Next month, we are celebrating the 90-year anniversary of Tswett's first report on chromatography, presented at the meeting of the Biological Section of Warsaw Society of Natural Scientists. On this occasion this two-part article outlines the steps in Tswett's research which led to the discovery of the chromatographic separation process and summarizes the most important points in his work.

The Editors

M. S. Tswett and the Discovery of Chromatography
I: Early Work (1899–1903)*

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M. S. Tswett

Summary

On the occasion of the 90th anniversary of the Warsaw lecture of M. S. Tswett representing the first report on the chromatographic technique, this two-part article outlines the evolution of the method. This part deals with Tswett's early work in 1899–1901 and his research in 1901–1903 leading to the lecture at Warsaw. The second part will deal with the fundamental twin papers of 1906, with his book of 1910, summarizing all his activities related to the investigation of plant pigments and to the development of chromatography, and with a few special questions related to Tswett's scientific activities in this field.

Introduction

In 1952 the Nobel Prize in Chemistry was awarded to A. J. P. Martin and R. L. M. Synge "for their invention of partition chromatography". On these occasions the recipients of the award present a lecture on their work. Martin started his Nobel Lecture with the following statement [1]:

> "If enough histories, written while the ideas are still fresh in the mind of people concerned, are available for a variety of discoveries of inventions, it may eventually be possible to lay down some of the principles required to facilitate the obtaining of fruitful results in scientific research in general."

In his lecture Martin gave an example of what he suggested and discussed the background of the discovery of partition chromatography: the problems they were facing and the considerations which finally led them to try partitioning between two phases, one stationary and the other moving, as the basis of separation. In doing this Martin provided a very important contribution to the history of chemistry in general, and specifically, to the history of chromatography.

The particular contribution of Martin and Synge was to extend chromatography which up to then was based on adsorption, to the utilization of partition as the basis of separation. This achievement, however, does not reduce the importance of the original work of Michael S. Tswett, the inventor of chromatography.

Unfortunately Tswett never had the opportunity to accept the applause of his peers and present a summary of his thinking leading to the new technique. In fact, his main concern in the decade following his discovery was to defend himself against his contemporaries who belittled the importance of his work. However, in his publications and in the polemical material there is enough information to permit the reconstruction of his thinking and the sequence of events which led to the development of chromatography, one of the most important scientific achievements of the twentieth century. Our goal in this two-part article is exactly what Martin suggested: to reconstruct Tswett's thinking and summarize the events and steps during the development of chromatography from observation, through experiments trying to reproduce a natural process and

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utilize it for a meaningful purpose and finally to the development of a systematic laboratory separation method.

Next month we are commemorating the 90th anniversary of Tswett's lecture at the meeting of the Warsaw Society of Natural Scientists in which he first described the fundamentals of the technique he called three years later "chromatography". Our present discussion is dedicated to this anniversary.

It is not our aim to deal with the life of Michael S. Tswett; this had been treated in the past in numerous publications [2–12]. However, since for better understanding of the present discussion, knowledge of the major events of his brief life (he died one month after reaching his 47th birthday) is necessary, these are summarized in Supplement No. 1, with some additional information not found in earlier publications. We also use this opportunity to correct a few errors found in earlier publications.

The development of chromatography by Tswett can be best followed through his major reports published in the first decade of this century. In the present article we shall deal with his early work in Russia leading to his Russian master's (magister's) thesis of 1901 [14] which provided the background to this development, and with the first period of his research in Warsaw which was then summarized in the lecture presented in 1903 [15]. In Part II to be published next month we shall deal with his twin papers from 1906 [16–17] which described the fully developed new method and reported on some results in the investigation of plant pigments; and, finally, with his 1910 book [18] containing a systematic discussion of the state of chlorophyll chemistry, debating the misconceptions of his contemporaries and presenting his own results together with a concise description of the chromatographic technique. In addition, we shall discuss a few special questions related to Tswett's scientific activities in this field.

Background; The Beginning of Tswett's Research in Russia (1899–1901)

The subject of Tswett's Swiss Ph. D. thesis submitted in 1896 to the University of Geneva was cell physiology, dealing with the structure of cells in plants, the movement of the protoplasma and the structure of chloroplasts [13]. Contrary to remarks in some publications, it had no direct relationship to his future work except that, naturally, it was the first demonstration of his interest in the pigments present in plants and the mechanism of assimilation of solar energy by plant tissue. However, the development of the chromatographic method can be directly connected to Tswett's research in 1899–1901 in the laboratory of the Russian Imperial Academy of Sciences, in St. Petersburg, in conjunction with the preparation of his Russian magister's (master's) thesis [14]. He emphasized this connection a number of times. In his 1903 lecture [15] he stated that "the present work began with some experiments which I made a few years ago on the insolubility of chlorophyll in petrol ether or ligroin". Later, in the preface of his 1910 book [18] he again noted that "the first outline (of chromatography) is to be found in my Russian work of 1901". Thus, in order to understand the motives which led Tswett to the development of chromatography, we have to investigate his Russian master's thesis from this aspect.

The fundamental observation leading eventually to the development of chromatography was that petroleum ether or ligroin[a] which easily dissolve chlorophyll and other associated pigments when they are available in isolated form, cannot be used to extract the same pigments from leaves, while this can be easily accomplished when using ethanol as the solvent or as a component of it. After extensive investigations Tswett very correctly concluded that this behavior – which had also been observed earlier by other researches – cannot be ascribed to the "insolubility" of the chlorophyll pigments in petrol ether or ligroin vs. their "solubility" in ethanol, or to other assumed phenomena such as e.g., that during extraction with ethanol the chlorophyll pigments may undergo a chemical change rendering them soluble. He postulated that "this (behavior) is rather due to the interference of the molecular forces of the tissue, that is to say, to adsorption". As he summarized two years later [15]:

> "the dissolving power of ligroin for the pigments is lower than the adsorption force of the tissue. However, the latter force is overcome by certain other solvents, such as alcohol; hence the addition of even a small portion of absolute alcohol to ligroin leads to an immediate extraction of all the pigments."

After this explanation Tswett logically made the conclusion that if the insolubility of the chlorophyll pigments in the leaves is due to adsorption the forces of which are stronger than the dissolving power of one solvent but weaker than that of another solvent, then one should be able to imitate this process by using a substrate which would behave similarly to the tissue of plant leaves. He selected filter paper which consists of cellulose just as the plant leaves do. After extraction of the pigments from leaves by ethanol he evaporated the solvent and redissolved the residue in ligroin. Next, he impregnated the filter paper with this solution. The paper tainted with the pigments in this manner behaved toward the solvents exactly in the same way as the original green leaves: ligroin only extracted carotene but not chlorophyll, while the addition of traces of ethanol to ligroin freed all pigments, and this behavior could be readily observed by colour changes on the

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[a] These two solvents used most frequently by Tswett represented the most widely used non-polar solvents of that time. Both correspond to certain petroleum fractions. *Petroleum ether* (petrol ether, petrol, petroleum benzin) consists mainly of C_5–C_6 hydrocarbons with a boiling range of 35–80 °C. *Ligroin* (naphtha, solvent naphtha) mainly consists of higher paraffins, with more than 80 % boiling between 130 and 145 °C.

filter paper and in the solutions. As he expressed it, chlorophyll can be transformed in this way at will from the "soluble" to the "insoluble" state and *vice versa,* without altering the chemical nature of the pigment. Since there can be no chemical affinity between the cellulose of the filter paper and the pigment, the only explanation is that this is a typical case of adsorption.[b]

As a conclusion we can state that Tswett's research work reported in his magister's (master's) thesis [14] showed (among others) the following results:

- the retardation of chlorophyll in the leaves is due to adsorption;
- the adsorbed pigments can be easily desorbed, i.e., transferred from the "insoluble" to the "soluble" form by the addition of even small amounts of certain solvents which have a stronger dissolving power toward the pigment(s) than the adsorption force of the leaves;
- the process observed in leaves can be repeated (simulated) with the use of filter paper consisting also of cellulose;
- since the pigments are colored, the colour changes during adsorption-desorption provide an excellent means of detection.

The results of this work reported in his thesis led Tswett to postulate that if filter paper shows an adsorptive action toward chlorophyll pigments present in ligroin solution, then it might be possible that certain powdered substances would also behave similarly. He also felt that a systematic investigation of this question "would elucidate the nature of adsorption, and furnish a new physical method based on adsorption for the separation of substances".

We are not sure whether he already carried out some preliminary work in St. Petersburg along this line. It is not included in his master's thesis; on the other hand, Senchenkova, in her book [8], refers to a lecture Tswett presented in St. Petersburg on October 30, 1901 – one month after defending his master's thesis in Kazan – at the Congress of the Society of Russian Natural Scientists and Physicians on "Methods and Tasks of the Physiological Study of Chlorophyll." She evidently found the published abstract of this lecture according to which "the speaker draws attention ... to the adsorption method he has developed and demonstrates the underlying phenomena" (see also Note 27 in [11]). We have no further information on this. It is, however, clear that as soon as he settled in Warsaw, he started to work along these lines.

Start of the Development of a New Separation Method (1901–1903)

After arrival in Warsaw toward the end of 1901, Tswett spent the next 14 months to study in details the possibility of utilizing adsorption for the separation of plant pigments. The summary of these activities was presented by him in a lecture presented on March 8 (21),[c] 1903, at the meeting of the Biological Section of the Warsaw Society of Natural Scientists[d] [15]. Tswett's report can be divided into four parts: preparation of the pigment solutions, investigation of a large number of powdered substances concerning their adsorptive properties, adsorption of substances other than leaf pigments, and finally, the description of a separation method based on "filtration" and on adsorptive precipitation.

The first step in these investigations was the preparation of a solution of the pigments. The fresh leaves were powdered rapidly in a mortar together with glass powder and magnesia or calcium carbonate (added to neutralize the acids present in the leaves). Acetone was then added and the grinding continued under acetone. The resulting chlorophyll solution was filtered through a double filter paper or through a pad of asbestos to clear it from suspended particles.

The same process was also repeated but now carrying out the grinding under ligroin containing 10 % absolute ethanol. After filtration this solution was shaken with water; ethanol passes practically completely into the aqueous phase while the pigments remain dissolved in the ligroin phase.

In his report, Tswett presented the results of the investigation of over one hundred inorganic and organic adsorbents (Supplement No. 2). As stated by him, he tested such a wide variety of materials so that "it should be possible to arrive at a general adsorption law for matter, independent of its molecular structure" and to "ascertain various chemical effects of a range of substances on the chlorophyll pigments." The experiments were carried out in three ways:

(1) The adsorbent ground to a fine powder was poured into a small filter funnel made by drawing a narrow tube from the bottom of a test tube, adding a small

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[b] Although this question will be discussed as a separate section in Part II of our publication, we should already emphasize here that Tswett had a strong and in-depth knowledge of the theoretical treatments of adsorption which were very new at that time.

[c] Concerning the date see Note 1 to Supplement No. I.

[d] Hesse and Weil [19] translate the name as the "Warsaw Society of Natural Sciences" while it is given as "Warsaw Naturalists' Society" in [20]. Such associations were frequent in the 19th century in a number of European countries, encompassing learned people who were interested in various questions of natural sciences. The names of these associations represent an adaptation of the German "Naturforschende Gesellschaft" or "Gesellschaft für Naturforscher," i.e., association of people dealing with the exploration of Nature. In Russia, such societies existed both nationwide and locally, and sometimes they also had their own scientific journals. For example, both Tswett's master's thesis submitted to Kazan University [14] and his 1903 Warsaw lecture [15] were published in the journal of the respective local society.

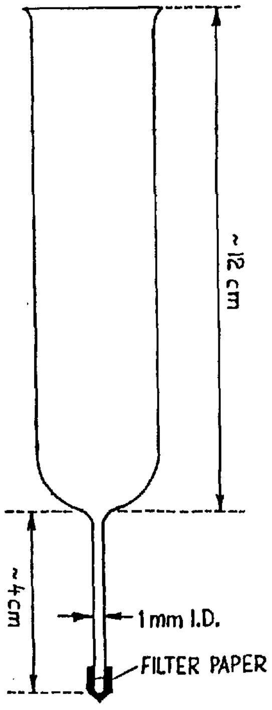

Figure 1
System used in Tswett's first experiments examining the behavior of adsorbents and the separation of pigments. After the description in [15].

filter paper cup to its end (Figure 1) and pressed in firmly and uniformly with a glass rod. Pure ligroin was first passed through the powder column to displace air, then the ligroin solution of chlorophyll was introduced into the tube. Filtration took place under a slight pressure or suction, and adsorption (or the lack of it) could be observed by colour changes.

(2) The chlorophyll solution and the adsorbent were introduced into a small test tube which was then vigorously shaken and centrifuged.

(3) In the case of hygroscopic substances the adsorbent was added to the chlorophyll solution in a mortar and then ground. From then on, version (2) was followed.

All tested substances were able to adsorb at least some of the chlorophyll pigments. The best results were obtained with inulin.[e] When the chlorophyll solution was shaken in the tube with inulin powder (particle size about 2 µm), part of the pigments were immediately adsorbed on the powder which, after precipitation to the bottom, looked green while the supernatant solution was yellow. If more inulin powder was used, the solution became less colored; if a sufficient quantity of inulin were added, only the carotenes remained in solution. Various solvents could be used to liberate the adsorbed pigments from the precipitated inulin powder: best results were obtained with ligroin containing 10 % ethanol.

Calcium carbonate, alumina and a number of other adsorbents gave results similar to those observed with inulin. In other cases, however, different behavior was observed. Some substances adsorbed not only the chlorophyll pigments but also the carotenes and xanthophylls; in other cases, interaction with the solid modified the chemical composition of the pigments. A special case was charcoal which adsorbed the chlorophyll pigments so strongly that they could not be extracted from it even with pure ethanol.

As mentioned above, in this early work Tswett carried out separation in two ways: either by precipitation of the adsorbent retaining the pigments, or by "filtration", having the pigment solution percolating through a short column of the adsorbent. Describing the latter version in Tswett's own words, using inulin as the adsorbent:

> "It is very instructive to observe the adsorption phenomena during filtration through a powder. First a colourless liquid flows out of the bottom of the funnel, then a yellow one (carotene), while a bright green ring forms at the top of the inulin column,[f] below which soon a well defined yellow ring appears. On subsequent washings of the inulin column with pure ligroin both rings, the green and the yellow, are considerably widened and move down the column ... If the powder column through which filtration took place was not sufficiently long to retain all the colouring matter by adsorption, then the yellow ring might reach the bottom opening of the funnel in its descent and a yellow ligroin solution flow out ... There are also differences within the green ring itself, consisting of a deep blue-green lower zone and a yellow-green upper zone."

This is clearly identical to chromatographic separation. However, evidently, Tswett did not decide as yet to propose this variation as the preferred new separation technique. At that time his favoured method was what he called precipitation by differential adsorption and extraction. This was a stepwise method consisting of mixing the pigment solution with the adsorbent and precipitation of the latter with the pigments adsorbed on it, then extracting the individual pigments from the adsorbent with a suitable solvent and carrying out partitioning between two solvents, ligroin and 80 % aqueous ethanol,[g] to isolate the individual pigments. The individual fractions were identified by their UV absorption maxima.

As an example for this method. Tswett illustrated in his lecture the separation of various pigments extracted

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[e] Inulin is a polysaccharide, a starch-like substance, isolated from the tubers of a number of plants such as dahlia or artichoke.

[f] It should be noted that at that time Tswett did not use the expression "column" to describe the tube filled with the adsorbent; we are using this term in the translations for convenience. He generally spoke about the "adsorbent pellet" or the "tube filled with the adsorbent." He first used the term "column" (*Säule*) in his twin 1906 papers; from then on, he also called the tube filled with the adsorbent a "chromatogram."

[g] This method was originally described by Kraus, an early pioneer in the investigation of chlorophylls. Tswett often refers to it as the *Kraus'sche Verfahren* (Method of Kraus). Tswett called the phenomenon "differential dissolution in two-phase systems" and not "partitioning".

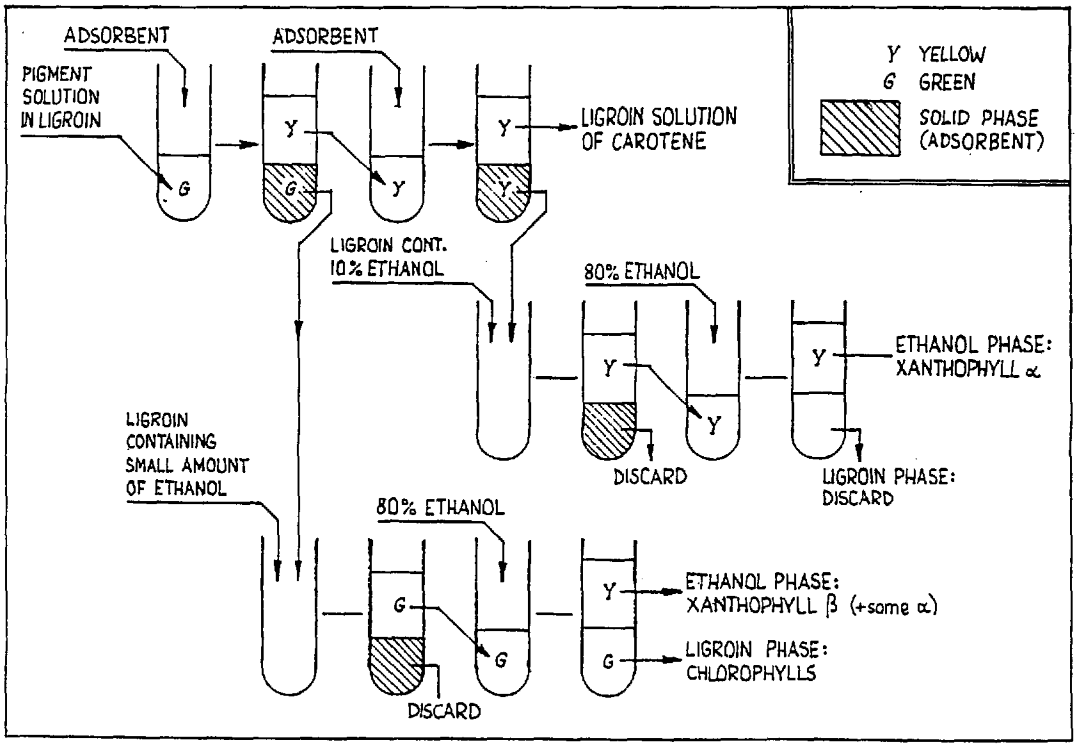

Figure 2

Separation scheme used by Tswett in the stepwise differential adsorptive precipitation and extraction method. After the description in [15].

from leaves. As visualized in Figure 2 this resulted in solutions of pure carotene, xanthophyll α and β, and the chlorophylls.

It should be mentioned here that at the time Tswett carried out his research the identification of fractions of the plant pigments was based mainly on differences in certain physical characteristics (e.g., crystal structure, solubility in different solvents and UV light absorption) and not necessarily on differences in their chemical composition. In general "carotin" (carotene) referred to hydrocarbons and "xanthophyll" to oxygenated compounds; the elementary compositions of these two compound groups were established in 1907 by Willstätter and Mieg, but the structural differences between the various isomers (indicated by suffixes to the general names) were not known until their molecular structures were established in the later 1920s onward. The term "carotenoids" as a collective name for the carotenes, xanthophylls and similar compounds was proposed by Tswett in 1911 [23], but it took about a decade until it was universally accepted. The same is true about the chlorophylls where, in Tswett's time, there was a great controversy concerning the existence of one or more chlorophyllic pigments in plants and whether the different fractions are native or are artifacts, products of chemical treatment. Thus, it is sometimes difficult to exactly identify the individual fractions indicated by Tswett (and others) by different suffixes or names with the chemically defined compounds.

Although Tswett's main interest was always directed toward plant pigments, he also tried to utilize selective adsorption fractionation for the separation of other substances. Thus, we have here the beginnings of chromatography as a universal separation method, without yet using this name.

As a conclusion we can summarize Tswett's 1903 lecture as the key link between his observation of the natural process in plants and the development of a laboratory separation method. It reported on the investigation of over 100 solid substances concerning their potential as adsorbents and described two techniques in which such adsorbents were used to separate plant pigments in the laboratory, with the potential of utilization for the separation of other compounds. At that time Tswett preferred the technique of differential adsorptive precipitation combined with selective extraction and partitioning between two solvents, but the lecture also clearly described the fundamentals of the technique we now call column chromatography, although at that time, he did not yet use this term.

Tswett's 1903 lecture was published only two years later and even then only in a local journal, in Russian [15]. Therefore, his results remained practically unknown until his two major papers were published in 1906 in the widely read transactions of the German Botanical Society. However, in spite of this, the date of presentation of this lecture – March 21, 1903, by our present calendar – should be considered as the birthday of chromatography.

Supplement No. 1. Major events in the life of Michael Semenovich Tswett[1]

February 1, 1829	Semen Nikolaevich Tswett, his father, was born in Chernigov, Malorussia (Ukraine)
1846	Maria de Dorozza, his mother, was born in Kütahya (Turkey), of Italian parents
1870	S. N. Tswett and Maria de Dorozza married
May 14, 1872	Birth of Michael Semenovich Tswett in Asti, Italy, at 11:30 AM; his mother apparently died soon after his birth[2]
1872–1896	Living in Switzerland, first in Lausanne (1872–1885), and then in Geneva
June 17, 1891	Graduates from high school in Geneva
1891–1896	Studying at the University of Geneva, majoring in botany
1894	While a student at the University of Geneva, he receives the Humphry Davy Prize[3] for original research on the anatomy of *Solanaceae*
February 1896	Completes the work for his doctorate thesis in botany and successfully finishes all the necessary examinations
Spring 1896	Moves to Russia, to his father who meanwhile settled in Simferopol (Crimea) as a high official of the Russian Government
July 1896	Mails the text of his doctorate thesis to the University of Geneva; it is published later in this year in the *Bulletins de Laboratoire Botanique Générale de l'Université de Genève* [13][4]
December 1896	Moves to St. Petersburg, starting to work in the laboratories of the Imperial Academy of Sciences; his Swiss doctorate is not accepted in Russia and he has to get new Russian degrees
April 24, 1900	His father dies in Yalta (Crimea)
September 23, 1901	Defends his Russian Master's Thesis at the University of Kazan [14]
October 30, 1901	Presents a paper on "Methods and Tasks of the Physiological Study of Chlorophyll" at the 11th Congress of the Russian Society of Natural Scientists and Physicians, in St. Petersburg
End of 1901	Moves to Warsaw, starting to work at the University as a laboratory assistant
1902	Becomes a "privat dozent" at Warsaw University
Summer 1902	Study trip to Germany; becomes a member of the *Deutsche Botanische Gesellschaft* (the German Botanical Society)
March 8 (21), 1903	Lectures at the meeting of the Biological Section of the Warsaw Society of Natural Scientists; this is the first public disclosure of the chromatographic technique [15]
Summer 1903	Study trip to Germany
September 1903	Applies unsuccessfully for appointment as the head of the Plant Physiology Department at the Institute of Agriculture and Forestry, in Novaya Aleksandriya[5]
1905–1906	Extended study trips to Germany, to the Universities of Berlin and Kiel
June 1906	Submits his two major papers on chromatography to the *Berichte der Deutschen Botanischen Gesellschaft* [16, 17]
May 30 and June 28, 1907	At the meetings of the German Botanical Society, in Berlin, he demonstrates the chromatographic technique and shows pure pigment solutions prepared in this way
August 1907	Becomes a lecturer at the Veterinary Institute of Warsaw
September 16 (29), 1907	Marries in Warsaw Helena A. Trusevich (born on May 14, 1874, in the Russian-occupied part of Poland)
October 1908	Becomes a senior lecturer at the Chemistry and Mining Department of the Polytechnic Institute of Warsaw
1908	Completes the work for his Russian doctorate thesis
1909	Study trip to Germany
December 31, 1909	At the 12th Congress of the Society of Russian Natural Scientists and Physicians, he presents a paper on "A New Physical Method for the Analysis of Pigment Mixtures and its Application to the Investigation of Chlorophylls", and demonstrates the chromatographic separation method

February 1910	The text of his thesis is finally submitted to the printer; it is published later in this year in book form [18]
November 28 (Dec. 11), 1910	Defends his doctorate thesis in Warsaw
1911	Applies unsuccessfully for appointment as professor of botany at Moscow University
1911	Extended study trip to Germany (Berlin), the Netherlands (Amsterdam, Leiden and Delft), Belgium (Brussels) and France (Paris)
December 1911	Receives the M. N. Akhmatov Prize of the Imperial Russian Academy of Science, for his book[6]
1914	Refuses an offer to become professor of botany at the University of Tomsk (Siberia), because of bad climate
Summer of 1914	For health reason, he spends some weeks in the Austrian alps, in Pürgg and Aigen[7]
June 1915	He leaves Warsaw with his wife and mother-in-law for vacation to Odessa. During his absence Warsaw is occupied by German troops[8] and he cannot return anymore there; they temporary move to Moscow
1915	Applies unsuccessfully for appointment as professor of botany at the University of Samara and at Novorossiisk University[9]
August 1916	Assumes his duties at the evacuated Polytechnic Institute of Warsaw, in Nizhii Novgorod
April-August 1917	Stays at Vladikavkaz (Caucasian Mountains) because of health reasons
March 24, 1917	Appointed as professor of botany and director of the Botanical Gardens at the University of Tartu (present-day Estonia)[10]
September 1917	Arrives in Tartu, to start his teaching duties
February 24, 1918	German troops occupy Tartu; temporary, the Russian professors remain in the town
August 31, 1918	Leaves Tartu with the second train evacuating the Russian professors to Voronezh where the university was to be reorganized as a new State University
November 12, 1918	The new Voronezh State University begins its activities
June 26, 1919	M. S. Tswett dies in Voronezh
June 1922	His wife dies[11] in the village of Glebovka, near Abrau-Dyurso, on the eastern shore of the Black Sea, just northwest of Novorossiisk

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[1] This table was mainly compiled from information contained in refs. [2-12]. In general full dates referring to events in Russia are given according to the old Julian calendar which was by about two weeks behind the Gregorian calendar used in the western world. This is the reason why the "Great October Revolution" of 1917 actually happened on November 7. If available, the corresponding date according to the Gregorian calendar is given in parenthesis.

In addition to his study trips, Tswett often spent vacation periods in Western Europe, mostly in Switzerland. These are not enumerated in this table.

[2] After the death of Michael's mother, Semen Nikolaievich Tswett remarried twice. His second wife was Adelaida Aristova, a Russian; they had two children, Natalya (born 1878) and Aleksandr (born 1882). His third wife was Ella Wilhelmina Ebner, a native of Bavaria; they had three children, Nadezhda (born 1889), Vera (born 1891) and Vladimir (born 1892).

[3] The Humphry Davy Prize was established in 1829 by Lady Davy in memory of her husband who died in Geneva.

[4] We do not know the exact date of his doctorate degree but we found information that he received the corresponding document on November 13, 1896.

[5] This town was located in Russian-occupied Poland, about 50 km from Lublin and 140 km from Warsaw.

[6] This prize was established in 1908 to honor selected important and original treatises written by Russians, in the Russian language, in three fields: science (physics and mathematics; chemistry and botany also belonged here), history and philosophy, and Russian language and literature. In each field, one major and three minor awards were given, with cash premiums of 1000 and 300 Rubles each. Tswett received in 1911 the major award in science. This prize was discontinued at the end of the first world war.

[7] Austria declared war on Serbia on July 28, 1914; however, while Germany declared war on Russia on August 1, Austria joined only on August 6. On July 24, Tswett and his wife were still in Austria, in Aigen (on the river Enns, about 60 km southeast of Bad Ischl), but evidently they managed to return to Russia at the last minute.

[8] Warsaw was occupied by German troops on August 4-7, 1915. The Polytechnical Institute was evacuated just before the occupation. Since the Tswett's were already absent, all his private property, manuscripts, scientific notes and library were lost.

[9] Novorossiisk University was not located in the city of Novorossiisk, which is on the eastern shore of the Black Sea, but in the town of Odessa, on its northwest shore: its name indicates that it is the university for "New Russia."

[10] This university was originally founded in 1632 by the Swedish king Gustavus II Adolphus; at that time Livonia (the Baltic shore, including present-day Latvia and Estonia) was part of Sweden and the town's name was *Dorpat*. In 1710 this area was annexed into Russia by Peter the Great and then, the university suspended its operation. It was reorganized in 1802 by Tsar Alexander I as an imperial university and soon it became a highly respected school. In this period the original German/Swedish name *(Dorpat)* or its Russian version *(Derpt)* was used for the town. Teaching language was German, but a sizeable

part of the students (in 1847: 32 %) came from other (non-Baltic) parts of Russia; e.g., S. N. Tswett, the father of M. S. Tswett, also studied here (in 1848–1851). Wilhelm Ostwald, the founder of physical chemistry, was also a student at the University of Dorpat in 1872–1875 and has served here as an assistant until 1881. From 1889 onward, a forced Russification took place, changing the teaching language to Russian; in 1893, the town's name was officially changed to *Yur'ev*, but Dorpat remained in use also. In 1918, during the (temporary) German occupation of the area, the Russian university moved to Voronezh. One year later, after the formation of independent Estonia, the University of *Tartu* (the Estonian name of the town) was reorganized, now as an Estonian university, and it remained this since then, even in the period of 1944–1989, when Estonia was part of the Soviet Union.

[11] She killed herself.

Supplement No. 2. Substances investigated by Tswett as potential adsorbents*

Elemental materials:	
	aluminum [Al], antimony [Sb], iron [Fe], lead [Pb], magnesium [Mg], silicon [Si], sulfur [S] and zinc [Zn]
Inorganic compounds:	
Oxides	*aluminum [Al_2O_3]*, antimony [Sb_2O_3], iron [Fe_2O_3], lead [PbO], magnesium [MgO], *manganese [MnO_2]*, mercury [HgO], *phosphorus [P_2O_5]*, silicon [SiO_2], silver [Ag_2O], uranium [U_3O_8] and *zinc [ZnO]* oxides; magnesium peroxide [MgO_2]
Hydroxides	aluminum [$Al_2O_3 \cdot H_2O$], barium [$Ba(OH)_2$], boric [$B(OH)_3$] and sodium [NaOH] hydroxides; soda-lime [$Ca(OH)_2 + NaOH$]
Halides	aluminum [$Al_2Cl_6 \cdot 12H_2O$], ammonium [NH_4Cl], barium [$BaCl_2 \cdot 2H_2O$], calcium [$CaCl_2$, dry], cobalt [$CoCl_2 \cdot 6H_2O$], copper [$CuCl_2 \cdot 2H_2O$], iron [$FeCl_2 \cdot 4H_2O$], magnesium [$MgCl_2 \cdot 6H_2O$], mercury [$HgCl_2$], potassium [KCl, dried at 180 °C] and sodium [NaCl] chlorides; potassium bromide [KBr]; mercuric [HgI_2] and potassium [KI] iodides
Sulfides	mercury [HgS] and potassium [K_2S] sulfides
Chlorates and iodates	barium [$Ba(ClO_3)_2 \cdot H_2O$] and potassium [$KClO_3$] chlorates; potassium iodate [KIO_3, dried at 180 °C]
Nitrites	*potassium [KNO_2]* nitrite
Nitrates	barium [$Ba(NO_3)_2$], calcium [$Ca(NO_3)_2 \cdot 4H_2O$ and dry], copper [$Cu(NO_3)_2$, dried], lead [$Pb(NO_3)_2$, dried at 180 °C], potassium [KNO_3, dried], silver [$AgNO_3$] and uranium [$UO_2(NO_3)_2 \cdot 6H_2O$] nitrates
Phosphates and molybdates	ammonium [$(NH_4)_3PO_4$], calcium [$Ca_3(PO_4)_2$], iron [$Fe_2(PO_4)_2 \cdot 4H_2O$], potassium [KH_2PO_4 and K_2HPO_4] and sodium [NaH_2PO_4 and Na_2HPO_4] phosphates; ammonium molybdate [$(NH_4)6Mo_7O_{24}$–$4H_2O$]
Permanganates	potassium [KMnO4] permanganate
Carbonates	calcium [$CaCO_3$], *copper [$CuCO_3 \cdot Cu(OH)_2$]*, iron [$FeCO_3$], magnesium [$(MgCO_3)_4 \cdot Mg(OH)_2 \cdot 5H_2O$], potassium [$K_2CO_3$, dry] and sodium ($Na_2CO_3$, dry] carbonates
Sulfates	ammonium [$(NH_4)_2SO_4$], barium [$BaSO_4$], calcium [$CaSO_4 \cdot 2H_2O$ and dry], copper [$CuSO_4 \cdot 5H_2O$ and dry], iron [$FeSO_4 \cdot 7H_2O$ and dry], magnesium [$MgSo_4$, dry], manganese [$MnSO_4 \cdot 7H_2O$], potassium [$K_{2S}O_4$] and zinc [$ZnSO_4 \cdot 7H_2O$] sulfates; ammonium copper sulfate [$(NH_4)_2Cu(SO_4)_2$]; sodium bisulfate [$NaHSO_4$]
Sulfites	*sodium [$Na_2SO_3 \cdot 7H_2O$]* sulfite; sodium [$NaHSO_3$] bisulfite
Silicates	potassium silicate [K_2SiO_3; dried]; asbetos
Cyanides	potassium ferro- and ferricyanides
Organic compounds:	
Acids	boric, citric, oxalic, picric, quinic, tannic, tartaric and uric acids
Salts of organic acids	lead and copper acetates; ammonium and magnesium oxalates; ferric saccharate
Amides	asparagine; urea
Phenols	hydroquinone, phenolphthalein, pyrogallol and resorcinol
Aldehydes	paraformaldehyde

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* Compounds given in Italics were added in Tswett's book [18] to the original list of his 1903 lecture [15]. The chemical formulae of the individual compounds are given according to the listing in [18].

Polyols	chloral hydrate; dulcitol (galactitol) and mannitol
Carbohydrates	dextrin, galactose, saccharose (sucrose), cellulose (filter paper), inulin and starch
Proteins	hemoglobin, ovalbumin, pepsin, peptone; egg white
Alkaloids	cinchona, quinine
Dyes	carmine, chrysoidine, fluorescein, gentian violet
Miscellaneous substances:	
	emery, garden soil, glass wool, *Kieselgur,* charcoal made of bone and blood, salicin

References

Titles in English, French or German are given in the original language; titles in Russian are given both in Russian and in English translation. Transliteration from Cyrillic is according to the rules of *Webster's New World Dictionary* which are practically identical to the rules of *Chemical Abstracts.* The only exception is the name of *Tswett* which we spell here in the way he used to do it in his non-Russian publications: according to the correct transliteration, it should be spelled as *Tsvet.* In the case of Russian publications, no abbreviations are used.

[1] *A. J. P. Martin,* "The Development of Partition Chromatography" (Nobel Lecture, December 12, 1952). In Nobel Lectures Including Presentation Speeches and Laureates' Biographies: Chemistry, 1942–1962. Elsevier, Amsterdam, 1964; pp. 359–371.

[2] *Ch. Dhéré,* "Michael Tswett – le Créateur de l'Analyse Chromatographique par Adsorption; sa Vie, ses Travaux sur les Pigments Chlorophylliens." Candollea (Genève) **10,** 23–73 (1943).

[3] *T. Robinson,* "Michael Tswett." Chymia **6,** 146–161 (1960).

[4] *K. I. Sakodynskii,* "M. S. Tswett – His Life." J. Chromatogr. **49,** 2–17 (1970).

[5] *K. I. Sakodynskii,* "The Life and Scientific Work of Michael Tswett." J. Chromatogr. **73,** 303–360 (1972).

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[7] *K. I. Sakodynskii,* "New Data on M. S. Tswett's Life and Work." J. Chromatogr. **220,** 1–28 (1981).

[8] *E. M. Senchenkova,* Mikhail Semenovich Tswett. Nauka Publisher, Moscow, 1973.

[9] *K. I. Sakodynskii,* Michael Tswett – Life and Work. Carlo Erba Strumentazione, Milan, undated (1981).

[10] *L. S. Ettre,* "Evolution of Liquid Chromatography: a Historical Overview." In *Cs. Horváth* (editor), High-Performance Liquid Chromatography – Advances and Perspectives, Vol. 1, Academic Press, New York, NY, 1980; pp. 1–74.

[11] *I. M. Hais,* "Tswett's Letters to Claparède." J. Chromatogr. **440,** 509–531 (1988).

[12] *K. Siilivak* (editor), History of Tartu University 1632–1682. Perioodika, Tallinn, 1985.

[13] *M. Tswett,* "Etudes de Physiologie Cellulaire: Contribution à la Connaissance des Mouvements du Protoplasme, des Membranes Plasmiques et des Chloroplastes" (Dissertation). Bull. Lab. Bot. Gén. Univ. Genève **1** (No. 3), 125–206 (1896).

[14] *M. S. Tswett,* "Fiziko-Khimicheskoe Stroenie Khlorofil'nogo Zerna. Eksperimental'noe i Kriticheskoe Izsledovanie" (The Physico-chemical Structure of the Chlorophyll Particle. Experimental and Critical Study). Trudy Obshchestva Estestvoispytatelei pri Imperatorski Kazanskom Universitet **35** (No. 3), 1–268 (1901). A summary of this thesis, written by Tswett, was published in Botan. Centralbl. **89,** 120–123 (1902).

[15] *M. S. Tswett,* "O Novoi Kategorii Adsorbtsionnykh Yavlenii i o Primenenii ikh k Biokhimicheskomu Analizu" (On a New Category of Adsorption Phenomena and their Application to Biochemical Analysis). Lecture at the March 8 (21), 1903, meeting of the Biological Section of the Warsaw Society of Natural Sciences. Trudy Varshavskogo Obshchestva Estestvoispytatelei; Otdelenie Biologii **14,** 20–39 (1905). For English translation see refs. [19] and [20a].

[16] *M. Tswett,* "Physikalisch-chemische Studien über das Chlorophyll. Die Adsorption." Ber. dtsch. botan. Ges. **24,** 316–323 (1906). For English translation see ref. [20b].

[17] *M. Tswett,* "Adsorptionsanalyse und chromatographische Methode. Anwendung auf die Chemie des Chlorophylls." Ber. dtsch. botan. Ges. **24,** 384–393 (1906). For English translation see refs. [20c] and [22].

[18] *M. S. Tswett;* Khromofilly v Rastitel'nom i Zhivotnom Mire (Chlorophylls in the Plant and Animal World). Karbasnikov Publishers, Warsaw, 1910. For partial English translation see ref. [20d].

[19] *G. Hesse, H. Weil* (editors), Michael Tswett's First Paper on Chromatography. M. Woelm, Eschwege, 1954.

[20] *V. G. Berezkin* (editor), Chromatographic Adsorption Analysis: Selected Works of M. S. Tswett. Ellis Horwood, New York, London, 1990; (*a*) pp. 9–19; (*b*) pp. 21–26; (*c*) pp. 27–34; (*d*) pp. 35–79.
It should be noted that (although this is not specifically mentioned in it) the translations of Tswett's Russian papers for this book were not prepared from the originals but from a Russian reprint edition published in 1946 [21]; even the titles of the two books are the same.

[21] *A. A. Rikhter, T. A. Krasnosel'skaya* (editors), M. S. Tswett: Khromatograficheskii Adsorbtsionnyi Analiz. Izbrannye Roboty (M. S. Tswett: Chromatographic Adsorption Analysis. Selected Works). Izdatel'stvo Akad. Nauk S.S.S.R., Moscow, 1946.
This book contains the text of Tswett's 1903 lecture [15], the Russian translation of the two fundamental papers of 1906 [16, 17] and the first part of Tswett's 1910 book [18] dealing with the analytical methods and their underlying physical chemistry, along with some biographical material, a listing of Tswett's publications and a discussion of chromatography since Tswett.

[22] *H. H. Strain, J. Sherma,* "Michael Tswett's Contributions to Sixty Years of Chromatography." J. Chem. Ed. **44,** 235–242 (1967).

[23] *M. Tswett,* "Über den makro- und mikrochemischen Nachweis des Carotins." Ber. dtsch. botan. Ges. **29,** 630–636 (1911).

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