# Capillary Electrophoresis/ Electrochemistry

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*Capillary electrophoresis (CE) has received a great deal of attention in the analytical research community. While it is not yet widely accepted for routine bioanalytical chemistry, it shows great promise. One development attracting attention is the combination of CE with electrochemistry. This review considers the technology and several applications to neuroscience and biotechnology.*

Capillary electrophoresis (CE) is proving to be a powerful separation technique. Electrochemical (EC) methods offer significant advantages over classical spectroscopic techniques for CE separations because electrochemistry occurs directly at an electrode surface and, thus, the limits of detection are not compromised by the small dimensions inherent in CE. This is in contrast to spectrophotometric detectors, in which the signal is pathlength-dependent and therefore influenced by the i.d. of the separation capillary. Moreover, electrochemical detectors do not require an optical carrier, and as a result, are much less costly than absorption and fluorescence detectors. This is especially significant when one considers that electrochemical detectors are easily tunable without such components as monochrometers and filters. While laser-induced fluorescence detection offers comparable sensitivity, it is restricted by the limited number of wavelengths of commercially available lasers. In addition, derivatization (either pre- or postcolumn) is often required, which further limits its usefulness.

### *System Design*

Although electrochemical detection offers both sensitivity and selectivity for CE, it is not routinely employed. This is due primarily to the difficulty in performing electrochemistry in the presence of the high voltage associated with CE separations. Therefore, the main design requirement in interfacing an electrochemical detector to a CE system is the electrical isolation of the electrophoretic current from the electrochemical cell. Typically, the current generated by the application of the high voltage separation current is several orders of magnitude greater than that measured at the electrochemical detector.

Wallingford and Ewing (1) were the first to report the design and use of a CEEC system. They employed a porous glass sleeve in which two sections of capillary column were joined near the cathodic end of the capillary. This conductive joint (rather than the end of the column) was immersed in the cathodic buffer reservoir where it permitted the flow of ions but not bulk electrolyte flow. This made it possible to ground the CE system ahead of the electrochemical detector. Buffer and solute zones were pumped by the electroosmotic flow (EOF) generated in the separation capillary past the joint, through the detection end of the column, and into the electrochemical cell, where detection was performed without the adverse effects of the applied electrical field. A schematic diagram of this system is illustrated in ***F1***.

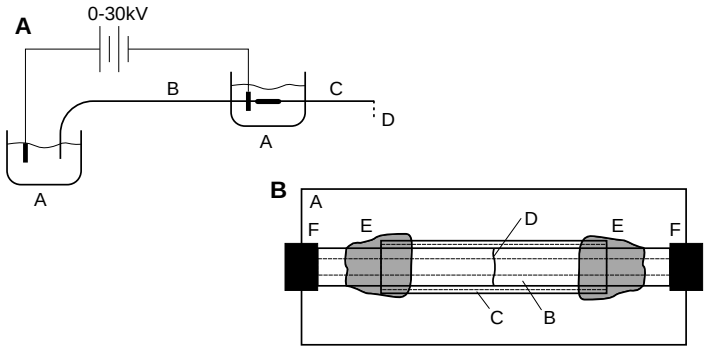
A more rugged and easily constructed version of this type of design was reported by O'Shea et al. (2), who employed the ion-exchange membrane Nafion as the conductive material. The use of this flexible material rather than the fragile porous glass eliminated the necessity to realign the capillary sections after fracture. Other materials that have been successfully implemented in this fashion include

porous graphite (3), cellulose acetate (4), and palladium (5). An alternative decoupling device was reported by Huang and Zare (6), who used a $CO_2$ laser to drill a hole (ca. 40 μm in diameter) in the side of a capillary, which was subsequently filled and sintered in place by a mixture of solder glass and powdered fused silica. The glass frit allowed electrical contact to be established, facilitating its use either in the reported application of sample collection or for grounding prior to electrochemical detection.

Amperometric detection for CE can also be accomplished without the use of a decoupling device (7). This is termed end-column detection, and in this case the working electrode is placed at the end of the capillary (but not inside). However, for this mode of detection, it is necessary to employ capillary columns of very small i.d. (< 20 μm) and low-conductivity buffers to assure that virtually all the voltage associated with the separation is dropped across the capillary. One limitation of this approach is that, because the detector is not isolated from the separation voltage, the actual detection potential is influenced by the electrical field present at the end of the capillary. The magnitude of this electrical field is proportional to the diameter of the capillary, and thus, best results are obtained with capillaries of less than 20 μm i.d. Additional disadvantages of this design are that small fluctuations in the voltage of the power supply used for the separation can translate into noise at the electrochemical detector, and that the electrode must be equilibrated each time the separation voltage is switched on at the start of a run. Due primarily to these factors, concentration detection limits using this design are consistently higher than those obtained with decoupling devices.

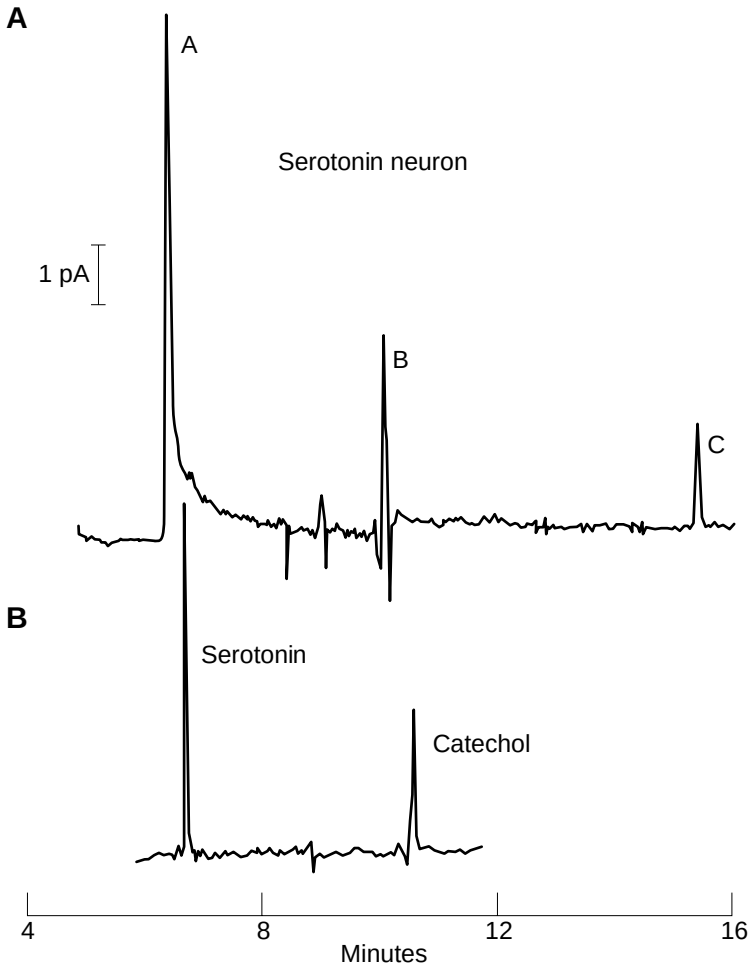
***F1***

*(A) Schematic diagram of coupled CZE system. A, buffer reservoirs; B, separation capillary; C, detection capillary; D, eluent. (B) Microscope slide; B, fused silica capillary; C, porous glass capillary; D, joint; E, epoxy; F, polymer coating. (Adapted with permission from ref. 1.)*




***F2***

*Comparison of electropherograms of a cytoplasmic sample obtained from a large serotonin neuron of* Planorbis corneus *(A) to an electropherogram of serotonin and catechol obtained from a standard solution (B); separation capillary length, 77 cm; capillary i.d., 5 μm; buffer, 25 mM MES (pH 5.65); injection 5 s at 10 kV; separation potential 25 kV. (Adapted with permission from ref. 9.)*




## *Applications*

### Direct amperometry

*Carbon electrodes.* Pioneering research in the area of CEEC was done by Ewing's group (8–11) and was focused primarily on the analysis of single cells. The small sample requirements of CE make it ideal for this application. Sampling of the cytoplasm was accomplished using a capillary glass microinjector (8) or, alternatively, the anodic end of the capillary itself as the injector (9). An electropherogram of a cytoplasm sample obtained from a snail neuron is shown in ***F2***. This represented the first direct detection of neurotransmitters in a single nerve cell. Detection limits using CEEC were in the range of $10^{-7}$ to $10^{-9}$ M for a variety of neuroactive indoles and catecholamines (8–11).

Another application of CEEC which exploits the small sample volume requirements of CE is the analysis of microdialysis samples (12–14). In microdialysis, recovery is a function of flow rate, and the best recoveries are obtained at flow rates of less than 1 μL/min. Therefore, in order to obtain good temporal resolution, a technique having the capability to analyze submicroliter samples with high sensitivity is desirable. Since CEEC consumes sample volumes of nanoliters or lower with detection limits in the nM range for most compounds, enhanced temporal resolution is possible. In one report of CEEC employed for microdialysis studies, L-dopa was administered ip to a rat, after which the levels of the drug were continuously monitored iv over a 2-hour period (12). In comparison to LCEC analysis, CEEC provided better resolution and sample utilization. With the LC method, it was necessary to dilute samples in order to obtain usable injection quantities. The small sample requirements of CE are also advantageous because multiple analyses can be performed on a single dialysate sample. Therefore, it is possible to perform voltammetric characterization of L-dopa and its metabolites for confirmation of peak identity and purity by running the same sample at several different detection potentials.

CEEC and microdialysis were also combined to monitor the release of excitatory amino acids in the brain of a rat (13,14). Extracellular levels of aspartate, glutamate, and alanine in the frontoparietal cortex of the brain were determined. The effect of an influx of high concentrations of potassium ion on the overflow of the amino acids was also monitored. Since these and the majority of other amino acids lack electrochemically active moieties necessary for direct detection, derivatization with naphthalene-2,3-carboxaldehyde (NDA), which reacts with primary amines to produce electroactive derivatives, was employed. Detection limits reported for the derivatized amino acids were in the $10^{-7}$ to $10^{-8}$ M range.

CE is an especially useful technique for the separation of peptides. Most CE separations employ UV detection at 210 nm. Complexation with Cu(II) has been shown to be a sensitive method for the selective detection of peptides by LEEC (15). A similar approach was attempted by CEEC in which Deacon and co-workers (16) employed a Cu(II)-coated capillary for the determination of peptides by CEEC; this permitted direct detection without the need for precolumn derivatization. The Cu(II)-coated capillaries were prepared by flushing fused-silica capillaries with the Biuret reagent. The Cu(II) in the reagent associates with the negatively charged silanol groups on the walls of the capillary. Under alkaline conditions, peptides complexed with Cu(II) on-column and were oxidatively detected at a carbon fiber microelectrode. Di-, tri-, tetra-, and pentaglycine were determined with detection limits ranging from $7.0 \times 10^{-7}$ to $5.5 \times 10^{-6}$ M. It was found that sensitivity decreased with increasing peptide size.

Amperometric detection has also been used with micellar electrokinetic capillary chromatography (MECC). In MECC, the separation is based primarily on partitioning of analyte with a hydrophobic micelle rather than on electrophoretic effects (17). Wallingford and Ewing (10) employed MECC using sodium dodecyl sulfate (SDS) with CEEC for the determination of neutral and cationic catecholamines. It was reported that the sensitivity of the electrochemical detector was affected by the presence of SDS due to fouling of the electrode surface. However, in a later study, it was demonstrated that modification of the electrode surface with Nafion alleviated this problem; however, no conclusive quantitative data were reported (11).

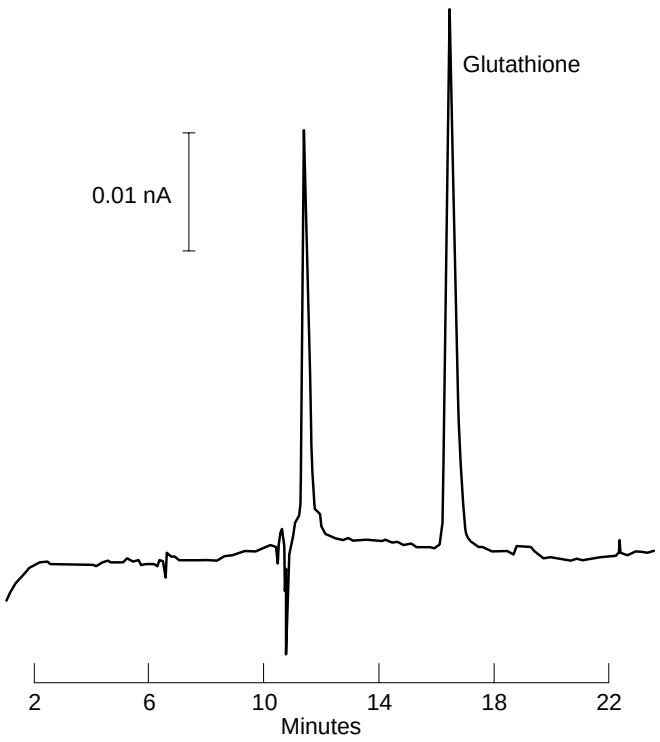
The application of reductive electrochemical detection in LC has been limited as a result of high background currents associated with dissolved oxygen and trace metals. To circumvent this problem, the mobile phase must be deoxygenated and all oxygen-permeable tubing must be eliminated. Reductive electrochemical detection for CE has recently been reported (18). Advantages of this approach compared to LC include rapid deoxygenation times (i.e., minutes vs. hours) and the absence of large amounts of plumbing that will need to be replaced. Analytes detected by reductive CEEC include dinitrophenyl amino acids and a series anthraquinones. The direct detection of the anticancer agent mitomycin C in plasma demonstrated the selectivity of this technique. Deoxygenation of the CEEC system by nitrogen allowed detection limits in the $10^{-7}$ M range to be achieved (18).

*Metal electrodes.* Other electrode materials in addition to carbon have been employed for direct amperometric detection in CE. The use of an amalgamated gold wire microelectrode for the detection of biological thiols, including cysteine and glutathione, down to the nanomolar range has been reported (19). Detection is based on the catalytic oxidation of mercury in the presence of thiols at +0.1 V vs. Ag/AgCl, and is therefore very selective. This system was demonstrated for the determination of glutathione in a rat brain homogenate. The high degree of selectivity is shown in ***F3***, where only one other peak was detected in this complex sample. The applicability of the system can be extended to the detection of thiol-containing drugs, including D-penicillamine, captopril, and 6-mercaptopurine, at the submicromolar level.

This methodology was further advanced by Lin et al. (20) in the development of a dual electrochemical detector for the analysis of both disulfides and free thiols by

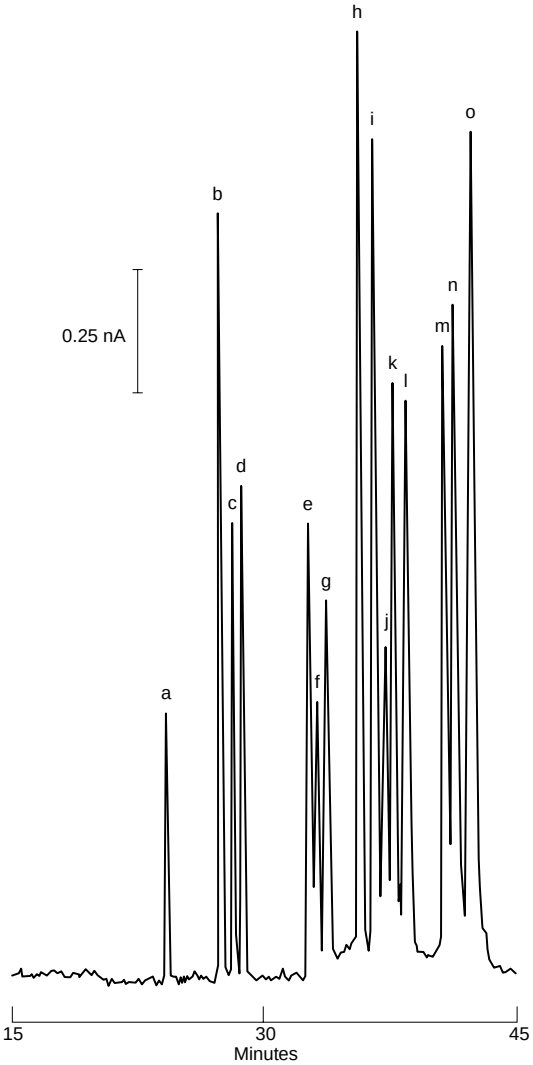
**F3**

*Electropherogram of rat brain homogenate. Peak corresponds to 58 fmol of GSH. Separation buffer, 10 mM MES (pH 5.5); separation voltage, 30 kV; detection potential, +150 mV vs. Ag/AgCl. (Adapted with permission from ref. 19.)*




**F4**

*CZE/ADCP electropherogram of a mixture containing 15 different carbohydrates (80–150 µM). Conditions: separation electrolyte, 100 mM NaOH; fused-silica capillary, 50 µm i.d. × 73 cm; injection 10 s by gravity (10 cm height); separation voltage, 11 kV; carbohydrates (a) trehalose, (b) stachyose, (c) raffinose, (d) sucrose, (e) lactose, (f) lactulose, (g) cellobiose, (h) galactose, (i) glucose, (j) rhamnose, (k) mannose, (l) fructose, (m) xylose, (n) talose, (o) ribose. (Adapted with permission from ref. 23.)*




CE. This detector employed two gold/mercury amalgam microelectrodes operated in series. The first, or upstream, electrode reduced the disulfide to its corresponding thiol. The second, or downstream, electrode detected the resulting thiols as well as endogenous free thiols in the sample. The method yielded detection limits in the micromolar range, and was used for the analysis of cystine and cysteine in urine samples.

The application of a mercury liquid film on a gold microdisk electrode for CEEC determination of 14 metal ions has also been reported (21). In this case, the complexation agent was added to the buffer system, and the ions were detected as their α-hydroxyisobutyric acid complexes. A limitation associated with the Hg-film electrode was its lack of long-term stability. This was demonstrated by a decrease in response of up to 50% for certain complexes over a 5-hour period.

A copper wire microelectrode-based amperometric detector has been described by Engstrom-Silver and Ewing (22). The detection principle was based on the complexation of certain analytes, including amino acids, with a copper oxide film on the surface of the electrode. This complexation and subsequent dissolution of copper oxide resulted in an oxidation current that is dependent on analyte concentration. The detection of nonelectroactive amino acids and dipeptides and electroactive catechols using a 10-µm copper wire electrode was demonstrated.

Copper wire microelectrodes have also been used for the direct detection of carbohydrates at high pH (23). In this report, 15 different sugars were separated at pH 13 (***F4***), and no apparent deterioration of the microelectrode was seen. The response of the detector was found to be linear over three orders of magnitude (µM–mM), and limits of detection for the carbohydrates studied were in the fmol range.

*Chemically modified electrode.* Direct amperometry of many important analytes is often compromised by slow electron transfer kinetics at the electrode surface, which causes oxidation (or reduction) to occur at a potential greater than the expected thermodynamic potential. Chemically modified electrodes (CMEs) can overcome this problem through the use of surface-bound mediators that catalyze the redox reaction of specific solution species at substantially reduced potentials. The use of such CMEs for CEEC has recently been reported (24,25). The first type of modified electrodes employed were based on the immobilization of an electrocatalyst or enzyme into a carbon paste matrix. Electrodes were constructed from a short length of 150-µm i.d. fused silica capillary packed with carbon paste containing the modifier at one end. The disc electrode was then aligned with the end of the CE capillary in a wall-jet configuration. One type of electrode that was investigated incorporated the electrocatalyst cobalt phthalocyanine. This electrode was used to detect several analytes, including cysteine, glutathione, and thioguanine. Detection limits were in the $10^{-8}$ M range. The enhanced selectivity achieved with this electrode was demonstrated by the analysis of cysteine in urine at +450 mV vs. Ag/AgCl. A second design was based on immobilization of an enzyme, glucose oxidase, in carbon paste; this electrode was employed for the selective detection of glucose in blood (24).

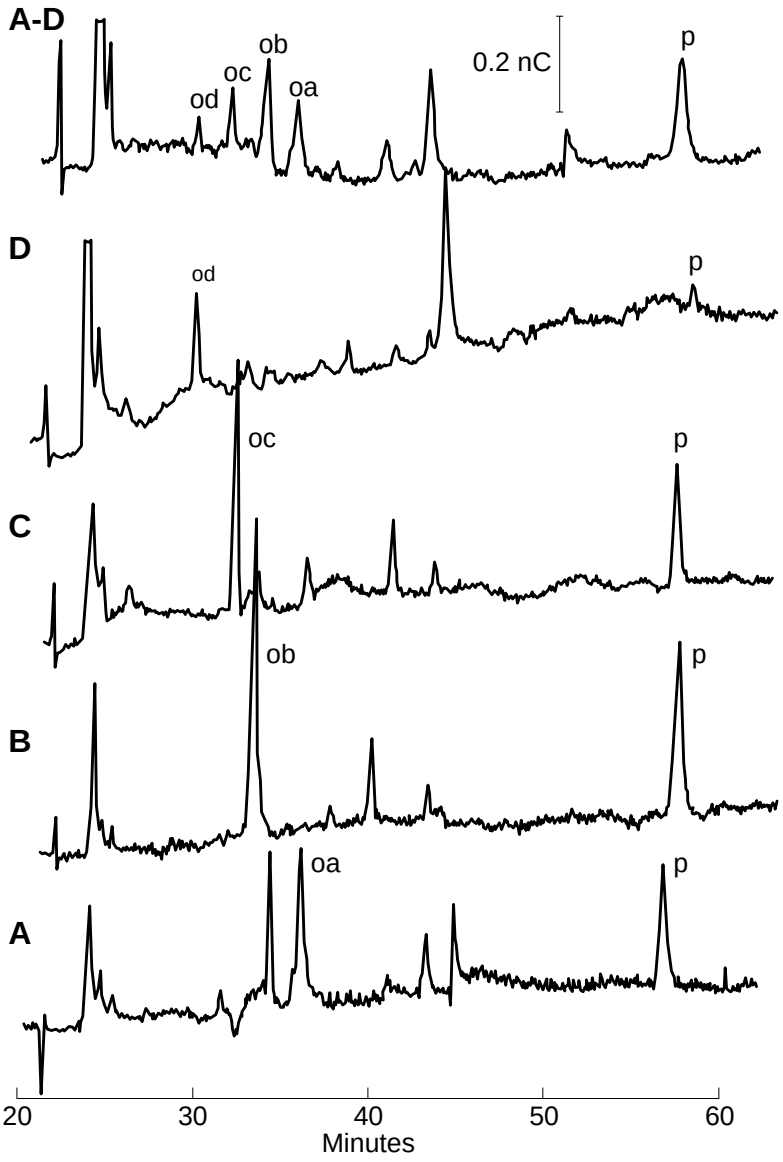
A limitation of CMEs for CE is that the optimal run buffer for the CE separation is often not compatible with the electrolyte conditions necessary for maximum detector response. The use of a RuCN-based modified electrode in conjunction with CE separation for simultaneous determination of thiols and disulfides has recently been reported (25). However, this electrode requires solutions of low pH and high ionic strength for maximum sensitivity and stability. This problem has been addressed by the development of a membrane-based on-column mixer for CE that makes it possible to perform the separation and detection using different buffer conditions (26). This mixer consisted of a cellulose acetate tube and permitted diffusion of ions through the membrane into the detection end of the separation capillary. By employing either HCl or NaOH in a reservoir surrounding this mixer, the pH of the run buffer could be altered just prior to the detector to achieve more desirable detection conditions.

### *Pulsed Amperometry*

Pulsed amperometric detection (PAD) in conjunction with liquid chromatography is a well-established technique, particularly for the analysis of carbohydrates. In the first report of CE-PAD a 50-µm diameter gold wire microelectrode was employed as the working elec-

***F5***

*CZE-PAD analysis of PNGase F-treated glycopeptide fractions isolated from recombinant coagulation factor VIIa with the amino acid backbone Val-Gly-Asp-Ser-Pro-blank-Ile-Thr-Glu-Tyr. Peak p in each electropherogram is the decapeptide, while oa, ob, oc, and od are the oligosaccharides released from glycopeptides A, B, C, and D, which contain 4, 2, 1, and 0 sialic residues, respectively. (Adapted with permission from ref. 28.)*



trode (27). Several carbohydrates of physiological interest, including glucose, were determined by CE-PAD, with detection limits of 0.9 μM or 22.5 fmol. This technique has more recently been employed for the detection of glycopeptides and carbohydrates isolated from recombinant coagulation factor VIIa (28) (***F5***). One of the advantages of CE-PAD for the analysis of glycoproteins is that both the carbohydrates and the peptides generated by PNGase F treatment can be determined in a single electrophoretic run.

Lu and Cassidy (21) employed a two-step pulse waveform for the detection of $Tl^+$, $Pb^{2+}$, and $Cu^{2+}$. The procedure was based on the application of a negative potential to reduce the analyte ions, followed by a positive potential in which the current was sampled from the oxidation of the reduced metal.

### Indirect Amperometry

Although direct and pulse amperometry can provide very sensitive measurements, many analytes are not electroactive and therefore cannot be detected by this approach. Indirect detection can circumvent this limitation; it is based on the analyte displacing a mobile phase additive in the eluted band. The displacement of the additive by the analyte causes a decrease in the signal because the concentration of the additive in the analyte zone is lower than its steady-state concentration. Olefirowicz and Ewing (29) were the first to demonstrate the feasibility of indirect amperometric detection for CE. Dihydroxybenzylamine (DHBA), an electroactive compound, was used as the cationic electrophore, providing a stable background by its continuous oxidation at +700 mV vs. SCE. Zones of nonelectroactive cations displaced the DHBA during the electrophoretic run and were detected as negative peaks. Several amino acids and peptides were detected in this manner with detection limits in the subfmol range. In addition, direct amperometric detection of catechols could be performed simultaneously, allowing both electroactive and nonelectroactive analytes to be detected in the same electrophoretic separation.

### Conclusions

CE and electrochemical detection have emerged as a powerful combination for trace measurements of electroactive compounds. Electrochemical detection has been demonstrated to be particularly well suited to CE, as it is concentration-sensitive and therefore not compromised by the small volumes associated with CE. There is little doubt that only the "tip of the iceberg" has been seen in terms of applications of CEEC. The literature now contains the basic elements for the design of such a system, which should make possible a viable commercial detector in the future. Such an instrument would certainly address the sensitivity limitations currently restricting the applications of CE.

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