Dual Conductivity/Amperometric Detection System for Microchip Capillary Electrophoresis

Joseph Wang* and Martin Pumera

Department of Chemistry and Biochemistry, New Mexico State University, Las Cruces, New Mexico 88003

The performance characteristics and advantages of a new dual electrochemical microchip detection system based on simultaneous conductivity and amperometric measurements are described. The system relies on the combination of a contactless conductivity detector with an end-column thick-film amperometric detector. Such coupling of the conductivity and amperometric detection modes in a single separation channel greatly enhances the sample characterization to offer simultaneous measurements of both ionic and electroactive species, improved reproducibility, and confirmation of peak identity. The simultaneous measurement of nitroaromatic and ionic explosives is used for demonstrating the ability to detect both electroactive and ionic species. Major improvements are also observed for analytes responding at both detectors. For example, the generation of dual response ratios can be used to improve the reproducibility and confirm the peak identity/integrity. Such dual response ratios reflect the distinct redox and conductivity properties of the individual analytes. The independence of the two detectors is reflected in the absence of "cross-talk" effects. The behavior of the dual detector is comparable with those of the individual detectors. Such a dual electrochemical detection system is easy to implement and requires inherently portable low-cost instrumentation.

Chip-based separation devices, particularly micromachined capillary electrophoresis (CE) systems, have witnessed a dramatic growth in recent years. Such miniaturized devices represent the ability to shrink conventional "benchtop" analytical systems with major benefits of speed, integration, portability, high performance, reagent economy, and low cost. As the field of microfluidic devices continues its rapid growth, there are urgent needs for developing compatible detection modes. Much of the work on CE microchips relies on the use of laser-induced fluorescence (LIF) detection. Yet, due to their relatively large supporting instrumentation, most LIF systems are not compatible with the creation of truly miniaturized systems.

Electrochemical detection, in contrast, offers great promise for such microsystems, with features that include inherent miniaturization of both the detector and control instrumentation, compatibility with advanced micromachining technologies, high sensitivity, independence of optical path length or sample turbidity, and low cost and power requirements.[1,2] The majority of chip-based electrochemical detectors have relied on fixed-potential amperometric measurements of electroactive species. Conductivity is another electrochemical detection mode that has attracted considerable recent interest.[3−5] A conductivity detector can sense all ionic species having conductivity different from the background electrolyte. Conductivity detection has thus been shown to be a sensitive method for the detection of inorganic[3,4] and organic[5] ions in microchip separations.

The present paper reports on a new dual amperometric/conductivity detection strategy for CE microchip systems. The coupling of flow systems with multiple detection principles can facilitate the measurement of analytes with different physical properties. A variety of dual detection schemes have thus been proposed for conventional liquid chromatography, capillary electrophoresis, or flow-injection systems,[6−12] but rarely in connection with microfluidic devices.[13−15] Schrum et al.[13] demonstrated the simultaneous fluorescence and light-scattering monitoring in microchip flow cytometry. Martin et al.[14] reported on a dual-electrode amperometric detection in microchip formats, and Lapos et al.[15] have just described the simultaneous LIF and amperometric measurements on CE microchips.

The present coupling of conductivity and amperometric detection methods in a single microchip separation offers a greatly improved sample characterization. For example, the simultaneous amperometric and conductivity electropherograms allow more migrating components to be identified and quantified. The ability to monitor both electroactive and ionic components is illustrated for the simultaneous detection of redox-active nitroaromatic and

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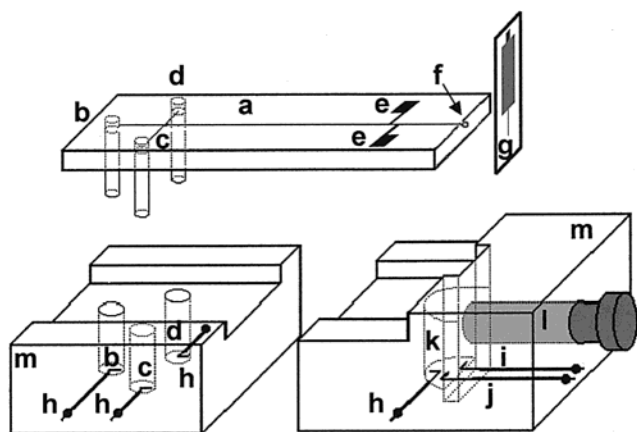* Corresponding author. E-mail: joewang@nmsu.edu.

Figure 1. CE microchip system with a dual amperometric/conductivity detection system: (a) glass microchip, (b) run buffer reservoir, (c) sample reservoir, (d) unused reservoir, (e) aluminum electrodes for contactless conductivity detection, (f) channel outlet, (g) thick-film working electrode for amperometric detection, (h) high voltage power electrodes, (i) counterelectrode, (j) reference electrode, (k) outlet reservoir, (l) plastic screw, (m) Plexiglas holder.

ionic explosives. Such simultaneous microchip monitoring of low- and high-energy explosives addresses the growing demands for innovative and effective tools for on-site detection of major explosives. Dual response ratios provide unique characterization of the individual components based on their distinct redox and conductivity properties and can be used for improving the precision. A schematic depiction of the dual conductivity/amperometric CE microchip configuration is shown in Figure 1. The system relies on the coupling of a contactless conductivity detector[4] with an end-column amperometric detector.[16] Its attractive behavior is reported in the following sections.

EXPERIMENTAL SECTION

Reagents. Histidine (His), 2-(N-morpholino)ethanesulfonic acid (MES), lithium dodecyl sulfate (LiDS), sodium acetate, ammonium nitrate, and sodium perchlorate were purchased from Sigma. Stock solutions of 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB), 2,4-dinitrotoluene (2,4-DNT), and 2-amino-4,6-dinitrotoluene (2-Am-4,6-DNT) (1000 mg/L in acetonitrile) were obtained from Cerilliant (Austin, TX). *p*-Aminophenol (*p*-AP), *o*-aminophenol (*o*-AP), and *m*-aminophenol (*m*-AP) were obtained from Aldrich. Methylamine solution was purchased from Fluka, and acetic acid was obtained from Fisher Scientific. The run buffer for the simultaneous analysis of low- and high-energy explosives (a mixture of 20 mM MES and 20 mM His containing 15 mM LiDS, pH 6.1) was prepared by dissolving LiDS, MES, and His in deionized water. The acetate buffer (pH 5.0) for the determination of aminophenols was prepared by mixing 20 mM sodium acetate and 20 mM acetic acid solutions at a 70:30 volume ratio. Stock solutions of the target analytes (ammonium; methylammonium; sodium; TNB; TNT; 2,4-DNT and 2-Am-4,6-DNT; or *p*-AP, *o*-AP, and *m*-AP) were prepared by dissolving the compounds (or their salts) in the corresponding run buffer. All chemicals were used without any further purification.

Apparatus. The homemade high-voltage power supply had an adjustable voltage range between 0 and +4000 V. The CE

microchip, with the dual amperometric/conductivity detection system, is shown schematically in Figure 1. The simple cross glass microchip was obtained from Micralyne (model MC-BF4-001, Edmonton, Canada); its original waste reservoir was cut off, leaving the channel outlet available for the end-column amperometric detection.[16] The cover plate of the glass chip was polished to a thickness of 150 μm to facilitate the contactless conductivity detection.[4] The microchip had a four-way injection cross-connected to the three reservoirs and the separation channel. The effective lengths of the separation channel for the on-column contactless conductivity detection and for the end-column amperometric detector were 51 and 75 mm, respectively. Short pipet tips were inserted into the holes of the buffer and sample reservoirs. A Plexiglas holder was fabricated to house the separation chip, the amperometric detector, and the buffer/sample reservoirs.[16]

Contactless Conductivity Detector. An Agilent 33120A function generator (Agilent, Palo Alto, CA) was used for generating the sinusoidal AC waveform for the conductivity detection. The contactless conductivity detection was accomplished using a frequency of 200 kHz with a peak-to-peak amplitude of $5V_{p-p}$. The electronic circuitry of the conductivity detector was placed on the chip cover.[4] The circuitry was designed in accordance with a previously reported scheme.[4,17] This scheme allows convenient interface to data acquisition systems (chart recorder or a computer DAQ). The electronic components were purchased from local suppliers. The electronic circuit was placed in a shielding box to protect the electronics from external electric fields. The open side of the box was placed as close as possible to the sensing electrodes (on the chip) so that the box acts also as a shield for the electrodes. The rectangularly shaped conductivity electrodes (0.8 × 10 mm) were fabricated from two 10-μm-thick aluminum foil strips. The end side of the electrode was widened to 4 mm to facilitate the electrical connection. The electrodes were fixed to the top of the 150-μm-thick cover plate of the glass microchip using a common epoxy at a distance of 24 mm from the end of the microchannel and with a distance of 1.3 mm between them (Figure 1e). Thin copper wires (0.1-mm diameter, 25-mm long, Aldrich) were attached to the electrodes using a conducing epoxy (Chemtronics, Kennesaw, GA) and were tin-soldered to the detector electronics. The electrodes were placed in an "antiparallel" orientation to minimize the stray capacitance between them.[4,18]

Amperometric Detector. The thick-film carbon electrodes were printed with a semiautomatic printer (model TF 100, MPM, Franklin, MA). A carbon ink (Electrodag 440B; Catalog no. 49AB90; Acheson Colloids, Ontario, CA) was used for printing the working electrodes. Details of the printing process and dimensions were given elsewhere.[16] The gold-modified thick-film electrode was prepared by applying a potential pulse waveform (square-wave pulse between −0.2 and +0.75 V vs Ag/AgCl, with a pulse width of 0.6 s) for 40 min in a 1000 mg/L Au(III) solution.[19] The resulting end-column thick-film amperometric detector (Figure 1, g) was described earlier.[16] The electropherograms were recorded with the 620 Electrochemical Analyzer (CH Instruments, Austin, TX) using a time resolution of 0.1 s while applying the

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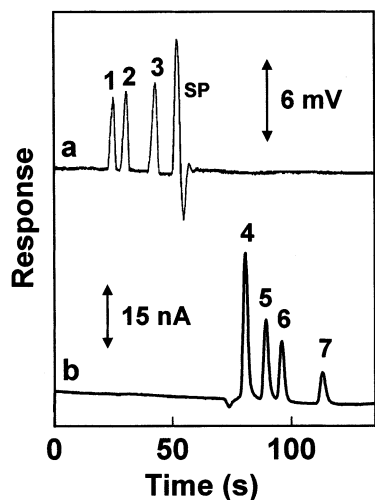
Figure 2. Electrophoregrams showing the simultaneous measurement of low- and high-energy explosives, as recorded with the conductivity (a) and amperometric (b) detectors. Analytes: ammonium (1), methylammonium (2), sodium (3), TNB (4), TNT (5), 2,4-DNB (6), and 2-Am-4,6-DNB (7), system peak (SP). Explosive concentration, 2 mM (1,2,3) and 15 ppm (4,5,6,7). Conditions: MES/His buffer (20 mM, pH 6.1) containing 15 mM LiDS as the run buffer; separation field strength, +250 V/cm; injection field strength, +250 V/cm for 2s; detection at 200 kHz, 5 V_{p-p} (a), and at −0.5 V (b).

desired detection potential (usually −0.5 V vs Ag/AgCl wire). Sample injections were performed after stabilization of the baseline.

Procedure. The channels of the glass chip were treated before use by rinsing with 0.1 M NaOH and deionized water for 10 min each, respectively. Reservoirs b, d, and k in Figure 1 were filled with the electrophoretic run buffer solution, and reservoir c was filled with the sample mixture. After the initial sample loading (in the injection channel), the sample was injected by applying a field strength of +250 V/cm for 2 s between the sample reservoir (c) and the grounded outlet reservoir (d). This drove the sample "plug" into the separation channel through the intersection. The analytical separation was performed by applying the separation potential to the run buffer reservoir (a) with the outlet reservoir (d) grounded and all other reservoirs floating.

Safety Considerations. *The high-voltage power supply should be handled with extreme care. The chemicals are mutagenic/toxic/irritants. Skin contact and accidental inhalation or ingestion should be avoided.*

RESULTS AND DISCUSSION

The two orthogonal detection modes allow measurements of a wider range of analytes per separation. Measurements of low- and high-energy explosives were employed for demonstrating the simultaneous profiling of redox and conductivity properties. Figure 2 displays typical dual-detection electropherograms for a sample mixture containing the nitroaromatic explosives TNB (4), TNT (5), 2,4-DNB (6), and 2-Am-4,6-DNB (7), as well as the explosive-related ammonium (1), methylammonium (2), and sodium (3) ions. Although the conductivity detector (a) profiles only the ionic species (1−3), the amperometric one (b) responds to the redox-active nitroaromatic components (4−7). Both detectors display well-defined and resolved peaks (half-peak widths of 2−3 s), flat baselines, and low noise levels. Total assay of this 7 explosive-
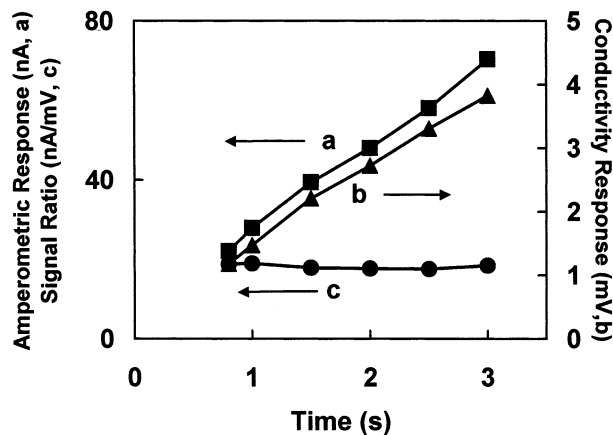

Figure 3. Effect of the injection time upon the simultaneous amperometric (a) and conductivity (b) response for 350 μM p-aminophenol, as well as upon the corresponding peak ratio (c). Conditions: acetate buffer (20 mM, pH 5.0) as the run buffer; separation field strength, +188 V/cm; sample injection at +188 V/cm for 2 s; detection at 200 kHz, 5 V_{p-p} (a), and at +0.8 V using a gold-coated thick-film carbon electrode (b).

related mixture can thus be performed within <2 min. Such microchip detection of a wide range of explosive components holds great promise for obtaining the desired forensic information in a faster and simpler manner.

In addition to a wider range of detectable analytes, the amperometric/conductivity detection scheme allows the attainment of two simultaneous signals for the same component. This offers several analytical advantages through the generation of dual response ratios (for components yielding response at both detectors). Such dual response ratios can be used for confirming the peak identity, estimating the peak purity, or improving the reproducibility. For example, a common problem with electrokinetic injection is the run-to-run variations in the injection time.[20] This is illustrated in Figure 3 for the detection of p-aminophenol in connection with a deliberate change of the injection time from 0.8 to 3.0 s. As a result, both the amperometric (a) and conductivity (b) aminophenol signals greatly fluctuate to yield large relative standard deviations (RSD) of 42.2 and 41.2%, respectively. Such irreproducibility can be addressed by the dual detection scheme. Since both peaks are similarly influenced by the injection time, the resulting amperometric/conductivity response ratio (c) is largely independent of the injection time. This is indicated from the dramatically improved reproducibility (a RSD of 3.3%). Note that such an improvement is achieved without the use of an internal standard. Similar improvements are also anticipated for variations in the migration time, as was illustrated recently for a dual fluorescence/amperometric detection.[15]

The peak ratios also provide a real-time fingerprint of each component and can facilitate the peak assignment (through comparison to ratios for a standard mixture). The dual response ratios of the new detector reflect the distinct redox and conductivity properties of the individual analytes. Figure 4 shows dual-detection electropherograms for a sample mixture containing three different aminophenols (that yield well-defined and resolved peaks at both detectors). Peak ratios of 17.8, 21.9, and 5.4 nA/mV have thus been obtained for p-aminophenol (1), o-aminophenol

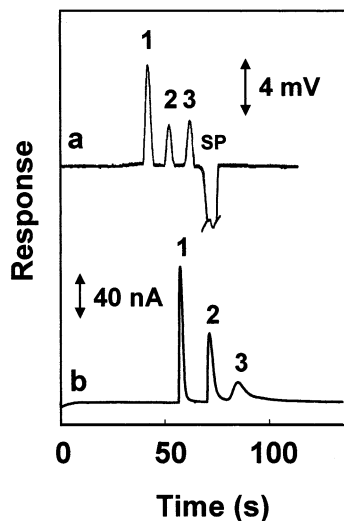(20) Dose, E.; Guiochom, G. A. *Anal. Chem.* **1991**, *63*, 1154.

Figure 4. Electrophoregrams showing the simultaneous measurement of aminophenols as recorded with the contactless conductivity (a) and amperometric (b) detectors. Analytes: *p*-aminophenol (1), *o*-aminophenol (2), *m*-aminophenol (3), system peak (SP). Concentration, 900 μM (*p*-AP) and 450 μM (*o*-AP and *m*-AP). Other conditions as in Figure 3.
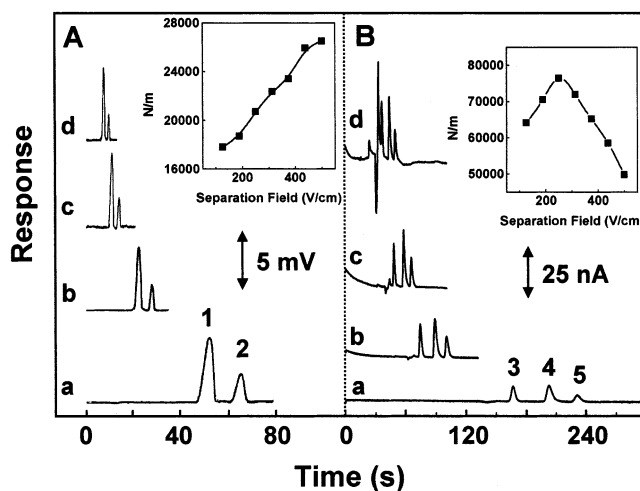

Figure 5. Influence of separation field strength upon the simultaneous conductivity (A) and amperometric (B) response for a mixture containing 2 mM ammonium (1), 700 μM methylammonium (2), 30 μM TNB (3), 60 μM TNT (4), and 60 μM 2,4-DNB (5). Separation field strength +125 (a), +250 (b), +375 (c), +500 (d) V/cm. Also shown (as insets), the influence of the separation voltage upon the number of theoretical plates of the ammonium (A) and TNT (B) peaks. Other conditions as in Figure 2.

(2), *m*-aminophenol (3), respectively. The improvement in the peak identity information is analogous to that of dual-potential amperometric detection schemes,[7] which rely solely on redox properties. Note also the shift in the migration times, associated with the placement of the two detectors at different locations along the separation channel (on-column vs end-column; Figure 1). Accordingly, the amperometric peaks show more tailing than the conductivity ones.

Experimental parameters affecting the dual conductivity/amperometric detection of explosive components were evaluated and optimized. The influence of the separation field strength upon the simultaneous conductivity and amperometric response is displayed in Figure 5. As expected, increasing the separation field from +125 to +500 V/cm dramatically decreases the migration time of the ammonium (1) and methylammonium (2) species from 51.0 to 7.0 s and from 64.5 to 9.5 s, respectively (Figure 5A) and of the TNB (3), TNT (4), and 2,4-DNT (5) explosives from 166.6 to 35.5 s, from 202.6 to 42.6 s, and from 231.3 to 48.3 s, respectively (Figure 5B). The two detectors display different plate numbers vs separation field profiles (Figure 5, inset). As expected, the plate number of the ammonium ion conductivity peak increases substantially from 17 675 to 26 309 over the 125–500 V/cm range (A). In contrast, the plate number of the amperometric TNT peak increases to a maximum value of 76 436 upon raising the separation voltage field from +125 to +250 V/cm and decreases gradually to 49 757 upon raising the field strength to +500 V/cm (B). Although one would expect a larger number of theoretical plates at higher voltage fields, the plate number of the amperometric detector is influenced by other factors, such as end-column broadening and incomplete isolation from separation field.[21,22] Such coupling lowers the apparent detection potential, hence slowing the reduction and reducing the ability to detect the peak. The

separation voltage field has a negligible effect upon the peak-to-peak background noise level of both conductivity and amperometric detectors for voltages ranging from +125 to +312 V/cm. Separation field strengths higher than +312 V/cm resulted in higher background and noise levels (associated with Joule heating effects) of the conductivity detector. Yet, a flat baseline is observed even at +500 V/cm, reflecting the effective isolation of the on-column contactless conductivity detector. The initial baseline slope of the amperometric detector, observed for voltages ranging from +375 to +500 V/cm, indicates an incomplete isolation of this end-column detector from high separation voltages.

The dual conductivity/amperometry microchip detection system displays a well-defined concentration dependence for simultaneous measurements of both low- and high-energy explosives. For example, a calibration experiment involving sample mixtures containing increasing levels of ammonium ion and TNT (in steps of 500 μM and 8 μM, respectively) yielded well-defined peaks proportional to the analyte concentration (not shown; conditions as in Figure 2). The resulting calibration plots were highly linear with sensitivities of 2.76 mV/mM and 0.32 nA/μM for ammonium and TNT, respectively (correlation coefficients >0.999). Detection limits of 0.32 μM TNT and 50 μM ammonium ion were estimated from assays of a mixture containing 2 μM TNT and 200 μM ammonium (not shown).

Good reproducibility is another property of the CE conductivity/amperometry microchip system. For example, Figure 6 displays electropherograms for a series of six repetitive injections of a five-component explosive-related mixture containing 2 mM ammonium (1), 700 μM methylammonium (2), 25 μM TNB (3), 60 μM TNT (4), and 60 μM DNB (5). Stable response peaks are observed during this simultaneous operation, with relative standard deviations of 3.5 (1), 3.8 (2), 3.2 (3), 3.1 (4), and 3.5% (5). The stability of the response is attributed in part to the absence of unwanted surface-passivation reaction associated with the contactless-conductivity operation.

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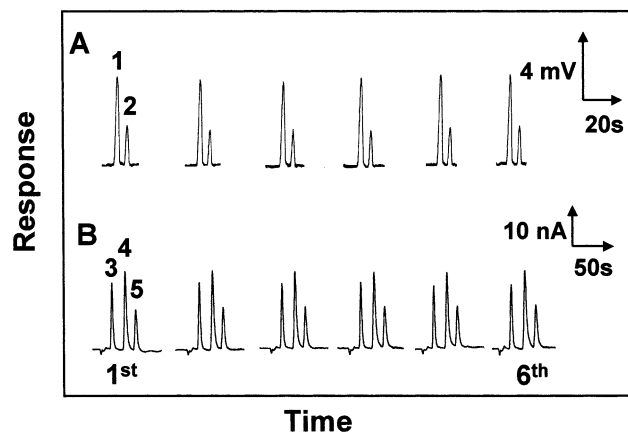
Figure 6. Repetitive simultaneous measurements of a mixture containing 2 mM ammonium ion (1), 700 μM methylammonium ion (2), 30 μM TNB (3), 60 μM TNT (4), and 60 μM 2,4-DNB (5), as recorded with the conductivity (A) and amperometric (B) detection modes. Other conditions as in Figure 2.

The possibility of "cross-talk" between the two detectors was also examined. No apparent difference in the conductivity signal was observed in the presence and absence of the amperometric detector. For example, similar conductivity peaks were observed for 1 mM ammonium and methylammonium ions upon turning the amperometric detector on and off (not shown). Similarly, a mixture containing 10 μM TNB, 16 μM TNT, and 36 μM 2,4-DNB yielded similar current signals when the conductivity and amperometric detection systems were operated simultaneously and upon switching off the conductivity detector. The background characteristics of the amperometric detector were also not affected by the conductivity operation. Similarly, no cross talk was observed for compounds (e.g., aminophenols) responding at both detectors (not shown). The absence of cross-reactivity effects reflects the independence of the two detectors associated with the placement of the conductivity detector on the chip cover and of the amperometric one at the end of the channel.

CONCLUSIONS

We have demonstrated a single-channel simultaneous conductivity/amperometry detection that enhances the power of CE microchip systems. Such dual electrochemical microchip detection system greatly improves the sample characterization, because it offers simultaneous monitoring of electroactive and ionic species, confirms the peak identity/integrity, and improves the reproducibility. Improved resolution of comigrating components (in which only one species is electroactive or ionic) is also anticipated. The distinct sample characterization associated with the use of independent amperometric and conductivity detectors makes this strategy a powerful tool in the armory of microchip detection schemes. The ability to simultaneously detect both nitroaromatic and ionic explosives holds great promise for monitoring major explosives at the sample source. Although the system was illustrated in analyses of explosives and aminophenols, it is applicable to a wide range of analytes. Additional improvements could be achieved by combining the conductivity detection with a dual-electrode amperometric detector (that would add the potential dimension of information). The dual electrochemical detection scheme is easy to implement, requires an inherently portable low-cost instrumentation, and holds great promise for the creation of truly miniaturized analytical systems.