CHARPTER V

MICROCHIP CAPILLARY ELECTROPHORESIS COUPLING WITH ELECTROCHEMICAL DETECTION

5.1 Introduction

The concept of microchip-based assays integrating all the components required for chemical and biochemical analysis, while utilizing the microfluidic elements to guide the sample automatically from one place to another is important towards realizing micro total analysis systems. The development of various assays is necessary to create new generation technologies in the fields of clinical and environmental sciences, and to create biomedical devices for a better future.

Currently, laboratory based analyses provide more precise and accurate data than most on-site processes. However, attracted by the promise of on-site analyses in time efficiency, cost reduction, and potential real-time decisions, efforts are being made in developing analytical devices that are stable, and reliable. There is a great need to monitor environmentally hazardous materials and biological and chemical warfare agents. Safeguards are necessary to protect the health and safety of society, ensure food safety, and protect water supply systems. In particular, there is a need for devices that are able to monitor and identify hazardous materials rapidly as an early warning system or post-analysis fingerprinting tool. It is also important that these devices are portable for on-site use. Compact systems, suitable for on-site measurement of environmental pollutants are performed, since they afford the option of rapid warning and avoid errors and delays inherent to lab-based analyses. New types of miniaturized analytical instrumentation are therefore required to meet all the demands of on-site analyses, matching the performance of laboratory based assays. Such demands include reduced sample/reagent consumption and waste production, simple operation, size of the instrument and data processing/storage steps, and faster and cheaper analyses.

Miniaturized analytical systems (referred to as "lab-on-a-chip" devices) are attracting considerable interest, due to their potential in greatly enhancing the speed of analytical separations, while dramatically reducing the system size or weight and

consumption of samples and reagents. "Lab-on-a-chip" technology offers great promise for converting large and sophisticated instruments into powerful filed-deployable analyzers. Microchip systems will enable novel investigations in many areas as mentioned, such as clinical diagnostics, environmental monitoring or forensic investigations. These miniaturized devices represent the ability to shrink the conventional 'bench-top' separation systems with the major advantages of speed, cost, portability and solvent/sample consumption. As the field of chip-based micro-scale systems continues its rapid growth, there are urgent needs for developing compatible detection modes. As with other analytical systems, sensitive detection schemes are also required for micro-instrumentation. While microchip technology is maturing very rapidly, the development and availability of effective detectors has also been demonstrated and documented.

Various types of detection modes have been employed to monitor separations on these devices. Laser-induced fluorescence (LIF) has been the most popular mode for microchip capillary electrophoresis (CE), due to its inherently high sensitivity [69-70]. However, most compounds are not naturally fluorescent and must be derivatized with a fluorophore to be detected by LIF. Furthermore, it is necessary to select the excitation wavelength specific for the particular fluorophore. Mass spectrometry (MS) has also been employed as a detection mode for miniaturized devices. The primary advantage of coupling MS with microchip CE devices is increased throughput of samples. Unfortunately, commercially available MS systems are costly and not inherently portable [167-168].

Electrochemistry (EC) offers great promise for such microsystems, with features that include remarkable sensitivity (approaching that of fluorescence), inherent miniaturization of both the detector and control instrumentation, independence of optical path length or sample turbidity, low cost, low-power requirements and high compatibility with advanced micromachining and microfabrication technologies. Recent reviews presenting electrochemical detection illustrated attractive properties in and advantages of adopting such a detection scheme for microsystems [169-174].

Mathies *et al.* first demonstrated the suitability of electrochemical detection for microchip electrophoretic systems in connection with fixed-potential amperometric measurements [175]. Such controlled-potential detectors are ideally

suited for monitoring analytes that are electroactive at a modest potential, i.e. easily oxidizable or reducible compounds. Various electrode materials (carbon-based electrode materials such as carbon fibers, glassy carbon, and graphite; metallic electrodes such as gold, platinum, copper and silver; and other chemically modified electrodes) have been used by various authors for their use in microsystem technology.

In environmental analysis, hydrazines, phenols, and explosives are of primary concern because of the adverse human health affects associated with acute exposure to these compounds. Analytical methods were developed to document the presence of these pollutants in the environment and describe the extent to which an area was contaminated. The monitoring of these compounds continues to be of great interest in order to understand the long-term health effects of chronic exposure to low concentrations of pollutants. As instrumental detection limits improve and new detection techniques become more widely used, there is a need for developing fast and sensitive methods to detect these hazardous analytes. Such methods must, however, be field-tested and in compact size with integrated sample extraction, sample preparation, and analyte detection modules. In environmental monitoring, delayed analytical results could lead to more serious contamination. However, on-site analysis can significantly improve real-time decisions, such as those concerning environmental remediation or site characterization. In addition to saving time and enabling on-site decision making capabilities, on-site analysis can dramatically reduce the cost of operations and eliminate errors associated with transportation and storage of samples.

This chapter focused on the development of microsystems with electrochemical detectors suitable for the detection of environmentally important compounds. Some of the examples of significance are discussed in the following sections combining the versatility of microchips with the simplicity of electrochemical detectors to automate, miniaturize, and simplify both analytical tools and processes so as to solve analytical problems in an efficient and convenient fashion.

5.2 Experimental Section

5.2.1 Electrode Preparation

5.2.1.1 Capillary Electrode Construction

The unmodified carbon paste was prepared by thoroughly hand-mixing 5 g of graphite powder and 3 mL of mineral oil (Aldrich). The CoPc-modified paste was prepared in a similar pattern except that the graphite powder was mixed with the desired weight of CoPc before adding the mineral oil. Mixing of these two components was accomplished by adding 10 mL of diethyl ether to 5 g of the CoPc/graphite mixture and sonicating until all the diethyl ether evaporated. The CoPc loading in the carbon paste was described in terms of percent basis of the weight of CoPc added to the graphite powder. A copper wire (10 cm long and 0.5 mm diameter) was inserted into a piece of fused silica capillary (5 cm long, 0.53 mm id, 0.68 mm od) and a 1.0 cm opening was left in the capillary for the subsequent filling of the paste material. A portion of CoPc/graphite mixture was then packed into one end of a fuse silica capillary. Finally, the surface was smoothed by polishing on a piece of weighing paper to form a disc electrode. Electrical contact was obtained using internal copper wire.

5.2.1.2 Screen Printed Carbon Electrode Fabrication

The thick-film carbon screen printed electrodes (SPE) were printed on 33 x 10 mm ceramic substrates with a semi-automatic screen printer (Model TF 100, MPM, Franklin, MA). A carbon ink (Acheson Colloids, Electrodag 440B, Cat. No. 49AB90, Ontario, CA) was used for printing the working electrode strips. Details of the screen-printing process were described elsewhere [176]. Bare screen-printed carbon electrodes (0.30 x 6.0 mm) were used for measurements of the nitroaromatic explosives. The gold-coated carbon working electrodes, used for detecting of the phenolic compounds, were prepared by electrodepositing gold for 30 min at -0.2 V (vs. Ag/AgCl) onto the carbon electrodes from a solution containing 300 ppm Au(III), 0.1 M NaCl and 1.5% HCl.

5.2.2 Microchip Apparatus

5.2.2.1 Apparatus for Normal Chip Layout

The simple-cross glass microchip, obtained from Micralyne Inc.(AMC, Model MC-BF4-001, Edmonton, Canada), was fabricated by means of wet chemical etching and thermal bonding techniques. The 90 mm × 16 mm × 2 mm chip had a four-way injection cross (50 μm × 50 μm), with a 72 mm long separation channel and side arms, each 5 mm long. The detection reservoir of the chip was cut off to facilitate the end-column electrochemical detection. The channels had a maximum depth of 20 μm and a width of 50 μm.

Details of the integrated microchip CE/electrochemical detection were described elsewhere [176]. The CE microchip was placed in a laboratory-built Plexiglas holder for housing the separation chip and electrochemical detector. The holder consisted of a sample, running buffer, and unused reservoirs. Platinum wires, inserted into each reservoir, served as contacts for the high-voltage power supply. Short pipettes were inserted into the three holes on the glass chip for providing solution contact between the channel on the chip and the corresponding reservoir on the chip holder. The electrochemical detection compartment, on the channel outlet side, consisted of a platinum wire, an Ag/AgCl wire counter and reference electrodes. The home-made high-voltage power supply (with an adjustable voltage range of between 0 and +4000V) was used for applying injection and separation voltages.

5.2.2.2 Apparatus for Continuous Monitoring

Details of the integrated capillary electrophoresis (CE) glass chip microsystem and its electrochemical (EC) detector were described previously [176]. Briefly, the homemade high-voltage power supply had an adjustable voltage range of between 0 and +4000 V. The simple-cross single separation channel glass microchip was obtained from Micralyne (Model MC-BF4-001, Edmonton, Canada). The glass microchip had a four-way injection cross that was connected to three reservoirs and a separation channel. The sample and run-buffer reservoirs were cut off

and the corresponding chip areas cut with a diamond saw (in a local glass shop) to sharp inlets facilitating the continuous introduction of the sample and run-buffer solutions from 2 mm i.d. Tygon tubing mounted over the inlets (and sealed with epoxy). The detection reservoir was also cut off to facilitate the end-column amperometric detection. The channels had a maximum depth of 20 μm and a width of 50 μm at the top. A piece of PDMS sheet was used for sealing the remaining reservoir.

A Plexiglas holder was fabricated for housing the separation chip and the electrochemical detector compartment, while allowing a convenient replacement of the chip and working electrode. The hemispherical detection compartment (d) housed the platinum-wire grounded electrode (h) and the three-electrode detection system. The latter consisted of the screen-printed working electrode (i), a platinum wire auxiliary electrode (k), and an Ag/AgCl wire reference electrode (j). Two platinum wires (l_1,l_2) were inserted into the sample (e) and run-buffer (f) inlet tubing [close to the sample (b) and buffer (c) inlets, respectively] to serve as contacts to the high-voltage power supplies. The platinum wires were sealed to the tubing by extra fast-setting nonconductive epoxy (Elementis Specialties, Inc., Belleville, NJ). A FIAlab peristaltic pump (P) (Alitea Instruments USA, Medina, WA) was used to drive the sample solution at a fixed rate from a 50 mL beaker flow through the 2 mm i.d. Tygon polymer tubing (e) over the sharp inlet tip connection (b), and continuing to a 1 mm i.d. Teflon tubing outlet (g) (Upchurch Scientific Inc., Oak Harbor, WA) that was inserted into the sample tubing 1 mm from the sharp inlet tip (b). The sample was injected into the separation channel by holding the platinum wire electrode (l_1) at a positive potential (2000 V for 3 sec), while maintaining the platinum wire grounded electrode (h) in the detector compartment (d) at a ground potential. The running buffer was injected simultaneously into the separation channel in a similar (electrokinetic) fashion. The running-buffer solution was initially introduced to the side sharp buffer inlet tip (c) by filling it into a 2 mm i.d. Tygon tubing (f) with a syringe and holding the end of the tubing in the running buffer reservoir (RB). The running buffer flow in the tubing was maintained by capillary action.

5.2.3 Electrophoresis Procedure

Before use, the channel was treated with 0.1 M sodium hydroxide and deionised water for 15 and 10 min, respectively. For hydrazine detection: The running buffer was 10 mM phosphate buffer (pH 6.5), prepared by dissolving the required amount of sodium phosphate monobasic and sodium phosphate dibasic in deionized water. Each of the reservoirs in the chip holder and corresponding pipette tips on the microchannel chip were filled with their respective solutions. The sample reservoir was filled with the mixture of hydrazines, while the "buffer" reservoirs were filled with the CE running buffer solution. A potential of 1,000 V was applied to the "sample" reservoir for 3 s with the detection reservoir grounded and other reservoirs floating. The separation was performed by applying 1,000 V to the running buffer reservoir with the detection reservoir grounded and other reservoirs floating. By switching the high voltage contacts, the separation potential was subsequently applied to the "running buffer" reservoir for the separation of the hydrazine compounds. All experiments were carried out at room temperature.

For phenolic compounds: to obtain the 'total' signal, the running buffer was 10 mM phosphate buffer (pH 5). A 10 mM phosphate buffer (pH 8) solution served as the running buffer for the separation of phenolic compounds. The reservoirs were filled with the 10 mM phosphate buffer (pH 5) (a), 10 mM phosphate buffer (pH 8) (b), and the sample (c) solutions, respectively. The flow-injection and separation operations were depicted in details. Flow control was accomplished by applying the selected voltage to the corresponding reservoir. Samples were injected by applying a voltage of +1,500 V between the sample (c) and grounded reservoir for 3 sec while floating all remaining reservoirs. 'Total' and 'individual' assays were performed by switching the high voltage contacts and applying separation potentials of +4,000 and +1,500 V, respectively, to the corresponding reservoir (a or b), with the detection reservoir grounded and all other reservoirs floating. Switching from the acidic running buffer solution to the basic one was accomplished by applying +4,000 V to the corresponding reservoir (b) for 150 sec.

For plastic explosives detection: the running buffer for the separation of the plastic explosives was 20 mM MES (pH 4) containing 25 mM SDS. Each of the reservoirs in the chip holder and corresponding pipette tips on the micro channel chip

were filled with their respective solutions. The sample reservoir was filled with a mixture of explosives. The potential of 1,500 V was applied to the 'sample' reservoir for 3 s with the detection reservoir grounded and other reservoirs floating. The separation was performed by applying 1,500 V to the running buffer reservoir. By switching the high voltage contacts, the separation potential was subsequently applied to the 'running buffer' reservoir for the separation of the explosive compounds. All experiments were carried out at room temperature.

For continuous monitoring: The detection cell (d) was filled with the running buffer solution, which was continuously loaded into the separation channel by applying the desired potential (usually +2,000 V) to the platinum wire electrode (l_2) and a ground potential to the platinum wire grounded electrode (h). The sample solution was flowing continuously onto the sharp sample inlet tip at the required rate (usually 0.5 mL/ min) and injected periodically into the separation channel at the desired potential in a manner similar to the buffer injection.

5.2.4 End-column Amperometric Detection

The working electrode was placed opposite the outlet of the separation channel through a plastic screw. The 20-μm distance from the working electrode to the chip outlet was controlled by the plastic screw for the disk electrode, and the 50-μm distance from the band working electrode to the chip outlet was controlled by a thick tape spacer.

The high voltage power supply had switchable voltage ports between the running buffer and sample injections, with an adjustable voltage range of between 0 and 4,000 V. Amperometric detection was performed with an Electrochemical Analyzer 621A (CH Instruments, Austin, TX) using the "amperometric *i-t* curve" mode. The electropherograms were recorded with a time resolution of 0.1 s while applying a detection potential versus Ag/AgCl wire. Sample injections were performed after stabilization of the baseline. The raw data of electropherograms were digitally filtered using built-in 15-point least-square smoothing by CHI Version 3.27 software (CH Instruments).

5.2.5 Safety considerations

The high voltage power supply and associated open electrical connections should be handled with extreme care to prevent electrical shock. Phenolic compounds are toxic/irritant/dangerous for the environment, and should be handled in the fume hood. Skin and eye contact, and accidental inhalation or ingestion should be avoided. The explosive compounds cause headache, weakness, anemia, and liver injury, and the vapor of such chemicals is very dangerous. Stock solutions of explosives must be prepared and handled in the fume hood. The stock solutions must be stored in closed small glass containers, and isolated from any reducing reagents. These explosives are also toxic and in part carcinogenic and mutagenic. Therefore, special care must be taken to dispose of waste solution.

5.3 Results and Discussion

5.3.1 Microchip Capillary Electrophoresis/Electrochemical Detection of Hydrazine

Since the conception of a microchip–based capillary electrophoresis (CE) system was described, the number of research laboratories developing microchip CE devices has increased dramatically. Miniaturized CE analysis systems have brought numerous advantages to working over large-scale analyzers, including fast analysis, high efficiency, low reagent consumption, and portability. Therefore, microchip systems offer great promise for important applications such as environmental or industrial monitoring and clinical diagnostics. Electrochemical detection was ideally suited for miniaturization and compatible with modern microfabrication, and it offers high selectivity and sensitivity.

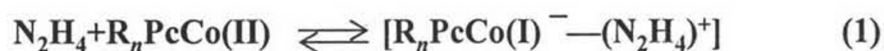
The focus of this article was on a microchip CE/amperometric system for the separation and detection of hydrazine compounds. Hydrazine and its derivatives were important compounds of interests in the chemical industry and pharmaceutical processes. They are applied in many areas such as fuel cells, herbicides, catalysts or rocket propellants [177-180]. Moreover, hydrazine and its derivatives were suspected to result in a number of adverse effects on mammals,

including damage to the liver and kidneys, production of various blood abnormalities, irreversible damage to the nervous system, alteration in human behavior after exposure and carcinogenic effects on humans. Due to the environmental and toxicological significance of hydrazine compounds, many efforts have been devoted to develop reliable and effective detection methods for this family of compounds. Amperometric detection was a powerful technique for monitoring redox species. Unfortunately, hydrazines were difficult to detect electrochemically because of their high overpotentials at ordinary electrodes [181]. In order to enhance the amperometric detection of hydrazines, electrochemical flow detectors based on various modified electrodes have been used for monitoring hydrazine compounds in conventional liquid-chromatography [182], flow injection [183], and capillary electrophoresis systems [184]. Chemically modified electrodes can improve the power of amperometric detection in both conventional CE [185-186] and microchip CE [187]. The specific chemically modified electrode used here consisted of a conventional carbon paste mixture to which cobalt (II) phthalocyanine (CoPc) was added as a modifier. CoPc was well-known for its electrocatalytic capabilities in numerous organic oxidations [184]. The utility for CoPc-modified carbon paste electrodes for CE microchips has not been demonstrated. Here, the attractive performance of microchip CE and the electrocatalytic properties of renewable CoPc-modified electrodes were combined for detecting hydrazine compounds. Utilizing these merits, hydrazine, 1,1 dimethylhydrazine and phenylhydrazine were rapidly separated and detected by CE/amperometry. The performance characteristics of the CE/amperometric microsystem and electrode design are reported in the following sections.

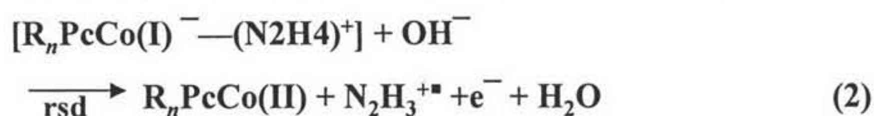
5.3.1.1 Comparison of Unmodified and CoPc-Modified Carbon Paste Capillary Electrodes

Hydrazines were difficult to oxidize at conventional/bare carbon electrodes because of their large overpotentials. Several surface-confined catalysts have been shown as useful for accelerating the oxidation of hydrazine compounds and facilitating their anodic detection. Earlier reports found that using the modification of carbon electrodes by electrodeposition of palladium was particularly

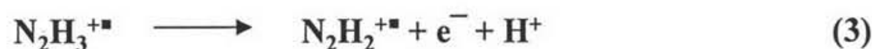
useful for minimizing the overvoltage effect. The use of various chemically modified electrodes has been explored to overcome this problem. A simple modification of the carbon paste electrode matrix with CoPc was found to be an effective way of minimizing the oxidation potential of hydrazines and facilitating their amperometric detection. Figure 5.1 compared the typical electropherograms for hydrazine (a), 1,1 dimethylhydrazine (b), and phenylhydrazine (c) at the unmodified (A) and CoPc-modified (B) carbon paste detectors. The marked electrocatalytic activity of CoPc-modified electrode towards hydrazines offerd enhanced sensitivity, low noise, and well resolved peaks. The three peaks can be separated from well-defined peaks within 130 s, with CoPc-modified carbon paste electrodes. The sensitivity of hydrazine detection at CoPc-modified carbon paste electrodes was much higher than that at the unmodified electrode under the same conditions. Such accelerated anodic detection was attributed to mediation or catalysis by the Co (II) state of the modifier [20]. When the reaction occurs at potential removed from the Co (II) / (I), the surface concentration of Co (II) sites was practically constant and independent of potential and the kinetics are controlled simply by the transfer of the first electron involving the hydrazine molecule. So the data can be explained according to the following mechanism [188].



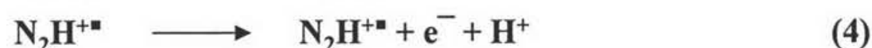
$$N_2H_4 + R_nPcCo(II) \rightleftharpoons [R_nPcCo(I)^- —(N_2H_4)^+] \qquad (1)$$



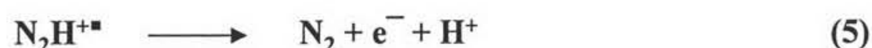
$$[R_nPcCo(I)^- —(N2H4)^+] + OH^- \xrightarrow[rsd]{} R_nPcCo(II) + N_2H_3^{+\bullet} + e^- + H_2O \qquad (2)$$



$$N_2H_3^{+\bullet} \longrightarrow N_2H_2^{+\bullet} + e^- + H^+ \qquad (3)$$



$$N_2H^{+\bullet} \longrightarrow N_2H^{+\bullet} + e^- + H^+ \qquad (4)$$



$$N_2H^{+\bullet} \longrightarrow N_2 + e^- + H^+ \qquad (5)$$

Hence, the electron-transfer rate on the electrode occurred quickly. The flat baseline and low noise level at this separation voltage showed an effective isolation from the high separation voltage. These, along with the well-defined peaks, indicated convenient quantitation down to the micromolar level (see data in Figure 5.1).

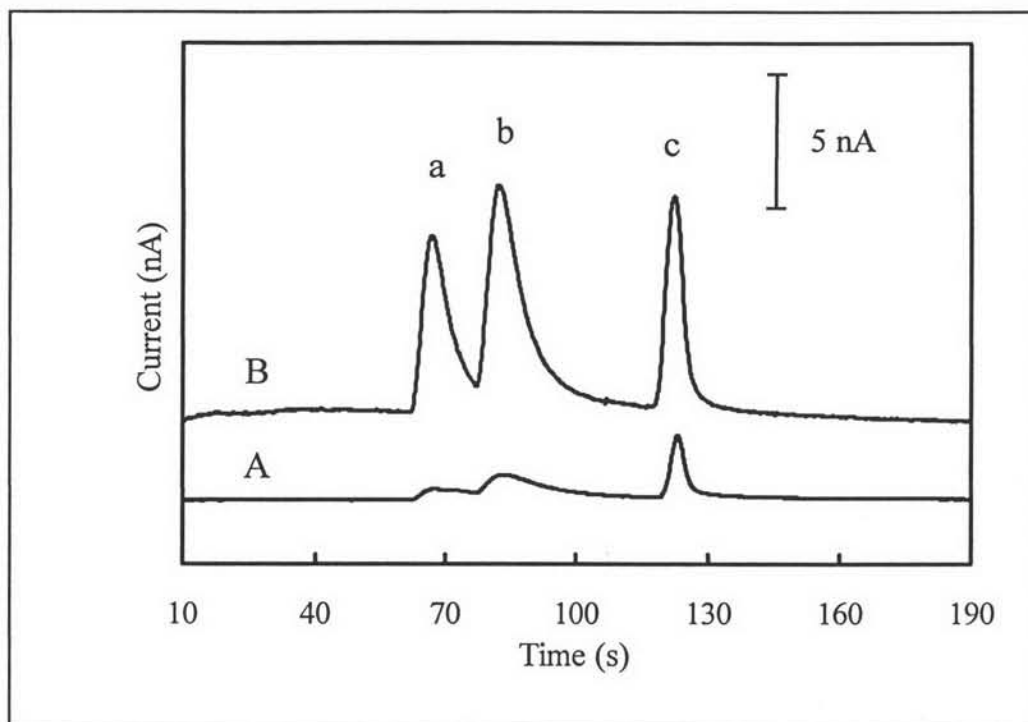


Figure 5.1 Electropherograms for a mixture containing 50 μM hydrazine (a), 100 μM 1,1 dimethylhydrazine (b), and 100 μM phenylhydrazine (c), obtained at unmodified (A) and 5 % CoPc-modified (B) carbon paste electrodes. Separation and injection voltages, + 1,000 V; injection time, 3 s; detection potential, +0.5 V (vs Ag/AgCl). Phosphate buffer (10 mM and pH 6.5) was used as a running buffer.

5.3.1.2 Capillary Electrophoresis Optimization

In any CE separation, the buffer pH had a significant impact on the ionization and electrophoretic mobility of each analyte. A pH optimization was carried out for the separation of hydrazine compounds. The pH values on the migration time were examined in the pH range of 6.2 – 7.6, as shown in Figure 5.2. All buffers contained a 10 mM phosphate buffer. Hydrazine, 1,1 dimethylhydrazine and phenylhydrazine could not be well separated at pH ≥ 6.8. This may be attributed to the low ionization of hydrazine, while pH approaching the p*Ka* (p*Ka* = 8.0, 7.7, 5.21 for hydrazine, 1,1 dimethylhydrazine and phenylhydrazine, respectively) led to a more neutral form of each analyte. From the results, the pH 6.5 buffer was found to

be optimal because it led to a good resolution of the first two peaks when compared to pH 6.2 buffer (for pH 6.5, $R_s = 0.86$ and pH 6.2, $R_s = 0.76$). Moreover, it was evident that a pH 6.5 buffer exhibited the highest current signal using CoPc-modified carbon paste electrodes. Accordingly, this buffer was selected for all subsequent work.

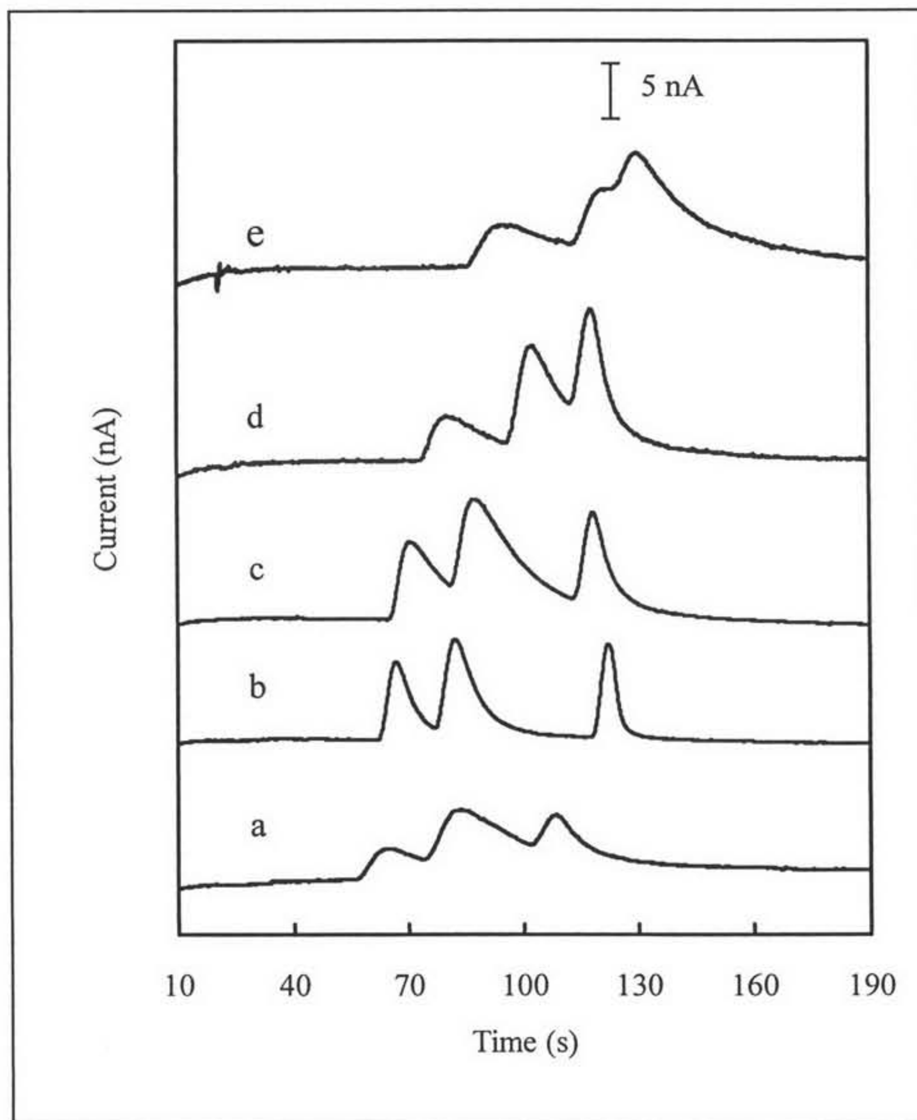


Figure 5.2 Influence of pH value of 10 mM phosphate run buffer on the separation and detection of mixture at the 5 % CoPc-modified carbon paste capillary electrodes. pH 6.2 (a), pH 6.5 (b), pH 6.8 (c), pH 7.3 (d), and pH 7.6 (e). Other conditions were the same as those in Figure 5.1.

5.3.1.3 Influence of CoPc Loading

The effect of the CoPc loading on the analytical performance of a modified capillary electrode was investigated over a 1 to 10% range. Figure 5.3 shows the current response versus CoPc composition of 50 μM hydrazine and 100 μM phenylhydrazine using 10 mM phosphate run buffer (pH 6.5). As this figure illustrates, the hydrazine oxidation currents were enhanced between 1 to 5% of the modifier and then decreased when the amount of the modifier was increased, owing to decreasing the conductivity of the carbon paste. The highest sensitivity was obtained using a loading of 5% modifier. Loading lower than 5% also enhanced peak currents; however, the electrode-to-electrode reproducibility was significantly inferior (%RSD (n=6) > 15% for hydrazine and phenylhydrazine). Overall, a 5% modifier loading offered the most favorable performance and was therefore used throughout this study.

5.1.3.4 Hydrodynamic Voltammetry

Figure 5.4 showed the typical hydrodynamic voltammograms of 50 μM hydrazine (a) and 100 μM phenylhydrazine at this modified electrode. As expected, the detection potential affected the sensitivity and detection limits of this system. Figure 5.5 illustrated the enhanced electrocatalytic activity of the CoPc-modified carbon paste electrodes in comparison with the unmodified carbon paste electrodes. It depicted the hydrodynamic voltammograms for the oxidation of 50 μM hydrazine at unmodified (A) and CoPc-modified (B) carbon paste electrodes.

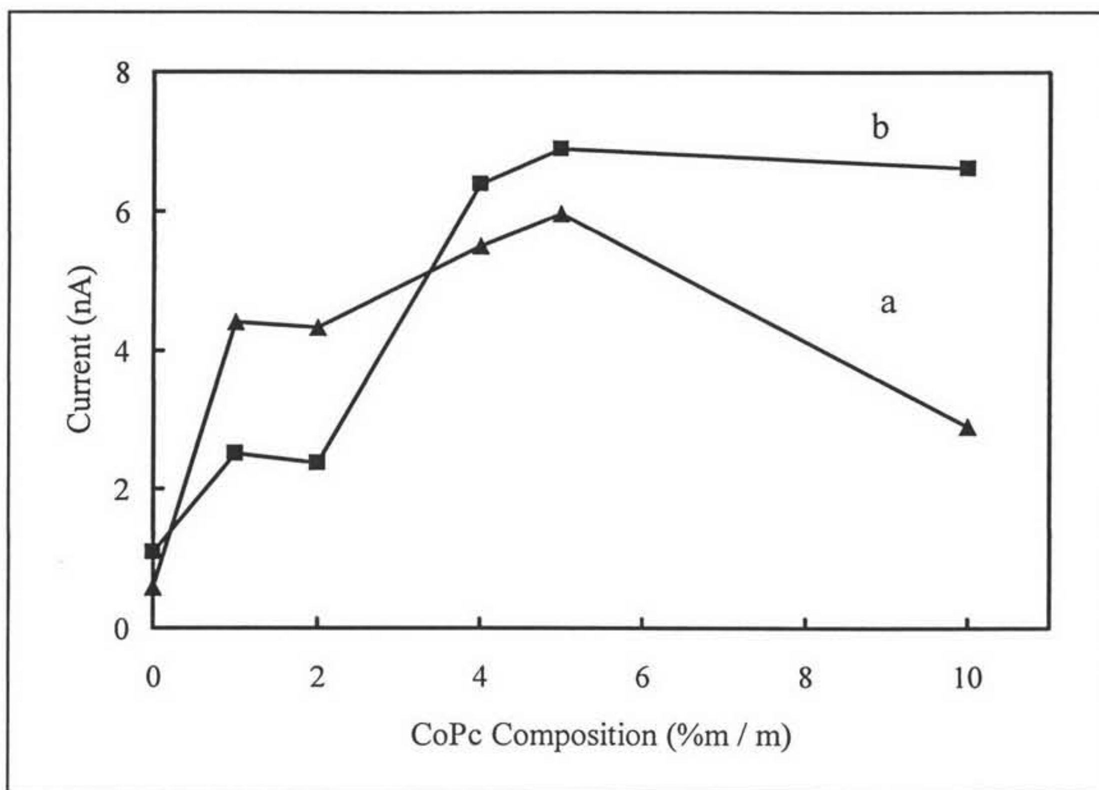


Figure 5.3 Effect of CoPc loading in carbon paste on the current response of 50 μM hydrazine (a) and 100 μM phenylhydrazine (b). Other conditions were the same as those in Figure 5.1.

The curves were recorded step by step (in steps of 0.1 V) over the 0.0 to +0.9 V range, using a separation voltage of 1,000 V. At CoPc-modified carbon paste electrodes, hydrazine displayed a defined wave starting at around +0.0 V and leveling off at above +0.5 V. The half-wave potential for hydrazine at the unmodified (A) and CoPc-modified (B) carbon paste were +0.48 and +0.35 V, respectively. The electrocatalytic property toward the investigated analyte, pronounced as the half-wave potential on the CoPc-modified carbon paste electrode, decreased by approximately 130 mV in comparison with that on the unmodified electrode. These voltammograms indicated that the CoPc-modified carbon paste-based detector provided a greatly improved performance, with a substantially higher sensitivity over the entire potential range and a moderate lowering of the operating potential (the geometric areas of both electrodes are the same). Because of the low background current at the lower potentials, the stability of the electrode can be

enhanced so that the reproducibility is improved. All subsequent amperometric work employed a constant potential of +0.5 V. Higher operating potential would be required while working with higher separation voltages that may shift the voltammetric profile in the anodic direction.

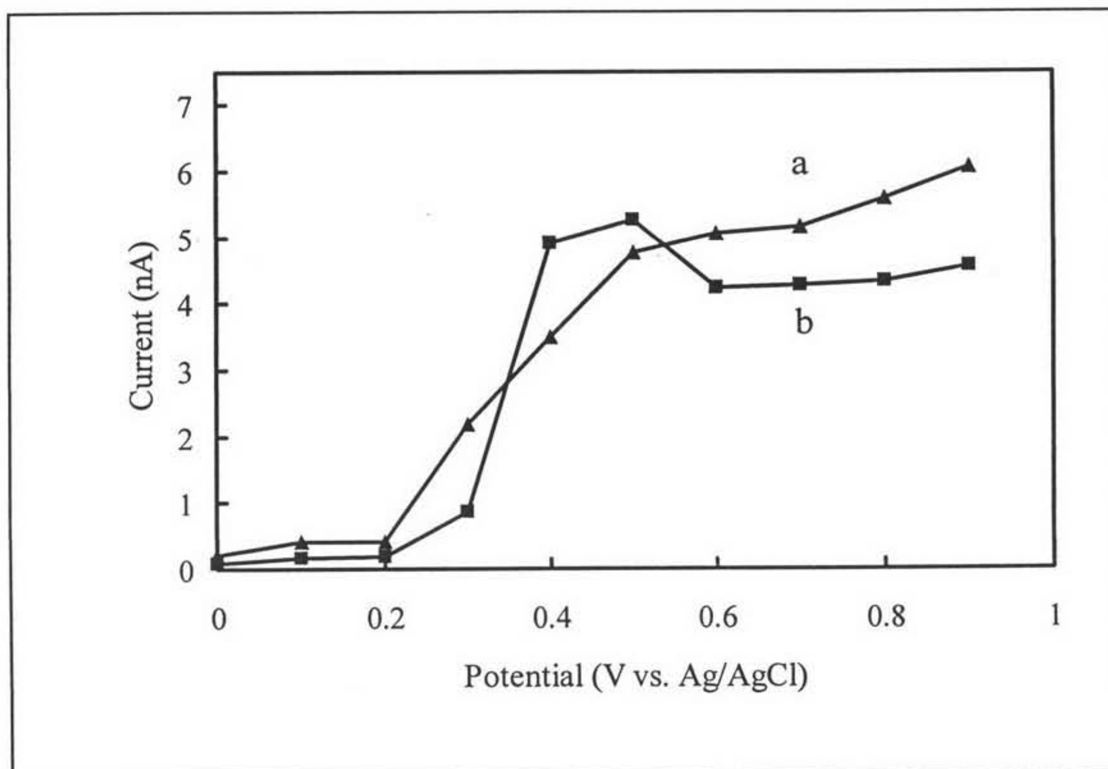


Figure 5.4 Hydrodynamic voltammograms for 50 μM hydrazine (a) and 100 μM phenylhydrazine at 5 % CoPc-modified carbon paste electrodes. Other conditions were the same as those in Figure 5.1.

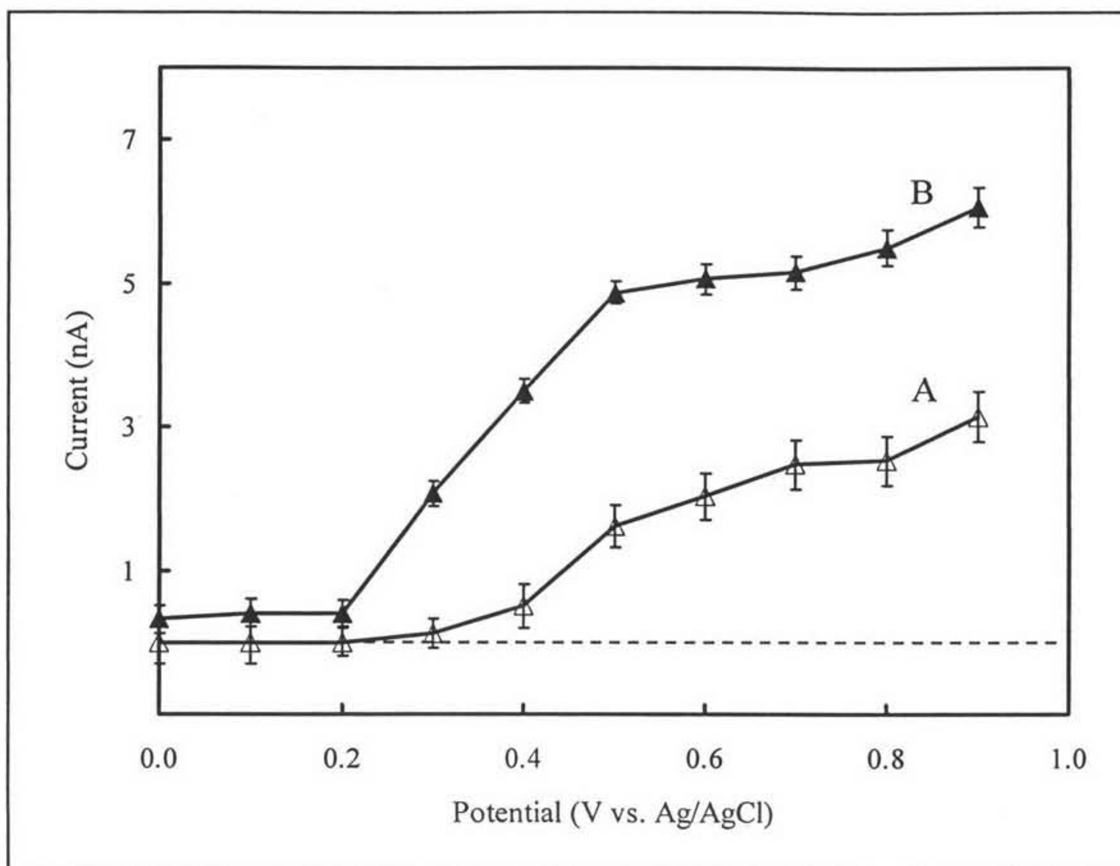


Figure 5.5 Hydrodynamic voltammograms for 50 μM hydrazine at unmodified (A) and 5 % CoPc-modified (B) carbon paste electrodes. Other conditions were the same as those in Figure 5.1.

5.3.1.5 Analytical Figures

The CoPc-modified carbon paste-based detector offers a well-defined concentration dependence. Electropherograms for mixtures containing increasing levels of hydrazine (a) and phenylhydrazine (b) in increments of 20 μM and 40 μM, respectively, were shown in Figure 5.6. Defined peaks with a current proportional to the analyte concentration were observed. The resulting calibration plots (Figure 5.6B) were linear with the sensitivity of 173 and 85 nA/mM for hydrazine and phenylhydrazine, respectively (correlation coefficients, 0.9977 and

0.9981). The detection limits, based on a signal-to-noise ratio (S/N) of 3, were found to be 0.5 μM for hydrazine and 0.7 μM for phenylhydrazine.

Good precision was another attractive feature of the chemically modified electrodes. The precision of microchip CE/CoPc-modified carbon paste electrodes was examined from a series of six repetitive injections of a sample mixture containing 50 μM hydrazine and 100 μM phenylhydrazine. Reproducible signals were obtained with relative standard deviations (%RSD) of 4 % and 7% for the peak current of hydrazine and phenylhydrazine, respectively. Such good precision indicated negligible surface fouling of the CoPc-modified carbon paste electrode. An inferior precision, with RSD of 20%, was observed in prolonged operation ($n \geq 10$), possibly due to a gradual loss of CoPc from the electrode surface. The paste design allowed rapid renewal. Different electrode surfaces after surface renewal also displayed good precision, %RSD, between electrode-to-electrode of 2.53% for hydrazine and 2.55% for phenylhydrazine (n=3).

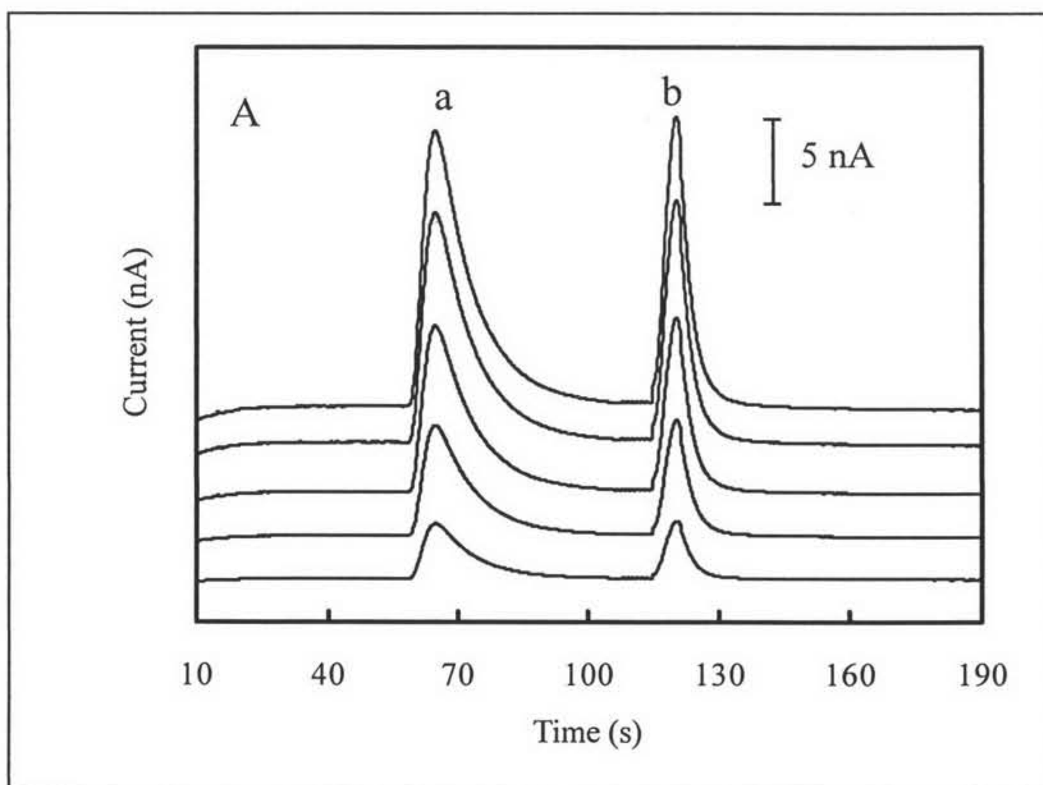


Figure 5.6 (A) Electropherograms detailing the current response to increase concentrations of hydrazine (a), and phenylhydrazine (b) in steps of 20 μM and 40 μM, respectively.

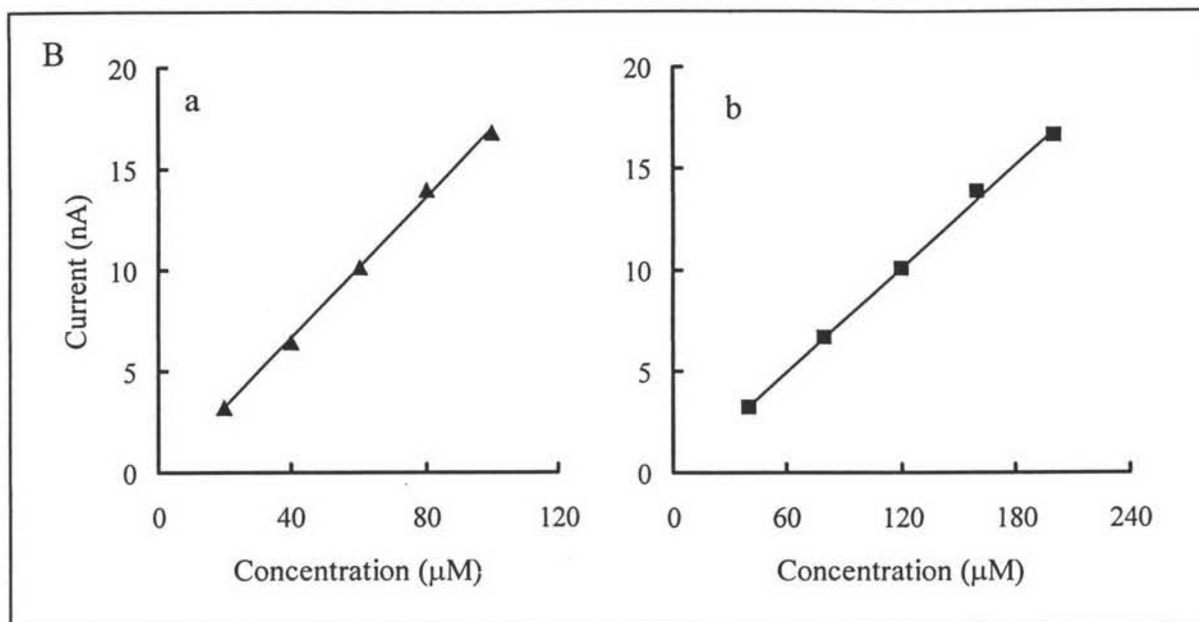


Figure 5.6 (B) The resulting calibration plots. Other conditions were the same as those in Figure 5.1.

5.3.1.6 Summary

This is the first demonstration of CoPc-modified electrode utility for detecting hydrazines in microchip CE devices. The above results showed that the hydrazines can be successfully separated and detected with the proposed method. The electrocatalytic activity of the CoPc-modified carbon paste electrode allowed low-potential detection of this group of hydrazines when compared with the unmodified carbon paste electrodes. Moreover, these CoPc-modified electrodes exhibited good stability in the flowing system, enhanced sensitivity, and good reproducibility in amperometric detection. Combining the accelerated anodic detection of renewability, bulk modification, and versatility of CoPc-modified electrodes with the advantages of the microchip CE format, has offered attractive analytical applications. The resulting microchip CE held great promise for rapid field screening of hydrazine contaminants and other pollutants. The negligible solvent/sample consumption (compared, for example, to current liquid chromatographic protocols) of microchip CE made it suitable for on-site

environmental detection. Other types of catalytic modifiers were currently being examined for improving the microchip detection of different classes of compounds.

5.3.2 Microchip Device for Rapid Screening and Fingerprint Identification of Phenolic Pollutants

The dramatic downscaling and integration of chemical assays made microfabricated chip devices extremely attractive as environmental-monitoring screening tools and they held considerable promise for effective field monitoring of priority pollutants [189-190]. Small dimensions of microchip devices were particularly attractive for on-site environmental applications, with their minimal solvent/reagent consumption and waste production, speed and high degree of integration. The significant improvement in the rate of waste generation and material consumption had enormous implications in green chemistry [190]. Electrochemical detection offered great promise for chip-based environmental monitoring systems, with features that include inherent miniaturization and integration of the detector and control (potentiostatic) instrumentation, high sensitivity, low-power requirements, low cost, and high compatibility with advanced microfabrication and micromachining technologies [169-171]. Such properties made electrochemical detection extremely attractive for creating truly portable field-deployable microsystems. The versatility of microchip devices held great promise for developing novel protocols for efficient environmental monitoring.

This article reported on an attractive single-channel microchip manifold that offered convenient distinction between 'total' and 'individual' phenolic pollutants. Phenols were a group of pollutants expelled into environmental waters and soils by a wide variety of industrial processes [191]. Increasing environmental legislation, which controlled the release and levels of phenolic compounds in the environment, has created a need for reliable monitoring of these substances in air, soil and especially water. Because of the toxicological significance of phenolic compounds, a reliable method was required for their environmental monitoring. Compact systems, suitable for field measurements of phenols, were preferred since they afforded the option of rapid warning and avoided errors and delays inherent to

laboratory-based analyses. Since most phenols were oxidizable at moderate potentials, electrochemistry can serve as a highly sensitive tool for their microchip detection. In the present work, switching between 'total' and 'individual' phenol measurements was accomplished by rapidly changing the pH of the run buffer from an acidic one (where the phenols are neutral and not separated) to a basic one (that led to the ionization and separation; Figure 5.7). 'Total' measurements of repetitive flow injection were thus performed for providing a timely warning and alarm. Switching to the 'separation' mode took place only when the 'total' flow-injection screening assay indicated the presence of phenolic compounds. Such microchip warning-alarm/identification capability has met the demands of many practical environmental monitoring scenarios. Analogous microchip monitoring of 'total' and 'individual' explosive compounds, based on switching between SDS-free and SDS-containing buffers, was described [192].

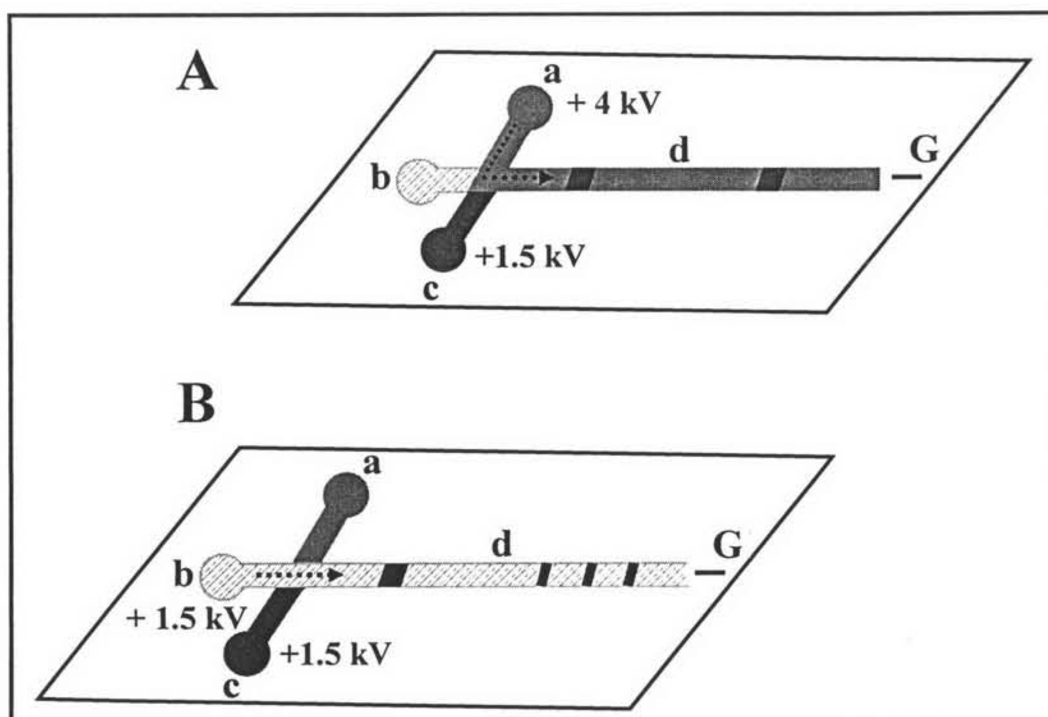


Figure 5.7 schematic diagrams of the 'total' (A) and 'individual '(B) on-chip assays of phenolic compounds. (a) Reservoir with 10 mM phosphate buffer (pH 5), (b) reservoir with 10 mM phosphate buffer (pH 8), (c) sample solution, (d) separation

channel, (e) amperometric detector. Initial operation using the acidic buffer led to rapid 'total' measurements (A) followed by switching to the basic buffer for separating and detecting 'individual' phenols.

The goal of this work was to demonstrate the characteristics and advantages of a microchip device for fast switching between 'total' and 'individual' measurements of phenolic pollutants. The 'total' phenolic content was obtained using an acidic run buffer where the compounds were neutral and not resolved. This results in a rapid 'flow-injection' like the operation that served as fast screening of phenolic compounds. Once the presence of these toxic compounds has been detected, the system was switched from the screening mode to the separation mode, through the introduction of a basic run buffer (present in a second reservoir) to the separation channel that led to ionization and separation of the phenols.

5.3.2.1 pH Effect

Key factors affecting the performance of the CE/FIA-microchip switching protocol were examined and optimized. Control of the running buffer pH was an important parameter for optimizing the separation of ionizable analytes in CE because the buffer pH affected the degree of ionization and mobility of solutes. The influence of the pH was investigated over the 5.0 to 9.0 range (Figure 5.8). The resolution between the four phenols decreased gradually upon lowering the pH. As expected, these analytes had a similar migration time at the lower pH, and their mixture yielded a single peak (within ca.100 sec) that reflected the 'total' amount. Switching to basic buffers (a,b) led to well-resolved peaks, and good resolution between four peaks was observed at pH 8, with the entire assay requiring around 185 sec.

5.3.2.2 The Effect of Separation Voltage

The electrical field had a profound effect upon the speed and sensitivity of the 'total' assay. As expected, the migration time decreased upon increasing the separation voltage (Figure 5.9). Increasing the separation potential

from 1,500 V to 4,000 V (in 500 V increments) dramatically decreased the migration time from 81 s to 30 s for the phenol/2-chlorophenol mixture. Hence, the 'total' flow injection screening assay can be performed at a fast rate of 120 samples/hr. The peak height increased rapidly upon increasing the voltage from 1,500 V to 3,000 V and from then on more slowly. Increasing the separation voltage had a negligible effect on the baseline noise or slope, indicating an effective isolation from high separation voltages.

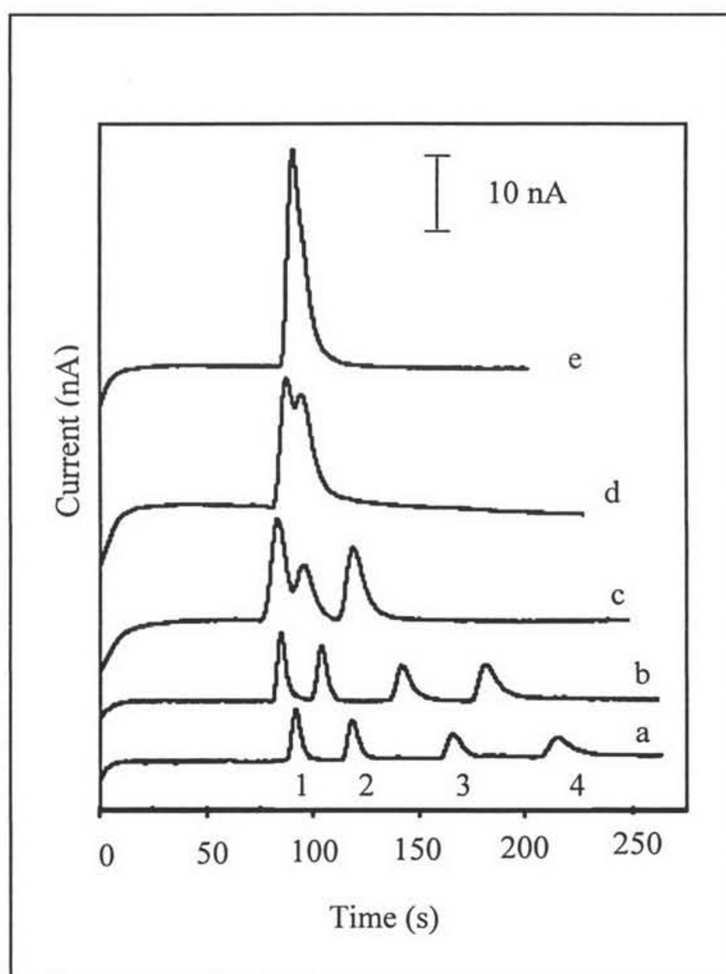


Figure 5.8 Influence of the pH of the buffer on the amperometric response for a mixture of (1) phenol (50 μM), (2) 2-chlorophenol (100 μM), (3) 2,3-dichlorophenol (200 μM) and (4) 4-nitrophenol (200 μM). pH buffer: (a) 9.0 , (b) 8.0, (c) 7.0, (d) 6.0 and (e) 5.0; buffer concentration 10 mM; separation voltage 1,500 V; injection voltage 1,500 V for 3 sec; detection potential +1.1 V vs. Ag/AgCl wire.

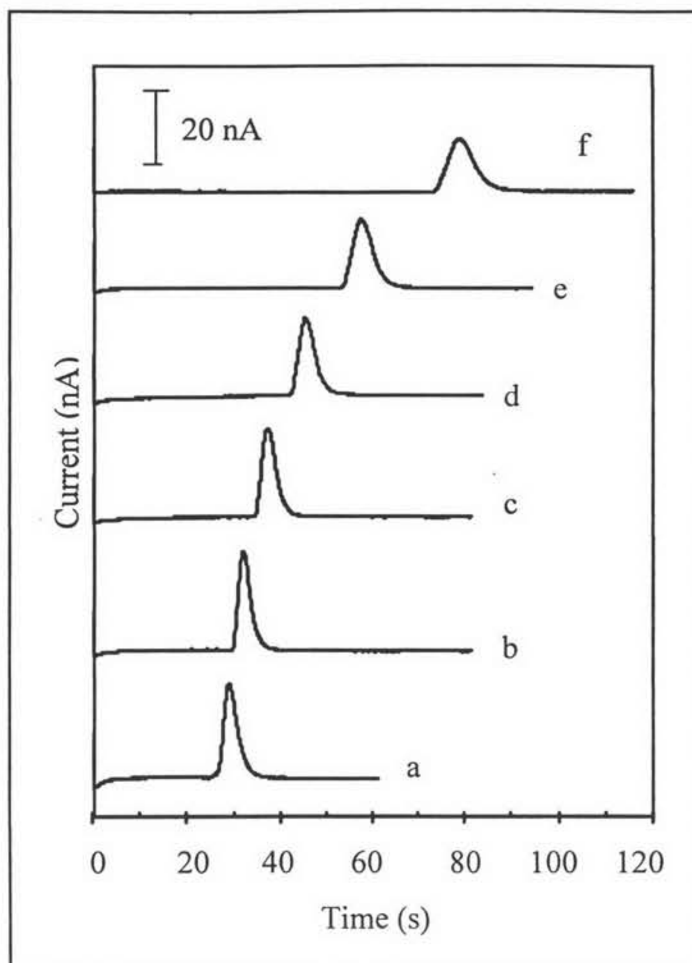


Figure 5.9 Effect of the separation voltage on the flow-injection response for a mixture of phenol (50 μM) and 2-chlorophenol (100 μM). Separation voltages: (a) 4,000, (b) 3,500, (c) 3,000, (d) 2,500, (e) 2,000 and (f) 1,500 V. pH buffer, 5.0. Other conditions were the same as those in Figure 5.8.

5.3.2.3 The Influence of Washing Time and Switching Voltage

Switching between the 'flow injection' and 'separation' modes was strongly affected by the washing time or potential. Increasing the washing time facilitated the exchange of the buffers in the separation channel. Figure 5.10A displayed the dependence of the phenol resolution and 2-chlorophenol peaks upon

increasing the washing time between 25 sec and 150 sec (a-f), using a 'switching potential' of 4,000 V and a separation voltage of 1,500 V during the measurement. Before each experiment, the separation channel was filled with 10 mM phosphate buffer (pH 5). Complete replacement of this acidic buffer with the basic (pH 8) one was obtained using a 150 sec washing time, and led to well resolved peaks ($R_s = 1.68$). Also shown in Figure 5.10B, electropherograms for a mixture of phenol and 2-chlorophenol were recorded using a switching time of 150 sec, along with different switching voltages (1,500-4,000 V, a-e). While switching voltages lower than 3,000 V yield incomplete resolution, higher voltages offered well-resolved peaks. Most subsequent work thus employed a switching voltage of 4,000 V for a 150 sec period. A similar time was found to be sufficient for switching back from the basic phosphate buffer (pH 8) to the acidic (pH 5) one upon returning to the 'total' assay.

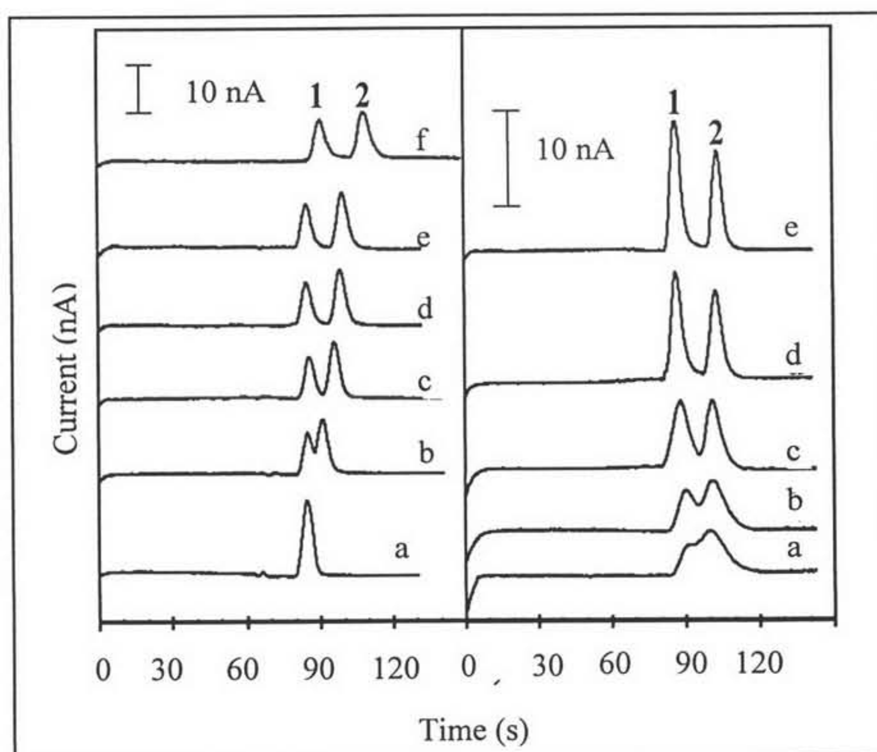


Figure 5.10 Influence of the switching time and the switching voltage upon the peak resolution between (1) phenol (50 μM) and (2) 2-chlorophenol (100 μM). (A) Electropherograms recorded using a switching voltage of 4,000 V and a washing time of (a) 25, (b) 50, (c) 75, (d) 100, (e) 125 and (f) 150 sec. (B) Influence of the switching voltage; electropherograms recorded using a 150 sec washing time and

switching voltages of (a) 1,000, (b) 1,500, (c) 2,000, (d) 3,000 and (e) 4,000 V. Running buffer of pH, 8.0. Other conditions were the same as those in Figure 5.8.

5.3.2.4 Analytical Figures

Such switching conditions led to highly reproducible measurements of 'total' and 'individual' phenolic compounds. Figure 5.11 displayed the precision of such switching between 'total' (A, B, C) and 'individual' (D, E, F) assays of a sample mixture containing (a) 50 μM phenol and (b) 100 μM 2-chlorophenol. Such an operation resulted in good reproducibility, with a relative standard deviation (RSD) of 0.21 % for the migration time of the 'total' assay. The RSD for the migration times for individual phenol and 2-chlorophenol peaks were 0.42% and 1.16%, respectively. Clearly, the buffer replacement, as well as the flow injection, separation, and detection steps, were highly reproducible. The anodic detection of phenolic compounds was commonly prone to surface fouling effects associated with the deposition of polymeric films formed on the carbon electrode during the oxidation. However, the reproducible peak heights in Figure 5.11 indicated minimal surface fouling for a prolonged switching operation ($n > 10$) between 'total' and 'individual' measurement. The RSD for the 'total' peak current was 4.95% and for the 'individual' phenol and 2-chlorophenol signals 4.96% and 7.20%, respectively. Apparently, the ultrasmall sample zones characterizing the microchip operation led to a negligible film formation.

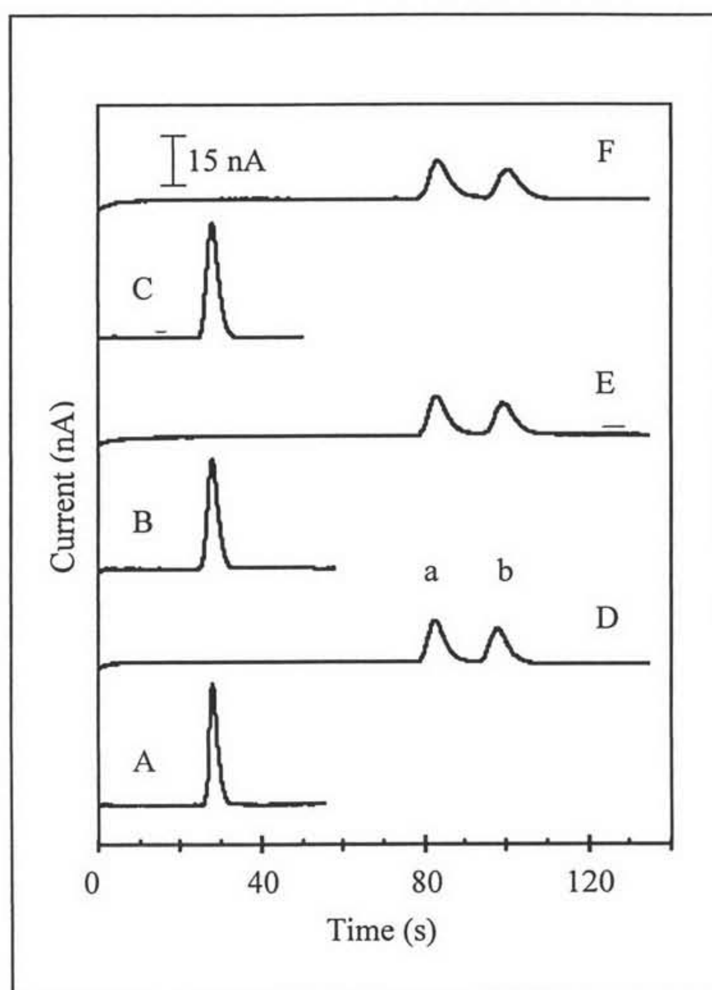


Figure 5.11 Electropherograms depicting the precision of alternate 'total' (A, B, C) and 'individual' (D, E, F) assays using a mixture containing (a) 50 μM phenol and (b) 100 μM 2-chlorophenol. Separation voltage at 4,000 (A, B, C) and 1,500 V (D, E, F). Running buffer of pH 5 (A, B, C) and pH 8.0 (D, E, F). Other conditions were the same as those in Figure 5.7.

5.3.2.5 Summary

A single-channel CE/FIA-microchip protocol has been demonstrated for the detection of both 'total' and 'individual' phenolic compounds. The microchip layout allowed placement of different running buffer solutions in two reservoirs and conducting sequential FIA and CE operations (in the same channel) upon exchanging the buffer. This represented another example of exploiting the versatility of microchip devices for performing novel analytical applications. The resulting CE/FIA-microchip operation offered rapid, convenient, and reproducible measurements for both total and individual phenols. Such ability to rapidly screen for target pollutants and switch for their detailed identification should have great impact on environmental protection and industrial process control.

5.3.3 Microchip for Separation and Detection of Plastic Explosives

Explosives have attracted attention because these important chemical species need to be detected in many field applications [193-195]. With the surge of international terrorism and increased use of explosives in terrorist attacks, law enforcement agencies throughout the world were faced with the problem of detecting bombs hidden in luggage, mail, vehicles, and aircraft, as well as the suspects themselves. This has become a major analytical problem, which requires highly sensitive, specific, and fast methods for its solution. Ideal explosives detection could be reliable and simple, and provide an unambiguous signal when detection was made. This report focused on detection of the plastic explosive - 'semtex'. Semtex is a specialized form of explosive material produced by mixing RDX (Cyclotrimetylene trinitramine) with PETN (Pentaerythol tetranitrate), as shown in scheme 1, and then adding binders and stabilizers [196]. In the US, RDX has been viewed as a military explosive. PETN is considered more as a commercial explosive, partly because of its private industrial manufacture and widespread use in detonating cord. On-site environmental detection and monitoring of traces of explosives and their degradation products is necessary. In areas suspected of contamination from toxic explosives, monitoring the quality of groundwater has been considered in order to prevent poisoning of human and animal populations. The need to separate and determine

explosives have sustained continuing investigations of different methods including capillary electrophoresis [197-199], LC [200-201] and gas chromatography [202-204]. Both optical and electrochemical detection were used in most relevant publications.

Since the reported development of miniaturized chemical analysis systems, particular attention has been given to the micro machined capillary electrophoresis (CE) chip, due to its powerful technique in separation and detection. Its unique properties consist of attainable high resolution, minimal sample volume requirement, short analysis time, and high separation efficiency [205-206]. Several groups, including ours, reported on microchip CE-based electrochemical microsystems for the separation and detection of nitro explosive compounds [207-209]. An end-column, low-potential reductive detection was employed for these classes of substances. Other groups described on-chip separations and optical detection of organic explosives [210]. Rapid identification of explosives would allow first responders and emergency personnel to make important decisions concerning barricading, evacuation, or efficient decontamination of particular sites and also prevent danger to the personnel themselves. Today's systems are too bulky or slow to meet the 'detect to warn' needs for civilians or soldiers. Designing miniaturized field portable devices that retain the high sensitivity and selectivity of sophisticated laboratory-based instruments represents a major analytical challenge. Moreover, the unique features of CE microchips make them particularly attractive as 'Green Analytical Chemistry' screening tools. These attractive and powerful performance characteristics were reported in the following sections.

5.3.3.1 Electrochemical Characteristics of RDX and PETN

The preliminary study of electrochemical reduction of RDX and PETN using a screen-printed carbon electrode was performed using the MES buffer solution (pH 4). Figure 5.12 displayed typical cyclic voltammograms for 200 ppm RDX (A) and 300 ppm PETN (B). The corresponding background voltammograms were also shown as dotted lines. An approximate but well defined reduction (with an E_p =-0.6 volt) of RDX was observed starting at around -0.4 volt. A less defined reduction peak (with an E_p =-0.7volt) was observed for PETN. No anodic peaks were observed on the reverse scan within the investigated potential

range (-1.0 to +0.5 V) for both analytes, reflecting irreversibility of nitro explosive compounds.

Figure 5.13 displayed the characteristic electropherogram recorded at -0.8 volt under optimal separation conditions for a mixture containing micromolar concentrations of RDX and PETN. Using an MES buffer (20 mM, pH 4) as a running buffer and a separation voltage of 1,500 V, the two peaks were well resolved, with the entire assay requiring ~2.3 min.

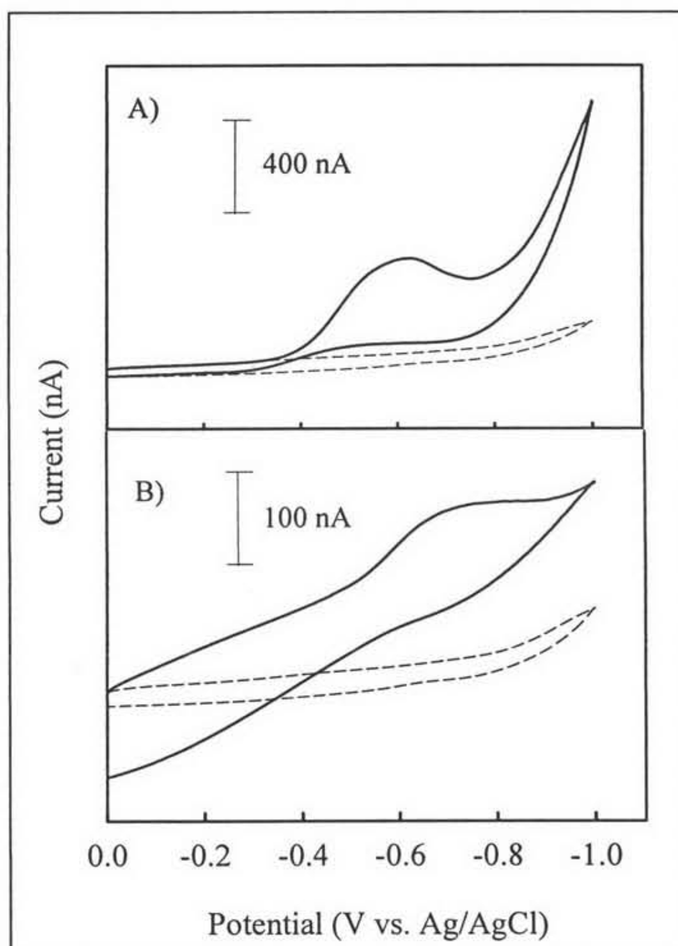


Figure 5.12 Cyclic voltammograms of 200 ppm RDX (A) and 300 ppm PETN (B) in 20 mM MES pH 4 (solid line) and 20 mM MES pH 4 (dash line), over the 0.0 and -1.0 V range. Scan rate, 50 mV/s.

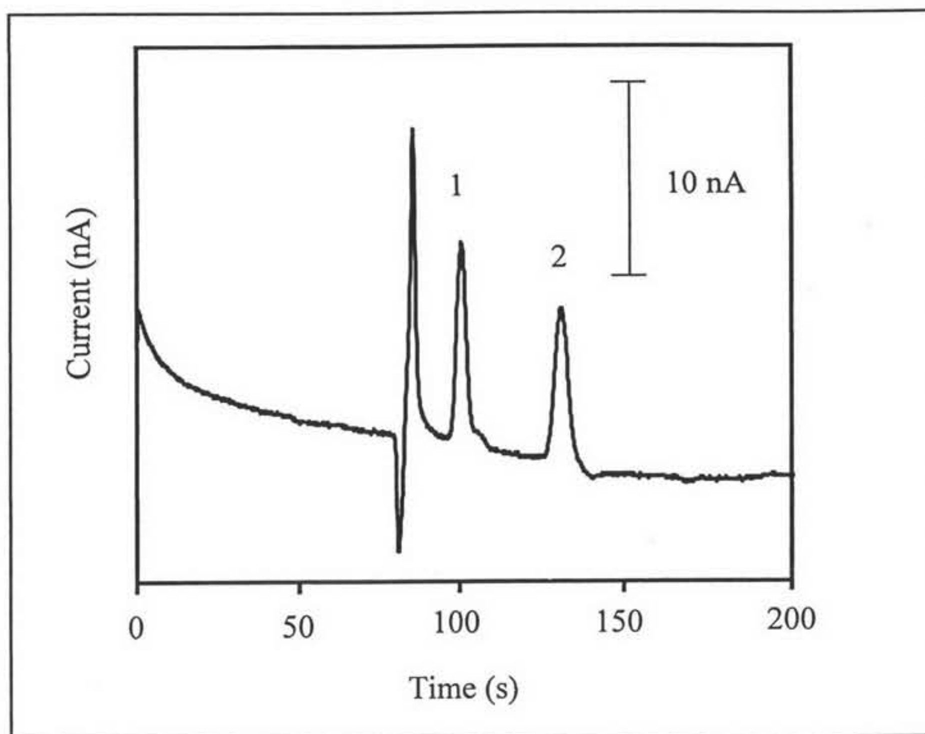


Figure 5.13 Separation and detection of Explosive compounds: (1) 10 ppm RDX, and (2) 200 ppm PETN. Separation buffer, 20 mM MES (pH 4) containing 25 mM SDS; separation and injection voltages, + 1,500 V; injection time, 3 s; detection potential, -0.8 V (vs Ag/AgCl wire) at bare carbon screen-printed electrode.

5.3.3.2 Conditions for Separation and Detection of RDX and PETN

In order to achieve a complete separation of RDX and PETN, and enable them to keep good electrochemical responses, several experimental parameters influencing the separation of RDX and PETN were examined and optimized.

Effect of buffer pH: In order to study the electrochemical detection, the pH buffer was one of the most important parameters because it determined the extent of ionization and reduction of each solute. For the detection of RDX and PETN under study a pH optimization was performed. The influence of pH was varied using buffer systems in the acidic, neutral and basic region: 20 mM MES buffer at pH 4.0, 6.0, 7.0,

and 8.0. Every buffer was 25 mM SDS. Figure 5.14 showed the results from a study of pH optimization. It was evident that at pH 8.0, a broad peak was reached, with no separation between two of these compounds. After decreasing the pH buffer, RDX and PETN showed a sharp peak appearing, with a high resolution on the baseline of the buffer. At pH 4, RDX and PETN provided a good sharp peak and the highest signal. Moreover, the analysis time was lower because of influence from the low ionic strength at this pH value. These results indicated that RDX and PETN can be determined by microchip capillary electrophoresis with amperometric detection. Subsequent pH buffer work, used at MES buffer pH 4, offered the most favorable condition for RDX and PETN.

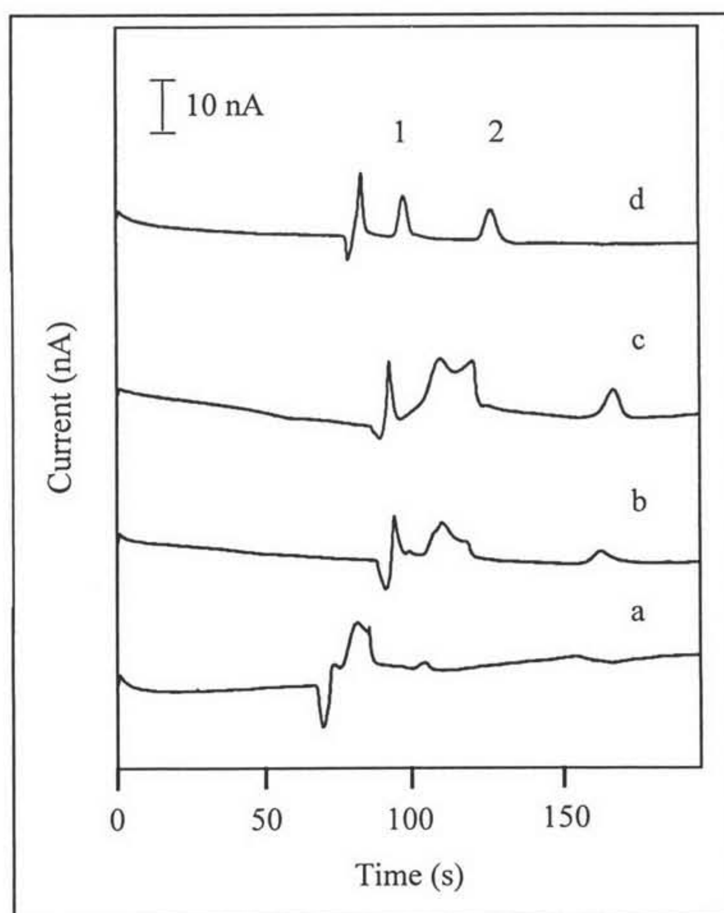


Figure 5.14 Effect of pH on running buffer upon the separation and response of 10 ppm RDX (1) and 200 ppm PETN (2), (a) pH=8.0, (b) pH=7.0, (c) pH=6.0, and (d) pH=4.0. Separation buffer, 20 mM MES containing 25 mM SDS; separation and

injection voltage, + 1,500 V; injection time, 3 s; detection potential, - 0.8 V (vs. Ag/AgCl wire) at a bare carbon screen-printed electrode.

Effect of the electrolyte concentration: The influence of a concentration of MES buffer in the range of 10-30 mM on the separation of explosive compounds was examined with 25 mM SDS at pH 4.0. For general analyte, the mobility diminished with increasing electrolyte concentration. On the other hand, mobility of some analyte would increase, and then decrease, before increasing again with electrolyte concentration. For explosive compounds the mobility increased slightly with increasing MES concentration. Better sensitivity for the separation was achieved when the MES concentration was increased. Figure 5.15 displayed the peak separation of these compounds at different concentrations of MES buffer. From the results, there was little difference between the analysis time of explosive compound at 30 mM and 20 mM MES. Therefore, a 20 mM MES concentration was selected as the optimal value to keep for a Joule heating minimum.

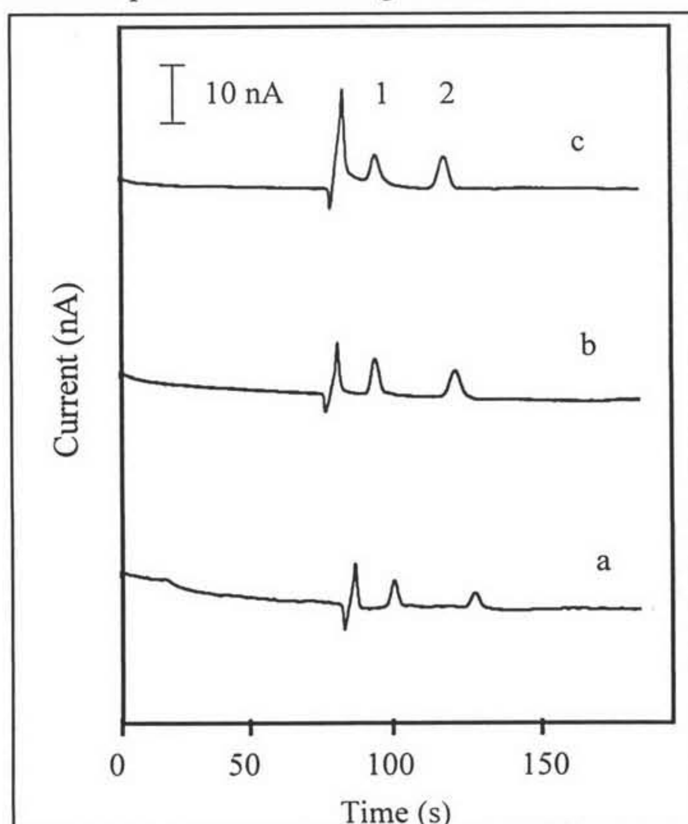


Figure 5.15 Effect of electrolyte concentration based on the separation and response of 10 ppm RDX (1) and 200 ppm PETN (2), (a) 10 mM, (b) 20 mM, and (c) 30 mM. Separation buffer, 20 mM MES (pH 4) containing 25 mM SDS. Other conditions were the same as those in Figure 5.14.

Effect of micelle concentration: Figure 5.16 showed the influence of SDS concentration in the separation of these compounds at different concentrations of surfactant, at a given concentration of 20 mM MES pH 4.0. Different behavior was observed when the concentration of surfactant was varied in the range of 10-30 mM. It can be seen that an increase in the analysis time was placed when SDS concentration was increasing. This behavior can be explained by both the increase of an ionic strength of the buffer separation and a higher interaction between analyte and micelles, due to the use of higher SDS concentration. At the concentration of 15 mM SDS, PETN was separated, but a poor resolution was obtained from RDX. Thus, an SDS concentration of 25 mM was chosen as the best condition with regards to resolution and analysis time. In the case of increasing the amperometric response using the higher separation voltage, a good resolution was attained at the SDS concentration of 20 mM. However, this condition may not be suitable, due to the closeness between the RDX and solvent peak.

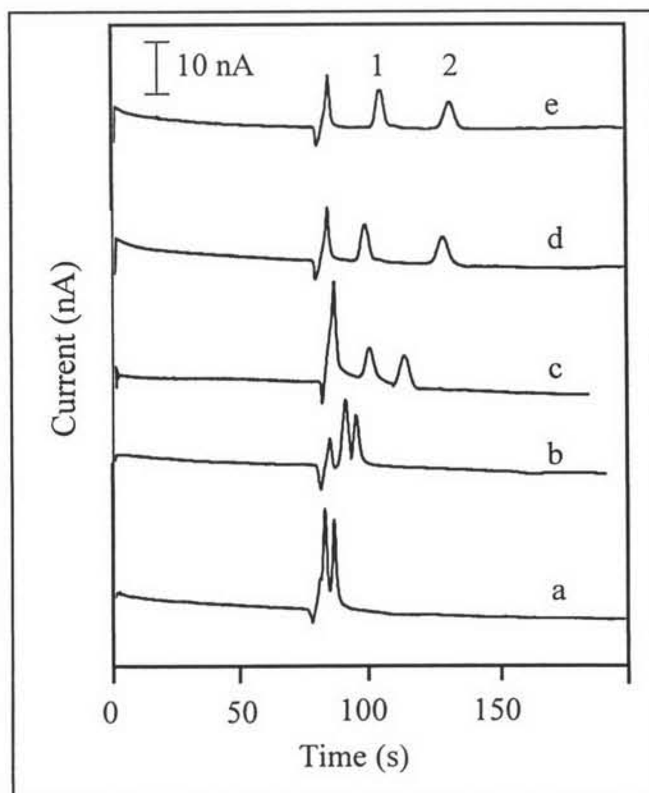


Figure 5.16 Effect of SDS concentrations upon the separation and detection of 10 ppm RDX (1) and 200 ppm PETN (2), (a) 10 mM, (b) 15 mM, (c) 20 mM, (d) 25 mM, and (e) 30 mM. Other conditions were the same as those in Figure 5.14.

5.3.3.3 Effect of Detection Potential

The potential applied to the working electrode directly have affected the sensitivity and detection limits of this method and it was necessary to determine the hydrodynamic voltammograms (HDVs) for the analytes to obtain the optimal potential. Figure 5.17 depicted HDVs for the reduction of RDX (a) and PETN (b) using the screen-printed carbon electrode detector. The curves were recorded step by step over -0.0 to –1.0 V. Both compounds showed similar profiles, with an increase of the response when increasing the applied potential. Although high applied potential gave results in higher peak currents, both the baseline noise and the background current increased substantially due to the solvent reduction. A high background current led to an unstable baseline, which was a disadvantage for sensitive and stable detection. Therefore, amperometric detection work was employed as a detection potential of -0.8 V to compromise the sensitivity of PETN.

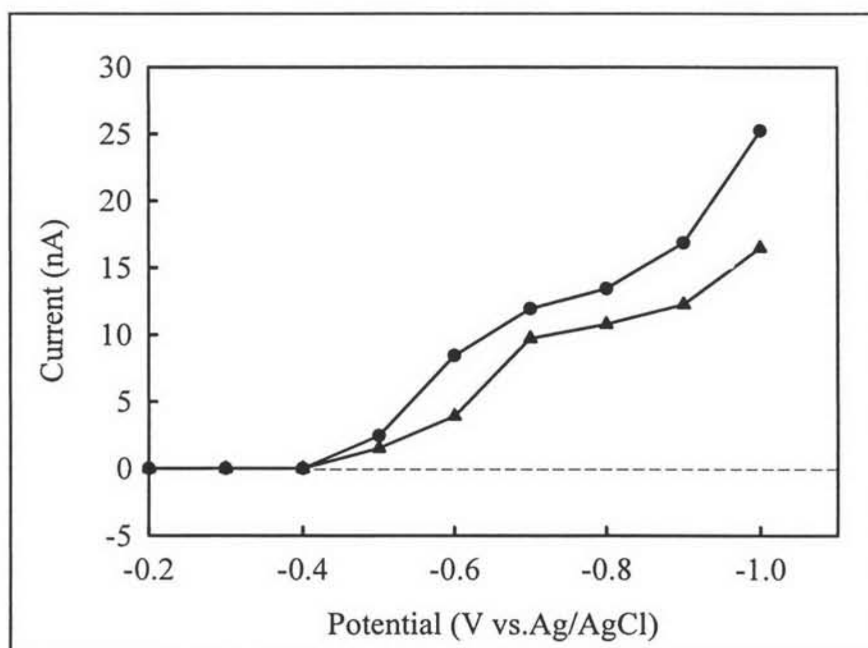


Figure 5.17 Hydrodynamic voltammograms of 10 ppm RDX (•) and 200 ppm PETN (▲). Conditions were the same as those in Figure 5.12.

5.3.3.4 The Analytical Figures

Electropherograms for sample mixtures containing increasing levels of RDX in 2 ppm steps, and of PETN in 25 ppm steps were also shown in Figure 5.18 (a-e). Defined peaks proportional to the analyte concentration wereobserved in both compounds. The resulting calibration plots (also shown inset) were linear with a sensitivity of 1.255 nA / ppm and 0.0416 nA/ ppm for RDX and PETN, respectively (correlation coefficients, 0.9982, 0.9995). The total assay time was around 150 s. The favorable signal-to-noise characteristics of these compounds indicated low detection limits of ca. 0.2 ppm for RDX and 2 ppm for PETN (based on S / N = 3).

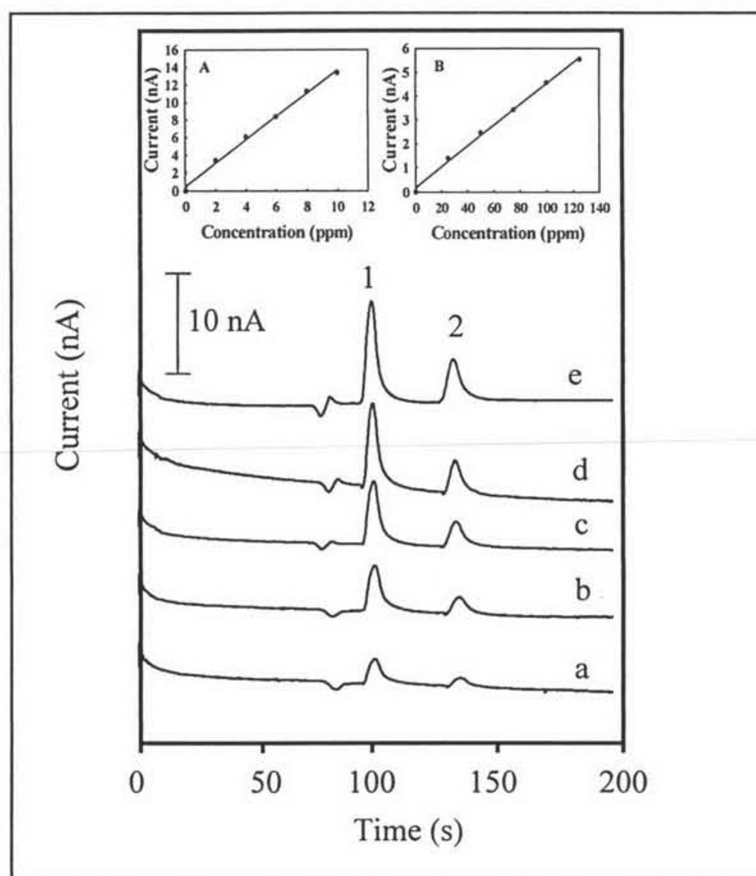


Figure 5.18 Electroprerograms for mixture containing increasing levels of (1) RDX, and (2) PETN in steps of 2 and 25 ppm, respectively (a-e). The calibration was also shown in the inset. Other conditions were the same as those in Figure 5.14.

The ability of reproducibility was demonstrated in Figure 5.19, which showed the electropherograms for 10 repetitive injections of 10 ppm RDX and 200 ppm PETN (using the same detector), resulting in relative standard deviations (% RSD, n=10) for peak current of 2.3 % and 2.4 % for RDX and PETN, respectively, and retention times of 0.4% and 1.1% for RDX and PETN, respectively. Such high stability was attributed to the use of extremely small sample volumes that resulted in a negligible accumulation of reaction products. The design of the microsystem also permitted rapid replacement of the detector in the case of surface fouling.

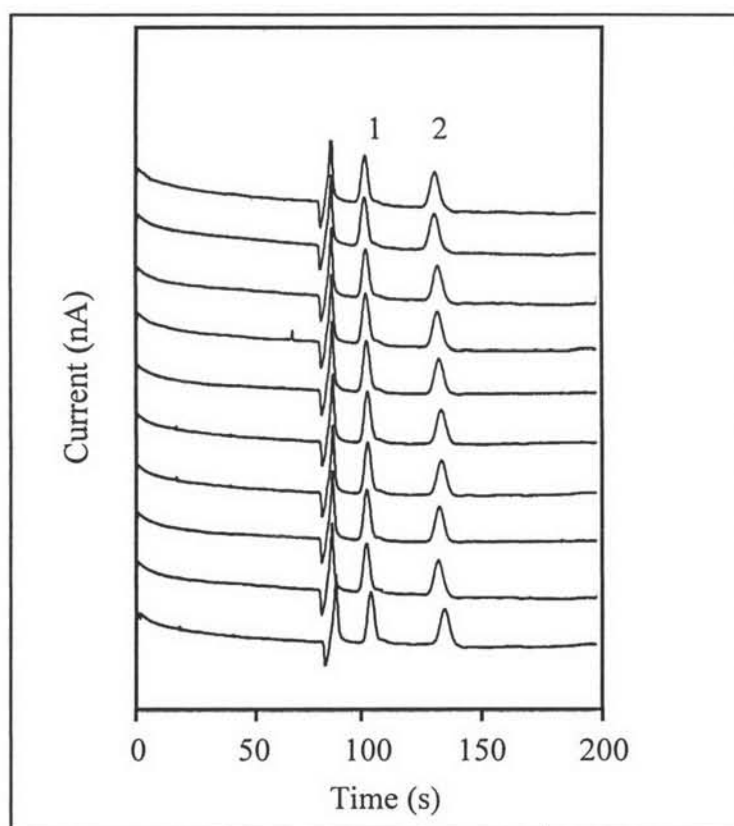


Figure 5.19 Repetitive simultaneous measurements of a mixture containing (1) 10 ppm RDX, and (2) 200 ppm PETN as recorded with amperometric mode. Other conditions were the same as those in Figure 5.13.

5.3.3.5 Summary

CE microchips with electrochemical detection have been used for the separation and detection of explosive compounds, with coupling offering great promise for environmental screening of toxic explosive compounds. In comparison to current liquid chromatographic protocol, the waste production was negligible, thus indicating in a very attractive green analytical chemistry method. The design was in contrast to disposable CE chips equipped with integrated thin-film or sputtered working electrodes that require replacement of the entire analytical system in the case of electrode passivation or severe electrode fouling. Therefore, this system reported here with was more applicable for analyzing hazardous compounds. Such a miniaturized explosive microanalyzer would enable transportation of the sample source to the forensic laboratory and offer significant advantages in terms of speed, efficiency, cost, small samples, and automation. These improvements could have a major impact upon the protection of first responders, on decision making and gathering of forensic data, or upon the prevention of terrorist activity. The new field testing capability should benefit other monitoring scenarios ranging from environmental protection to industrial process control.

5.3.4 Continuous Monitoring with Microfabricated Capillary Electrophoresis Chip Devices

Microfabricated microfluidic analytical devices, integrating multiple sample handling processes with the actual measurement step, represent a rapidly developing field in analytical chemistry and have therefore been of considerable interest recently [211-212]. Such devices offer tremendous potential for obtaining the desired analytical information in a faster, simpler, and cheaper manner compared to traditional laboratory-based instruments. Yet, despite extensive activity over the past 15 years, substantial work needs to be done if microfluidic devices were to be used for routine real-life monitoring scenarios. Such monitoring scenarios require the incorporation of a continuous sampling capability (from the external environment) through paying proper attention to the macroscopic/microfluidic ("world-to-chip") interface and elimination of the manual (pipetting, washout) steps.

Recent efforts have led to several useful approaches for introducing macroworld samples to microfabricated separation devices without manual intervention. These include the creation of a wide-bored sample introduction channel, [213] use of a flow-gated interface, [214-216] a falling-drop interface design, [216] use of a microfluidic matrix device,[217] or automatic sampling from an autosampler. Despite these elegant concepts, the "macro-to-micro" interfacing remains a weak challenging point that hinders the realization of on-line microchip monitoring [218]. Considerable work thus remains before such devices were used for routine monitoring applications.

Here, a simple and yet powerful approach was presented for delivering rapidly flowing samples from the macroworld to CE microchips, based on mounting a tubing containing the hydrodynamically flowing sample stream onto a sharp inlet tip. A sharp sample inlet was recently described in this journal for introducing a series of quiescent samples from discrete microliter vials into CE microchip devices [219]. The new sharp-inlet interface, developed in the present study, allowed reproducible injection of very small sample volumes from a continuously flowing liquid stream (that is larger by several orders of magnitude), and obviated the need for complex microchannel layouts. The resulting interface, illustrated in Figure 5.20, facilitated the electrokinetic loading of well-defined segments of a flowing stream directly into the separation microchannel without perturbing the separation. Such ability to continuously introduce flowing 'macro-world' samples into micrometer channels makes CE microchip devices highly compatible with real-life on-line monitoring applications.

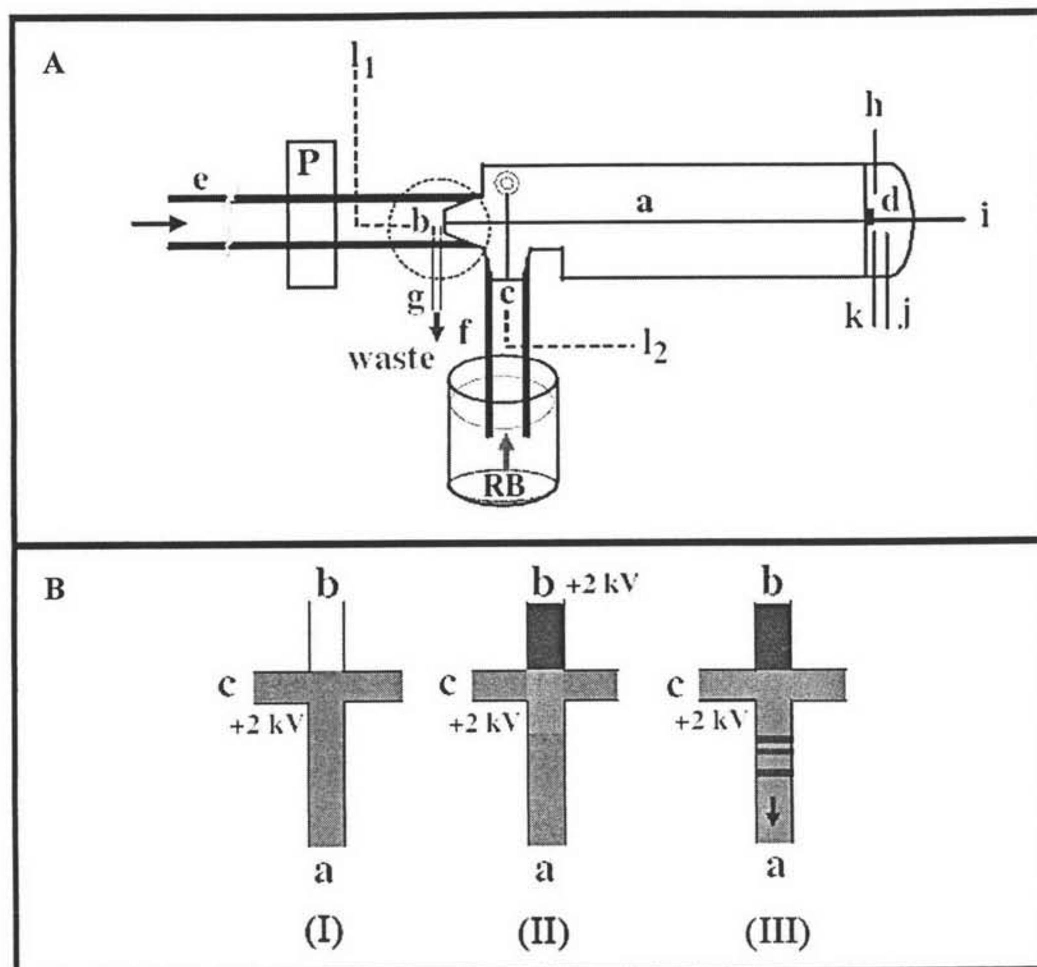


Figure 5.20 (A) Structure of the microfluidic chip system with on-line continuous monitoring capability. The separation channel (a) was joined by a sharp sample inlet tip (b), a side sharp running-buffer inlet tip (c) and a detector compartment (d). The sample was introduced by pumping from the reservoir with a peristaltic pump (P) through the Tygon tubing (e) onto the separation channel through a sharp inlet tip. The sample solution was drained through a plastic tubing (g) that was placed 1 mm from the sample inlet tip. The running buffer was introduced by capillary action through the plastic tubing (f). The detector compartment (d) was designed to accommodate the platinum wire ground electrode (h), the screen-printed carbon working electrode (i), platinum wire auxiliary electrode (k), and the Ag/AgCl wire reference electrode (j). Two platinum wires (l_1 and l_2) were inserted into the plastic tubing (e and f), respectively, serving as contacts to the high voltage power supply. (B) Steps involved in the new microchip assay: plug patterns during the (I)

introduction of the running buffer into the channel; (II) sample injection; (III) sample separation and detection.

The new sharp-inlet interface offered convenient, rapid and reproducible delivery of hydrodynamically flowing streams directly onto the separation channel of the CE microchips, without perturbing the separation or interrupting the sample flow. Electrokinetic loading was used for introducing very small sample volumes into the narrow separation microchannel from large 'macro-world' volumes in wide tubing. As desired for chip-based monitoring systems, the new interface led to a good temporal resolution and fast response to sudden concentration changes.

5.3.4.1 Carry -Over Effect

Figure 5.21A illustrated typical electropherograms for two injections of continuously flowing sample mixtures of nitroaromatic-explosive (left) and phenolic (right) compounds. Both samples displayed well resolved peaks within 145 and 170 sec, respectively, with reproducible peak heights and migration times. Such assay times led to a good temporal resolution (with a sampling frequency of approximately. 20-30 samples / hr). Higher separation voltages can be used to increase the temporal resolution further. The ability to rapidly respond to sudden changes in the concentration was demonstrated in Figure 5.21B, which showed electropherograms for four alternate flow-injection measurements of 10 (a) and 5 (b) ppm TNB solutions. Defined peaks with no observable carry over or cross contamination between the two explosive levels were observed.

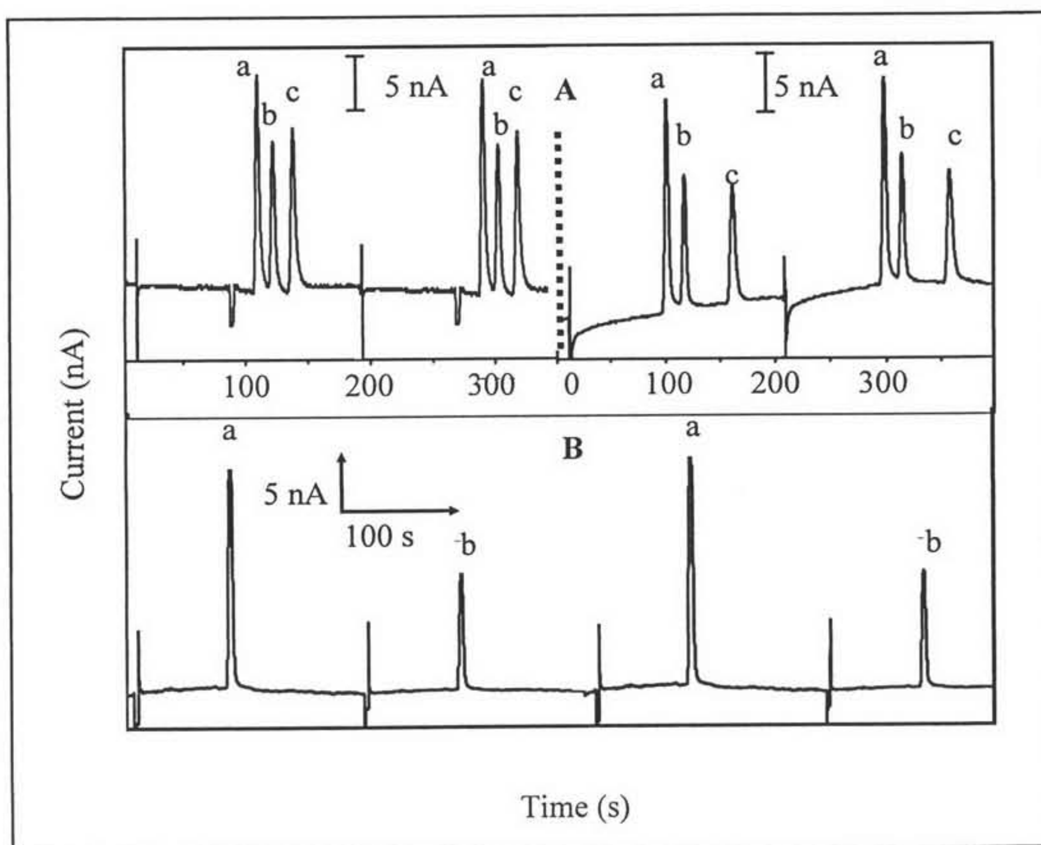


Figure 5.21 (A) Typical electropherograms showing the separation for an explosive mixture [Left; TNB 10 (a), DNB 15 (b) and TNT 20 (c) ppm] and phenolic compounds [Right; phenol 100 (a), 2-chlorophenol 100 (b) and 2,3-dichlorophenol 200 (c) μM]. Sampling time, 3 s; running buffer (for explosives), 15 mM sodium borate solution (pH 9.2) containing 15 mM SDS, and (for phenolic compounds), borate/phosphate buffer (10 mM each, pH 8.0). Sample flow rate, 0.5 mL /min; detection potential, -0.5 V (Left) and +0.90 V (Right) (vs. Ag/AgCl wire); injection and separation voltages, 2,000 V (Left) and 1,500 (Right) V. (B) Electropherograms for samples, which were introduced repetitively containing 10 ppm (a) and 5 ppm (b) TNB (connecting to 2 sample reservoirs and a T connector). Other conditions were the same as those in Figure 5.21A (Left).

5.3.4.2 Influence of Sampling Time

Variables affecting the performance of the new 'world-to-chip' interface were assessed and optimized. The volume (length) of the sample plug, introduced from the wide tubing into the separation channel, can be reproduced by controlling the sampling time. Figure 5.22 displayed electropherograms for a 10 ppm TNB solution obtained using different sampling times (1 - 20 sec, a-g) in connection to a continuously flowing sample stream (at 0.5 mL/ min). As expected, the explosive peak height increases with the volume of the injected sample at first and then it started to level off (above 5 sec, d). It was also worth noting that the peak width increased from 6 to 18 and 31 sec upon increasing the sampling time from 1 to 10 and 20 sec, respectively. The overall peak area was proportional to the injected sample volume. Obviously (as illustrated below), the sample zone was dependent also on the applied field strength. The rising edge of all peaks coincided and had the same shape, regardless of the sampling time. Overall, these profiles indicated that the new chip interface led to well-defined and controllable sample plugs.

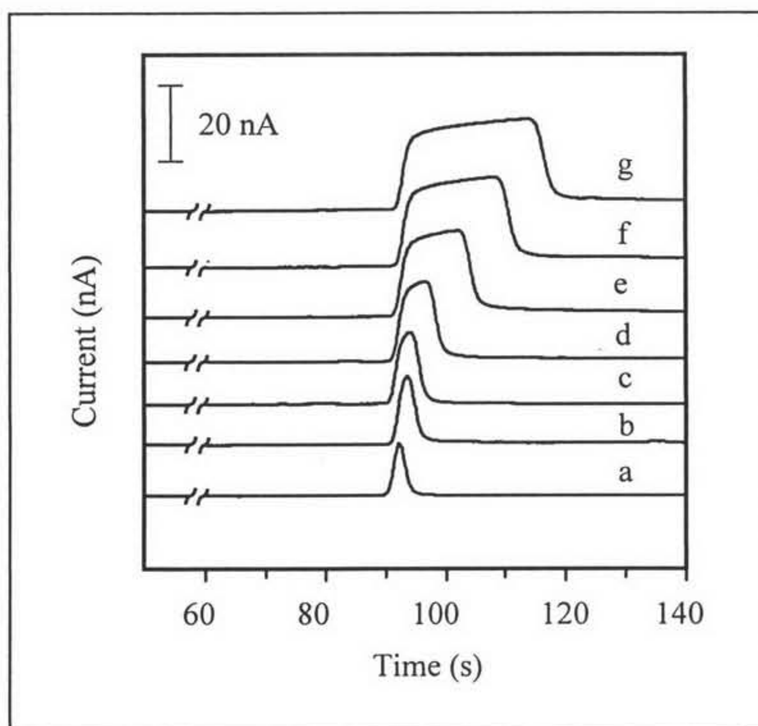


Figure 5.22 Effect of the sampling time upon the response for 10 ppm TNB. Sampling time, 1, 2, 3, 5, 10, 15, and 20 s (a-g); running buffer, 15 mM sodium borate (pH 9.2). Other conditions were the same as those in Figure 5.20A (Left).

5.3.4.3 Influence of Sample Flow Rate

Figure 5.23 examined the influence of the sample flow rate upon the response of 10 ppm TNB. Well defined explosive peak was observed for the different flow rates (0.2 – 1.8 mL/ min, a-e) in connection to a 3 sec sampling time (with electrokinetic delivery of sample from a continuous sample stream into the sample channel of the chip) and separation and injection voltages of 2,000 V. The peak current was independent of the flow rate over the entire range examined, indicating that the amount of sample entering the separation channel was also independent of the flow rate. In contrast, some increase in the peak-to-peak baseline noise level was observed for flow rates higher than 0.9 mL/ min. Overall, these data indicated convenient microchip monitoring of low explosive concentrations over a wide range of flow rates.

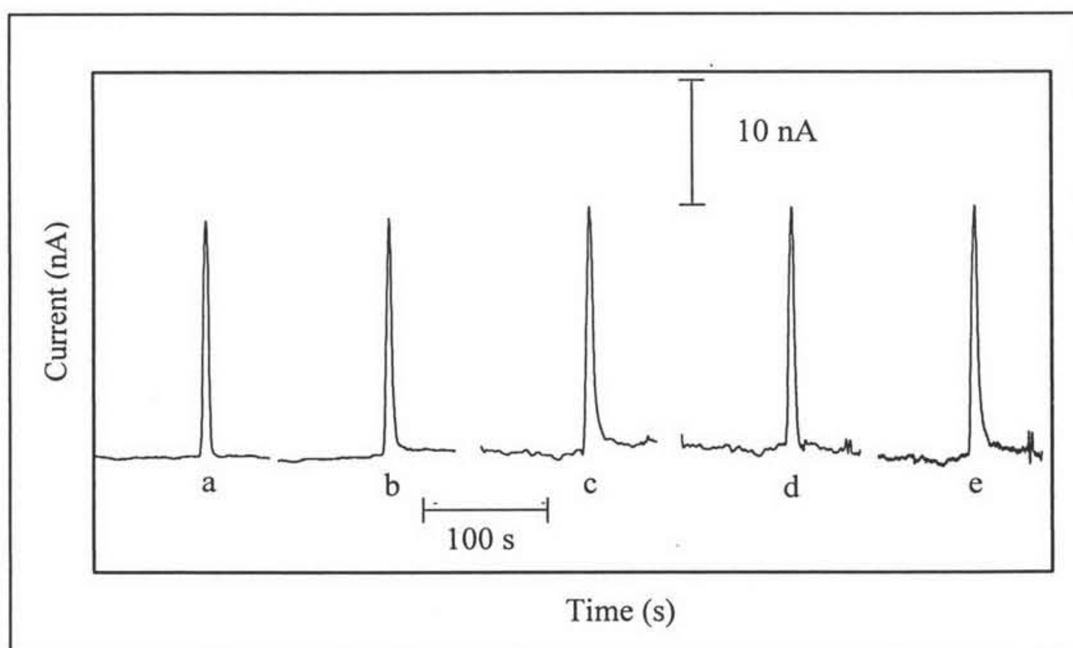


Figure 5.23 Influence of sample flow rate on the response for a 10 ppm TNB solution. Flow rate, 0.2 (a), 0.5 (b), 0.9 (c), 1.3 (d), and 1.8 (e) mL/ min. Other conditions were the same as those in Figure 5.21A (Left).

5.3.4.4 Effect of Separation Voltages

The effect of separation voltages upon the behavior of the CE microchip was examined using a constant sample flow rate of 0.5 mL/ min (Figure 5.24A). The peak intensity increased rapidly by 2-fold upon increasing the voltage between 1,000 and 2,000 V and levels off thereafter. The peak height also increased in a near linear fashion (by nearly 4 fold) upon raising the injection voltage to between 2,000 and 4,000 V (Figure 5.24B). These results also showed the convenience for detecting a low concentration sample.

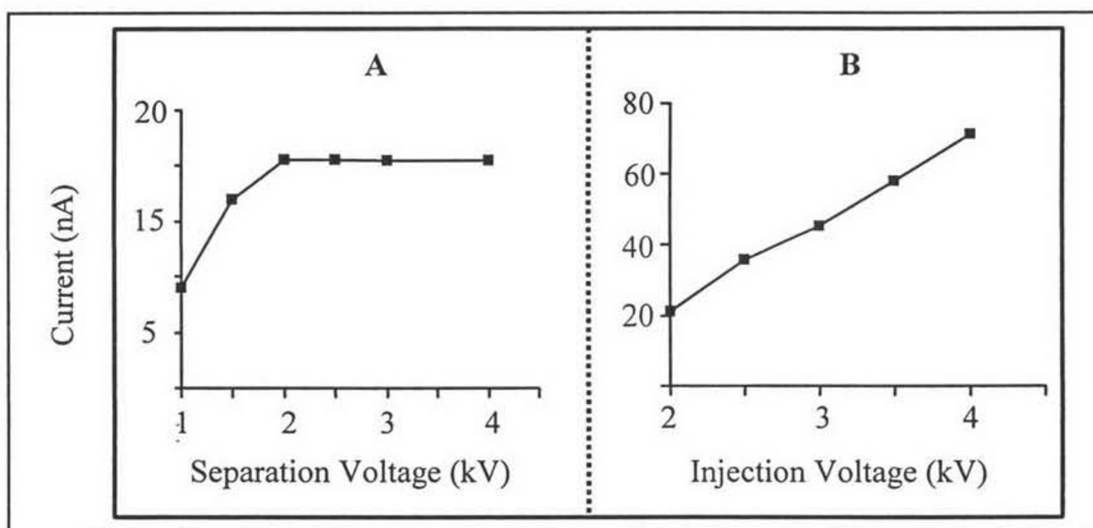


Figure 5.24 (A) Influence of the separation field strength upon the response of a 10 ppm TNB solution. Running buffer, 15 mM sodium borate (pH 9.2). (B) Effect of the sampling voltage on the response for 10 ppm TNB using a separation voltage of 2,000V. Other conditions were the same as those in Figure 5.21A (Left).

5.3.4.5 Analytical Performances

It was important for the new interface to offer reproducible and stable sampling from a continuously flowing liquid stream. The performance of the microsystem under conditions simulating continuous monitoring was tested by evaluating its behavior during a long run of 40 repetitive measurements, carried out

over a total time of 125 min (Figure 5.25). Such prolonged operation results in a highly stable TNB peak had relative standard deviations (RSD) of 3.7 % and 3.1 % for the current response and migration time, respectively. A highly stable baseline, with no apparent drift, was observed throughout this series. These data indicated reproducible sampling from a continuously flowing liquid stream (as well as a highly stable electrochemical detector).

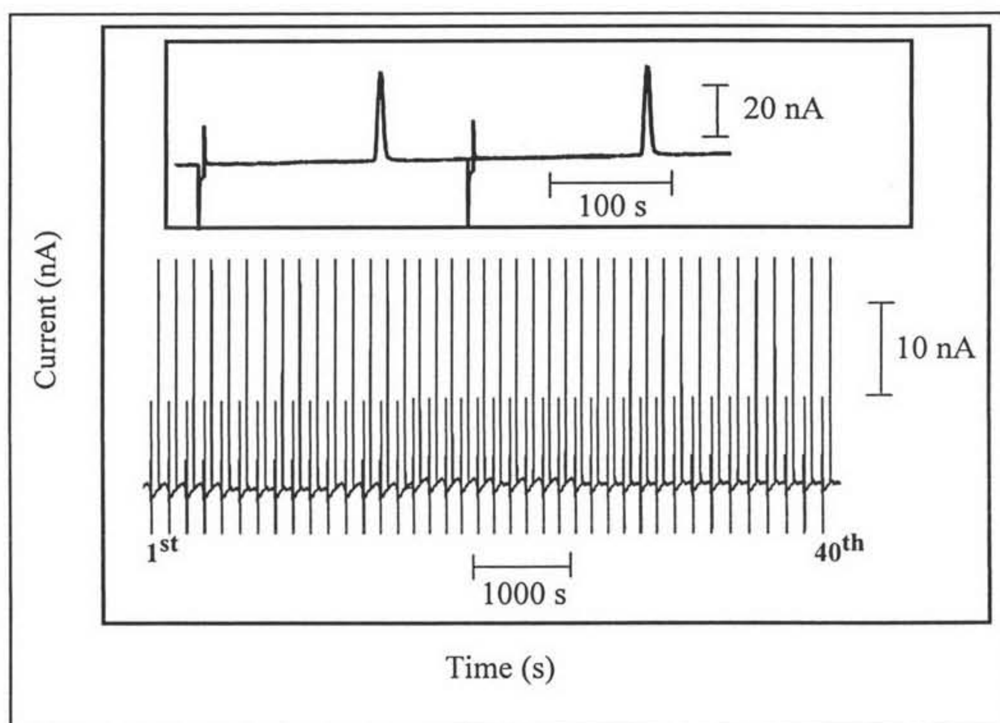


Figure 5.25 Response for 40 repetitive injections of a 15 ppm TNB solution. Sampling time, 2 s; running buffer, 15 mM sodium borate (pH 9.2); Top inset shows the response for two such injections. Other conditions were the same as those in Figure 5. 20A (Left).

The continuous sample introduction was coupled to well-defined concentration dependence. Figure 5.26 displayed electropherograms for mixtures containing increasing levels of different nitroaromatic explosives (A) and phenolic compounds (B). Well-defined peaks, proportional to the concentration of the five compounds, were observed. The resulting calibration plots (shown as insets)

were highly linear with sensitivities of 1.900 and 0.919 nA/ppm for TNB and TNT, respectively, and 2.406, 1.107 and 0.449 nA/ppm for phenol, 2-chlorophenol, and 2,3-dichlorophenol, respectively (correlation coefficients, 0.998-0.999). The favorable signal-to-noise characteristics of the response indicated low (sub-ppm and micromolar) detection limits.

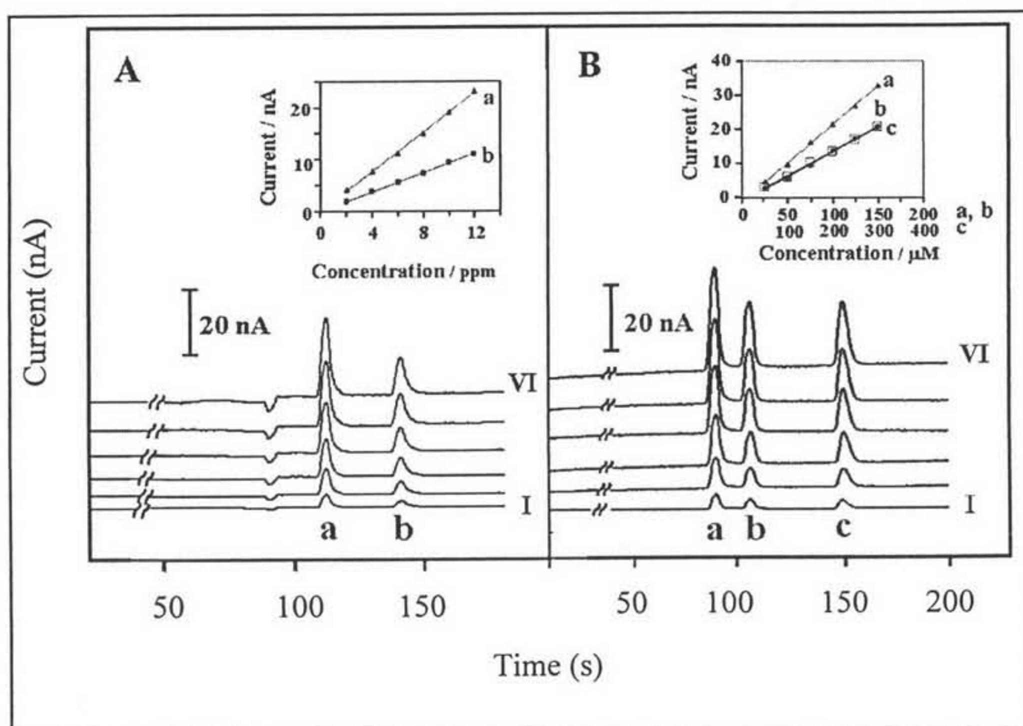


Figure 5.26 Electropherograms detailing the response to sample mixtures containing increasing concentrations of explosives [A; TNB(a) and TNT(b) in 2 ppm steps (I-VI)] and phenolic compounds [B; phenol (a), 2-chlorophenol (b) and 2,3-dichlorophenol (c) in steps of 25 µM (a,b) and 50 µM (c) steps (I-VI)]. Insets showed the corresponding calibration plots. Other conditions were the same as those in Figure 5.21.

5.3.4.6 Summary

A new 'macro-to-micro' chip interface based on a sharp sample inlet was developed that allowed rapid, convenient and reproducible introduction of a continuously flowing sample stream into narrow microchannels. Such simple and yet effective interface facilitated the use of hydrodynamically pumped large-volume samples without perturbing the CE separation, and hence the realization of chip-based on-line monitoring. While the new sharp-inlet interface has been presented in connection to electrochemical detection, it can be readily adapted to other detection modes. Further integration of additional on-chip functional elements and processes could lead to portable fully automated devices for on-site environmental monitoring, on-line monitoring of industrial processes and security surveillance.