Capillary Electrophoresis: A Product of Technological Fusion

An introduction to capillary electrophoresis (CE), its different forms, and its applications, and the history of CE research at HP, leading to the new HP CE instrument described in this issue.

by Robert R. Holloway

Electrophoresis is one of the most powerful, if traditional, analytical methods. Picton and Linder, Hardy, and Ellis were turn of the century pioneers in the analysis of biocolloids (proteins and carbohydrates). By electrophoretic methods they were able to work with these previously intractable materials. Arne Tiselius ushered in the age of instrumentation with a demonstration of the first cell for electrophoretic analysis in the 1940s, and in the 1990s electrophoresis is one of the most visible icons of science, with TV and newspapers daily displaying electrophoretic DNA spot patterns.

The fused silica capillary, a glass tube about the size of a hair, is a spin-off of the optical fiber. It exists more because it could be made (by heat-drawing a large glass tube into a tiny one) than because it was seen as a powerful analytical tool. It is not surprising that one of its grandest applications, capillary electrophoresis (CE), was not foreshadowed.

Various workers in the electrophoretic field (Everaerts, Hjerten, Mikkers, Virtanen), while aware of the benefits of going to small systems, did not employ the fused silica capillary. The growth of the method began when a few workers, including scholars Jorgensen and Lukacs of the University of North Carolina, industrial researchers McManigill and Lauer of HP Laboratories, and others, not necessarily sophisticated in electrophoresis but aware of the power of the capillary in gas chromatography (demonstrated by Ray Dandenau and Ernie Zerenner of Hewlett-Packard in 1979[1]), conducted the first experiments in fused silica.

Separation Science

In analytical chemistry, separation is a fundamental process. A chemical substance is generally intimately mixed with many other substances, and its determination and identification is made considerably easier by its physical separation from the mixture. Thus, analytical chemists have worked hard to understand the separation process and to develop many modes of separation.

The separation principle for a particular separation method is the physical or chemical property that varies in magnitude among the substances in a sample. In chromatography, for example, the separation principle is often chemical affinity for chromatographic materials. A feature of CE is that its separation principle is orthogonal to that of liquid chromatography (LC), or in other words, it has a completely different basis.

Separation implies physical movement or transport. In free solution capillary electrophoresis (FSCE), the simplest form of CE, transport of a particular chemical species is the resultant of several driving forces. The species moves in response to an electric field, which is important if it is charged. It moves in response to the flow in the channel, which can be caused by the electric potential difference across the fluid-silica interface (electroosmotic flow) or by mechanical pumping of the fluid. Finally, its migration is affected by the frictional drag it experiences, which depends on its size and shape and the viscosity of the fluid.

Modes of CE

CE is really a group of several procedures. CGE, or capillary gel electrophoresis, is an enhancement of an older technique. IEF, or isoelectric focusing, has also been done in other formats, and is still in the process of adaptation to the capillary. ITP, or isotachophoresis, has not found wide use but has considerable potential for preparing pure chemicals. MEKC, or micellar electrokinetic chromatography (also called by other acronyms), is a completely new method that combines electrophoresis and partition chromatography. The simplest and most characteristic mode, in terms of which all the others can be described, is FSCE. It is the form most practiced today.

Free Solution CE

In FSCE, a capillary channel, typically a few micrometers to two hundred micrometers in diameter, is filled with a conducting liquid, most often a water solution containing an acid or a base and its salt, termed a buffer, which has a pH that is insensitive to the addition of small amounts of acids or

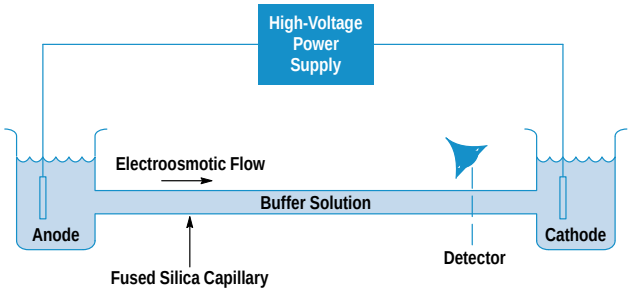

Fig. 1. Free solution capillary electrophoresis.

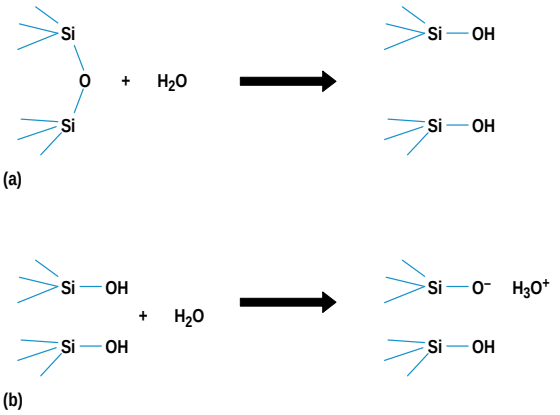

Fig. 2. (a) Hydration of a raw silica surface to polysilicic acid. (b) Negative charging of silica by ionization of the surface.

bases. The two ends of the channel are immersed in two reservoirs which are held at different electric potentials; in other words, a voltage is applied to the column. In the most usual situation, this will result in the flow of the liquid from the anodic reservoir to the cathodic reservoir. This is electroosmotic flow (Fig. 1).

Electroosmotic Flow

The surface of silica in contact with an aqueous medium with a pH no lower than 2.5 or so is loaded with negative charge. This is because silica hydrates and becomes an acid, releasing positive hydrogen ions into the medium (Fig. 2). The excess positive charge is physically localized within a very narrow zone close to the surface (Fig. 3).

Since capillaries enclose very small channels, and since electrophoretic currents are proportional to the cross-sectional area of the channel, FSCE involves small currents (from a fraction of a microampere to a hundred microamperes or so) and thus small amounts of heat relative to conventional scales of electrophoresis. As a result it is possible to apply much larger voltages, and axial electric fields in the channel are high—hundreds of volts per centimeter.

Water molecules in the narrow zone of positive charge are subjected to the pull of the positive charge as it moves to the cathode. In fact, the entire column of fluid is dragged. In contrast to what would happen if this same column of fluid were moved by a piston, there is no laminar flow, no wall drag tending to produce a parabolic flow profile (Poiseulle flow). The flow is proportional to the electric field, and can reach a velocity of millimeters per second.

Transport

At the anode a thin zone of a mixture of analytes in aqueous solution is introduced into the capillary. The molecules in the zone are immediately subject to the strong electric field and the bulk flow of the electrolyte. They are impeded by the molecules of electrolyte through which they must move.

The different species each move with a characteristic velocity. In general, they move toward the cathode, but if strongly negative, can actually be expelled into the anodic reservoir. As they move, they separate into bands enriched in the various species relative to the original mixture band.

The most spectacular feature of CE is that the molecules in a band are subject to very little dispersion while traveling along the column. The flow in the column does not appreciably stir up the band, because it is a flat-profile flow. Diffusion is inescapable and does spread the band, but because the experiment is very fast, not much diffusion occurs. In the CGE form of the technique, the medium is a gel, diffusion is drastically slowed, and prodigious resolution is achievable.

Somewhere along the tube, as close as possible to the cathode, a detector is mounted to visualize the bands as they pass. Since silica is transparent to ultraviolet radiation, the most convenient detector consists of a lamp on one side of the column and a photodetector on the other side. The bands absorb the light as they move into the path of the lamp rays, and the signal is displayed as a series of peaks similar to the familiar peaks of chromatography, although unlike chromatographic peaks the quantity of material represented by a particular peak depends on when it passes the detector as well as its size. Figs. 4 and 5 show typical electropherograms obtained by FSCE and CGE, respectively.

CE Applications

The mature practice of electrophoresis and the new technology of fused silica capillaries have been combined to perform previously unrealized analyses (CGE for the quantitation of oligonucleotide failure sequences and automatable DNA sequencing), to perform others with unprecedented speed and resolution (faster DNA restriction fragment length poymorphism (RFLP) analysis and peptide mapping than liquid chromatography), and to provide a badly needed orthogonal separation principle for all liquid-phase analysis (the combination of charge and frictional drag). CE is not just an incremental improvement on existing methods; rather, it allows entirely new things to be done. It is this paradigm-shifting character that makes CE attractive in an industry

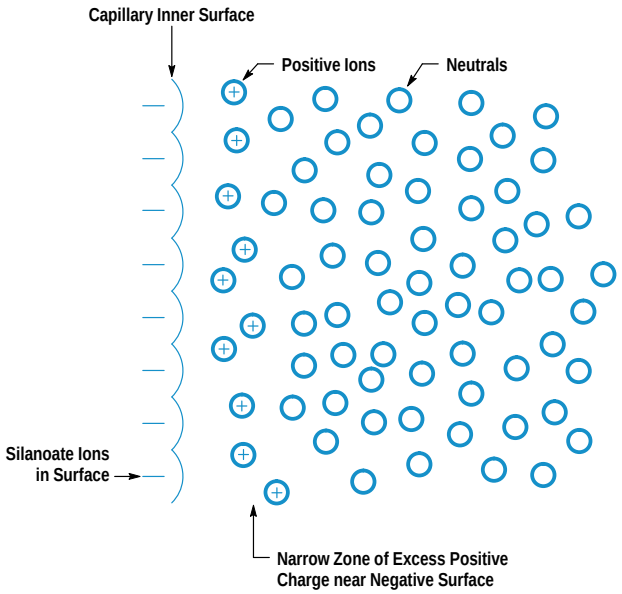

Fig. 3. In electroosmotic flow, a double layer of positive and negative charges forms near the surface of the capillary.

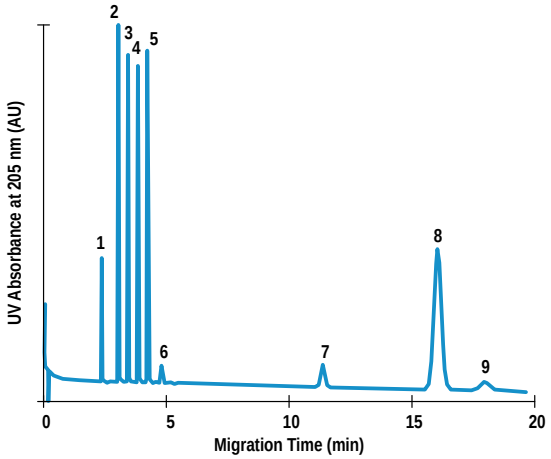

Fig. 4. An FSCE separation of an amine mixture. Peaks: 1. pyridine. 2. benzylamine. 3. diphenylamine. 4. adenine. 5. aminophenylboronic acid hemisulfate. 6. 4-aminoantipyrine. 7. xanthene. 8. 2-amino-5-nitrophenol. 9. hypoxanthine. Buffer: 0.1M phosphate, pH 2.7. Separation conditions: anode 20 kV, cathode 10 kV. Column: 40 cm. Injector-detector: 15 cm.

that, while not growing as explosively as the computer industry, has been changing rapidly, with products becoming obsolete in a matter of months.

Most of the separations performed in the mainstream chemical industry, as well as the food and drug industries, can be carried out and are being tried by CE. In some instances, notably the large-molecule areas of bioscience, good applications have already been found and are on their way to validation. In many others, sometimes surprising ones involving molecules with no charge differences, there is great interest. The high resolving power of CE sometimes outweighs an inappropriate separation principle, for example in the case of chiral drug molecules. These molecules are uncharged and have the same frictional drag and therefore cannot be separated with ordinary media. Their separation in CE comes from transport through a medium that is itself chiral, and the low dispersion of electroosmotic transport preserves it for examination.

CE and the Bioanalytical Market

In the biotechnical segment of the analytical chemical market HP has had only a few products. One of the most important is the liquid chromatography (LC) system, which is used to characterize chemical products and validate their identities (traditional quality control). LC is very powerful in the field of general chemicals, along with gas chromatography (GC), but the new bioengineered pharmaceuticals, the nucleic acids, and peptides and proteins challenge even its power. CE is a welcome partner method that shows promise in solving some of these challenges.

Oligonucleotide Failure Sequences

The oligonucleotide, which is a sequence of molecular letters of DNA code, is used in the research and development effort in the new biotechnical industry. These segments, a few to perhaps a hundred letters in length, are synthesized to be used as templates, or more properly stencils, for operations that create large amounts of the informational molecule (polymerase chain reactions or PCR) or as implements for the manipulation and recognition of critically important DNA molecules, such as those that cause inherited traits (genetic diseases) or those that can identify individuals. The synthesis of these molecules is carried out one letter at a time and is prone to mistakes at each step. After a series of steps, a population of failure sequences exists along with the desired materials. These mistakes are most easily detected and quantitated by capillary gel electrophoresis (CGE). Neither the traditional slab gel techniques of electrophoresis nor LC has the resolving power to distinguish the authentic material from these attendant failures.

Peptide Mapping

In FSCE, the most important mode in terms of the number of analyses carried out, an important bioanalytical target is the protein digest. Most of the bioengineered pharmaceuticals being produced or contemplated are proteinaceous. The identity of a protein product is determined by using specific proteins to break it down catalytically into a mixture of smaller proteins (peptides) which identifies its origin just as a geometrical pattern of closely spaced curved ridges identifies the fingertip on which it occurs. The peptide mixture can be analyzed with LC to produce the molecular analog of the fingerprint, called a peptide map. The mixture can also be analyzed with CE to produce an equally characteristic map more quickly. Both maps are useful, and at this stage of manufacturing practice, their complementarity is beginning to be widely appreciated.

The Future of CE: Integrated Liquid-Phase Analysis

CE will continue to find applications. Its growth rate as a liquid-phase analytical method will be higher than its more mature cousin, LC. Most of LC's applications are firmly entrenched and sufficient for immediate needs. Improvements in these will be incremental enhancements involving a variety of techniques including CE.

CE in the future will likely be a part of a complex strategy. Workers are now combining the unique separating power and efficiency of CE with the versatility of LC and the mass spectrometer's ability to identify unknowns. New forms of separation continue to spin off the CE experiment (most recently CEC, or capillary electrochromatography) and will also play a part in the integrated future of liquid-phase analysis.

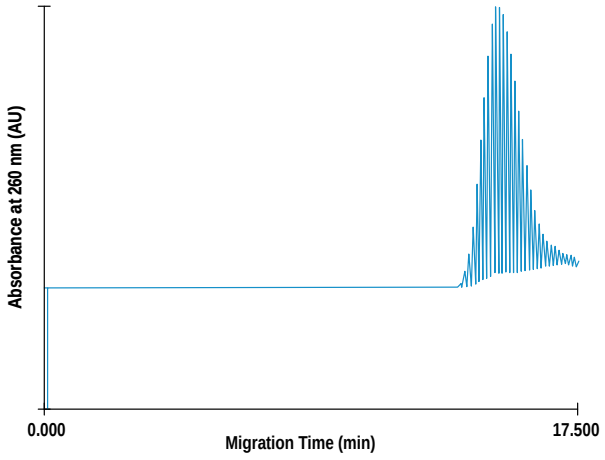

Fig. 5. A CGE separation of oligoadenylic acids. The oligomers range from 40 to 60 units in length.

HP Laboratories and CE

Douglass McManigill of HP Laboratories became interested in CE as a result of the publication of Professor Jim Jorgenson's first paper describing the use of fused silica capillaries for electrophoresis.[2] McManigill, in what is now the chemical systems department of the Analytical/Medical Laboratory of HP Laboratories, was working in the area of supercritical fluid chromatography, but was intensely interested in the physics of transport processes. Along with Henk Lauer of the HP Waldbronn Analytical Division, he built a CE apparatus and began an investigation of the CE separation process.

At roughly the same time, Paul Bente and Joel Myerson of HP Genenchem were working on an electrophoresis instrument to do gel separations of proteins and peptides. While they had limited success instrumentally, the pressurized gel synthesis they invented was the only reproducible technique for making the gels for several years.

In time, Lauer left the company, and over several years, McManigill led the project, working with many others, including Sally Swedberg, who later worked in Waldbronn on CE, mechanical engineer Stu Lerner, who is no longer with HP, Jim Young, mechanical engineer and designer whose contributions included a method of coating columns with a resistive layer, Mark Bateman, computer scientist and electrical engineer, who created the first software for the instrument, mechanical engineer John Christianson, who worked on the first prototype and is now at the HP Vancouver Division, the author, a chemist from the medical group at HP Laboratories, Don Rose from Jorgenson's group at the University of North Carolina, Jürgen Lux from Max-Planck Institut für Kohleforschung in Mülheim, Germany, Cathy Keely, an engineer who made hundreds of CE runs to explore the unexpected behavior of columns with a conductive coating, Tom van de Goor, a former student of Everaerts in Eindhoven, the Netherlands, who also joined the study of the control of electroosmotic flow, and Wes Cole, an electrical engineer who modeled electric fields at the ends of capillaries.

Another group at HP Laboratories, led by Gary Gordon, worked on several issues surrounding a CE instrument. Physicist Dick Lacey helped design a detector. Gary developed the "bubble cell," which offers a greater optical path length. An automated system for the fabrication of bubble cell capillaries was developed by Rich Tella, Henrique Martins, Bill Gong, and Frank Lucia of the manufacturing systems and technology department.

Christmas season 1989 saw the transfer of HP Laboratories technology into the capable hands of Alfred Maute, Fred Strohmeier, Martin Bäuerle, Fritz Bek, Franz Bertsch, Bernhard Dehmer, Monika Dittmann, Ulrike Jegle, Patrick Kaltenbach, Alwin Ritzmann, Werner Schneider, Klaus Witt, and Hans-Peter Zimmerman of the Waldbronn Analytical Division. The result is the instrument described in this issue.

The help of Doug McManigill is acknowledged in the construction of this history.

References

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