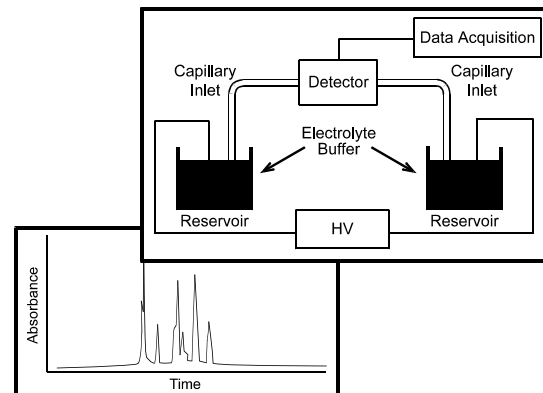




Capillary Electrophoresis for Environmental Monitoring



The Need

The Analytical Chemistry Research Program of the National Exposure Research Laboratory's Environmental Sciences Division (ESD) is developing new methods for determining toxic and hazardous chemicals in samples from hazardous waste sites. This research is guided by several goals for analytical methods:

- Shorter analysis time to reduce costs and improve quality control procedures.
- Improved separations performance and applicability to a wide spectrum of analytes, including nonvolatiles, as compared with current techniques based on capillary gas chromatography (GC).
- Field-screening capability to achieve faster results and better coordination between sampling and analytical workers.
- "Green" chemistry techniques that reduce the generation of laboratory waste (e.g., low solvent consumption) while simultaneously reducing personnel exposure to toxic chemicals.

- Simple technology, exportable to foreign countries and applicable to a broad range of analytes in a continuous monitoring format.

These goals are summed up by the phrase "cheaper, better, and faster," and are being met by an innovative separations technology called capillary electrophoresis (CE) that is new to environmental analysis.

Traditional methods for introducing a sample into an analytical device have various drawbacks. Liquid introduction of samples and liquid chromatographies and electrophoretic separations are the more universally applicable techniques since they do not depend on volatility of analytes or have molecular weight limitations. Thermally labile and polar compounds often deposit in the injector systems of gas chromatographs (even cold on-column retention gap systems) to degrade chromatography, precision, and quantitative accuracy. High performance liquid chromatography (HPLC) has attempted to fill the need for liquid state separations, but its application to ionic organics, neutral

hydrophobic compounds, and inorganic ions has not been universal. CE is a separations technique that can meet the goals stated above while filling a central, cross-cutting role in analytical chemistry for polar volatiles, most semivolatiles, nonvolatiles (e.g., herbicides), inorganic cations, inorganic anions, and biomarkers (i.e., indicators of exposure). Introduced in 1981, CE is now firmly established as the technique of choice for pharmaceutical and biomedical analysis.

CE is easily interfaced with optical detection methods based on Uv-visible absorption, indirect detection (UV or fluorescence), and laser-induced fluorescence (LIF) detection.

CE methods that are applicable to routine problems are emerging, and EPA-approved CE methods are anticipated shortly. CE technology is widely developed commercially, and EPA staff at ESD are confident that current CE methods are sufficiently robust to provide valuable contributions to environmental assessment at the present time.

The Use

CE instrumentation is simple (see illustration in the brochure header). A fused silica capillary (typically 0.050 or 0.075 mm x 27 to 57 cm) connects two buffer (elec-

trolyte) reservoirs. A high-voltage power supply (ca. 30 kV) connects the reservoirs via the buffer-filled capillary. The technique has been miniaturized as "CE on a chip",

and it is capable of adaptation to continuous monitoring applications based on fast separations (see page two of this fact sheet).

CE analyte bands travel with flat profiles that produce extremely high resolutions (see Figure 1). Reported

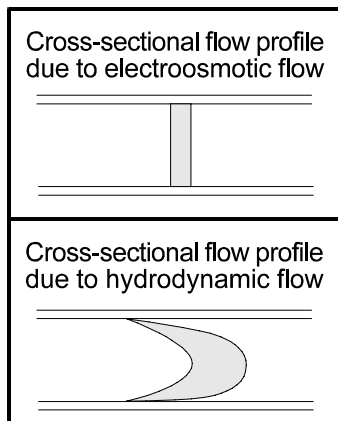


Figure 1. Flow Profiles

values usually range from 250,000 to 1,000,000 theoretical plates, with exceptional values up to 2.7 million. These values exceed those obtained with other liquid phase techniques such as HPLC, and equal or surpass the best capillary GC techniques. This extremely high resolution permits separation of many more analytes on a given column, eliminating chromatographic interferences.

Figure 2 illustrates some of the principles involved in CE and micellar electrokinetic chromatography (MEKC) which involves the addition of surfactant molecules to the buffer solutions. MEKC (also called MECC) was introduced by Terabe et al. in 1984. Terabe

et al. also introduced applications of cyclodextrins and urea in MEKC for improving separations involving hydrophobic molecules. Electrophoresis (i.e., the migration or mobility of ions in an electric field) accounts for the movement of ions of the appropriate charge toward the cathode or anode in narrow bands. The electrophoretic flow is shown in Figure 2 by a smaller, dark arrow. In addition, an electroosmotic (EO) flow exists that transports bulk liquid with buffer from one reservoir to the other depending on conditions. Usually, for bare silica, an excess of mobile positive charge exists in solution because of the ionization of silanol groups on the silica surface. The EO flow is illustrated by the large, white arrow. This flow is characterized by a flat, piston-like profile rather than the parabolic flow characteristic of pressure-driven systems. This flat-flow profile results in extremely narrow peaks and high efficiency in CE. The separation of neutral analytes under MEKC is based on their affinity for micelles (aggregates of surfactant molecules) that migrate under these conditions. These micelles are considered to form a pseudo-stationary phase. Another capillary format using EO flow, electrokinetic chromatography (EKC), involves the use of

packed capillary columns with C18 derivatized silica particles forming the stationary phase.

The fact that CE is based on electromigration of ions means that the technique can be of great value in determining inorganic ion concentrations. The U.S. EPA Region VII has approved a method for determining hexavalent chromium (Cr(VI)). Ionic organic applications developed in the pharmaceutical and biomedical areas include separation of proteins and amino acids. Applications to environmental organic ions include determination of acids, phenols, and amines. Dr. William Brumley and co-workers at the EPA ESD are actively pursuing research in CE and MEKC separations of such organic analytes as sulfonic acids, carboxylic acids, benzidines (substituted p,p'-diaminobiphenyls), phenols, anilines, and PNAs.

The combination of ion-based CE and MEKC CE provides a nearly universal analyte separation methodology. Two valuable characteristics of CE in developing routine methods are separation speed and sensitivity. Brumley and Brownrigg (1994) report high-speed CE separations of four substituted benzidines in a little over two minutes. For samples that do not require extensive preparation,

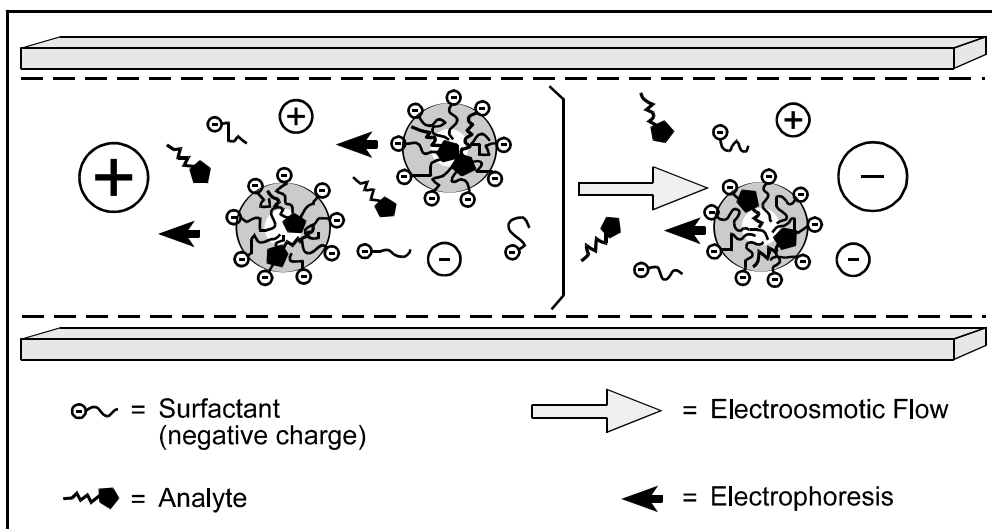


Figure 2. Illustration of the components of MEKC

The Use

Continued

replicate sample analyses can be performed to provide better confidence in analytical results. Because the detector is on-column, there is no band-broadening and signal loss due to "dead volume" mixing in the detector. This gives CE extremely low analyte mass detection limits.

Absolute mass sensitivity of detection by optical spectroscopy (no preconcentration or field amplification) is about 10^{-6} M to 10^{-7} M in a sample by UV or indirect UV or indirect fluorescence detection. For a relative molecular mass of 100, this calculates to 1 pg to 100 fg on-column for a 10 nL injection. In the case of LIF, the detection limit may approach 10^{-11} M to 10^{-14} M in a sample resulting in 10 ag to 10 zg injected on-column. Reaching these mass sensitivities presents one of the greatest challenges in the development of CE/MS.

Lack of detection sensitivity under UV is one of the major factors that has limited the development of environmental applications of CE. Although detection in CE is very mass

sensitive (i.e., the absolute amount of substance on-column), concentration limits of detection are substantially higher because of the small injection volume typically used (ca. 1 to 10 nL). This can be a serious limitation for environmental analysis, whereas for microenvironment studies (e.g., a single cell) it is an advantage. Various approaches for overcoming the limitations of nL injection volumes are discussed below. These include both sample handling and improving detector sensitivity (e.g., using LIF). One approach is to use preconcentration techniques such as solid-phase extraction before injecting samples. Additional approaches have coupled C18 columns with CE columns for preconcentration. An alternative approach is to use CE techniques to concentrate analytes. These can involve coupled columns and a technique called isotachopheresis. Another approach is to use field amplification during sample injection to concentrate analyte ions. These techniques can lead to factors of 100 to 1000 improvement in detection limits.

CE is a highly leveraged analytical tool in terms of investment because of biomedical and pharmaceutical research. It is thus assured of continuous and rapid development. Current improvements in absorption detection, such as degenerate four-wave mixing, promise lowering detection limits to 10^{-8} M.

Derivatization strategies to take advantage of LIF sensitivity (as low as 6 molecules on-column) are currently underway at ESD.

Additional leveraging in environmental applications is being sought through inter-agency agreements. One of the tasks currently being considered is parallel processing of sample streams via bundled capillaries. Sample throughput could be increased 10-fold, for example, with 10 capillaries operating simultaneously with detection. Partners with EPA are also being sought for development of CE/MS.

The Status

Current developments in CE/MS at ESD and elsewhere focus on electrospray ionization with quadrupoles, double focusing instruments, ion traps, and time-of-flight mass spectrometers. Currently, a cooperative agreement between ESD and an external institution is awaiting implementation (Ms. Tammy Jones, Project Officer).

Laboratory evaluations and research efforts have resulted

in at least one EPA CE method for hexavalent chromium that was approved in Region VII in March 1994. Dr. W.C. Brumley, Dr. Wayne Garrison, ERL-Athens, as well as other EPA and independent researchers, have performed considerable research into the application of CE to environmentally important analytes. The results have been sufficiently successful that the next step is to apply the technology to real-world samples. Once

the methods have been demonstrated on these types of samples, EPA staff are interested in soliciting requests to perform CE analyses in a field setting. To submit environmental samples for CE analysis or to be considered for a CE field demonstration, contact Dr. Brumley or Mr. Ken Brown, listed at the end of this sheet.

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