Introduction: One Hundred Years of Chromatography

Chromatography is 100 years old. M. S. Tswett, its inventor, started its development during the early years of the 20th century and presented the first report on his early investigations in March 1903, at a local meeting in Warsaw, to an audience of 41 including his colleagues and students of the university. At that time the technique was in a somewhat embryonic state and Tswett was not yet sure about the final methodology. It took him three more years to finalize and describe it for international audience in the famous twin papers of 1906.

At the beginning Tswett's method was ridiculed as an oddity and he was considered a parvenu who tries to intrude in a field where he does not belong. A remark by Leon Marchlewski, then an internationally respected Polish scientist from Cracow, immediately after Tswett's fundamental publications is typical: he warned that Tswett should not believe that "a simple filtration experiment" (this is how Marchlewski characterized chromatography) would be enough for him to "swing himself to the height of a reformer of chlorophyll chemistry".[1] Even the otherwise polite Richard Willstätter, professor at the University of Munich and the highest authority in chlorophyll research, considered chromatography an "odd way" to carry out pigment research.[2] It is thus not surprising that, in the first 25 years after Tswett's publications, the use of chromatography was tried only in about half a dozen laboratories. Even as late as in 1929, there were scientists who still expressed their negative opinion about the importance of Tswett's invention. It is suffice to cite here F. M. Schertz, an American agricultural chemist,[3] according to whom

> it is evident that Tswett was never at any time dealing with pure pigments, for not once were the substances crystallized.

The big breakthrough finally came during 1930–1931 in Heidelberg, in the laboratory of Richard Kuhn, followed almost immediately by Paul Karrer, in Zurich, László Zechmeister, in Pécs, Hungary, and then many others. It is worthwhile to quote here Karrer's statement during 1939, just a decade after Schertz' dictum[4]:

> It would be a mistake to believe that a preparation purified by crystallization should be purer than one obtained from chromatographic analysis. In all recent investigations chromatographic purification widely surpassed that of crystallization.

The meteoric rise of the application of chromatography in the 1930s can be best illustrated by comparing the number of publications in the decade following Tswett's invention with the decade following Kuhn's activities. Between 1906 — Tswett's twin papers — and 1914 — the outbreak of the First World War — we can find a total of only nine publications (in addition to those of Tswett) in the international literature describing applications of chromatography. At the same time, the bibliography section in the second edition of Zechmeister's chromatography book published in 1938 lists a total of 550 publications for the period of 1930–1938.[5]

One of the reasons for the delay in the acceptance of chromatography was that Tswett's method represented a radical change in the existing philosophy of how complex natural substances are investigated. Instead of obtaining a single compound in crystal form, he separated all the individual compounds from the matrix and from one another. In other words, instead of isolating one single compound and discarding the rest, chromatography provided all (or at least most) compounds present in pure form, and permitted to do it by using only a small amount of the starting complex mixture. This change in the philosophy needed to appreciate the superiority of chromatography was best characterized in 1937 by G. M. Schwab, professor at the University of Munich. According to him[6]

> only after biochemistry, pressed by new problems, demanded methods for the reliable separation of small quantities of similar substances, could chromatography celebrate a rapid and brilliant resurrection.

These citations, the last three within a period of less than a decade, are given here to illustrate Tswett's struggle for acceptance and then the sudden change in the attitude of the international scientific community.

In descriptions chromatography is usually considered a technique. However, it is more than a simple technique: it is an important part of science encompassing chemistry, physical chemistry, chemical engineering, biochemistry, and is cutting through different fields. When introduced 100 years ago, it represented a new paradigm, and provided the theory and practice of interactions between two different phases. Also, while we primarily consider it a laboratory method, the amounts handled by chromatography cover many orders of magnitude. It is true that most of the samples analyzed are very small in the domain of microchemistry — let us not forget that it was gas chromatography which initiated the development of microsyringes, with capacities of less than one microliter (10^{-3} g) — and today, we routinely determine amounts in the nanogram (10^{-9} g) to picogram (10^{-12} g), and even to the femtogram (10^{-15} g) level; at the same time, however, we know of real industrial plants constructed in the former Soviet Union in the 1970s, using gas chromatography columns of 15–200 cm diameter, for the production of 200–1200 metric tons/year of pure compounds,[7] and in February 2007 the 19th International Symposium on Preparative (Liquid) Chromatography was held in Baltimore, Maryland, discussing mainly applications for biochemical and pharmaceutical separations.

Steps in the Evolution of Chromatography

The subject of our book is the evolution of chromatography. It actually starts well before Tswett and we may even contribute one of Moses' miracles to "chromatographic separation," probably as a natural process. Although the use of adsorption-type (partial) separation had been

reported in the second part of the 19th century, Tswett is without any question the true inventor of the technique. His life and activities are a fascinating subject and we try to capture as much of it as possible, particularly since earlier discussions and publications included some errors. We shall also report in detail on the activities of the five pioneering scientists — Kränzlin, Dhéré, Palmer, Lippmaa, and Coward — who, in the two decades following Tswett's work introduced chromatography in their investigations.

As already mentioned the situation suddenly changed in 1930–1931, after the first publication from Kuhn's laboratory: chromatography was reborn and within a decade it became widely used, first in laboratories dealing with the study of natural substances, but soon also in chemical and biochemical laboratories, where separation of various substances was desired.

In the 1940s the technique of chromatography was also further extended, adding partitioning and ion-exchange as a means of separation to adsorption–desorption, and demonstrating that chromatography can be performed not only in a column, but also on a planar surface (paper chromatography).

In the early 1950s chromatography underwent another quantum leap by the introduction of gas chromatography (GC). For many of us the next two decades represented the most interesting period of our lives, when the development accelerated and almost every day brought something new. At that time gas chromatography even eclipsed liquid chromatography (LC), and was on its way to dominate alone the field of analytical chemistry.[8] The evolution of gas chromatography was also accompanied by a detailed study of its theoretical background, and finally the technique was based on a sound theoretical foundation. Then, as the next step, this gain in the theory of GC was used to investigate the possibilities of improving classical LC. As a conclusion, while the 1950s and early 1960s saw the meteoric evolution of GC, the second part of the 1960s saw the introduction of a new, more sophisticated version of LC. Its principles remained the same as used by Tswett in the first decade of the 20th century, but the results were much improved by systematically applying the theoretical conclusions learned in GC to the separation process in LC. In fact, the difference

was so striking that even a new term, high-performance liquid chromatography (HPLC) was adapted for its characterization. This new version of liquid chromatography resulted in the unparalleled rise of its application, within about two decades even surpassing GC. This evolution is still continuing.

In addition to gas chromatography the 1950s also saw the development of size-exclusion (gel filtration) chromatography, the extension of planar chromatography into thin-layer chromatography, and the development of the automated amino acid analyzer, which we may consider as the first sophisticated liquid chromatography instrument.

Before the advent of GC, chromatographers put together their "chromatograph" using simple laboratory hardware. This would not have been possible anymore with GC: construction of the needed instrument was beyond the capabilities of a standard laboratory. Fortunately the decade after the Second World War saw the establishment of the new scientific instrument industry which in turn became involved in the development, manufacturing, and marketing of the sophisticated and increasingly automated instruments, first making gas and then liquid chromatography everybody's tool.

The second half of the 20th century also had one interesting development that greatly accelerated the rapid spreading of the newest innovations, both in equipment and applications: the organization of frequently held symposia where the newest developments were reported to a truly international audience. At the end of these meetings the participants rushed home, to try and apply all the new innovations they have learned at the symposium, both from the formal presentations and during the intensive formal and informal discussions, characteristic of these gatherings. In the last chapters of this book we report on the most important early symposia both in GC and LC, setting the trend.

The 32 chapters of this book guide the reader through the fascinating evolution of chromatography in the 20th century. In addition to the development of the most important milestones of the technique, the background of the individual inventions is also provided and information is given on the scientists' life and activities.

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