Capillary Electrophoresis: Focus on Undergraduate Laboratory Experiments

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Capillary electrophoresis (CE) is a versatile technique well suited to teach concepts that are fundamental to a chemistry degree program. The applicability of CE to metabolites, DNA, proteins, pharmaceutical compounds, environmental contaminants, or components of foods and beverages makes it a desirable tool in the chemical laboratory to teach students how to use different instrumentation for sample analyses. The simplest mode of CE separates analytes according to their electrophoretic mobilities. Any species with a charge is a potential candidate for CE analysis. But variations on the theme can hone the separation to exploit analyte differences such as hydrophobicity, size, and chirality, thereby greatly expanding the applicability of the technique. Most analytical chemistry textbooks now include a section discussing this technique and some of its more common variants. The instrument can be used to solidify a working understanding of the mechanism of separation and the detection scheme. CE is rapid, which makes it feasible to complete several separations within the time frame of a typical undergraduate laboratory period. Two considerations for including CE in the teaching laboratory are access to instrumentation and the implementation of effective learning exercises.

The Instrument

At the time of publication, two primary global vendors of CE instruments are Agilent Technologies and Beckman Coulter (see Figure 1A for an image of the PA-800). These and other vendors are listed in Table SI-1 in the supporting information. If purchasing a CE instrument is not an option, several groups have documented procedures for constructing benchtop custom-built instruments (1–5) and even microfluidic instruments (6). A custom-built instrument, such as the one shown in Figure 1B, is less expensive to assemble and deepens students’ understanding of the principles of instrumental operation. However, a student who completes the laboratory exercise using a custom-built instrument will not be able to boast experience on a commercial instrument commonly placed in industrial, government, and academic laboratories. A commercial instrument is easier to use and is programmable, which standardizes the execution of the method and streamlines the learning process. Custom-built instruments lack robotic automation and require a higher level of skill to operate. Instruments can be constructed or purchased with different modes of detection, including UV—visible absorbance detection, laser induced fluorescence, and mass spectrometry. Other instrument features to be considered include thermal control, injection options, and the number of samples and running buffers that can be accommodated. Custom-built instruments can be assembled for perhaps as little as $15,000 depending on the detector, computer, and software.

Commercial instruments can vary greatly in price, quickly climbing in cost to over $70,000, depending on the various options, especially detection. As always, interested users are encouraged to carefully consider their specific needs. Finally, the ease of purchase of some instruments may be dependent upon distribution or worldwide availability.

The Laboratory Exercise: Quantitative Analysis

Peer-reviewed resources in the literature document a variety of experimental protocol and laboratory exercises. Alternatively, vendors sell an assortment of ready-made kits that can be easily applied to solve specific separation problems. For practitioners of CE familiar with method development, unique experiments can be tailored to meet departmental and educational themes. Many published laboratory experiments designed for the teaching laboratory focus on qualitative or quantitative analyses of real-world samples. These exercises include: the determination of vanillin in a food product (7);
ions in water samples (8–10) or soil (11); water soluble vitamins (5, 12); analgesics in over-the-counter medicine (13); caffeine in beverages (4, 14–16); quinine in tonic water (17); disinfectants in cleaning products (18); preservatives in food and cosmetics (19); potassium in fertilizer (20); calcium in a dietary supplement (20); and the amino acid composition of peptides and proteins (21, 22). CE can be used to verify reaction products and used in conjunction with a chemical synthesis experiment, for example, of acetylsalicylic acid (23), sulfonium ions (24), and substituted benzoic acids (25).

Teaching Fundamental Principles as Well

While many educators prefer to engage students in hands-on analyses during the laboratory period, some may stress activities that promote critical thinking required to predict transport and migration. The theory of migration in free solution CE is simple to explain; however, students often lack a conceptual understanding of these transport mechanisms and are not able to predict the order of analyte migration prior to beginning a laboratory experiment. To strengthen students’ comprehension of the fundamental mechanisms of CE separations, several prelaboratory tutorials or dry labs may be utilized (2, 3, 10, 12, 13, 20, 22, 26–29). Laboratory activities that stress the analyte migration order may be administered before sample analyses. For example, by selecting appropriate charged analytes and a neutral marker, the students can determine the electrophoretic mobilities and electroosmotic flow. Classic separations of a cation (norephedrine HCl), a neutral compound (caffeine), and several anions (acetaminophen, acetylsalicylic acid, salicylic acid) are shown in Figure 2A using a background electrolyte buffered at pH 9 (13). This standard separation is to be completed before sample analyses of over-the-counter medicines (see for example the electropherogram shown in Figure 2B). The students can be instructed to predict migration before coming to the laboratory and then complete a number of experiments if the separation time is kept short. For example, a sample containing a cation (atenolol) and neutral marker (dimethylformamide) separated using a background electrolyte buffered at pH 7 required less than 2 min for each run (2, 3). With these rapid separations, the effects of injection volume on efficiency and repeatability as well as response factor and linear calibration with external standards could easily be studied in a single laboratory period. To keep these introductory experiments timely, the running buffer, the protocol for sample introduction, and the detection parameters, all of which are optimized prior to beginning the experiment, must be provided to students. This approach can be used to demonstrate transport in CE or even $pK_a$ values (30). These exercises support troubleshooting and prepare students for method development once they understand the fundamentals.

Beyond the Traditional Laboratory Experiment

Lab courses with more time available might easily include additional experiments. Other separation mechanisms can be taught in the laboratory, by incorporating additional selection reagents in the running buffer, such as pseudostationary phases that support hydrophobic partitioning. These micelle electrokinetic capillary chromatography separations are effective for neutral (7, 13, 14, 31) or charged compounds (21, 22) and can be compared with reversed-phase liquid chromatography separations as both are based on partitioning (7, 14, 22). Undergraduate experiments that incorporate chiral separations are feasible with the addition of a selector such as cyclodextrin (24, 32). CE is also amenable to other essential concepts, such as diffusion coefficient (33). Experiments have been designed to evaluate the utility of different instruments by comparing results obtained using CE with UV spectrophotometry (14, 15), liquid chromatography (7, 13–15, 19, 22), nuclear magnetic resonance (24), or atomic absorption (20). CE is applied with increasing frequency to benchtop analyses, and it can be used as a prelude to other instrumental topics and applications such as microfluidics, single or subcellular analyses, and biomolecular sequencing and identification. The flexibility and benefits of CE make this instrument a powerful tool to capture the imagination and excitement of the next generation of scientists.

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Literature Cited


Supporting Information Available

A table listing vendors and their Web site URLs is available via the Internet at http://pubs.acs.org.