# Capillary Electrophoresis, A New Technique for the Semiconductor and Plating Industries

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Capillary electrophoresis (CE) is revolutionizing analytical separations. It is used routinely to separate drugs and impurities, proteins, nucleic acid derivatives, and other large organic molecules in complex biological matrices. CE separations of inorganic anions and cations, low molecular weight organic acids, amines, and other small molecules are achieved in minutes with extremely high peak efficiencies, often reaching 100,000 plates or more.

Because the mechanism of separation in CE is different than that of chromatographic separations, the techniques are complementary. In CE, charged analytes separate based on their different velocities in a capillary when an electric field is applied. The migration order of inorganic ions in CE is different than typical elution orders in ion chromatography (IC). Separations that are troublesome by IC, are often simple by CE and *vice versa.* In addition, sample matrices that require substantial dilution or pretreatment because they foul chromatographic columns, can often be injected directly into a capillary.

CE when done in uncoated fused silica capillaries is often referred to as capillary zone electrophoresis (CZE). To perform a CZE separation, the ends of the capillary are placed in reservoirs containing electrolytes and electrodes. The total volume within a capillary is on the order of hundreds of nanoliters and a day's worth of separations consumes only milliliters of electrolyte. A voltage is applied to the capillary and separation of individual analytes occurs based on differences in charge to mass ratios. Separated analytes are detected on column as they migrate past the detector window. The electrolyte solutions may contain charged carrier ions, flow and selectivity modifiers, and buffers. Since most inorganic ions lack chromophores, indirect detection is used. A UV absorbing carrier ion provides the background signal for detection of the analytes. Fast analysis times, optimized selectivity, and high peak efficiencies are achieved with appropriate modifiers.

The intent of this paper is to describe the basic theory and terminology of capillary electrophoresis with an emphasis on separations of small inorganic ions. Several applications are shown to demonstrate the utility of CE for the separation of inorganic anions and cations, low molecular weight organic acids, and amines.

## Instrumentation

CE requires only simple instrumentation (Figure 1), two buffer reservoirs, a power supply, a capillary, and a detector. The capillaries are made of fused silica and of varied lengths, typically 25 to 100 cm in length with internal diameters of 50 or 75 µm. The ends of the capillary are placed into the buffer reservoirs that contain electrolyte and the electrodes. The power supply provides the high voltages necessary to produce separations, typically 15 to 30 kV.

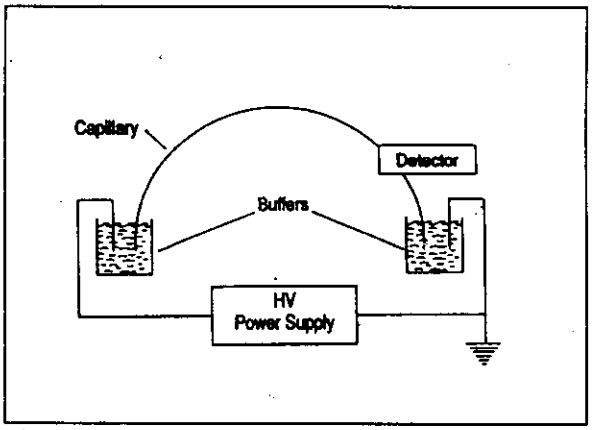


***Fig. 1–CE Basic Schematic. The components used for CE are a capillary, buffers (or electrolytes), high voltage power supply, and a detector.***

Detection of separated analytes is accomplished on column by a detector that is placed toward the outlet end of the capillary. Commercial instruments, in addition to the basic components, often include autosamplers, means of multiple injection modes, multiple detectors, power control, and sample and capillary temperature control.

Sample is introduced into one end of the capillary, usually termed the source or inlet side, by one of several different methods; electrokinetically, by using an applied voltage, or hydrostatically, by using gravity, pressure, or vacuum. With electrokinetic (or electromigration) injection, a potential is applied to the capillary and the sample migrates into the capillary as a result of the electrophoretic mobilities of the analytes and as a result of electroosmosis. Electrophoretic mobility and electroosmosis are discussed below. With hydrostatic injection modes, a difference in pressure caused by raising the sample vial above the outlet (or destination) vial, applying pressure to the sample vial, or applying a vacuum to the outlet side of the capillary. The difference in pressure is used to introduce sample into the capillary. Hydrostatic injection is sometimes referred to as hydrodynamic injection. Sample volumes in CE are typically on the order of nanoliters to picoliters.

Because of the small size of a capillary and the resultant small sample and electrolyte volumes, detection in CE is accomplished on column. No flow cells are used as in liquid chromatographic detectors. The polyimide coating on the fused silica capillary that renders the capillary flexible is removed from the section of the capillary that is aligned with the detector window.

**Separation, How Does It Happen?**

The inside surface of a silica capillary is negatively charged due to the silanol groups. Positively charged electrolyte ions are attracted to the negatively charged walls creating a charged double layer (Figure 2). When an electrical potential is applied to the capillary, the inner most cationic layer moves toward the cathode carrying the electrolyte in the capillary with it. The phenomenon of bulk liquid flow in a capillary is called electroosmosis or electroendoosmosis.

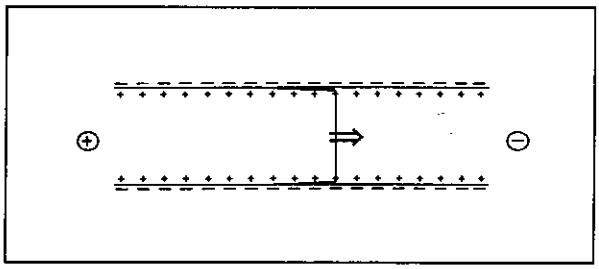

*Fig. 2–Electroosmosis is caused by the charged capillary wall.*

After a sample is injected into the capillary and a potential is applied, analyte ions begin to migrate toward the electrode of opposite charge. The rate of migration of each analyte species reaches a constant value that is dependent on the analyte's charge to mass ratio. Under these conditions, the velocity of an ion is directly proportional to the electric field. This proportionality constant is called the electrophoretic mobility (μ). Different ions will have different mobilities, depending not only on their charge to mass ratios, but also on their shape and the solvating medium.

Both electrophoretic mobility and electroosmosis contribute to analyte migration. If the instrument is configured such that the detector side of capillary is cathodic, the cations pass the detector first and their migration is supplemented by the eo flow. The neutral species are then carried passed the detector by the eo flow. The anions will pass the detector window only if the eo flow is great enough to overcome their electrophoretic mobilities that cause migration towards the anode (Figure 3).

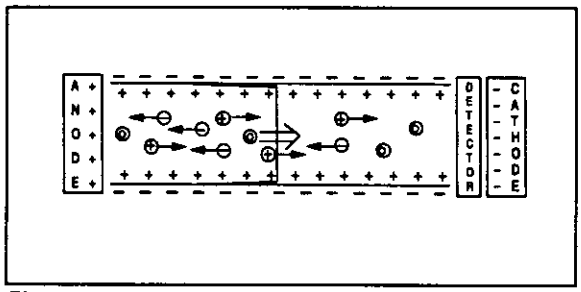


*Fig. 3–Electroosmotic flow ($\mu_{eo}$) is greater than electrophoretic mobility (μ).*

For most small, inorganic anions, electroosmotic flow is barely sufficient to

overcome their electrophoretic mobilities. Poor peak efficiencies and long analysis times result. In order to achieve rapid anion separations, an electroosmotic flow modifier is added to the electrolyte to promote the formation of a second double layer, making the capillary wall cationic. Negatively charged buffer components align themselves with the positively charged wall. When a potential is applied, the negatively charged layer moves the bulk liquid in the capillary towards the anode. Thus, the electroosmotic flow is reversed. In order for anions to move past the detector window, the polarity of the instrument is configured such that the detector side of the capillary is anodic.

Alkyl ammonium salts such as cetyltrimethylammonium bromide[1] (CTAB), tetradecyltrimethylammonium bromide[2] (TTAB), and hexamethonium bromide[4] (HMOH), and amines such as diethylenetriamine[3] (DETA) have been used to suppress or reverse electroosmotic flow. The alkyl ammonium salts are especially attractive eo flow modifiers because they are quaternary ammonium species and their ability to modify the capillary wall is not changed as a function of pH, providing more consistent eo flow. An additional advantage of hexamethonium bromide is that it is easily converted to the hydroxide form where as CTAB and TTAB cannot be. The use of CTAB and TTAB would not permit accurate determination of bromide in a sample because it is also in the electrolyte.

## Indirect Detection

Since most inorganic ions lack chromophores and commercially available CE instruments use UV/Visible or fluorescence detection, indirect detection is employed. For the case of indirect UV detection, the electrolyte used for separation contains a UV absorbing ion that produces a background signal. A nonabsorbing analyte displaces absorbing electrolyte resulting in the absence of an absorbing species in the background as the analyte zone migrates past the detector window. A negative peak is observed (Figure 4).

The absorbing (or visualizing) ion must have a large extinction coefficient to maximize the decrease in signal resulting from its displacement by the analyte[5]. In addition, the wavelength at which the chromophore of the visualizing ion absorbs should be well away from any wavelength at which analyte ions absorb to prevent direct analyte UV absorption from disrupting the indirect detection mechanism. An attractive consequence of indirect detection is that all separated analytes passing the detector produce a signal, i.e. indirect detection is universal.

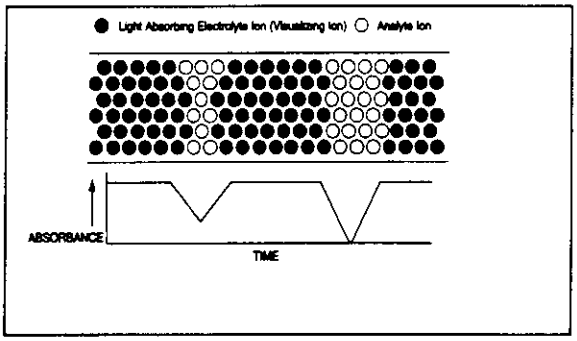


***Fig. 4—Indirect Detection. Displacement of a UV absorbing carrier ion by a non-UV absorbing analyte ion produces a negative peak.***

## UV Absorbing Carrier Ion

The UV absorbing ion in the electrolyte must also have an electrophoretic mobility that is closely matched to that of the analytes of interest and be present in the electrolyte in sufficient concentration to provide relatively symmetric peaks[6]. Peak asymmetry in capillary electrophoresis is predictable and to a certain extent unavoidable. Dispersion of an analyte zone into the carrier ion zone when the sample contains ions of different mobility than that of the carrier results in either fronting or tailing peaks. When the electrophoretic mobility of the analyte ion is greater than that of the carrier ion, the analyte zone migrates with a diffuse leading edge and the resultant peak fronts. A tailing peak is observed for the reverse case in which the mobility of the analyte ion is less than that of the carrier ion. This phenomenon is shown in Figure 5 for anionic analytes. Ferrocyanide, an anion of higher mobility than the phosphate carrier ion exhibits fronting, while phthalate, with a lower mobility than phosphate, tails. Ions such as pyromellitate (1,2,4,5-benzene tetracarboxylate) and trimesate (1,2,5-benzene tricarboxylate) which have similar electrophoretic mobilities at this pH to that of

phosphate exhibit good peak symmetry and high efficiency. In order to obtain high resolution separations of closely migrating peaks, the fronting and tailing behavior must be minimized by proper choice of the carrier ion.

For indirect detection of anions, pyromellitate is an excellent choice for a UV absorbing carrier ion[4]. The electrophoretic mobility of pyromellitate when fully ionized is similar to the mobilities of many common inorganic anions and low molecular weight organic acids. In addition, pyromellitate has the necessary spectral qualities required for indirect detection, a high extinction coefficient (7.81 x $10^3$ liter • $mol^{-1}$ • $cm^{-1}$ at pH 7.5)[4] UV absorbance at 250 nm, a wavelength well removed from interference of direct absorbance of several inorganic anions. Inorganic anions such as bromide, nitrate, nitrate, iodide, azide, and thiosulfate absorb at lower UV wavelengths.

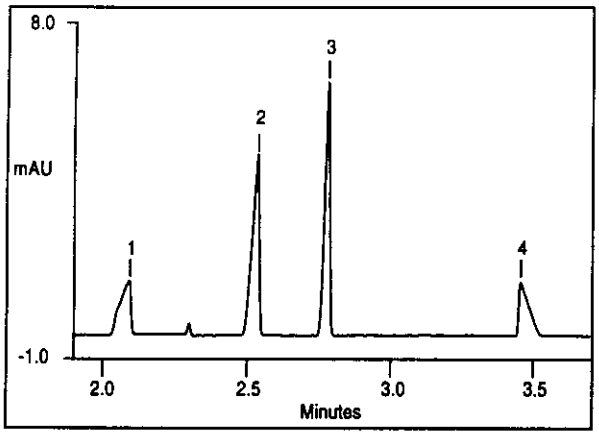


***Fig. 5–Characteristic Peak Shapes in CE. Capillary: 50 µm i.d., 50 cm total length. Electrolyte: 5.0 mM $Na_2HPO_4$, 5.0 mM $NaH_2PO_4$, 0.75 mM hexamethonium hydroxide, pH 7.0. Applied voltage: 30 kV (600 V/cm). UV direct detection at 254 nm. Gravity injection, 100 mm for 30 seconds. Peaks: 1=ferrocyanide, 2=pyromellitate, 3=trimesate, 4=phthalate.***

For cation indirect detection, copper (II) at pH less than 5 has an electrophoretic mobility well matched to most inorganic cations and low molecular weight amines, and also has the required spectral characteristics for indirect UV detection[7]. The pH of the electrolyte is maintained below 5 to ensure that the copper is present as a free divalent cation. Copper (II) hydrolyzes at pH greater than 5, increasing the effective mass of copper and reducing its electrophoretic mobility. The electrophoretic mobility of copper (II) at pH 4 matches the mobilities of the analytes of interest resulting in high efficiency cation separations.

**Other Electrolyte Components**

To provide reproducible electrolyte pH as well as consistent pH throughout the course of an electrophoretic separation, a buffered electrolyte must be used. For anion separations, the pyromellitate - hexamethonium electrolyte is buffered at pH 7.7 with triethanolamine. At this pH, the pyromellitate is fully ionized and it's electrophoretic mobility is closely matched to that of the analyte anions of interest. The copper (II) based electrolyte for cation separations is buffered at pH 4.0 with formic acid. In this case, not only does a buffered electrolyte provide stable pH during electrophoresis, but also prevents the hydrolysis of copper (II).

The copper (II) electrolyte system also contains 18-crown-6. The use of 18-crown-6 to achieve separation of ammonium and potassium, which have nearly identical electrophoretic mobilities[8], has been documented by other researchers[9,10].

**Experimental**

*Equipment*

All electropherograms are obtained using a Dionex CES I system (Dionex Corporation, Sunnyvale, CA, USA). Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) of 50-µm i.d., 375-µm o.d., and various lengths are used. The detection window is located 5 cm from the outlet end of the capillary. Data collection at 10 points/second is accomplished with a Dionex AI-450 data acquisition system. Unless otherwise stated, sample is injected hydrostatically by raising the sample vial 100 mm above the level of the destination vial for 30 seconds. The polarity of the analog output of the UV/Visible detector is reversed to obtain positive peaks. The wavelength for indirect UV detection of anions using the pyromellitate electrolyte is 250 nm. A wavelength of 215 nm is used for cation indirect detection with the copper (II) electrolyte.

*Reagents and Standards*

All reagents and standards are of analytical - reagent or ACS grade unless otherwise specified, and prepared in 18 MΩ deionized water. For preparation of the anion electrolyte; pyromellitic acid, 96% is from Sigma Chemical Company (St. Louis, Missouri, USA), hexamethonium bromide, monohydrate, 97% is from Aldrich Chemical Company (Milwaukee, WI, USA), triethanolamine, >99%, is from Fluka Chemical (Ronkonkoma, NY, USA), and sodium hydroxide, 50% aqueous solution is from Fisher Scientific (Pittsburgh, PA, USA). Anion standards are prepared from sodium salts from Fisher Scientific. Octanesulfonic acid (0.1 M) is from Dionex Corporation. OnGuard™ A cartridges (Dionex Corporation) containing high capacity anion exchange resin and converted to the hydroxide form are used to convert the hexamethonium bromide to hexamethonium hydroxide.

For cation separations, copper (II) sulfate, pentahydrate, is from MCB (Norwood, OH, USA). Formic acid is obtained from Fluka Chemical and 18-crown-6 from Aldrich Chemical Company.

## Applications

*Anion Separations*

A separation of anion standards using the pyromellitate - hexamethonium electrolyte is shown in Figure 6 and demonstrates the high efficiency and fast analysis times achievable with CE. The electrolyte, which is buffered with triethanolamine, provides excellent run to run reproducibility with RSDs of 0.5% or less on migration time and of 2% to 5% on peak area (for n=15). The anions are separated according to their electrophoretic mobilities. For example, chloride migrates past the detector before fluoride because chloride has a higher velocity in the presence of an electric field than fluoride.

Trace analysis is also possible with CE. In Figure 7, low µg/L (ppb) anions are detected in high purity water using the pyromellitate - hexamethonium buffer system and an electrostacking technique. In electrostacking, electromigration injection for a long duration is used to isotachophoretically preconcentrate the analytes of interest in the capillary prior to separation[5,11]. The electrostacking technique

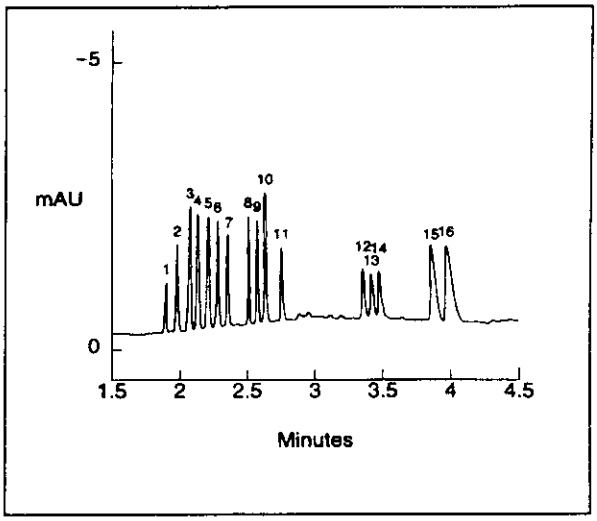


***Fig. 6–CE Separation of Anion Standards. Capillary: 50 µm i.d., 50 cm total length. Electrolyte: 2.5 mM pyromellitic acid, 6.5 mM NaOH, 0.75 mM hexamethonium hydroxide, 1.6 mM triethanolamine, pH 7.7. Applied voltage: 30 kV (600 V/cm). Indirect UV detection at 250 nm, signal output polarity reversed to produce positive peaks. Gravity injection, 100 mm for 20 seconds. Peaks: 1=dithionate (3 mg/L), 2=thiosulfate (5 mg/L), 3=bromide (8 mg/L), 4=chloride (3 mg/L), 5=sulfate (3 mg/L), 6=nitrite (3 mg/L), 7=nitrate (3 mg/L), 8=molybdate (5 mg/L), 9=azide (4 mg/L), 10=thiocyanate (3 mg/L), 11=chlorate (3 mg/L), 12=fluoride (0.5 mg/L), 13=bromate (3 mg/L), 14=formate (2 mg/L), 15=phosphate (3 mg/L), 16=phthalate (10 mg/L).***

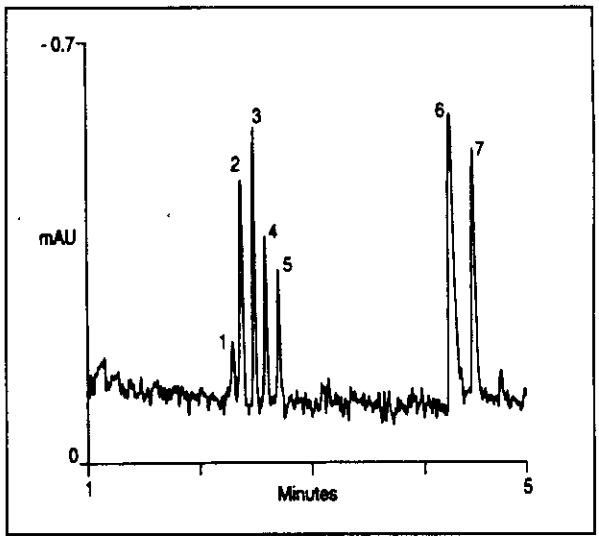


***Fig. 7–Trace Anions in High Purity Water. Electrophoretic conditions the same as in Fig. 6. Electromigration injection, 5000 V for 45 seconds. Sample contains 50 µM octanesulfonate, the isotachophoretic terminating ion. Peaks: (all at 5 µg/L) 1=bromide, 2=chloride, 3=sulfate, 4=nitrite, 5=nitrate, 6=fluoride, 7=formate.***

is also used to analyze for trace anionic contaminants in 30% hydrogen peroxide, shown in Figure 8. With CE, undiluted sample can be injected directly. For IC analysis of hydrogen peroxide for trace anions, substantial dilution of the sample is required to prevent oxidation of the chromatographic resin. In Figure 9, trace iodide is determined in the presence of chloride and sulfate in a sodium hydroxide sample matrix. A pyromellitate - hexamethonium electrolyte containing methanol was used to separate iodide and chloride which have nearly identical electrophoretic mobilities[8] and co-migrate when using the standard pyromellitate - hexamethonium electrolyte. The methanol provides a slightly different selectivity and fully resolves the two peaks.

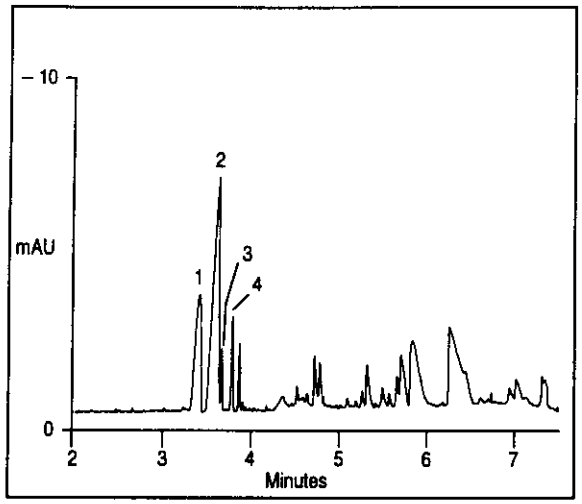


***Fig. 8—Trace Anions in 30% Hydrogen Peroxide. Conditions the same as in Fig. 7. Peaks: 1=chloride (150 µg/L), 2=sulfate (372 µg/L), 3=nitrite, 4=nitrate (61 µg/L).***

*Cation Separations*

A separation of cation standards using the copper (II) electrolyte with 18-crown-6 is shown in Figure 10. Migration time reproducibility with the buffered electrolyte is excellent; less than 0.3% RSD (for n=18). Good peak shapes and high peak efficiencies are achieved, indicating that the electrophoretic mobility of copper (II) is well matched to the electrophoretic mobilities of the analytes of interest. The separation of a series of alkyl amines is shown in Figure 11. The amines in this separation all have a +1 charge, and migrate according to their mass.

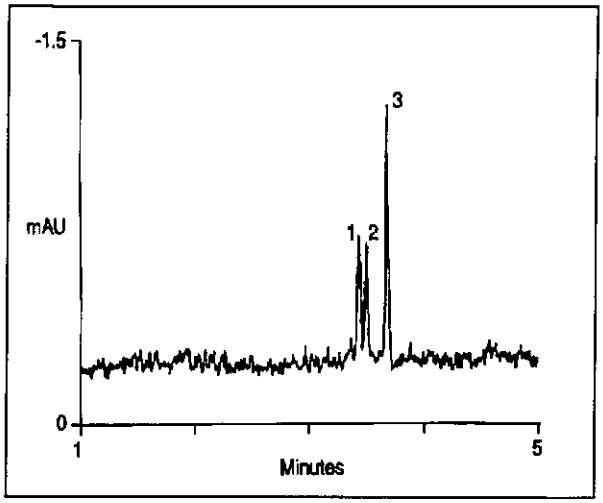


***Fig. 9—Trace Iodide in 100 mM Sodium Hydroxide. Conditions are the same as in Figure 7, except that the sample was diluted (1/1000) with 50 µM octanesulfonate. Peaks: 1=iodide (20 µg/L), 2=chloride (5µg/L), 3=sulfate (10 µg/L).***

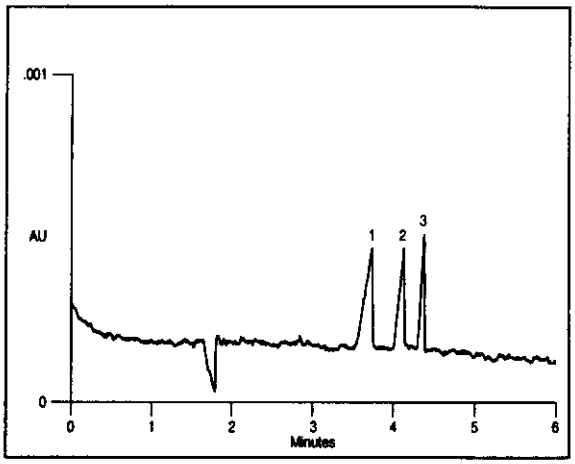


***Fig. 10—CE Separation of Cation Standards. Capillary: 50 µm i.d., 50 cm total length. Electrolyte: 4.0 mM cupric sulfate, 4.0 mM formic acid, 4.0 mM 18-crown-6, pH 3.0. Applied voltage: 20 kV (400 V/cm). Indirect UV detection at 215 nm, 1000 mV = 0.05 AU. Signal output polarity reversed to produce positive peaks. Gravity injection, 100 mm for 30 seconds. Peaks: 1=ammonium (3.6 mg/L), 2=potassium (7.8 mg/L), 3=sodium (4.6 mg/L), 4=calcium (4.0 mg/L), 5=magnesium (2.4 mg/L), 6=strontium (15 mg/L), 7=lithium (0.69 mg/L), 8=barium (27 mg/L).***

## Conclusion

The separation and detection of small ions, both inorganic and organic is accomplished with free zone capillary electrophoresis and indirect detection. Optimized electrolyte systems provide fast, high

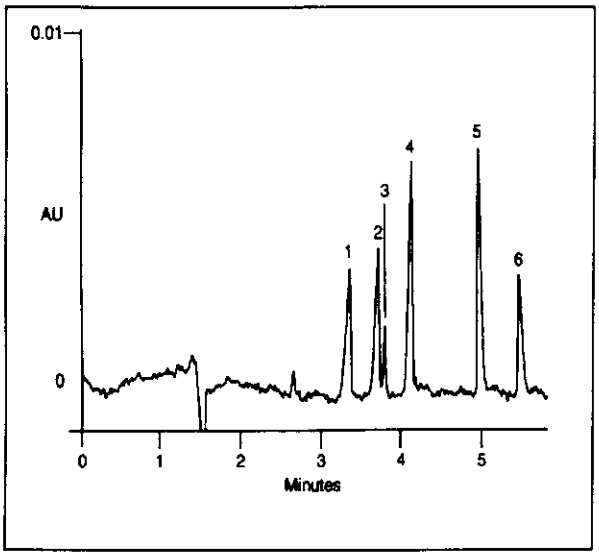


***Fig. 11—CE Separation of Amines. Conditions are the same as in Fig. 10. Peaks: (all at 20 mg/L) 1=monomethylamine, 2=dimethylamine, 3=trimethylamine, 4=monoethylamine, 5=diethylamine, 6=triethylamine.***

resolution separations. CE separations with indirect detection can be used to screen complex samples for analytes of interest because all separated analytes, if present in sufficient concentration, will produce a signal. Using electrostacking, trace analysis (µg/L) is possible. In addition, because of the absence of a stationary phase, as in chromatography, difficult sample matrices can be injected directly. CE and IC are complementary techniques, so CE can be used to confirm chromatographic results or as an alternative to chromatography.

CE is a low cost technique. Minimal amounts of electrolyte are consumed and small amounts of waste are produced. Savings are realized by reduced preparation time and waste disposal costs. A capillary that breaks or becomes unusable costs dollars to replace not hundreds of dollars as with chromatographic columns. The simplicity of the instrumentation makes it easy to use and rugged. The high resolution, low operating cost, and simplicity, make CE the separation technique of the 90's.

**References**

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