Starting with the description of the main components of a chromatographic unit, Chapter 5 gives an overview of process concepts available for preparative chromatography.

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. After a short explanation of numerical solution methods, a major part is devoted to the consistent determination of the parameters for a suitable model, especially those for the isotherm. Methods of different complexity and experimental effort are presented that allow a variation of the desired accuracy, on the one hand, and the time needed on the other hand. It is shown that an appropriate model can simulate experimental data within the accuracy of measurement, which permits its use for further process design as approached in Chapter 7. The necessity for optimization of chromatographic processes in the pharmaceutical and biotechnological industries results from high separation costs, which represent a major part of the manufacturing cost. However, due to the multitude of parameters and the complex dynamic behavior, pure empirical design and optimization of chromatographic processes are hardly possible. Mathematical modeling of the process is an essential requirement for this purpose.

Chapter 7, therefore, deals with model-based design and optimization of a chromatographic plant, where the already selected chromatographic system and concepts are applied. First, basic principles of the optimization of chromatographic processes will be explained. These include the introduction of the commonly used objective functions and the degrees of freedom. To reduce the complexity of the optimization and to ease the scale-up of a plant, this chapter will also emphasize the application of dimensionless parameters and degrees of freedom respectively. Examples for the

scale-up of an optimized plant are also given. Subsequently, methods for the model-based design and optimization of batch and SMB (Simulated Moving Bed) processes are introduced. For this purpose dynamic simulations of an experimentally validated model are used. Especially in the design and optimization of the complex SMB processes, a quick design and optimization method for SMB will be introduced. Finally, the performance of batch and SMB processes are compared.

Chapter 8 introduces chromatographic reactors that combine the chromatographic separation, discussed in the earlier chapters of this book, and a chemical reaction. The elementary reaction type $A \rightleftarrows B + C$ is used to explain the operating principle of the different chromatographic reactors. An overview over the different reactor types as well as the investigated types of reactions is given, and their influence on process design and operation is discussed. Rigorous modeling is, so far, the only tool available to describe the behavior of chromatographic reactors. Therefore, the modeling approach presented in Chapter 6 is extended to chromatographic reactors. Finally, the design of chromatographic reactors is discussed, using the examples of the esterification of β-phenethyl alcohol with acetic acid as well as the isomerization of glucose.

The benefits of model-based control strategies for the operation of SMB processes are demonstrated in Chapter 9. This is a rather new concept as, in today's industrial practice, SMB processes are still „controlled" manually, based on the experience of the operators. A nonlinear model predictive (NMP) controller is described that can deal with the complex hybrid (continuous/discrete) dynamics of the SMB plant and takes hard process constraints (e.g. the maximal allowable pressure drop) and the purity requirements into account. The NMP controller employs a rigorous process model, the parameters of which are re-estimated online during plant operation, thus changes or drifting of the process parameters can be detected and compensated. The efficiency of this novel control concept is proven by an experimental study.