Chromatography is 100 years old. On 8 March 1903 M.S. Tswett, an assistant at Warsaw University, presented a lecture at the meeting of the Biological Section of the Warsaw Society of Natural Sciences, titled “On a New Category of Adsorption Phenomena and Their Application to Biochemical Analysis.” In this lecture, he discussed his wide-ranging investigations of leaf pigments performed during the previous couple of years. These investigations led to the development of a special adsorption technique that permitted the separation of the leaf pigments. In subsequent years, he further refined this technique, which eventually became known as chromatography.

On the occasion of this centenary, I would like to investigate the stages of Tswett’s thinking that led to the development of chromatography and consider the way chromatography eventually became the most widely used laboratory technique. I will start with a brief summary of Tswett’s life and the events and pitfalls that influenced his activities.

The Life of M.S. Tswett
Mikhail Semenovich Tswett was born on 14 May 1872 in the small Northern Italian town of Asti. His father, a Russian, planned to spend an extended holiday in one of the resorts at the Lake Maggiore, the beautiful lake in Northern Italy. He arrived in Genoa, Italy, by ship from Russia with his pregnant wife and continued travelling by train to his destination. The condition of his wife forced him to interrupt their journey in Asti. Mikhail’s mother died soon after the birth of her son, and his father took him to Lausanne, Switzerland, as a weak infant.

During the next 24 years Mikhail lived in Switzerland, first in Lausanne and then in Geneva. His father returned to Russia but each year spent an extended vacation in Switzerland with his son. In 1884, he moved permanently to Switzerland with his family (he had remarried and had additional children). Finally, at the end of 1895, Mikhail’s father returned to Russia where he became a high financial officer of Taurida (the Crimean peninsula). He died in 1900 at the age of 71.

After finishing high school, Mikhail studied at the University of Geneva and majored in botany. He received his PhD in 1896. His thesis dealt with investigations of the structure of plant cells, the movement of the protoplasm and the structure of chloroplasts. After finishing his studies, he decided to repatriate to Russia to join his father. He had high hopes to find an appropriate academic position quickly; however, he apparently was unaware of the strict Russian system. According to this system, a junior academic position required a Russian magister’s (master’s) degree, and a senior position required a Russian doctor of science degree. In this respect, his Swiss PhD was irrelevant. I might add to this that at that time his knowledge of the Russian language was far from satisfactory: his mother language was French, and he learned Russian from his father only as a teenager. According to reports from contemporaries, even later when he became fluent in Russian, he spoke it with a definite French accent, and after he married in 1907, he preferred to speak French with his wife.

In December 1896, Tswett found a temporary position in a laboratory in St. Petersburg. He first tried to resubmit his Swiss
which also consists of cellulose. After extracting the pigments similarly to the tissue of plant leaves. He selected filter paper, to imitate the process by using a substrate that would behave on the basis of these molecular forces. Pigments easily. Tswett correctly identified adsorption as the reason for this behaviour might be the interference of some molecular forces binding the pigments to the leaf substrate and that these forces depend upon the individual pigments; for some, such as chlorophyll, they are stronger than for others. Only solvents with a dissolving power stronger than that of the binding molecular forces can be used for the extraction of a particular pigment. On the other hand, after the pigment is extracted and these molecular forces no longer exist, even the weaker solvents can dissolve all the pigments easily. Tswett correctly identified adsorption as the basis of these molecular forces.

After drawing this conclusion the next logical step was to try to imitate the process by using a substrate that would behave similarly to the tissue of plant leaves. He selected filter paper, which also consists of cellulose. After extracting the pigments from the leaves with ethanol, he evaporated the solvent and redissolved the residue in ligroin; next, he impregnated the filter paper with this solution. The paper taints with the pigments behaved exactly in the same way as the original green leaves: ligroin extracted only the carotenes, but after the addition of a small amount of ethanol, all pigments could be easily retrieved.

The title of his master’s thesis submitted to the University of Kazan’ was “The Physico-Chemical Structure of the Chlorophyll Particle: Experimental and Critical Study”,2,3 and it represented a detailed report of these studies. This degree finally qualified him for an appointment at a university, and he applied immediately for a position at the University of Kazan’. Meanwhile, however, an acquaintance of his from St. Petersburg, D.I. Ivanovskii (the discoverer of the tobacco mosaic virus) had just been appointed a professor of botany at Warsaw University, and he invited Tswett to join him there. Tswett accepted his invitation and moved to Warsaw at the end of 1901.

**The Development of Chromatography**

As a continuation of his thesis work, Tswett decided to perform systematic investigations to see whether plant pigments would be selectively adsorbed on other substances. Also, he postulated the possibility that by the proper selection of the solvents and adsorbents, he might develop a general method to separate various plant pigments. As soon as he settled in Warsaw, he started to explore these questions systematically.

Tswett investigated more than 100 inorganic and organic solid substances to study their adsorption characteristics toward chlorophyll. The powdered substance was packed into a small, narrow tube, and the ligroin solution of the pigments was added. Adsorption of the pigments could be observed by colour changes of the adsorbent powder and the pigment solution flowing out of the tube. After adsorption on the powder, the pigment could be desorbed (dissolved) by the selection of a suitable solvent. The best results were obtained using inulin (a polysaccharide) calcium carbonate, and alumina. His studies also showed that soon after the pigment solution

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**Figure 1: Separation scheme of the stepwise differential adsorptive precipitation and extraction method, used by Tswett in his early work. Drawn after the description in (4).**

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was poured into the small tube containing the adsorbent, green and yellow rings started to form. After adding more solvent to the column, the rings became separated, widened and moved down the column, and sometimes even separated into additional rings with different shades of colour, which indicated the presence of additional compounds. By the proper selection of the solvent, it also was possible to elute them successively from the column, which resulted in the solution of the separated individual pigments. This process, of course, is identical to chromatographic separation, although Tswett did not use this term at that time.

Tswett also devised another way to separate plant pigments by stepwise selective adsorption and extraction. He added the adsorbent powder to the pigment solution; it adsorbed the pigments, which then could be extracted selectively from the filtered adsorbent. Repeating the process several times with different solvents resulted in separate solutions of the pure pigments, which could be identified by the colour of their solution and by their UV-absorption spectra. Figure 1 (based upon his description) illustrates the multistage process used by Tswett in 1902–1903 for the separation of chlorophylls, carotenones and xanthophylls.

By the beginning of 1903, Tswett had advanced well in his investigations and was able to summarize his results in the famous lecture the centenary of which we celebrate this year. He considered this lecture to be only an interim report; therefore, he did not publish his results in any widely read journal. His lecture was printed only two years later in the periodical of the local society, thus, at that time, Tswett’s results and the possibility of a new separation technique remained unknown outside his immediate circle of colleagues.

Continuing his investigations, Tswett realized that the multistage process was too complicated, so he concentrated on the possibility of a one-step separation process on a small column in a continuous solvent flow. In the next two years, he further refined the technique and started to use it for the investigation of pigments present in various plants. Then, in 1905, he became involved in a scientific dispute.

At that time Hans Molisch, a professor at the University of Prague and one of the most respected European botanists of the period, published his investigations of the pigments of brown algae in a widely read German botanical journal. Evidently, Tswett also investigated these pigments, and his results differed from those of Molisch. Therefore, he immediately submitted a letter to the journal that stated his disagreement with Molisch. However, he did not provide any details about his own results but only stated that they were obtained with the help of “a new reliable method” that he soon would describe in detail. This letter initiated a couple of polemic publications by Molisch and F.G. Kohl, then a professor at the University of Marburg/Lahn, Germany, and the author of a book about the carotene pigments. Both scientists criticized Tswett for referring to results that were obtained by an undisclosed method. Tswett immediately answered with a more detailed description of his results, and, within one month, he submitted two major papers to the Berichte der Deutschen Botanischen Gesellschaft, which was the journal of the German Botanical Society and the leading European journal in this field. In these two papers, Tswett discussed his results and described the chromatographic method for the separation of plant pigments in detail, using the term “chromatography” for the first time. The second paper contains the most famous, widely quoted statement: “Like light rays in the spectrum, the different components of a pigment mixture, obeying a law, are resolved on the calcium carbonate column and then can be qualitatively and quantitatively determined. I call such a preparation a chromatogram and the corresponding method the chromatographic method.”

The word chromatography is composed of two Greek roots, chroma (colour) and graphein (to write), and its verbatim translation means “colour writing,” which refers to visualizing the separated multicoloured rings on the column. Another interpretation of this term links it to Tswett’s surname, the meaning of which is “colour” in Russian. According to this interpretation, “chromatography” actually could mean “Tswett’s writing.”
In Tswett’s opinion, a scientist always must consider the whole sample and separate all the substances present.

Tswett’s two fundamental papers describe the various aspects of liquid-adsorption chromatography in detail. They discuss the proper selection of the adsorbent and the solvent, the possibility of improving a separation by changing the solvent during a run or adding another solvent to the one used, and the possibility of two-dimensional chromatography by developing the column with another solvent after the first separation. He also emphasized that although his examples refer to plant pigments as the sample components, other substances also could be separated by chromatography and stated that in spite of its name, chromatography was not restricted to the separation of coloured compounds.

Figure 2 shows the illustrations included in Tswett’s 1906 paper.6,10,11 His fairly simple laboratory setup consisted of 2–3 mm i.d. vertical columns, the upper parts of which were widened to serve as reservoirs for the solvent. The length of the packing was approximately 30–40 mm; a small cotton-wool wad was placed at the bottom to hold it in place. As many as five columns were connected to a manifold that comprised a horizontal glass tube connected to a larger vessel serving as a buffer volume. Using a small hand pump (a rubber bulb), he could exert a small pressure on the system. When separation was completed, the columns were removed, the adsorbent column — the packing with the separated multicoloured bands — was pushed out of the tube carefully with a wooden rod, and the individual bands were cut off with a scalpel. From these fractions, the adsorbed pure substances could be dissolved with a suitable solvent. If larger amounts were needed for additional fractions, the adsorbed pure substances could be dissolved with a suitable solvent and collecting the separated compound with the solvent and collecting the individual fractions was introduced much later, in the second time.

Tswett’s 1910 Book

Following the publication of his twin 1906 papers, Tswett continued to refine his technique and also to investigate various plant and animal pigments. He also demonstrated the chromatographic technique at meetings of the German Botanical Society in Berlin, in this way advocating its use.

By 1908, Tswett decided to summarize all the accumulated knowledge in a book, which finally was published in 1910 in Russian by a Warsaw publisher. It was titled Chlorophylls in the Plant and Animal World,12 and it also served as his thesis for the Russian Doctor of Science degree (Figure 3). The excellence of his book is best demonstrated by the fact that the Imperial Russian Academy of Science honoured it in 1911 with its M.N. Akhmatov Prize, a major scientific award with a fairly high honorarium.

As its title shows, the primary subject of Tswett’s book was the investigation of the various pigments occurring in nature. For him, chromatography was only a means for this study, but he realized the importance of the new technique as a fundamental improvement in the ways of separation. This philosophy was in contrast to the opinion prevailing at that time that the most important goal was the isolation of a single substance for further study: in Tswett’s opinion, a scientist always must consider the whole sample and separate all the substances present. He also emphasized that a chromatographically separated substance is at least as pure as one obtained by traditional means such as chemical reactions, distillation and crystallization.

With respect to chromatography, Tswett’s book discussed all the information included in his previous publications in a systematic way and also added significant new material. For example, he further extended the list of suitable solvents and emphasized that each has advantages, disadvantages and particular fields of application. He also illustrated the use of solvent mixtures and the possibility of gradually changing the solvent during the chromatographic process. When dealing with the mechanism of separation, he treated the process theoretically and divided the adsorbent into consecutive segments, considering the adsorption equilibrium in each segment. This concept is not far from the theoretical plate concept. He also showed that the rate at which each sample component travels along the adsorbent bed depends upon its adsorption coefficient and is independent of the other components present. In the discussion of the various suitable adsorbents, Tswett always indicated whether the particular material was inert relative to the compounds to be separated or interacted chemically with them — advice some of his followers ignored.

He also emphasized that a chromatographically separated substance is at least as pure as one obtained by traditional means.

With regard to the chromatographic system, Tswett extended the size of the column to tubes of 30-mm diameter, with a packing bed length of approximately 80 mm, and he used these tubes for preparative purposes. He continued to prefer to end the separation while the separated rings were still on the column. The method of washing out (eluting) the separated compounds with the solvent and collecting the individual fractions was introduced much later, in the second
In 1911, Tswett published seven papers in German and French scientific journals. One of these papers included a very detailed discussion of the various carotenoids; in fact, this term was proposed by him in this paper and soon was generally accepted.

Struggle for Acceptance
As mentioned previously, Tswett’s twin 1906 papers were published as a result of his dispute with Molisch and Kohl. However, their publication did not satisfy his opponents, who soon were joined by Leon Marchlewski, a professor at the University of Cracow in the Austrian part of Poland. (Marchlewski’s aversion to Tswett might have had a nationalistic reason: Marchlewski, the patriotic Pole, against Tswett, the Russian, living in the Russian-occupied part of Poland.) Their dispute concerned both Tswett’s chlorophyll research and the new separation technique. To characterize the acerbic tone used by Marchlewski, it is enough to quote one of his comments that Tswett should not believe that he can “with the help of a filtration experiment swing to the height of a reformer of chlorophyll chemistry”.

The second main antagonist of Tswett was Richard Willstätter, at that time a professor at the Federal Technical University in Zurich, Switzerland. He was the most respected German organic chemist of the period, and later received the Nobel Prize in chemistry for his chlorophyll research. Willstätter carefully followed Tswett’s results; he even ordered a subsequent publication. He was so impressed by the technique that he immediately applied it to his own research and reported his results in both his thesis and a subsequent publication.

In Tswett’s lifetime, his technique was used by only three researchers: Gottfried Kränzlin in Germany, Charles Dhéré in Switzerland, and Leroy S. Palmer in the United States. Kränzlin was a graduate student at the Botanical Institute of the University of Berlin, where he worked on his PhD thesis of investigations of variegated plants. He was halfway through the planned research when he read Tswett’s 1906 papers. He was so impressed by the technique that he immediately applied it to his own research and reported his results in both his thesis and a subsequent publication. Charles Dhéré was professor at the University of Fribourg, Switzerland, and his main interest was the investigation of biologically important substances. In his work, the proper preparation of the substances of interest was of vital importance. Dhéré started to use chromatography in 1911 in conjunction with the thesis work of one of his graduate students, Władysław de Rogowski (who was from Warsaw, and it is not unlikely that he actually possessed a copy of Tswett’s 1910 book).

Finally, I should mention Leroy S. Palmer, working at the University of Missouri, Columbia, USA. After graduation in 1909, Palmer continued his studies in agricultural chemistry and received his PhD in 1913. His thesis work concerned the pigments present in milk and milk products and their relationships to the food intake of the animals. Instead of the assiduous methods existing at that time for the isolation of the individual pigments, Palmer chose to follow Tswett’s methodology — in other words, chromatography. It should be mentioned that even after graduation Palmer remained active in this field, and in 1922 he authored a major book about the carotenoids and related pigments in which he also discussed the methodology of chromatography in detail.

Acceptance and Triumph
When Tswett started his work, the prevailing methodology in the study of complex mixtures was the isolation of a single substance and its purification using extraction and crystallization. In contrast, the emphasis of Tswett’s work was upon separation of all individual substances present from both the matrix and from one another. This change was fundamental and contrary to the belief of most of his contemporaries. The situation changed only in the third decade of the twentieth century. By that time, the philosophy of organic and biochemical research started to change to the point that it required total separation (i.e., separation of all components present) and not just isolation of one or two major constituents, and it had shifted from working with larger amounts to small quantities. This change in philosophy prepared the ground for the “rapid and brilliant resurrection” of chromatography.

Chromatography was resurrected in 1931 in Heidelberg, Germany, by Edgar Lederer, a young researcher in Richard Kuhn’s laboratory. He learned about the existence of the technique from Palmer’s book (see above) and successfully used it to separate carotenoids present in egg yolk. This research was the start of the phenomenal rise of chromatography; within a decade, chromatography became an indispensable tool in organic and biochemical research, and, within a few decades, it was the most widely used laboratory technique.
To Tswett’s merit, he recognized the importance of chromatography 30 years before its wide acceptance. I take the opportunity on the occasion of the centenary of the technique to honour the genius of its inventor.

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