Anal. Chem. **2001,** *73,* 1804−1808

Capillary Electrophoresis Microchips for Separation and Detection of Organophosphate Nerve Agents

Joseph Wang* and Madhu Prakash Chatrathi

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

Ashok Mulchandani and Wilfred Chen

Department of Chemical and Environmental Engineering, University of California, Riverside, California 92521

A miniaturized analytical system for separating and detecting toxic organophosphate nerve agent compounds, based on the coupling of a micromachined capillary electrophoresis chip with a thick-film amperometric detector, is described. Factors influencing the on-chip separation and detection processes have been optimized. Using a MES buffer (20 mM, pH 5.0) running buffer, a 72-mm-long separation channel, and a separation voltage of 2000 V, baseline resolution is observed for paraoxon, methyl parathion, fenitrothion, and ethyl parathion in 140 s. Such miniaturization and speed advantages are coupled to submicromolar detection limits and good precision. Applicability to spiked river water samples is demonstrated, and the implications for on-site environmental monitoring and rapid security screening/warning are discussed.

Organophosphate (OP) compounds are among the most toxic substances and are thus commonly used as chemical warfare agents and pesticides. In view of major concerns regarding the toxicity of these compounds, there are urgent needs for innovative analytical tools for detecting OP pesticides and chemical warfare agents. To meet these requirements of rapid warning and field deployment, such devices should be compact, fast, and reliable. Various inhibition and noninhibition biosensor systems, based on the immobilization of acetylcholinesterase or organophosphorus hydrolase onto various electrochemical or optical transducers, have been proposed for field screening of OP neurotoxins.[1−5] Yet, such enzyme-based "class" biosensors cannot discriminate among individual OP substances, hence compromising the reliability of the assay.

In this article, we demonstrate rapid on-chip micellar electrokinetic chromatographic (MEKC) separation and sensitive electrochemical detection of organophosphate nerve agents. Miniaturized analytical systems (referred to as "lab-on-a-chip" devices) are attracting considerable interest[6−8] due to the potential for greatly enhancing the speed of analytical separations, while dramatically reducing the system size or weight and the consumption of samples and reagents. Separation of OP compounds, particularly OP pesticides, has traditionally been carried out using conventional liquid[9] or micellar[10] chromatographic systems, in connection with electrochemical or optical (UV) detection. An analogous on-chip separation/detection microsystem that can meet the requirements of on-site environmental monitoring or rapid field detection of chemical warfare agents has not been reported. The present microsystem combines the attractive features of chip-based MEKC separations and amperometric detection.[11−13] Electrochemistry (EC) offers considerable promise for microchip separation systems, with features that include remarkable sensitivity (approaching that of fluorescence), inherent miniaturization of both the detector and control instrumentation, low cost, low-power requirements, and high compatibility with advanced micromachining technologies.[11−13] Amperometric flow detection has been proposed recently for monitoring other classes of toxic compounds, including nitroaromatic explosives[12] and hydrazines.[14] The inherent redox activity of nitro-containing OP compounds makes them ideal candidates for amperometric detection. The analytical features of the new MEKC/EC nerve agent microchip, reported in the following sections, make it very attractive for addressing the needs of various environmental and defense scenarios.

(1) La Rosa, C.; Pariente, F.; Hernandez, L.; Lorenzo, E. *Anal. Chim. Acta* **1994**, *295*, 273.
(2) Mulchandani, A.; Kaneva, I.; Chen, W. *Anal. Chem.* **1998**, *70*, 5042.
(3) Wang, J.; Mulchandani, A.; Chen, L.; Mulchandani, P.; Chen, W. *Anal. Chem.* **1999**, *71*, 2246.
(4) Mulchandani, A.; Mulchandani, P.; Kaneva, I.; Chen, W. *Anal. Chem.* **1998**, *70*, 4140.
(5) Wang, J.; Mulchandani, A.; Chen, L.; Mulchandani, P.; Chen, W. *Electroanalysis* **1999**, *11*, 866.
(6) Figeys, D.; Pinto, D. *Anal. Chem.* **2000**, *72*, 330A.
(7) Soper, S.; Ford, S.; Qi, S.; McCarley, R.; Kelly, K.; Murphy, M. *Anal. Chem.* **2000**, *72*, 643A.
(8) Jacobson, S. C.; Hergenroder, R.; Moore, A. W., Jr.; Ramsey, J. M. *Anal. Chem.* **1994**, *66*, 4127.
(9) Martinez, R. C.; Gonzalo, E. R.; Garcia, F. G.; Mendez, J. H. *J. Chromatogr.* **1993**, *644*, 49.
(10) Aguilar, M.; Farran, A.; Serra, C.; Sepaniak, M. J.; Whitaker, K. W. *J. Chromatogr., A* **1997**, *778*, 201.
(11) Woolley, A. T.; Lao, K.; Glazer, A. N.; Mathies, R. A. *Anal. Chem.* **1998**, *70*, 684.
(12) Wang, J.; Tian, B.; Sahlin, E. *Anal. Chem.* **1999**, *71*, 5436.
(13) Martin, R. S.; Gawron, A. J.; Lunte, S. M.; Henry, C. S. *Anal. Chem.* **2000**, *72*, 3196.
(14) Wang, J.; Chatrathi, M. P.; Tian, B.; Polsky, R. *Electroanalysis* **2000**, *12*, 691.

10.1021/ac001424e CCC: $20.00
Published on Web 03/16/2001

EXPERIMENTAL SECTION

Reagents. Paraoxon, methyl parathion, ethyl parathion, and fenitrothion were obtained from Supelco. 2-(*N*-Morpholino)-ethanesulfonic acid (MES) hydrate was obtained from Lancaster (Pelham, NH), dodecyl sodium sulfate (SDS) was purchased from J. T. Baker, and acetonitrile was obtained from Aldrich. All the chemicals were used without any further purification. The electrophoresis buffer was a MES buffer (20 mM, pH 5.0). A 20 mM acetate buffer (pH 5.0) was used for comparison. Stock solutions were prepared in acetonitrile, and subsequent dilutions were performed daily in electrophoresis buffer and filtered with 0.45-μm filter (Gelman Acrodisk). The river water sample was collected from Rio Grande River at Las Cruces, NM. The sample was prepared by adding the required amounts of MES hydrate, SDS, and sodium hydroxide (to yield 20 and 7.5 mM levels and pH 5.0, respectively) to the Rio Grande water and filtered.

Apparatus. Details of the integrated chip/detection microsystem were described previously.[12] The glass microchip, fabricated by Alberta Microelectronic Co. (AMC, model MC-BF4-001, Edmonton, Canada), consisted of two crossed channels and three reservoirs, including a four-way injection cross (connected to the three reservoirs), and a 72-mm separation channel. The original waste reservoir was cut off leaving the channel outlet at the end side of the chip, thus facilitating the end-column amperometric detection.

A Plexiglas holder was fabricated for holding the separation chip and housing the detector and reservoirs. A short pipet tip was inserted into each of the three holes on the glass chip for solution contact between the channel on the chip and corresponding reservoir on the chip holder. The amperometric detector was placed in the waste reservoir (at the channel outlet side) and consisted of a Ag/AgCl wire reference, a platinum wire counter, and a screen-printed carbon working electrodes. The screen-printed working electrode was placed opposite to the channel outlet, at a 50-μm distance (controlled by a plastic screw and a thin-layer spacer). Platinum wires, inserted into the individual reservoirs, served as contacts to the high-voltage power supply. Such power supply had switchable voltage ports between running buffer and sample injections with a voltage range between 0 and +4000 V. Amperometric detection was performed with an electrochemical analyzer 621 (CH Instruments, Austin, TX) using the "amperometric $i-t$ curve" mode.

The thick-film carbon electrodes were printed with a semiautomatic printer (model TF 100, MPM, Franklin, MA). The Acheson ink (Electrodag 440B; Catalog No. 49AB90; Acheson Colloids, Ontario, CA) was used for printing electrode strips. Details of the printing process and dimensions were described elsewhere.[12]

Procedure. *Electrophoresis Procedure.* The channels were treated before use by rinsing with 0.1 M NaOH and deionized water for 20 and 5 min, respectively. The electrophoresis buffer was a MES buffer (20 mM, pH 5.0). The "buffer" reservoirs were filled with electrophoresis running buffer solution, while the "sample" reservoir with the mixture of organophosphorus pesticides. A potential of +1500 V was applied to the "sample" reservoir for 20 s in order to facilitate the filling of the injection channel (between the separation channel and the sample reservoir), with the detection reservoir grounded and all the other reservoirs floating. Separations were usually performed by applying +2000 V to the running buffer reservoir with the detection reservoir grounded and all other reservoirs floating.

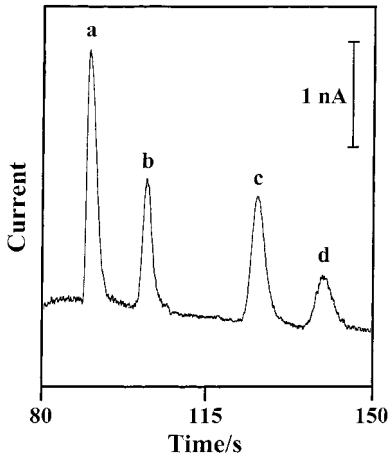

Figure 1. Separation and detection of organophosphate nerve agent compounds: (a) 1.0×10^{-5} M paraoxon, (b) 1.0×10^{-5} M methyl parathion, (c) 2.0×10^{-5} M fenitrothion, and (d) 4.0×10^{-5} M ethyl parathion. Separation buffer, 20 mM MES (pH 5.0) containing 7.5 mM SDS; separation voltage, +2000 V; injection voltage, +1500 V; injection time, 3 s; detection potential, −0.5 V (vs Ag/AgCl wire) at bare carbon screen-printed electrode.

The injection was affected by applying +1500 V between the "running buffer" reservoir and the grounded detection reservoir for 3 s. This drove the sample "plug" into the separation channel through the intersection. By switching the high-voltage contacts, the separation potential was subsequently applied to the "running buffer" reservoir for the separation of the pesticide compounds.

Safety Considerations. The high-voltage power supply should be handled with extreme care to avoid electrical shock. OP pesticides are highly toxic and should be handled in a fumehood. Skin and eye contact and accidental inhalation or ingestion should be avoided.

Amperometric Detection. The electropherograms were recorded with a time resolution of 0.1 s while the detection potential (usually −0.5 V vs Ag/AgCl wire) was applied. Sample injections were performed after stabilization of the baseline. The raw data of electropherograms were digitally filtered by the built-in 15-point least-squares smoothing option of the CH electrochemical analyzer (Software CHI version 2.05). All experiments were performed at room temperature.

RESULTS AND DISCUSSION

The present study targeted mainly priority OP pesticide pollutants, in connection with micofabricated CE glass chips with planar thick-film amperometric detectors.[12] Figure 1 displays a characteristic electropherogram obtained for a mixture containing micromolar concentrations of paraoxon, methyl parathion, fenitrothion, and ethyl parathion. Using a MES buffer (20 mM, pH 5.0) as running buffer and a separation voltage of 2000 V, the four peaks are well resolved, with the entire assay requiring ~2.5 min. Significantly longer separations (~20 min) were reported for analogous conventional liquid chromatographic assays that display a similar elution order.[9] A relatively flat baseline and a low noise level are observed, despite the negative detection potential (−0.5 V), use of nondearated running buffer, the high-separation voltage,

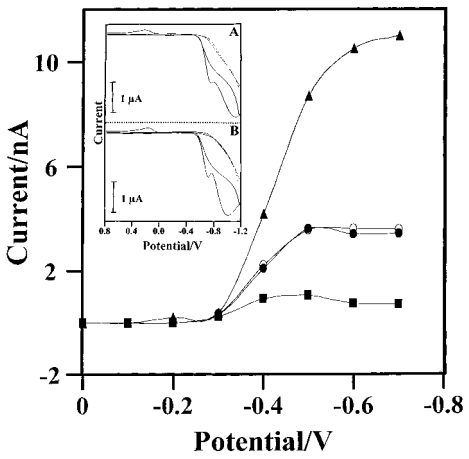

Figure 2. Hydrodynamic voltammograms of 7.1×10^{-5} M paraoxon (▲), 7.5×10^{-5} M methyl parathion (●), 1.41×10^{-4} M fenitrothion (○), and 1.34×10^{-4} M ethyl parathion (■). Running buffer is 20 mM MES (pH 5.0) with 7.5 mM SDS. Separation voltage +2000 V; injection voltage +1500 V; injection time 3 s; detection potential −0.5 V (vs Ag/AgCl wire). Also shown (as inset) are cyclic voltammograms for 5.7×10^{-5} M paraoxon (A) and 6.0×10^{-5} M methyl parathion (B) over the +0.8 and −1.2 V range, using a scan rate of 50 mV/s, along with the corresponding blank voltammograms (broken line).

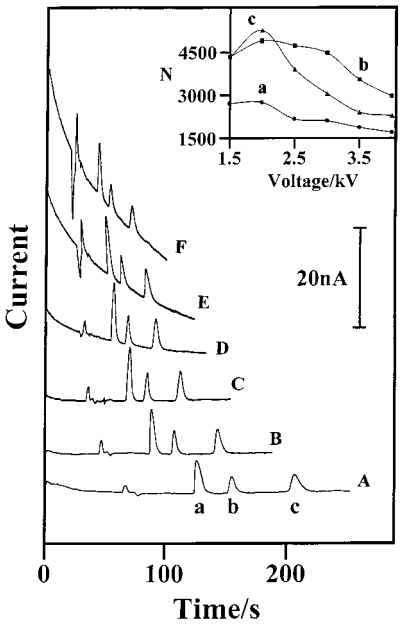

Figure 3. Influence of the separation voltage upon the response for a mixture containing 3.0×10^{-5} M paraoxon (a), 3.0×10^{-5} M methyl parathion (b), and 6.0×10^{-5} M fenitrothion (c). Separation performed using (A) +1500, (B) +2000, (C) +2500, (D) +3000, (E) +3500, and (F) +4000 V. Also shown (inset) are the resulting plots of plate number (*N*) versus separation voltage. Other conditions, as in Figure 1.

and the absence of a decoupling mechanism. These, along with the well-defined response peaks, indicate convenient quantitation down to the micromolar level. Detection limits of 6×10^{-7} M paraoxon, 1.2×10^{-6} M methyl parathion, 3×10^{-6} M fenitrothion, and 1.2×10^{-5} M ethyl parathion (i.e., 0.21, 0.40, 1.06, and 4.48 ppm, respectively) can be estimated based on the favorable signal-to-noise characteristics of Figure 1(S/N = 3). The high speed and sensitivity of the MEKC/EC microchip system are consistent with various on-site environmental and security needs. Lower detection limits are expected in connection with a dual electrochemical detection, i.e., via anodic measurements of the reduction product in a series (upstream−downstream) configuration.[9]

Figure 2 depicts typical hydrodynamic voltammograms for the reduction of submillimolar concentrations of four different OP nerve agent compounds. The curves were developed pointwise by making 100-mV changes in the applied potential over the 0.0 to −0.7 V range and using a separation voltage of 2000 V. All four compounds display similar current−potential profiles, with defined waves, starting around −0.3 V, and leveling off above −0.6 V. The half-wave potentials are −0.35, −0.37, −0.38, and −0.43 V for paraoxon, methyl parathion, fenitrothion, and ethyl parathion, respectively. All subsequent amperometric work employed a potential of −0.5 V that yielded the optimal detection. More negative potentials compromise the signal-to-noise characteristics, due to rising background signals. It should be pointed out that the exact position of the wave depends on the separation voltage (with higher fields shifting the voltammetric profile to the anodic direction).[15] The voltammetric waves of Figure 2 reflect the reduction of the nitro moiety of the OP compounds. The corresponding cyclic voltammograms for paraoxon and methyl parathion are also displayed in Figure 2 (as the inset). Two peaks, corresponding to the reduction of the nitro group to hydroxylamine, followed by conversion to an amine group, are observed (at potentials around −0.7 and −1.0 V, respectively). A small anodic peak, corresponding to the oxidation of the hydroxylamine to the nitroso derivative, is observed in the reverse scan. These profiles are consistent with those described elsewhere for OP pesticides.[9] Notice also the rapidly rising (oxygen reduction) background response, at potentials more negative to −0.75 V (broken line). Comparison of the hydrodynamic and cyclic voltammograms indicates a ∼200 mV shift in the response for the OP compounds (that starts around −0.3 and −0.5 V, respectively). Such a shift is attributed to the use of different reference electrodes (wire vs conventional Ag/AgCl) and to the influence of the separation voltage.

The effect of the separation potential upon the separation efficiency and amperometric response is shown in Figure 3. As expected, increasing the separation potential from 1500 to 4000 V (in 500-V increments, A−F) dramatically decreases the migration time for paraoxon (a), methyl parathion (b), and fenitrothion (c) from 123 to 41, 150 to 51, and 202 to 68 s, respectively. The paraoxon peak width (at half-height) decreases from ∼5.6 s at 1500 V to ∼2.4 s at 4000 V. Also shown in Figure 3 (inset) is the effect of separation voltage upon the number of theoretical plates (*N*) for paraoxon (a), methyl parathion (b), and fenitrothion (c). As can be seen from these profiles, maximum *N* values are obtained at a separation voltage of +2000 V. The plate number increases for all compounds to a maximum value of 2765 (a), 4900 (b), and 5263 (c) upon raising the separation potential from +1500 to +2000 V and then decreases gradually (to 1716 (a), 2983 (b), and 2300 (c) at 4000 V). The decreased separation efficiency at high field strengths can be attributed to nonideal effects such as Joule heating. The peak currents increase rapidly with the voltage between 1500 and 3000 V and decrease slowly at higher voltages. Such a decrease is attributed to the anodic shift of the hydrody-

(15) Wallenburg, S. R.; Nyholm, L.; Lunte, C. E. *Anal. Chem.* **1999**, 71, 544.

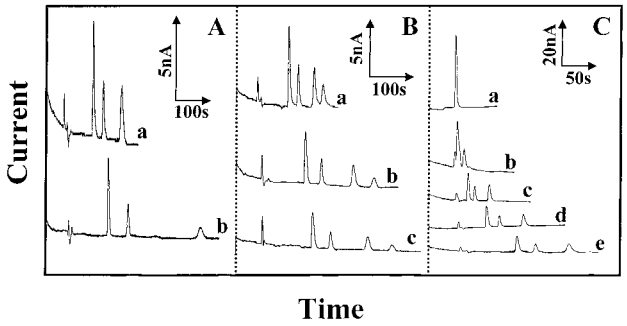

Figure 4. Effect of running buffer (A), pH (B), and SDS concentration (C) upon the separation and response of 7.1×10^{-5} M paraoxon, 7.5×10^{-5} M methyl parathion, 1.4×10^{-4} M fenitrothion, and 1.3×10^{-4} M ethyl parathion (in the order of appearance). (A) Effect of the buffer: MES (a) and acetate (b) buffers (20 mM, pH 5.0), containing 10 mM SDS. (B) Influence of pH: MES buffers (20 mM), containing 10 mM SDS, of pH 5.0 (a), 6.0 (b), and 7.0 (c). (C) Effect of SDS concentration: MES buffers (20 mM, pH 5.0), containing 0 (a), 2.5 (b), 5.0 (c), 7.5 (d), and 10 mM (e) SDS. Other conditions, as in Figure 1.

namic voltammogram[15] that results in operation below the potential-independent transport-limited plateau region. Flat baselines are observed using the low separation voltages; yet, a larger initial baseline slope, coupled to increased background noise, is observed for voltages ranging from 2500 and 4000 V, indicating an incomplete isolation from high separation voltages. Most subsequent work thus employed a potential of 2000 V.

The composition of the running buffer has a profound effect upon the MEKC microchip separation of OP compounds (Figure 4). A MES buffer resulted in a faster separation and a more sensitive detection than an acetate buffer, with the total assay time decreasing from 450 to 200 s (Figure 4A). Increasing the pH of the MES buffer between 5.0 and 7.0 led to longer migration times and smaller peaks (Figure 4B). Basic media were not examined due to the degradation of OP compounds. The MEKC separation of neutral compounds requires an addition of a surfactant (most commonly SDS). The influence of the SDS concentration is displayed in Figure 4C. Increasing the surfactant concentrations from 0 to 10 mM (a−e), in 2.5 mM steps, resulted in significantly shorter migrations times and larger signals; yet, the resolution was greatly compromised above 7.5 mM SDS. [The critical micellar concentration (cmc) for SDS is ~7.3 mM.] All subsequent work thus employed a 20 mM MES buffer solution (pH 5.0), containing 7.5 mM SDS.

The amperometric detector displays a well-defined concentration dependence over the range of analyte concentrations examined. Figure 5 displays electropherograms for sample mixtures containing increasing levels of paraoxon (A), methyl parathion (B), and fenitrothion (C) in steps of 7.1×10^{-6}, 7.5×10^{-6}, and 1.41×10^{-5} M, respectively. Defined peaks proportional to the analyte concentration are observed for all three compounds. The resulting calibration plots (also shown) are linear with sensitivities of 240, 98, and 62 nA/mM for paraoxon, methyl parathion, and fenitrothion, respectively (correlation coefficients, 0.998, 0.994, and 0.995).

As our future goal is to implement a fast-responding field-deployable microsystem, it is important that the device can be used for analyzing relevant environmental samples. Figure 6

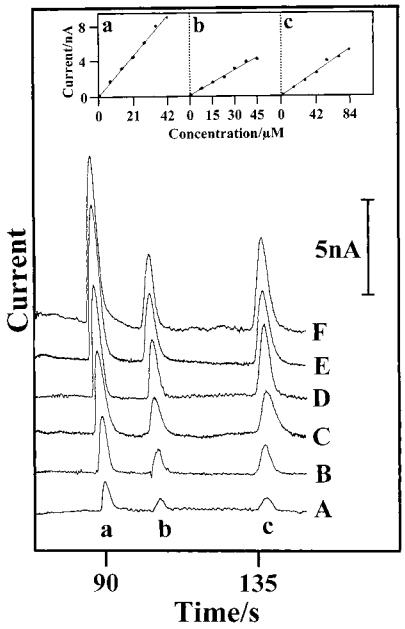

Figure 5. Electropherograms for mixtures containing increasing levels of paraoxon (a), methyl parathion (b), and fenitrothion (c) in steps of 7.1×10^{-5}, 7.5×10^{-5}, and 1.4×10^{-4} M, respectively (A−F). Also shown (as insets) are the resulting calibration plots. Other conditions, as in Figure 1.

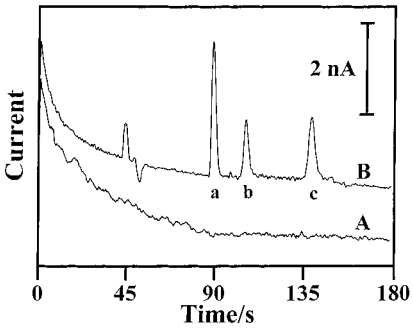

Figure 6. Electropherograms for river water sample before (A) and after (B) the addition of 1.4×10^{-5} M paraoxon (a), 1.5×10^{-5} M methyl parathion (b), and 2.8×10^{-5} M fenitrothion (c). The river water was sampled from Rio Grand River at Las Cruces, NM. Other conditions, as in Figure 1. The untreated sample was filtered and spiked with the required amounts of MES hydrate, SDS, and sodium hydroxide (to yield 20 and 7.5 mM levels, and pH 5.0, respectively).

demonstrates the suitability of the MEKC/EC microchip for separating and detecting three OP compounds, spiked into a river water sample [1.4×10^{-5} M paraoxon (a), 1.5×10^{-5} M methyl parathion (b), and 2.8×10^{-4} M fenitrothion (c)]. The electropherogram is characterized with well-defined and baseline resolved peaks; the total assay time is ~140 s. The favorable signal-to-noise characteristics of these real-sample data indicate low detection limits of 9×10^{-7} M paraoxon, 2.4×10^{-6} M methyl parathion, and 3.8×10^{-6} M fenitrothion (based on S/N = 3). Such values are slightly higher than those observed for synthetic samples (e.g., Figure 1). The absence of response peaks for the unspiked sample (electropherogram A) reflects the high selectivity of the amperometric detection and indicates great promise for assays of relevant environmental samples.

The precision was examined from a series of eight repetitive injections of a 7.5×10^{-5} M methyl parathion−1.4×10^{-4} M

fenitrothion sample mixture that yielded relative standard deviations (RSDs) of 5.4 and 6.5%, respectively (not shown; conditions, as in Figure 2). Yet, both peaks decreased gradually over a longer operation (e.g., >50% after 20 successive runs), reflecting the existence of surface fouling processes.[9] A similar current decay was reported for amperometric detection of OP substances following conventional liquid chromatographic separations.[9] The design of the microsystem permits rapid (5−10 s) replacement of the detector strip in the case of such surface passivation. Alternately, a simple electrochemical pretreatment can be used for enhancing the reproducibility.[9]

CONCLUSIONS

We have demonstrated the utility of CE microchips with thick-film electrochemical detectors for the separation and detection of toxic OP compounds. The integrated microsystem offers rapid (∼2.5 min) simultaneous measurements of micromolar levels of four organophosphorus compounds and holds great promise for a timely warning and alarm in case of a sudden change in the level of OP neurotoxins. While the concept of a nerve agent microchip has been demonstrated in connection to OP pesticides, the miniaturized system should be attractive for monitoring electroactive OP warfare agents. Additional OP neurotoxins may be monitored in connection with other electrochemical detection schemes (e.g., conductivity or potentiometry) or using on-chip enzymatic (OP hydrolase) assays. The integration of on-chip preseparation preconcentration should lead to further improvements in the sensitivity, as desired for various defense and environmental scenarios. Work is in progress in these directions and toward the integration of an on-chip potentiostatic circuitry. The latter would facilitate the implementation of a fast-responding field-deployable hand-held "nerve agent" microanalyzer.

ACKNOWLEDGMENT

Financial support from the U.S. EPA, USDA, ONR, and the DOE-WERC program is acknowledged. Mention of trade names or commercial products does not constitute endorsement by EPA for use.

Received for review December 5, 2000. Accepted February 6, 2001.

AC001424E