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Key moments in the evolution of liquid chromatography

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ABSTRACT

The paper is focusing on the work and activities of a few key scientists, from the very beginning to the mid-1940s, who had a key role in the evolution of liquid chromatography, and who laid the foundation on which present-day high-performance liquid chromatography could be developed.

INTRODUCTION

Today, with all the excitements related to the new developments, it is worthwhile to stop for a second and recall the work and activities of the pioneers in our discipline. Obviously, I would not have time to discuss the evolution of liquid chromatography from its inception to this symposium. Rather, I want to deal with the activities of a few people. In their selection I try to follow the principle used by Stephan Zweig (1881–1942), an Austrian writer famous mainly for his biographies. In one of his books entitled *Die Sternstunden der Menschheit*[a] [1] he tried to deal with the "star moments" of the human race: with key situations when, for a moment, the decision of a single person had a major influence on future events. Such a case was for example near Waterloo, on that faithful morning of June 18, 1815, when the French General Grouchy, whose orders were to find the army of the Prussian General Blücher, heard that a battle is under way, just a few miles from his troops. However, instead of turning around and joining Napoleon —which would undoubtly have tilted the balance in his favor— he continued to march to nowhere. He missed Blücher's troops who reached Waterloo at the decisive moment and were aiding Wellington in the final defeat of Napoleon.

In this paper, I am also dealing with such *Sternstunden,* key moments. I will discuss the activities of five people or groups who, at a certain moment during their professional life, were faced with a decision to select the proper method for the solution of their problem, and their decision influenced the future of chomatography.

[a] This is an almost untranslatable title. The English edition published in 1940 translated it as *The Tide of Fortune*. However, in my opinion, this translation of the original German title does not reproduce its real meaning.

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D. T. DAY AND THE "FILTRATION THEORY"

Let us start with David Talbot Day (1859–1925). He was a graduate of The Johns Hopkins University and has served between 1886 and 1914 as one of the principal scientists of the United States Geological Survey. This is the early period in the evolution of the American petroleum industry when the major petroleum fields were in Pennsylvania and Ohio. At that time a theory had been developed according to which there existed a "primary petroleum" which then migrated through the earth. During this migration the higher-boiling compounds were retained by the limestones and shales. Thus, some of the petroleum wells, located closer to the source of the primary petroleum will contain more high-boiling components than the petroleum of other wells further down from the original source, which are enriched in the lower-boiling components. This was called as the "filtration theory" and Day was one of its proponents. In 1897 in a paper entitled "A suggestion as to the origin of Pennsylvania petroleum" [2] he even suggested that the validity of this theory could be easily demonstrated by some experiments, which, of course, we would call today as frontal chromatography:

> *"... by experimental work it may be easily demonstrated that if we saturate a limestone such as the Trenton limestone with the oils characteristic of that rock and exert slight pressure upon it, so that it may flow upward through finely divided clay, it is easy to change it in its color to oils similar in appearance to the Pennsylvania oils, the oil which filters through being the lighest in color and the following oils growing darker."*

In the next years Day evidently carried out some experiments, with partial success; encouraged by these results, he presented a paper at the *First World Petroleum Congres* held in Paris, August 16–28, 1900, in conjunction with the International Exposition (where he was in charge of the petroleum exhibits of the U.S.A.). Day's paper was entitled "The variation in the characters of the crude oils of Pennsylvania and Ohio" and it was published in 1902, in the proceedings of the congress [3]; it is a relatively short paper, less than four printed pages long, written in a highly personal, first-singular mode and in a very eloquent French. Contrary to some reports[a] the "filtration experiments" are described only in general terms stating that

> *"the practical value of the process lies in the promise which inspired us to develop a scientific process for the separation of the various oils with which we now deal",*

without giving any information on the system he may have used, or on any results. The key statement in Day's paper is that

> *"the filtration method offers good hope; I wish to communicate to the world of scientists as the voice of the Congress, and I believe that before the next winter, I will be able to accomplish complete separations."*

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[a] For example, Weil [4] refers to this paper as one which supposed to have shown partial separation of petroleum fractions. However, his English translation of the quoted key sentence significantly differs from the original French text.

In the decade after the congress Day and his associates and colleagues published a few papers [5–8] showing that if crude oil is passed through a column (they used tubes about 5.5 ft. long, with 1.25 in. I.D.) packed with Fuller's earth or clay, then there will be some differences in the physical properties (specific gravity and boiling range) of the fractions present at different distances along the column. However, there is no mentioning in these papers on any "complete separation" of various petroleum constituents; and even Day himself, in an 1911-paper summarizing his activities in the last 15 years [9], does not mention at all that real separation would have achieved.

Obviously Day was very close to "chromatography" and even the system used by him is not much different than the one used by Tswett about the same time (although Tswett's columns were shorter and generally had a smaller internal diameter). Tswett also relied on the separated fractions remaining on the column; we are still about 30 years away of collected fractions. The major differences were that Tswett carried out elution chromatography, with a finite sample dissolved in a solvent, and used a mobile phase for elution, while Day and his colleagues simply sucked the whole oil through the column; and that, by the skillfull selection of the adsorbent and the solvents, Tswett could separate pure compounds while Day only achieved some distribution of the petroleum's components along the column. Here is our key moment: Day was at the treshhold of inventing a new separation method. As correctly stated by Zechmeister [10] —himself a pioneer in chromatography— Day and his colleagues "might well under favorable circumstances developed (the filtration experiments) into systematic chromatography." However, this did not happen and thus, chromatography was invented by another scientist, M. S. Tswett, a contemporary of Day.

M. S. TSWETT AND THE DISCOVERY OF THE CHROMATOGRAPHIC SEPARATION TECHNIQUE

We now arrived at M. S. Tswett (1872–1919), the true inventor of liquid chromatography. Tswett's life and activities have been the subject of many papers and even books (see, *e.g.*, refs. 11–17); what I want to discuss here is the important moment in his life which represented the difference between playing in his laboratory or becoming the esteemed inventor of a new technique.

By his job description. Tswet was a botanist (this is what he was teaching between 1902 and 1916 in Warsaw) although I would rather consider him a physicochemist. He was interested in plant pigments and he tried to isolate them from the plants; but he also realized that there are many compounds present simultaneously and thus, not only isolation of a single compound is needed but also separation of the compounds present together. Being a botanist by training, he was also interested in the natural systems represented by the plants in which these pigments are present. He considered adsorption as one of the natural forces fixing the chloropast pigments in plants and thus, his interest turned to this process and to the behavior of the various adsorbents. He postulated that the reason why polar solvents can extract the pigments from plant material while non-polar solvents cannot is that the former can break down the original adsorption complex while non-polar solvents do not have this ability. He even carried out some model experiments showing how pigment mixtures became adsorbed to various materials; as the next step he tried to utilize controlled adsorption to separate pigments extracted from plants.

Evidently Tswett started to utilize the technique we now call chromatography around 1902. We have the text of a lecture he presented in March 1903, at a meeting of the Biological Section of the Warsaw Society of Natural Sciences, entitled "On a new category of adsorption phenomena and their application to biochemical analysis" [18]. He used inulin as the adsorbent, packed in a small column. A ligroin solution of a plant extract was added onto the column and washed down with the solvent, creating well-defined and separated green and yellow rings. Obviously this has already been chromatography, although in the paper, Tswett did not use this name as yet.

It is interesting to note that although Tswett had a number of publications in western (German and French) journals in this period, he did not describe the new technique in them. It is difficult to explain the reason for this: either he did not feel it ready for publication, or considered it just a routine laboratory technique, not important enough for publication. Let me repeat: Tswett was a botanist, dealing with plant pigments, and not with separation methods, and most of his western publications were in botanical journals.

He finally changed his attitude in 1905. In September of that year Tswett published a paper [19] in which he criticized a report [20] on the pigments of brown algae written by Hans Molisch (1856–1937), an important and highly respected botanist in the Austro-Hungarian Monarchy who, at that time, was professor at the University of Prague and head of the Institute of Plant Physiology. In this paper Tswett mentioned that his more correct data were obtained with help of a "new, reliable method" he developed during the past few years. A controversy evolved in which everybody important in this field ridiculed Tswett's comments; after all, he did not give any details. In his reply [21] Tswett rejected the arguments of his opponents and promised that he will now really publish a report on his method; and indeed, within a month, in June 1906, he submitted two papers to the Bulletins of the German Botanical Society which represent the fundamental description of the chromatographic separation method. Due to their importance we should list here the titles of these two papers: "Physical-chemical studies on the chlorophyll. The adsorptions" [22], and "Adsorption analysis and the chromatographic method. Application to the chemistry of chlorophyll" [23].

This was the moment when Tswett had to make a decision: keep his method for himself or disclose it to the international scientific community. He selected the latter and, of course, the rest is history: chromatography was born.

L. S. PALMER: CHROMATOGRAHY EXPLAINING WHY THE BUTTER IS YELLOW

In the next 25 years, Tswett had only a few followers from whom I mention three, two in Europe and one here in the United States. The two Europeans are Charles Dhéré (1876–1955) at the University of Fribourg, in Switzerland, and Theodor Lippmaa (1829–1944) at the University of Tartu, in Estonia. For both Dhéré and Lippmaa chromatography was simple a tool for separation, they did not even describe the technique in their publications; therefore, from the point of the evolution of chromatography, their activities are only secondary. There was, however, one American scientist in this period who had a key role both as an original researcher expanding the utilization of chromatography and as the transmission between Tswett and the next generation. He is Leroy Sheldon Palmer (1887–1944).

Leroy Sheldon Palmer graduated in 1910 as a chemical engineer at the University of Missouri, in Columbia; however, in graduate school he switched to agricultural chemistry. After receiving his Ph.D., in 1913, Palmer joined the faculty of the College of Agriculture at the University of Missouri; in 1919, he moved to the University of Minnesota where he became Professor of Agricultural Biochemistry and later the head of that Division. During his long and distinguished career Palmer became one of the most important scientists of his time in the field of dairy chemistry and nutrition.

Palmer started to work on his Ph.D. thesis in 1910, finishing it in 1913. The work dealt with the carotenoid pigments in milk and milk products and their relationship to the pigments present in the food intake of the animal. He started these investigations at the time when the information available was very little; let us not forget that, *e.g.*, even the elementary composition of "carotin" and "xanthophyll" were established only in 1907 by Willstätter and Mieg [24] as $C_{40}H_{56}$ and $C_{40}H_{56}O_2$ respectively. At that time no structural information was available except that "carotin" is a hydrocarbon and "xanthophyll" has hydroxy groups. Even the fact that we are faced with a group of similar compounds was not a generally accepted fact. The name "carotenoids" was proposed in 1911 by Tswett [25] to indicate their apparent close relationship to the most important member of the group, but the final nomenclature of these compounds was not established until the late 1930s.

It is simply amazing to recognize how quickly Palmer adopted Tswett's method, chromatography, for his own research. He started his graduate research only four years after Tswett's publications and he fully utilized it for the separation of the various pigments present in milk and milk products. In this thesis [26] which was also published in four parts in the *Journal of Biological Chemistry* [27], coauthored with C. H. Eckles (1875–1933), the head of the Department of Dairy Husbandry at Columbia, he described in detail the technique and showed a number of practical applications.

Palmer's graduate work represents without any question the first systematic investigation to show that there is a definite relationship between the yellow pigment present in the plants and in the animals' fat. In fact he made it clear that the plant pigments are directly transferred into the animals' fat: in other words, the animals do not produce them. In connection with this work, I should mention an interesting finding. Recently, in a storage room of the University of Missouri, in Columbia, we have found a large poster, obviously prepared by Palmer in the 1913–1919 period [28]. It had the title "why the butter is yellow" and explained in a popularizing way that the grass the cows are eating contain among others yellow pigments which are transferred into the milk and milk products, causing the yellow color of the butter. Most likely this poster was prepared as part of the exhibition of the University's School of Agriculture or of the Missouri Agricultural Experient Station at various county fairs.

Another interesting point in Palmer's work was that —similarly to Tswett— he used ultraviolet spectroscopic measurements for the identification of the pigments present in the individual fractions of various samples. If two fractions occurring in different samples had the same absorption maxima, he considered them being chemically identical. In this, again, he was ahead of his time: UV identification of the chromatographic fractions was systematically used only over 20 years later, in the laboratory of Richard Kuhn, in Heidelberg.

In the years following his graduation Palmer continued to be engaged in

investigations relating the pigments present in the animal feed to the pigments present in milk and milk products, and further refining the methodology used for the separation of these pigments. This work finally culminated in a book on carotenoids [29] in which he again gave a detailed description of the chromatographic technique. This book is particularly important because, as we shall see below, Edgar Lederer learned chromatography from it.

As explained in the Introduction, our criterion for inclusion in this discussion was whether, at one point during his activities, a person had a key moment, facing the need for a decision which, in turn, significantly influenced the evolution of chromatography. This was certainly the case with Palmer: when he started his investigations on the pigments present in plants and in milk and milk products, chromatography, a very new and yet unproven technique, was certainly not the obvious choice: he could have utilized the techniques used at that time by Willstätter and others studying these pigments, which were based on selective extraction and crystallization. In this respect it is worthwhile to quote from a paper by Schertz of the U.S. Department of Agriculture (*i.e.*, another agricultural chemist), published in 1929 [30]:

> *"While Tswett's methods have been shown to be unreliable in identifying and distinguishing carotin and xanthophyll, the methods of Willstätter have been shown to be more reliable for this purpose."*

Even 20 years after Palmer's investigations, the reliability of chromatography was questioned!

To be fully objective, I must also mention that Palmer has another *Sternstunde*, key moment, in his later professional life, but there, he apparently did not recognize the opportunity. This refers to his nutrition studies: for a long time Palmer refused to accept the physiological and chemical relationship between the carotenoids and vitamin A, and he did not believe that vitamin A can be produced in the animal's body from carotene. This discovery was done in the late 1920s and early 1930s by Hans Euler-Chelpin (Stockholm University), Thomas Moore (Dunn Nutrition Laboratory, Cambridge, U.K.) and Paul Karrer (University of Zürich, Switzerland). Palmer had years earlier many data indicating this, but he did not consider them conclusive.

EDGAR LEDERER AND THE REBIRTH OF CHROMATOGRAPHY

By the late 1920s, chromatography was almost forgotten in Europe. Its "rebirth" can be accredited to Edgar Lederer, in the laboratory of Richard Kuhn, in Heidelberg, and we have here again a *Sternstunde* in the evolution of chromatography. The story had been told a number of times (see, *e.g.*, refs. 31–33); therefore, just a brief summary is given here.

Edgar Lederer (1908–1988) was born and studied at Vienna, Austria. After receiving his Ph.D., he joined in September 1930 the Institute of Chemistry of the Kaiser Wilhelm Institute for Medical Research at Heidelberg, Germany, headed by Professor Richard Kuhn, a pupil of Willstätter. There, Lederer's first job was to investigate whether the yellow pigment in egg yolk is not a mixture of some pigments found in plant material. As usual with young postdocs, Lederer carefully checked the literature the most important of which was Palmer's carotenoid book [29], and in it, he

found reference to Tswett, description of his chromatograhic separation method and illustrations for its possible use. Continuing his search, he also found a personal copy of the German translation of Tswett's 1910 book [34] (prepared specially for Willstätter). Based on all this information, Lederer decided —and this is that fateful moment— to try out chromatography, a technique which up to then was not used by anybody at Heidelberg.

In December 1930, he prepared a small (15 cm × 1 cm I.D.) column containing powdered calcium carbonate, and added the solution of a pigment mixture in carbon disulfide. By adding additional solvent to the top of the column, four well-separated rings were obtained. This experiment was then followed by others, separating the xanthophylls of egg yolk. The results were summarized first in a single-page report [35] followed by two detailed papers [36, 37], representing the rebirth of chromatography. From here on, the evolution of our technique is uninterrupted and straightforward.

THE MANHATTAN PROJECT AND THE PREPARATIVE SEPARATION OF RARE EARTHS BY ION-EXCHANGE CHROMATOGRAPHY

As the last example for a key development in he evolution of chromatography a little known application of the technique is mentioned which was the first application of chromatography for preparative (or, probably, more correctly, for production) purposes.[a] Here, again, we have a case where a problem evolved and the scientists involved in it had to make a decision how to solve it. Chromatography was not the obvious solution, but nevertheless it was a right decision.

We have to go back 50 years, to the start of the Manhattan project. Obviously this project consisted not only of the direct development of the atomic bomb, but there were also many parallel investigations on various theoretical and practical aspects. One of these had to do with the need to identify the fission products of uranium which in turn led to the project dealing with the separation of rare earth elements. This work started in 1942 at the Metallurgical Laboratory of the University of Chicago, but within a year moved to Clinton National Laboratories at Oak Ridge, Tennessee (the present-day Oak Ridge National Laboratories). From the many possibilities they decided to try the new synthetic ion-exchange resins which were commercially available just about that time (see *e.g.*, the work of Samuelson [38, 39]). The project was very difficult and also had to deal with basic investigations on ion exchange. They advanced rapidly and by December 1944, a second group at the Institute for Atomic Research at Iowa State College, at Ames, Iowa (the present-day Iowa State University), headed by F. H. Spedding, started to use ion-exchange chromatography on the preparative scale.

Obviously, because of the confidential nature of the work, nothing could be published during the war: they received the green light for this only after the war. Their first report was at a special Symposium on Ion-Echange Separations held during the Fall 1947 National Meeting of the American Chemical Society, in New York City, followed by the publication of 13 articles as a single issue of the *Journal of the American*

[a] Both Tswett and Lederer had already used chromatography for the preparation of pure fractions. However, their work involved only relatively small (up to maybe a few grams) quantities.

TABLE I

PURE RARE EARTHS PREPARED AT THE INSTITUTE FOR ATOMIC RESEARCH, IOWA STATE COLLEGE- AMES, IOWA, AS PART OF THE MANHATTAN PROJECT

After Spedding *et al.* [40,43].

Element		Quantity prepared (g)	Purity[a]
No.	Name		
59	Praseodymium	35	99%
		160	90%
60	Neodymium	800	99.9%
		770	98%
62	Samarium	160	>99.9%
		600	99%
69	Thulium	15	"Very rich"
70	Ytterbium	300	"Pure"
71	Lutetium	15	"Spectroscopically pure"

[a] Purity expressed as percent rare earth oxide.

Chemical Society in November 1947 of which one paper [40], by Spedding's group, discussed the "pilot-plant scale separations" of rare earths.

The "chromatographic plants" at Ames, Iowa, consisted of 24 columns, each 10 ft. long, with 4 in. diameter, and as one sample, 100 g of crude rare earth salts were introduced into each column. For one such sample over thousand liters of mobile phase (a 0.5% aqueous citrate solution, with a velocity of 0.5 cm/min) were used. The individual fractions were collected and then the halids of the rare earths were recovered and transformed into the rare earth metals by thermal reduction with calcium. In order to have some idea of the extent of these activities, in Table I, I list the amounts of the pure rare earths prepared during the project.

One of the most important chromatographic meetings ever happened was organized in September 1949, in England, by the Faraday Society [41]. At this meeting 43 papers were presented; from these two were given by members of the Manhattan Project team; the first, presented by Tompkins of Oak Ridge, dealt with the theoretical aspects and the use of ion-exchange chromatography for the separation of rare earth salts in very low concentrations [42], while the paper by Spedding reported on the large-scale separation of rare-earth salts and the preparation of the pure metals [43]. The highlight of the meeting was when Spedding opened his briefcase and took out rods of pure rare earth metals, showing them to the audience.

EPILOGUE

With this, we arrived close to our time. I could continue by pointing out additional key moments in the evolution of liquid chromatography such as, *e.g.*, the fundamental work by Martin and Synge published in 1941 and first describing liquid–liquid partition chromatography [44] for which they received the 1952 Nobel Prize in Chemistry; the development of paper chromatography in 1944 by Consden, Gordon and Martin [45]; or the first description of reversed-phase chromatography, in

1950, by Howard and Martin [46] and of gradient elution by Tiselius' group, in 1952 [47]. I could also deal with the pioneering work done in the mid-1960s by J. F. K. Huber [48] and Csaba Horváth [49] leading to HPLC [50]. But these are much too recent developments, and I leave their discussion to the next chronicler.

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