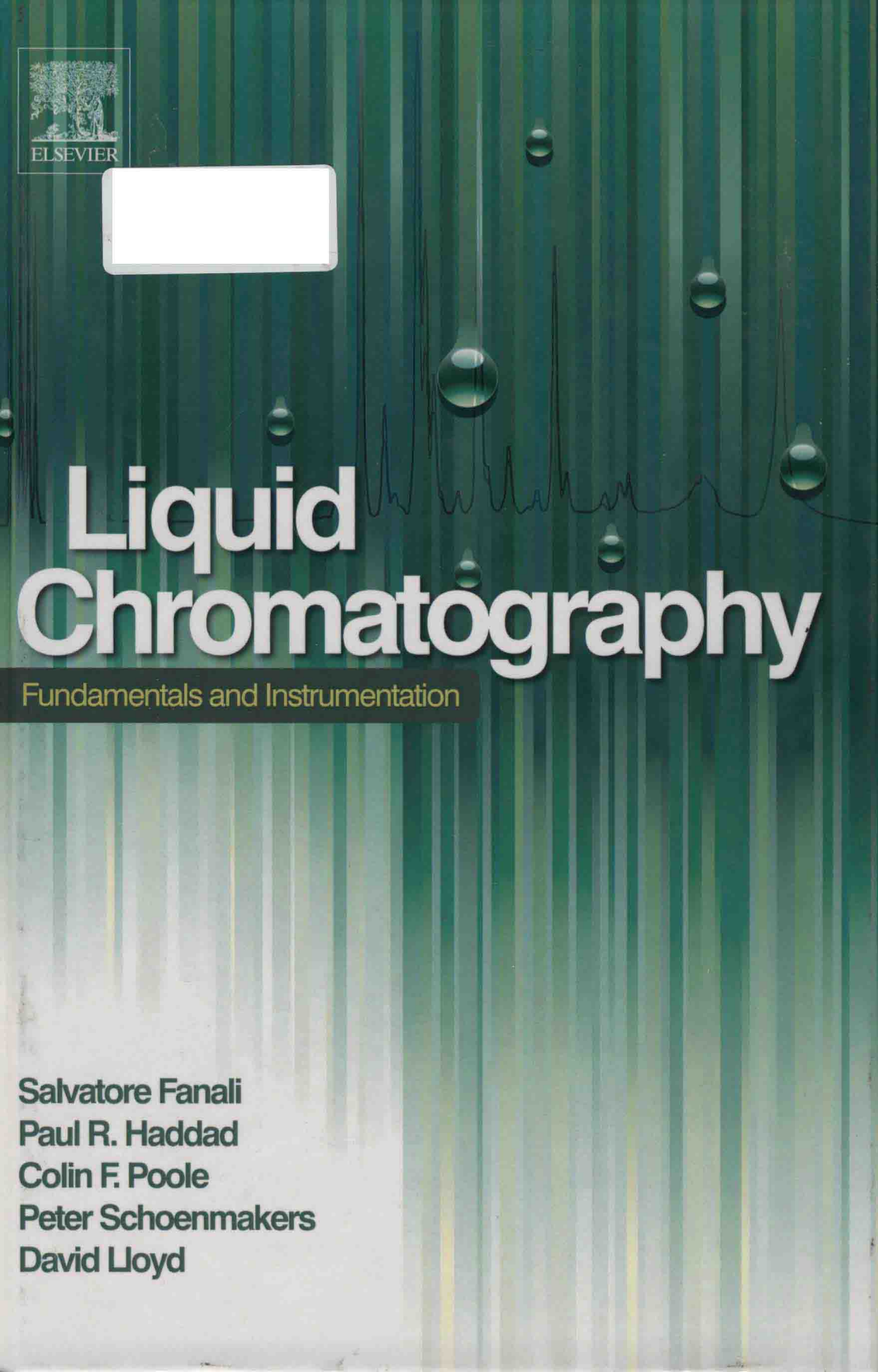
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Contents

CHAPTER

1

Milestones in the Development of Liquid Chromatography

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OUTLINE

The importance of liquid chromatography (LC), and especially high-performance LC (HPLC), in today's world hardly needs stating. It is the most widely used technique for the analysis of chemical mixtures and has contributed in a major way to science (especially the biological sciences) and everyday laboratory practice. Liquid chromatography is primarily a practical technique, so our story is limited to those innovations that

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contributed significantly to its present use in "working" laboratories. In reflecting on the history of LC, it appears to us that only a few "essential" actors are in this drama: single individuals whose absence might have delayed the technique by more than a year or two. Thus, the development of present-day LC has largely been an evolutionary, rather than a revolutionary, process. Furthermore, many important innovations within the past 50 years have occurred within industrial research and development (R&D) groups, where it is often not possible to assign credit for a final product to a single person. Finally, every attempt at history suffers from incomplete and conflicting accounts of who did what—and when. In the present "history," we try to emphasize "what" and "when" rather than "who."

1.1. INTRODUCTION

Several important innovations in the history of liquid chromatography are reviewed by Ettre [1]:

- Invention of chromatography in the early 1900s [2].
- Invention of partition and paper chromatography in the early 1940s [3].
- Development of ion-exchange chromatography [4] and the amino-acid analyzer during the 1950s [5].
- Invention of gel-filtration chromatography in the late 1950s [6,7].
- Development of the gel-permeation chromatograph in the early 1960s [8,9].
- Development of high-performance LC in the mid-1960s [8,10–17].

The present chapter emphasizes work on HPLC, while noting major, prior contributions that made this technique possible. Most advances in HPLC can be organized as follows:

- Development or application of basic theory, combined with empirical observations of the separation process.
- Invention of new chromatographic modes (e.g., ion-pair chromatography, hydrophilic-interaction chromatography) and the development of HPLC columns for new applications (chiral separation, large biomolecules).
- Development and improvement of equipment and columns.

1.1.1. Developments before 1960

A good account of the discovery of chromatography by Tswett is given in [2] and [14, pp. 4–6]. Despite a few subsequent applications of chromatography in other laboratories [14, pp. 7–9], this technique became generally accepted only after its reintroduction in 1931 by Kuhn,

Winterstein, and Lederer [18]. The invention of *liquid-partition* chromatography was reported by Martin and Synge in 1941, followed soon after by its extension to *paper* chromatography in 1944 and the first application of *two-dimensional* chromatography [3].

Significant work on ion-exchange separation began in the 1930s, with the subsequent development and application of ion-exchange chromatography (IEC) for separation of the rare earths and transuranium elements [4]. The extension of IEC to organic compounds was next, implemented by Cohn and Samuelson [14, pp. 17–21]. By 1958, Moore, Stein, and Spackman reported the automatic analysis of amino acid samples by means of IEC [5]. Their system was a precursor of HPLC that incorporated automatic pumping, efficient IEC columns, and continuous colorimetric detection. This system was later improved and commercialized as the Beckman-Spinco model 120B amino acid analyzer.

Still another major development, in the later 1950s, was the invention of gel filtration [6,7] for the separation of large biomolecules by molecular size; this was followed a few years later by the development of gel-permeation chromatography (GPC) for the similar separation of synthetic polymers [9]. The latter then led to the development of a commercial GPC system by Waters Associates (the GPC-100 [8]), which would morph into an early commercial HPLC system (the ALC-100).

1.1.2. HPLC at the Beginning

Prior to the development of the first HPLC systems, gas chromatography (GC) provided an example of what HPLC might be capable of: automation, speed, and detection sensitivity, as well as the separation of higher-boiling and thermally unstable compounds. By the early 1960s, the automation of LC had been demonstrated for amino-acid analysis and gel-permeation chromatography (Section 1.1.2). By then, it was appreciated that smaller particles in well-packed beds could increase both separation speed and efficiency. Fast separations with small-particle columns also require higher pressures to pump the mobile phase through the column. Detectors that could be used with LC for most samples presented a major challenge at this time—and for several years thereafter (Section 1.5).

Before 1965, the possibility of using HPLC for separating samples other than amino acids or polymers had undoubtedly occurred to many people. However, the exploitation of this idea required its reduction to practice, followed by the production of commercially available equipment, as in the case of the amino-acid analyzer and the gel-permeation chromatograph (Section 1.1.2). Viewed in these terms, *high-performance* LC was first reduced to practice in ~1964, in the United States under the direction of Csaba Horváth [16] and in Holland by Josef Huber (see [10, pp. 159–166

and 209–217]). The system developed by Horváth was subsequently the basis for the LCS 1000 Nucleic Acid Analyzer sold by Picker Nuclear (later acquired by Varian) and contributed to the first general-purpose HPLC (Waters ALC-100) [8]. Jack Kirkland had visited Huber's lab in 1964 and subsequently began an HPLC program at DuPont [19–22], which culminated in the Model 820 at about the same time as the ALC-100. By 1970, sales of HPLC systems were dominated by Waters Associates and Du Pont. Superficially porous Zipax [22] was initially the most popular column packing. In our opinion, Horváth, Huber, and Kirkland can be considered the "fathers" of HPLC. Some closely related work at this time by others [23–29] is also relevant to the origin of HPLC. For a description of the columns, equipment, and practice at that time, see [30].

1.2. HPLC THEORY AND PRACTICE

Separation as a function of experimental conditions was understood in general terms for GC, and similar principles were expected to apply to HPLC. Resolution, R_s, can be described by the Purnell equation [31] in terms of the plate number, N, separation factor, α, and retention factor, k:

$$R_s = 0.25(N^{0.5})(\alpha - 1)\,[k/(k+1)] \tag{1.1}$$

A semi-quantitative understanding of column efficiency (*plate number, N*) existed prior to 1965, based on the further development of the van Deemter equation for GC [32] and its extension to LC by Giddings [23]. Later work resulted in the highly useful and widely applied Knox equation [33]:

$$h = Av^{0.33} + B/v + Cv \tag{1.2}$$

where the reduced plate height, h, is related to the reduced velocity, v, of the mobile phase. The later development of "*Poppe*" (or "kinetic") plots further advanced our understanding and use of column efficiency [34]. For further details on Eq. (1.2) and values of N, see Chapter 2 and [35]. When developing an HPLC procedure, the main challenge has been the selection of separation conditions for the control of peak spacing, that is, "optimum" values of α (Section 1.2.2).

Basic theory played an important role in the development of HPLC, but its implementation was primarily the result of (a) the introduction of new separation modes or techniques (Section 1.2.1), (b) a better understanding of how best to vary conditions for a satisfactory separation (Section 1.2.2), and (c) improved columns (Section 1.3) and hardware (Sections 1.4 and 1.5).