

Capitalizing on Chromatography

LC and GC have been key to the central science.

KIMBERLY S. CLEAVES AND MARK S. LESNEY

In 2003, the global market for all types of materials characterization instruments was estimated at \$21.8 billion. Chemical characterization instruments were the largest sector, accounting for 59% of the global market. Chromatography was the fastest-growing subsegment, with a projected growth of 12.9% from 2004 to 2009 (Business Communications Co.).

Modern chromatography began more than 100 years ago when Russian botanist and plant physiologist Mikhail Tswett developed his differential migration method of liquid-solid chromatography, which he published in 1906. He succeeded in resolving complex mixtures of structurally similar yellow and green chloroplast pigments of various leaf extracts by using pure solvent to develop the chromatogram.

The colored pigments Tswett studied formed readily visible bands, which evolved from the application of selective adsorbents and extraction to purify the chlorophyll; hence he coined the term “chromatography.” Chromatography—“color writing” (translated from the Greek roots *chroma* and *graphein*)—earned Tswett the cognomen “father of chromatography.”

Paper and TLC

Contributions to chemistry during World War II had profound effects on the future of chemical instrumentation, especially chromatographic techniques developed by Archer J. P. Martin. Martin was instrumental in transforming chromatography into the invaluable laboratory tool that we know today for the separation and identification of compounds. In 1941, Martin and Richard L. M. Syngé developed liquid-liquid partition chromatography while working at the Wool Industries research laboratory in England. Then in 1944, he and his colleagues R. Consden and A. H. Gordon developed paper partition chromatography to separate mixtures of acetylated amino acids that defined proteins, one of the most important methods in the development of biotechnology. However, toward the end of the 1950s, thin-layer chromatog-

raphy (TLC) practically replaced paper chromatography as the most popular, routine chromatographic technique.

TLC evolved from the experiments of Dutch biologist M. W. Beyernick in 1889, with later improvements to the technique by H. P. Wijsman, who was the first to use a fluorescent indicator to detect a chromatographic zone. Shortly after World War II, American scientists J. E. Meinhard and N. F. Hall used the technique to separate terpenes found in essential oils and were the first to use a binder (cornstarch) to hold the adsorbent particles on the glass support. Building upon that work, Justus G. Kirchner and colleagues at the U.S. Department of Agriculture’s fruit and vegetable laboratory in California improved TLC in a technique that used adsorbent-coated glass plates that Kirchner called “chromastrips.”

Throughout the 1950s and into the 1960s, Kirchner and colleagues also perfected TLC techniques using square coated plates, carried out two-dimensional chromatography, and introduced the use of reactions on plates for identification purposes. TLC was also used for pesticide and pollution analysis, but its main benefit remained in the life sciences as a test run of solvent systems for GC and LC in research and production laboratories. The quest for new and improved solvents stimulated companies such as Orel Burdick and Bill Jackson’s Burdick & Jackson (now part of Honeywell) to begin to purify “distilled in glass” solvents to support the emerging analytical techniques of TLC, high-performance liquid chromatography (HPLC), and GC.

Recently, TLC has proved especially practical for separating enantiomers through the use of specialized cyclodextrin coatings. This process is the basis for chiral chromatography. In column form, Daicel chiral HPLC columns (developed by Y. Okamoto of Nagoya University, Japan) provided



Top: Ion-exchange system, Illco-Way ad, *Analytical Chemistry*, 1958

Center: Paper chromatography system, Schaar ad, *Analytical Chemistry*



by companies such as Chiral Technologies, are revolutionizing the production of biologically active drug enantiomers.

HPLC

Developments in partition chromatography led to the development of HPLC. In the early 1960s, commercial LC instruments were comparatively few; most were “homemade,” for example, by university glassblowers, and primitive by modern standards. Efficiency was low: Columns were typically a meter or more in length, and separations took hours and sometimes days to complete. However, building upon improvements in Martin and Synge’s classic 1941 paper that predicted HPLC would be possible if small stationary-phase particles were used and high-pressure differences were applied across the column, in the 1960s, J. Calvin Giddings confirmed their predictions using theoretical models.

In 1966, Csaba Horváth, working at Yale University with S. R. Lipsky (the GC pioneer who co-invented the electron-capture detector with James Lovelock), built the first high-pressure liquid chromatograph and gave it its name. And with others, he contributed greatly to the development of subsequent HPLC systems. HPLC today is synonymous with modern LC instrumentation (with the P now meaning “performance”). During the time Horváth developed HPLC, the Waters Corp. manufactured its LLS system, which became a prelude to its high-pressure liquid chromatograph. Shortly afterward, companies such as Barber-Colman marketed ionization detectors for LC, and DuPont manufactured and sold “high-pressure” LC systems with special packing materials and pressures for analyzing DDT and other pesticide sprays.

However, HPLC did not catch on until the early 1970s, when bonded phases made reversed-phase LC possible. Combined with the introduction of gradient elution in the 1950s by Arne Tiselius and co-workers, the new packing materials provided the basis for an invigorated HPLC, allowing for improved separation between very similar compounds. HPLC with UV absorbance detection was a major improvement over open columns, and it provided the more precise and rapid separations required for many areas of biotechnology and was especially useful for protein and nucleic acid determinations. Biochemical and pharmaceutical separations were also aided by the 1980 introduction of chiral stationary phases, used to separate enantiomers.

Computers and automation added to the convenience and appeal of HPLC. By 1977, microprocessor-controlled HPLC pumps became available, thereby vastly improving the sophistication and control of runs. Also in 1977, Finnigan (now Thermo Finnigan) produced the first commercial LC/MS.

By 1985, VG Instruments realized the benefit of integrating instrumentation with computers and

was one of the first manufacturers of a hyphenated laboratory data system. The following years also saw a vast undertaking in the development of the micro-, affinity, size exclusion, capillary electrophoretic, and chiral column as well as other specialized forms of columns adapted to HPLC (each with its own unique stationary phase medium). These years also saw the emergence of so-called fast HPLC. In the 1990s, “perfusion” columns, which are made of particles that have a mixture of large and small pores for high loading capacity and facile solvent and solute flow-through, were developed.

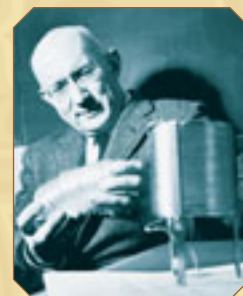
Monolithic columns were also developed, consisting of a rod of rigid, porous sorbent material, such as the metal-free polymerized silica used in Merck’s Chromolith line. They allow for high flow rates without extreme pressure and achieve efficient separations of proteins and peptides, polynucleotides, and small molecules.

Without pumps there would be no P in HPLC, whether it be pressure or performance. Syringe pumps, developed in the 1960s, use a motorized piston to push fluid from a reservoir into the column and have been commercially produced by companies such as Eldex and ISCO. Single-piston pumps, including the “fast-fill” variety (developed in the 1970s), have also achieved a measure of popularity. Fast-fill pumps use pistons made of sapphire or ruby; the single piston is driven through a housing into a Teflon seal using a motor-driven cam. They have been produced by Applied Biosystems, Beckman Coulter, Gilson, Thermo Electron, and Varian. Diaphragm pumps such as those produced by HP use a movable membrane to deliver fast-cycling flow that is useful for microbore column analyses.

IEC and IC

In 1917, Otto Folin and Richard D. Bell first utilized ion-exchange chromatography (IEC) to determine ammonia in urine. In 1927, the first zeolite mineral column was used to remove ions from solutions to determine the sulfate content of water. However, the full development of the application arose from the need for the technology to separate rare earth ions

CAPITALIZING ON CHROMATOGRAPHY



GOLAY AND OTC COLUMNS

In 1957, in a classic paper in *Analytical Chemistry* entitled “Vapor Phase Chromatography and the Telegrapher’s Equation,” Marcel Golay developed a mathematical theory that would be the start of his trail to a better theory of GC. Originally, packed columns were used exclusively for GLPC, but as a result of Golay’s work on chromatography theory in 1957, as well as that of Leslie S. Ettre, Abraham Savitsky, and co-workers at PerkinElmer, various open-tube capillary (OTC) columns were designed that allow for extremely large chromatographic efficiencies in small volumes across great column lengths.

One of the biggest problems of GC/MS performance historically was that of interfacing packed GC columns with the mass spectrometer. The high volumes of both sample and carrier gas in noncapillary columns proved overwhelming to the abilities of the mass spectrometer as a detector, and special interface packing columns had to be designed. With Golay’s OTC columns, the GC/MS interface could be dispensed with and the column eluent directly fed into the ion source of the mass spectrometer.

Overall, premade OTC columns have become the mainstay of modern GC, replacing packed columns for numerous applications. As might be expected, the production and marketing of capillary columns are highly competitive, and columns are produced by a wide variety of manufacturers, including Agilent Technologies, Alltech, Bio-Rad, PerkinElmer, Quadrex, Restek, SGE Analytical Science, and Supelco.

Above: Marcel Golay and early capillary column, *Luminaries of the Chemical Sciences*, 2002

CAPITALIZING ON CHROMATOGRAPHY



Above: Dionex ion chromatograph, *Made to Measure*, 1999

for the World War II Manhattan Project, which led to the atomic bomb.

In the early 1970s, Hamish Small and co-workers at Dow Chemical Co., as a novel method of IEC usable in automated analysis, developed ion chromatography (IC). IC uses weaker ionic resins for its stationary phase and an additional neutralizing suppressor, or stripper, column to remove background eluent ions. Since then, IC, marketed by companies such as Dionex and Metrohm-Peak, has developed into a sophisticated technique for the separation and determination of inorganic ions as well as low-molecular-mass organic acids and bases. It is a powerful technique for determining low concentrations of ions and is especially useful in environmental and water quality studies, among other applications.

GC and Company

In November 1945, the first analytical gas-solid (adsorption) chromatograph was developed in the chaos of postwar Germany by Fritz Prior, a graduate student under the direction of Erika Cremer, then head of the Institute of Physical Chemistry at Innsbruck University. Gas-solid chromatography (GSC) is based on the repeated adsorption and desorption of sample from the carrier gas to the solid adsorbent.

In 1950, in England, not content to rest on previous discoveries, Archer J. P. Martin, with his colleague Anthony T. James, developed gas-liquid partition chromatography (GLPC). Although Martin and Richard Synge had

predicted GLPC in their original paper on liquid-liquid chromatography in 1941, no one had taken them up on it, so Martin decided to try it himself. He and James used the new column with great success to separate a variety of natural products and reported their results in a series of papers and at meetings—including Martin's Nobel Prize lecture in 1952 (an award he received in conjunction with Synge).

In principle, GLPC is based on the separation of a sample by its partitioning between an inert carrier gas (mobile phase) and a liquid stationary phase that is held on a solid support. Differential interaction of sample components with the two phases leads to separation based on the heterogeneous nature of the phases and the temperature and pressure conditions of the chromatography run. Column geometry and the nature of the solid support for the liquid phase also can contribute significantly to the dynamics of the process.

Commercially available GLPCs were first produced in 1955 by Burrell Corp. (Kromo-Tog), PerkinElmer (Model 154), and Podbielniak

(Chromagraphette). Other companies followed rapidly, such as Beckman (GC-1) and Fisher Scientific (Fisher-Gulf Partitioner), both in 1956. F&M developed its Model 202 in 1959, which offered the first programmed temperature unit (F&M was ultimately purchased by HP, which spun off Agilent Technologies).

Use of a carrier gas provides some unique design requirements for gas chromatographs, including the need for pressure gauges, flow controls, and specially designed injector ports to ensure proper integration of sample with gas before column entry (see chapter, "Lab Equipment, Tools, and Widgets").

Doing with Detectors

A host of detectors have been used since the early 1950s, and to a great extent, improvement of detector technology has been responsible for much of the evolution of GC as a ubiquitous technique. Early instruments used thermal conductivity detectors, but in 1958, two ionization detectors were introduced: the argon ionization detector (originally incorporated into commercial devices by W. G. Pye & Co. and Barber-Colman in the late 1950s) and the flame ionization detector (FID). The FID was invented independently by researchers in Australia and South Africa. It evolved from the flame thermocouple detector first described by R. P. W. Scott in 1954.

Other detectors are also important, though less widely used. These include electrical conductivity detectors and electron-capture detectors. Many companies today, such as Shimadzu, offer a wide selection of these types of GC detectors alone or as part of integrated GC systems.

However, in the mind of most researchers, the ultimate in analytical detectors for comprehensive GC performance is, of course, the mass spectrometer, first linked to GC in the late 1950s. The key benefit of this coupling is the marvelous combination for each peak of a GC retention time and a corresponding mass spectrum.

In 1956, the first commercial GC/MS using a time-of-flight mass spectrometer (Model 12-101) was developed by the Bendix Corp. Other companies rapidly followed (see chapter, "Spectacular Spectrometry").

Today, advancements in GC can take a variety of approaches—from improving the column, the temperature control, and the support media to developing better injection and computational analysis techniques. But perhaps the use of GC in multidimensional hyphenated and tandem techniques, connected to everything from IR to NMR, has the most promise for expanding the repertoire of "vapor chromatography."

Chromatography remains a popular method of analysis because of its ease of use and adaptability to the volatility or stability of the analyte. Today, in all of its myriad forms, chromatography remains the most commonly used procedure in chemical analysis. ♦