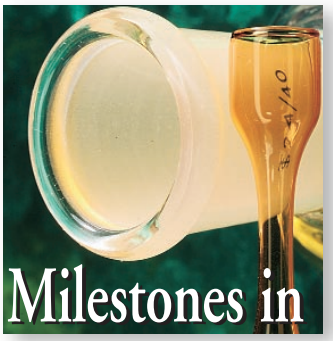


The Birth of Partition Chromatography

On the occasion of the 60-year anniversary of the invention of partition chromatography and the 50-year anniversary of the introduction of gas–liquid partition chromatography, this installment of "Milestones in Chromatography" discusses the circumstances that led to these developments and outlines the work of A.J.P. Martin, R.L.M. Synge, A.T. James and their co-workers, which opened new chapters in chromatography.

Leslie S. Ettre
Milestones in Chromatography Editor

In June of 1941, almost exactly 60 years ago, the (British) Biochemical Society held its 214th meeting in London, at the National Institute for Medical Research. At this meeting, A.J.P. Martin and R.L.M. Synge, two young chemists (Martin was 31 and Synge 26) presented a paper on the separation and determination of the mono-amino monocarboxylic acids present in wool using a new method (1). This lecture and its subsequent detailed publication represent the birth of partition chromatography (2).

Exactly 10 years later, Martin and another young scientist, 29-year-old A.T. James, submitted the manuscript of a major paper that described an extension of partition chromatography in which the mobile phase was a gas (3). This publication represents the birth of gas–liquid partition chromatography (GLPC).

On the occasion of these two anniversaries, we shall discuss in this "Milestones in Chromatography" column these milestones, probably the most important in the long evolution of chromatography since its discovery by M.S. Tswett almost 100 years ago. Without these inventions, this magazine would not exist and most of our readers would have some other job, maybe titrating or trying to isolate chlorophyll from some hundreds of kilograms of dried stinging nettle, as done in Willstätter's laboratory 96 years ago (4).

The invention of partition chromatography and the development of GLPC are fascinating stories. Fortunately, they are recorded fairly well in the Nobel Lectures of Martin (5) and Synge (6) and in the personal recollections of the principal players (7–10). I also had the honor of personally knowing them, discussing with them a number of times the background of their pioneering work. This column is based on these publications and personal information.

The Start at Cambridge University

Both Martin and Synge were students at Cambridge University in England — Martin graduated in 1932 and Synge in 1936. When still in high school, Martin became fascinated by fractional distillation and even built in the basement of his house some long distillation columns from empty coffee cans, soldered together. He originally planned to be a chemical engineer, but at Cambridge he changed to biochemistry upon the influence of Professor J.B.S. Haldane. As a teenager, Synge had already become fascinated by how living things functioned and, thus, he also majored in biochemistry at Cambridge.

After graduation, both Martin and Synge remained at Cambridge as graduate students, although their paths had not yet crossed. Martin joined the Dunn Nutritional Laboratory and was involved in research on vitamin E. He started separating carotenes by distribution between two solvents using separating funnels. Being always interested in engineering, he eventually built a very complicated laboratory machine, consisting of 45 5-foot-long tubes connected to one another and serving as the extraction funnels: 90 ball valves rattling loudly on their seats prevented the liquid from dropping back to the previous tube. In this machine (details of which have never been published but were only included in his doctoral thesis) Martin could carry out very efficient countercurrent extraction.

Meanwhile, Synge was active at the university's biochemical laboratory, and in 1938 he was offered an unusually generous scholarship by the International Wool Secretariat (IWS). Interestingly, both Martin and Synge had seen demonstrations of the potential of adsorption chromatography during this time. Martin participated in a lecture by Dr. Winterstein of Kuhn's laboratory (in Heidelberg, Germany) showing a chromatographic separation on a short $CaCO_3$ column. Although Martin immediately recognized the similarity between the chromatographic technique and the theory of distillation, he did not further pursue this subject at the time. However, he evidently stored this observation in his memory. About the same time, Synge also saw a demonstration of the chromatography of sea urchin pigments. According to his recollections, "Everybody

stood around and goggled at the pretty colors but no explanation was forthcoming from anyone present for how the undoubted separations had come about." Thus, it remained just a curiosity to him, inspiring no further interest in the technique.

Beginning of the Cooperation Between Martin and Synge

The IWS was maintained by the wool growers of Australia, New Zealand, and South Africa. Among other things, its aim was to fund research on various aspects of wool. It was proposed to Synge to study in detail the amino acid composition of wool, and also to try improving the methods of amino acid analysis, which were still done on a fairly primitive basis. This suggestion fit well his research activities at that time in the field of glycoproteins. One of the methods used was to acetylate digested egg albumin and remove the *N*-acetyl amino acids and peptides from the carbohydrate moiety by exhaustive extraction with chloroform.

Beginning his activities under the auspices of the IWS, Synge first measured the partition coefficients of acetyl amino acids between two phases: chloroform and water. Encouraged by the distinct differences found, the next logical step would have been to try to carry out separation by liquid–liquid extraction. At that time it was suggested to Synge to contact Martin, who obviously had a lot of experience in the extraction process and whose unorthodox large glass machine for countercurrent distribution was well known in the chemistry and biochemistry circles of Cambridge. With this step, their five-year and very successful cooperation started.

The Birth of Partition Chromatography

Because Martin's existing apparatus was not suitable for use with a chloroform–water system, he designed a completely different machine, now for continuous-flow, liquid–liquid countercurrent extraction. Meanwhile, he moved to the laboratories of the [British] Wool Industries Research Association in Leeds, United Kingdom. Synge joined him there, bringing with him the IWS scholarship. This new machine was set up there. As Martin mentioned, "It was a fiendish piece of apparatus," with 39 theoretical plates. The two operators had to watch it in 4-h shifts, continually battling drowsiness due to chloroform vapor. The machine was redesigned a number of times but problems always remained. Thus, although their work on the separation of the monoamino monocarboxylic acids present in wool using countercurrent extraction was published (11), it really could not be termed as satisfactory.

In 1940, Martin had a radically different kind of idea: to pack a glass tube with a mixture of wool and cotton, with the fibers parallel to the axis of the tube, and to have the chloroform flow above, and the water flow below, the packing. The idea was that the fibers would separate the two flows, but the amino acids would distribute differentially between the two solvent flows. However, it really did not work as hoped. Martin realized that the problem was related to creating equilibria in two liquids moving continuously in the opposite direction. Then, suddenly he found the solution: it was not necessary to move both liquids, but only one, keeping the other stationary in the tube. This was the birth of partition chromatography.

Martin and Synge decided that water should serve as the stationary phase and chloroform as the moving phase. They impregnated silica gel (used otherwise as the drying agent in a balance case) with water, packed the column with it, added the

acetylamino acid mixture onto the top, and poured chloroform down the column. Addition of methyl orange indicator to the water permitted it to follow the amino acids passing through the column as red bands. In the first experiment they separated acetylproline and acetylleucine, and collected the respective fractions.

This sounds very simple. However, it took Martin and Synge months of hard work to reproduce the conditions and obtain satisfactory results. The main problems were associated with questions that today sound trivial: the preparation of the proper silica gel, the use of the proper chloroform, and how to apply the indicator and initiate the fraction collection. During this time, Martin also developed the theory of chromatography, applying the theoretical plate concept from distillation (which he already learned while in high school).

As mentioned in the introduction, Martin and Synge presented the first report on their work in June 1941, at the meeting of the [British] Biochemical Society. They were slow with finishing the manuscript for publication; it was finally received on 19 November by the editor of *Biochemical Journal*. However, its publication was almost instantaneous: it was already included in the December 1941 issue of the journal (2). This paper, entitled "A New Form of Chromatogram Employing Two Liquid Phases," consisted of two parts. The first presented the theory of chromatography, and the second reported on the determination of the higher monoamino acids present in proteins.

It may be interesting to mention that at that time Martin and Synge preferred to speak of this new technique as liquid–liquid chromatography — the phrase *partition chromatography* was mentioned only once in the paper, between quotation marks. However, in subsequent years this expression became more and more used, and the citation of Martin and Synge's 1952 Nobel Prize in Chemistry specifically mentions their fundamental work resulting in the "invention of partition chromatography."

Paper Chromatography

An essential problem of the column used by Martin and Synge was that it could not be used for the analysis of dicarboxylic acids: the silica packing materials intended to serve only as a support for water adsorbed them. Thus, another material had to be found. The first thought was to use filter paper. As mentioned by Martin, he had seen "paper chromatograms" used by dyestuff chemists to check the purity of dyes (they did not call it a chromatogram). Martin and Synge tried the technique, placing a drop of the solution of two amino acids in the center of the paper, which was impregnated with water (the stationary phase). Butanol (the mobile phase) moved up the paper by capillary action and eventually reached its edge, moving the two amino acids at different speeds. Meanwhile, A.H. Gordon, a new addition to their team, was searching for a suitable color reaction that could reveal the amino acids on the paper, and he found a description in *Beilstein's Handbuch der Organischen Chemie* of the ninhydrin reaction that was adapted for this purpose. Subsequently they developed a more convenient setup that involved the use of paper strips placed in a closed container in which the air was saturated with water and the tops of the strips were dipped into troughs containing the mobile phase.

After this initial success, many different solvents were tried and the possibility of separating increasingly complex mixtures was investigated. However, no single solvent was able to resolve a mixture of all common amino acids. Therefore, they successfully tried what we now call *two-dimensional chromatography*. After developing the chromatogram on the paper strip in one dimension, they turned the paper 90° and used a different solvent to further separate the spots formed in the first development. Today, as electrophoresis enjoys its renaissance, it may be interesting to mention that in the very first experiment of two-dimensional chromatography, the first development was done by electrophoresis. However, they did not pursue this technique at that time.

Synge participated in the initial work (12,13), but he left Leeds in 1943 to join the Lister Institute of Preventive Medicine in London, thus ending his participation in the final development of the technique. Three scientists authored the main report on paper chromatography: Martin, Gordon, and R. Consden, a young chemist who meanwhile joined the team. This report was first presented on 25 March 1944 at the Annual Meeting of the Biochemical Society (14) and then published in the society's journal (15). This classic article represents the start of paper chromatography.

Although liquid–liquid partition chromatography carried out in a column had only relatively few followers, the use of paper chromatography advanced very rapidly. This mainly was due to the remarkable simplicity of the method. At that time, filter papers of standardized quality were commercially available and the necessary setup was within the reach of every laboratory. Naturally, this had not always been so, and Martin's group had had many difficulties at the beginning. The situation was amply characterized by Consden, in the preface he wrote 10 years later to the English edition of F. Cramer's textbook on paper chromatography (16): "Like other established methods, paper chromatography was not brought into the world without considerable birthpangs, and much could be written about these early adventurous days."

Using paper chromatography, separation required only a relatively short time and surpassed any techniques known at that time. A good characterization of the impact of paper chromatography was given by W.J. Whelan of the department of biochemistry and molecular biology of the University of Miami who, from 1945 to 1948, was a graduate student at the University of Birmingham with Professor N. Haworth, the winner of the 1937 Nobel Prize in Chemistry (17):

> "The technological advance the technique represented was astonishing. Amino acids, which were formerly separated by laborious techniques of organic chemistry and where large quantities of protein hydrolysates were needed, could now be separated in microgram amounts and visualized . . . [Paper chromatography] would allow one within the space of a week to carry out first a test for homogeneity and then a structural analysis of an oligosaccharide, which until then could very well have occupied the three years of a Ph.D. dissertation using Haworth's technique of exhaustive methylation, hydrolysis, and identification of the methylated monosaccharides."

As mentioned earlier, Synge left the team before the completion of the development of paper chromatography. However, he remained in contact with his former colleagues. Synge's interest at that time turned to the investigation of the amino acid composition of the antibiotics tyrocidin (18) and gramicidin S (19–21). The latter work was particularly important: Synge was the first who was able to elucidate the amino acid sequence in a polypeptide, and for this he used mainly paper chromatography. This work represented the basis of the more elaborate investigations of F. Sanger, which determined the entire peptide sequence of insulin and for which he received the 1958

Nobel Prize in Chemistry. An excellent summary of these investigations was given in Synge's Nobel Lecture (6).

Reversed-Phase Chromatography

In 1948, Martin joined the staff of the Medical Research Council, first at the Lister Institute and shortly thereafter at the National Institute for Medical Research in London. At the Lister Institute, the problem of separating longer-chain fatty acids by liquid chromatography (LC) arose. However, the existing system of having a polar stationary phase and a less-polar mobile phase was unsatisfactory for this purpose: the partition coefficients of such substances favored too much the less-polar phase. Therefore, Martin started to investigate the possibility of having a two-phase system in which the less-polar phase is stationary. Various attempts were tried, but these were not satisfactory: the problem was to find a hydrophobic support material for the less-polar stationary phase. Finally, this became possible by treating kieselguhr — a diatomaceous earth–based product — by dichlorodimethylsilane vapor, rendering it unwettable by the strongly polar solvents. Using such columns and systems, reversed-phase LC was introduced in 1949 and first used for the separation of lauric (C_{12}) to stearic (C_{18}) acids (22).

For more than two decades the widespread use of reversed-phase LC was hindered by the lack of suitable stationary phases and an understanding of the underlying physicochemical phenomena. In fact, a 1972 publication by the IUPAC (23) indicated that reversed-phase LC is "a technique of only historical interest." However, the situation changed in the first part of the 1970s, and today reversed-phase LC is the foremost liquid chromatographic technique.

Gas–Liquid Partition Chromatography

The introduction of the 1941 publication of Martin and Synge on liquid–liquid chromatography (2) contained the famous statement: "The mobile phase need not be a liquid but may be a vapour . . . Very refined separations of volatile substances should therefore be possible in a column in which permanent gas is made to flow over gel impregnated with a nonvolatile solvent." However, gas–liquid partition chromatography (GLPC) had to wait 10 years until it finally became reality.

We often find remarks in the chromatography literature questioning the reasons for the 10-year delay in picking up this very clear and unequivocal suggestion. However, this can be easily explained. Let us not forget that in 1941, World War II was raging and the United Kingdom was in her most difficult period. British journals could be received in only a few countries, and the whole of continental Europe was under German domination. In fact, even after the war, the 1941–1945 issues of many journals were missing from libraries. When communication was finally restored, paper chromatography was the most exciting new technique, and most people did not (or could not) go back to the basic 1941 publication.

There also was another reason why the analogy to gas-phase separation did not become obvious. At that time there was a general belief among prominent physicochemists (mainly in Germany) that longitudinal diffusion in a gas stream was so rapid that it would prohibit the existence of any small, discrete, and separate zones with different compositions. E. Wicke, a prominent German scientist, best articulated this belief in a lecture during the meeting of the German State Office for Economic Devel-

opment, held on 5 April 1940. The subject of his lecture was a summary of the possible chemical separation methods. Among these, he mentioned chromatography; however, he stated that the use of a chromatographic method with a gas as the means of elution seemed nearly without prospect, owing to the mixing in the direction of flow (24).

Thus, it was up to Martin to finally prove the validity of their original prediction. When in 1950 he moved to the National Institute for Medical Research, he invited A.T. James, a young scientist he met at the Lister Institute, to join him there. Martin's original idea was to develop a countercurrent column procedure using crystallization as the basic distribution system. However, after a few months of intensive work, no results were obtained, and James became very discouraged. Therefore, "to improve James' morale" (as said in Martin's personal recollections), Martin suggested that they go back to the 1941 prediction and try using a gas as the mobile phase in chromatography. The impetus to this was actually given by an inquiry from one of Martin's colleagues, looking for a more refined method than paper chromatography to separate fatty acids.

In the unsuccessful crystallization experiments, long columns packed with Celite were used. Celite lso was used for the gas chromatography (GC) columns, with a silicone oil used as the stationary phase to coat the Celite particles. They first tried the lower fatty acids, but these gave considerable trouble due to dimerization on the column. To prevent this, 10% of a nonvolatile acid (stearic acid) was added to the stationary phase. The column's end was dipped into a test tube containing an indicator solution, and the amounts of the eluted compounds were determined by titration, first manually; however, soon Martin constructed a very elegant automatic titration machine for this purpose. It was also realized that for higher-boiling compounds the column must be heated: therefore, a steam jacket was used for the column. Parallel to these investigations, Martin also expanded the theory of partition chromatography by considering the compressibility of the gas used as the mobile phase.

Their preliminary report on "liquid–gas partition chromatography" was presented at the 20 October 1950, meeting of the Biochemical Society (25). At that time, manual titration was still used for detection; the automated titrator was developed by Martin in the subsequent months, and its description was included in their final paper also discussing the theory and the separation of C_1–C_{12} acids. The manuscript of this paper was submitted on 5 June 1951 to the editor of *Biochemical Journal*; it is, however, interesting that while the 1941 paper by Martin and Synge was published within one month after receiving the manuscript, now it took almost 10 months. Then a few months later, two additional papers were published demonstrating the use of GLPC for the separation of ammonia, aliphatic amines, and pyridine homologues (26,27).

The impact of GLPC on analytical chemistry was tremendous and almost instantaneous. It was the right method introduced just at the right time, when the processes in petroleum refining and in the petrochemical industries required improved analytical controls that were no longer possible by the old laboratory techniques. GC provided the ideal way to solve these problems; within a couple of years, however, it was used for the analysis of almost every type of organic compound. Martin personally facilitated the rapid spread of the technique. He had early contact (even before the publication of their seminal paper) with scientists from major industrial organizations and not only demonstrated the technique but also advised them on how to simplify it by, for example, using syringe injection and a thermal conductivity detector.

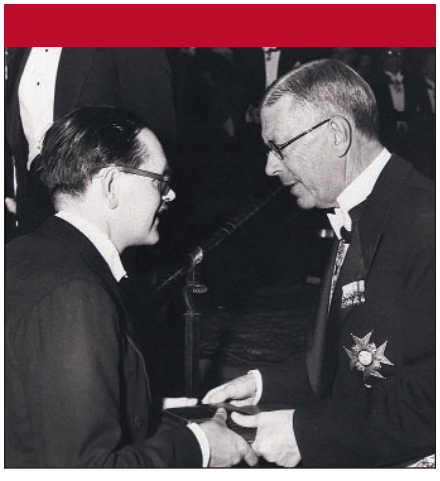
The 1952 Nobel Prize in Chemistry

The invention of partition chromatography earned Martin and Synge the 1952 Nobel Prize in Chemistry, a richly deserved recognition. The announcement of this award in *Nature* concluded with the statement:

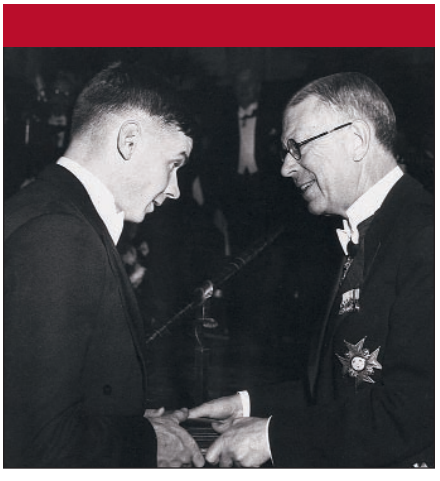
> "The methods evolved by Martin and Synge are probably unique by virtue of simplicity and elegance of conception and execution, and also by the wide scope of their application. It is likely that their invention will be considered by future generations as one of the more important milestones in the development of chemical sciences" (28).

In fact, the impact of partition chromatography has not been restricted to the chemical sciences. It also laid the foundation for the explosion of our knowledge in biochemistry and biology, which is still continuing with no slowdown in sight. All these developments are living proof of the genius of the inventors of partition chromatography, particularly that of A.J.P. Martin.

At a symposium held in 1969, Yale University Medical School Professor S.R. Lipsky, himself a pioneer in extending the use of GLPC into biochemistry, finished his



A.J.P. Martin receiving the 1952 Nobel Prize in Chemistry from King Gustav VI Adolphus of Sweden. (Courtesy of the Nobel Foundation, Stockholm, Sweden.)



R.L.M. Synge receiving the 1952 Nobel Prize in Chemistry from King Gustav VI Adolphus of Sweden. (Courtesy of the Nobel Foundation, Stockholm, Sweden.)

On the occasion of the centenary of the Royal Institute of Chemistry, the British Postal Service issued four stamps on 2 March 1977 honoring British achievements in chemistry. This stamp honored the 1952 Nobel Prize of Martin and Synge. The text on the stamp incorrectly mentions "starch chromatography."

lecture titled "Gas Chromatography: The Anatomy of a Scientific Revolution" (29) with the following words of tribute to Dr. Martin:

"He has twice made outstanding contributions to this field — in his discovery of partition chromatography and in his pioneering work on gas chromatography. He has thus altered, for the better, the lives of many of us. We, his scientific colleagues, thank him for allowing us to share with him this wonderful adventure."

There is nothing I could add to this tribute.

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