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Integrated Automated Analyzer for Monitoring of Explosives in Groundwater

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Integrated Automated Analyzer for Monitoring of Explosives in Groundwater

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1. BACKGROUND

The closure and remediation of former ammunition plants and military facilities requires accurate characterization of soil and groundwater contamination. Some of the pollutants found at these facilities are nitroaromatic and nitramine explosives and their biological and photolytic degradation products [1]. It has been found that the distribution of contamination is often highly heterogeneous, requiring numerous samples and analyses for these sites to be adequately characterized [1]. A number of different methods have been applied to the analysis of explosives, including gas [2], liquid [3-5], thin-layer chromatographies [6], Raman spectroscopy [7], electrochemical sensor [8], and immunoassay techniques [9]. It would be highly advantageous to develop a field analyzer capable of reliable and cost-effective analysis of explosives in environmental samples. Groundwater in contaminated sites usually contains several related compounds and their degradation products: 14 compounds in the U.S. Environmental Protection Agency (EPA) 8330 method. Therefore, simultaneous detection of these explosives in complex environmental matrixes by Raman spectroscopy, immunochemical sensors, or electrochemical sensors would be difficult and requires a chromatographic separation technique.

The EPA specifies SW-846 Method 8330 for the trace analysis of explosive residues in water, soil, or sediment matrixes [10]. Following sonication, extraction with acetonitrile, and preconcentration, analysis for 14 species is performed using high-performance liquid chromatography (HPLC) and ultraviolet (UV) absorption. Isocratic HPLC separations using commercially available C18 columns typically take over 30 minutes and are unable to separate the two aminodinitrotoluene isomers and two of the three dinitrotoluene isomers [11]. To fully identify each of the 14 compounds, an additional HPLC run must be performed using a cyano column, leading to an increase in analysis time and sample handling complexity. These disadvantages have led to the search for alternative liquid chromatographic techniques to the traditional isocratic HPLC separation of explosives. Emmrich and co-workers [12] investigated the use of mobile-phase gradients using a single C18 column. Using a C8 stationary phase under isocratic conditions and photodiode array detection, Bouvier and Oehrle [4] were able to identify all of the Method 8330 components in 25 minutes, but were unable to achieve baseline resolution. Crockett et al. [1] reported the cost for analyses by the EPA Method 8330 to be $250 to $350 per sample for turnaround times of 30 days and $1,000 per sample for turnaround times of 3 days. Thus, there is interest in the development of inexpensive and rapid methods for the determination of explosives, in particular those that can withstand the rigors of field usage for process monitoring. HPLC methods, however, utilized large-scale instrumentation that requires high pressure and therefore is not suitable for developing a field-portable analyzer.

Effort has also focused on developing highly efficient capillary electrophoretic (CE) procedures, particularly capillary electrochromatography (CEC) and micellar electrokinetic chromatography (MEKC) to improve the resolution for several explosive compounds that cannot be resolved by LC. Bailey and Yan [13] reported the separation of 14 explosives using CEC. A separation with baseline resolution is achieved for 14 compounds within 7 minutes. Using more aggressive running conditions, 13 compounds are separated within 2 minutes. A MEKC procedure using a sodium dodecyl sulfate (SDS)-phosphate, pH 7, electrolyte was first attempted by Oehrle [14-15] to separate 14 common explosives including 2,4-DNT

(dinitrotoluene), 2,6-DNT, 2-NT (nitrotoluene), 3-NT, and 4-NT. The resolution power of MEKC is thus well established, but the UV measurement is not sufficiently sensitive for the detection of explosives in environmental samples. Furthermore, many extractable components in contaminated soils, particularly soils that have supported vegetation, often interfere with absorbency measurement.

Electrochemical detectors, which promise high sensitivity, simplicity, and low cost, have been coupled with MEKC to detect the explosive content of soil extracts and groundwater, yielding results in good agreement with those obtained by the EPA HPLC method. The detection limit of explosives for MEKC with an electrochemical detector is 10-fold lower than that of an UV detector [16].

MEKC offers all the advantages of a miniaturized separation technique, including low solvent consumption (~ 100 nL/min) and waste generation, low sample-volume requirements, increased mass sensitivity, low power requirements, low operational costs, and extremely high separation efficiency. It also offers the potential for retention mechanisms and selectivities normally afforded by HPLC but with electrically driven flow, which reduces band broadening associated with pressure-driven parabolic flow profiles. In summary, the MEKC method offers advantages of high-resolution separations and sensitive detection as required for the analysis of explosives in environmental matrixes. These techniques can be implemented on a miniature scale and in an integrated devices with low power requirements. Because of these advantages, CE is well suited for further developed into a portable instrumentation for onsite fast separation and detection.

There has been a worldwide research effort over the last several years to use microfabrication technology to integrate whole laboratory systems onto microchips. These systems have been termed microscale total analytical systems (μ-TAS), or lab-on-a-chip devices. Numerous μ-TAS systems have been developed and reported in the recent literature with apparently very promising capabilities [17-20]. It is believed that lab-on-a-chip technology will revolutionize the new generation of analytical instrumentation. The advantages of μ-TAS include the capability to analyze small-volume samples with increased analysis speed, reduction in reagent consumption, and consequent reduction in waste disposal. Since these devices are small, they could potentially be integrated into micro-chemical systems for real-time monitoring.

Analyte detection remains an important issue for microchip devices. Most analyses on μ-TAS devices have been conducted using large detectors, such as laser-induced fluorescence (LIF), ultraviolet-visible (UV-Vis), and mass spectrometry [21-22]. Electrochemical detection is sensitive, compact, and easily integrated into a small device. Furthermore, electrochemical detectors can be microfabricated at a low cost relative to the above detectors [23-24]. However, two problems often encountered when using electrochemical detectors with complex sample matrices are 1) the presence of competing compounds that result in response peaks overlapping with the analyte of interest and 2) a limited detector lifetime due to surface fouling with some components in the sample. In addition, for effective detection at very low levels (sub ppb) of explosives in water, preconcentration of a large amount of sample is required. Microanalytical devices based on the lab-on-a-chip concept have shown some promising results for biomedical application. Microchips can handle only a very small amount of sample (nL to μL), and require that the sample is relatively clean. Environmental

samples, however, are relatively "dirty," and usually are relatively large in size (mL to L) in order to represent actual contamination level. Interfacing micro-world, CE-on-chip, and real world, "dirty" environmental samples is a challenging technical issue. Based on these considerations, there is a need for an automated sample processing before separation and detection with CE-on-chip.

2. OBJECTIVE

The objective of this project is to develop a prototype of a portable analytical system based on the on-line coupling of a miniaturized solid-phase extraction (SPE) device or on-chip integration of a micro-SPE with a microfabricated capillary electrophoresis/electrochemical detector for fast preconcentration/separation/detection of explosives and their degradation products in groundwater. Such a system has the potential to provide reliable, cost-effective characterization of groundwater contamination at U.S. Department of Defense (DoD) sites that are undergoing closure and remediation. Primary cost-saving benefits are based on minimizing routine sampling and analysis of groundwater samples, which will result in significantly lower cost associated with sampling, disposal of purgewater, and analysis of samples collected.

3. TECHNICAL APPROACH

CE-on-chip offers a fast separation capability. However, for the analysis of real samples, labor- and time-intensive manual pretreatment of the samples is necessary. Sample pretreatment and handling by an automated micro SPE system directly coupled to the CE-on-chip unit will eliminate the need for manual sample handling and thus will greatly increase the sample throughput and analysis sensitivity. One of the issues that must be addressed for successful coupling of the automatic SPE system and CE analysis is the small injection volume (nL range) required in the CE analysis. None of the commonly used injection techniques available in most commercial CE instruments allows a direct interfacing with a SPE system.

A SPE-CE system includes three parts: the SPE part, the CE part, and a specially designed interface. The SPE part provides efficient pre-treatment options and can be tailor-made for specific applications. The CE separation technique is characterized by high resolution, short analysis time, and multiple analyte capability. In this project, the interface between the SPE system and the CE-on-chip is achieved by using an interface flow channel with a volume-flow resistance that is much lower than that of the electrophoresis separation channel. An electrolyte stream carries a sample plug eluted from the mini-column toward the CE-on-chip. The dimension of the sample flow channel is designed to be much larger than that of the separation channel. While the sample plug passes along the separation channel, a small fraction of the sample is electrokinetically introduced into the separation channel. The application of electrokinetic injection for introducing a sample in the CE system elegantly solves the problem of small injection volumes required for the CE system. Moreover, it permits multiple injections into the separation channels while continuously applying the high voltage (HV) between the separation channels.

To develop a prototype of the automated analyzer for explosives, several specific activities have been performed. This includes the fabrication and evaluation of a microchip CE/electrochemical detector, interfacing the CE-microchip with the SPE system, and evaluation of the CE-microchip with mixtures of explosives.

4. BENEFITS/PAYOFF

If the technical goals of this project are achieved, there will be a significant payback to the DoD. The proof-of-principle results from this project will lead to the development of highly integrated, automated, and compact analyzers. The analyzers will be broadly applicable to many hazardous waste sites owned by DoD. Although the target analytes in this project are explosives, the analyzer can also be extended to other pollutants. It will be used for onsite/real-time analysis, eliminating the costs of sample packaging and shipping and reducing the waste produced. Since this technique combines the automated sample pretreatment with fast separation and detection, it will greatly increase the sample throughputs and reduce labor costs.

5. PROJECT ACCOMPLISHMENTS

5.1. Development of CE-Microchip with Electrochemical Detector

5.1.1. Design and Fabrication of CE/Microchip with Electrochemical Detector

A CE microsystem, based on the combination of microphotolithographically fabricated separation chips and thick-film electrochemical detector strips, has been developed. The microsystem consists of a screen-printed carbon-line electrode mounted perpendicular to the flow direction. Such coupling obviates the need for permanent attachment of the detector, allowing easy and fast replacement of the working electrode.

The thick-film (screen-printing) microfabrication technology is commonly used for large-scale production of extremely inexpensive and yet highly reproducible electrochemical sensors. The CE-microchip microsystem couples the microphotolithographically fabricated CE glass chips with the planar thick-film electrodes on ceramic wafers (Figure 1). Rather than fixing the detector permanently to the chip, the new design permits convenient and rapid replacement of the detector wafer. Such an easily exchangeable detector adds great versatility to the CE/electrochemistry operation, particularly in applications requiring a frequent electrode replacement. For example, it allows a convenient surface modification (in a separate/optimal electrochemical cell), a fast replacement of passivated electrodes (within 5 to 10 seconds), or the use of different electrode materials for comparison purposes. Despite its remarkably low cost, the thick-film detector displays an attractive analytical performance, with lower detection limits than those of analogous thin-film electrodes [25]. The microfabricated detector has no adverse effects on the CE separation and requires no decoupling mechanism and time-consuming alignment procedures, making it superior over the end-column detector of a conventional capillary CE [26].

The homemade high-voltage power supply had an adjustable voltage range between 0 and +4000 V. The glass microchannel separation chips were fabricated at Microlyne Inc. in Alberta, Canada, using standard microphotolithographic technology which includes wet-

chemical etching and thermal bonding techniques. The original waste reservoir had been cut off by the company, leaving the channel outlet at the highly flat end of the chip. The glass chip, shown in Figure 1, consisted of a glass plate (120 × 87 mm), with a 77-mm-long separation channel (between a deliberately blocked/unused reservoir and the channel outlet at the detection reservoir) and a 10-mm-long injection channel (between the sample reservoir and the buffer reservoir). The two channels crossed each other halfway between the sample and the buffer reservoir and 5 mm from the blocked reservoir to yield a separation channel with an effective length of 72 mm. The channels had a half-circle cross section, with a maximum depth of 20 μm and a width of 50 μm at the top. Pipet tips were inserted into the holes of the buffer and sample reservoirs (see Figure 1).

The glass chip was fixed in a laboratory-built Plexiglass holder (Figure 1), with silicone grease providing proper sealing. The holder contained reservoirs for the sample and buffer solutions, a detection reservoir, and an unused reservoir. A platinum wire was inserted into each reservoir and served as a contact for the high-voltage power supply. An additional platinum wire and an Ag/AgCl wire were also inserted into the detection reservoir, serving as the counter and reference electrodes, respectively, for the amperometric detection. The Ag/AgCl wire was prepared by electrochemical oxidization of a silver wire in 0.10 M hydrochloric acid. The detection reservoir has a special groove into which the screen-printed electrode strip fits exactly to allow reproducible and stable positioning, perpendicular to the flow direction. The screen-printed electrode strip was further held in place by a plastic screw pressing the strip against the channel outlet.

Amperometric detection was performed with an Electrochemical Analyzer 621 (CH Instruments) connected to a Pentium 166 MHz computer with 32 MB RAM.

Screen-Printed Electrodes. The screen-printed electrodes were printed with a semiautomatic printer (model TF 100, MPM, Franklin, MA). Printing was performed through patterned stencils (100-μm-thick, Specialty Photo-Etch, Inc., Texas) onto 100 × 100 × 0.64 mm alumina ceramic plates. Each plate consisted of 30 strips (33.3 × 10.0 × 0.64 mm) with each strip being defined by a laser pre/semi cut. The printing procedure consisted of the following steps. A carbon ink working-electrode layer (0.3 × 8.0 mm) was first printed on each of the strips of the ceramic plate and was cured at 100 °C for 30 minutes. Then, a silver ink (Ercon R-421[DRE-68]) contact layer (1.5 × 21.0 mm), partially overlapping the carbon layer, was printed and cured at 100 °C for 30 minutes. An insulating ink (Ercon R-488CI-G1 Insulator Green) layer was subsequently printed to cover the carbon-silver junction and to define the working-electrode area (0.30 × 2.5 mm) on one end and to expose the contact area on the other side. The strips were then cured at 100°C for 120 minutes. The final strip is shown schematically in Figure 1 (P-T). The cured layers of carbon, silver, and insulator had thicknesses of 10, 28, and 70 μm, respectively. Pieces of tape (Scotch, Magic Tape 810), with thicknesses of 60 μm each, were placed as shown in Figure 1(T) before using the strip. These tapes served as spacers controlling the distance between the strip and the channel outlet.

Electrophoresis Procedure. Before use, the channels were treated by rinsing them with a 1.0-M sodium hydroxide solution for 20 minutes, followed by deionized water for 1 min, 1.0% hydrochloric acid for 20 min, and finally with deionized water for 1 minute.

For the separation, the buffer and sample reservoirs (in the chip holder) and the corresponding pipet tips on the microchannel chip were filled with 250 μL of buffer and sample solutions, respectively. The chip was then placed in its holder with the pipet tips pointing downward into the reservoirs, and the detection reservoir was filled with buffer solution. Finally, the high-voltage power supply was connected to the reservoirs. To fill the injection channel between the separation channel and the sample reservoir with sample solution, +1500 V was applied for 30 seconds to the sample reservoir with the detection reservoir grounded and the buffer reservoir floating.

A buffer solution, containing 15 mM borate (pH 8.7) and 25 mM SDS, was used to separate the explosives. For this purpose, the injection was carried out by applying +1500 V to the sample reservoir for 3 seconds, with the detection reservoir grounded and the buffer reservoir floating. Before use, all buffer solutions were filtered through a 0.45-μm filter and sonicated for 20 minutes.

Separations were usually carried out by applying +1500 V to the buffer reservoir with the detection reservoir grounded and the sample reservoir floating. The solutions were not deaerated.

5.1.2. Characterization and Optimization

The CE microchip was integrated with an electrochemical detector. The working electrode was positioned at the outlet of the separation channel. Optimization of the integrated microchip CE/electrochemical detector has been investigated. Relevant parameters of separation and detection processes were optimized using a mixture containing 10 ppm of 1,3-dinitrobenzene (DNB) and 2,4,6-trinitrotoluene (TNT), which are relevant to the Strategic Environmental Research and Development Program (SERDP) mission.

Figure 2 shows the influence of the separation voltage upon the response of TNT and DNB (using a 60-μm spacing between the channel outlet and the electrode surface). The separation efficiency, the current signals, and the baseline slope are all affected by the separation voltage. Well-defined peaks for both DNB and TNT were observed at all separation potentials. As expected, increasing the voltage from +1000 V to +4000 V dramatically decreases the retention times for both analytes. The data of Figure 2, particularly the initial charging-current baseline rise, indicate incomplete isolation from the higher separation voltages. The separation voltage has a small effect upon the background noises. The peak-to-peak noise level increased from 40 to 55 pA upon changing the voltage between 1000 and 4000 V. The resolution of two peaks improves at the lower separation voltage. Therefore, the optimized separation voltage should compromise both the speed and the resolution.

The effect of the spacing between the working electrode and the outlet of the separation channel was also studied. Using the +1500 V separations, the amperometric signal decreases dramatically (~10-fold) upon increasing the spacing between 60 and 240 μm. The spacing also influences the separation efficiency between 60 and 240 μm, respectively. Such changes in the separation efficiency reflect the increased post capillary diffusional broadening at large channel-electrode distances. Longer channels can be used for improving the

separation efficiency. The peak broadening at the larger spacing is coupled to a slight increase in the retention times.

The present detector resembles the wall-jet design (with the channel/nozzle dimensions being much smaller than the detector wall). For conventional HPLC hydrodynamic wall-jet detectors, the jet issuing from the nozzle remains intact up to 10 mm. In view of the different velocity profile at the end-column CE detector (electro-osmotic flow "pushing" the analyte), it is not clear whether the liquid breaks up before impinging the detector. Compared with wall-jet-type detectors for conventional CE systems [26] that involed a fixed-disk electrode opposite to a circular capillary outlet, the present replaceable detector strip consists of a printed carbon-line electrode (300-μm width) positioned and centered across from a half-circle channel (of 50-μm diameter).

The electrochemical detection of nitroaromatic explosives is based on their low-potential reduction process. The effect of the detection potential on the amperometric detection of DNB and TNT was studied. Figure 3 depicts hydrodynamic voltammograms (HDV) for the detection of DNB (a) and TNT (b). The curves were taken in a stepwise fashion as the detection potential was changed incrementally at 100 mV, in connection to a 1500-V CE separation. As expected for the reduction of the nitro- moiety, both compounds display no response between 0 and -0.20 V. The response rises gradually between -0.20 and -0.50 V, and then levels off. The half-wave potentials are -0.38 V (DNB) and -0.33 V (TNT). Subsequent amperometric detection work employed a potential between -0.50 V and -0.70 V that offered the best signal-to-noise characteristics. A dramatic increase in the baseline current, its slope, and the corresponding noises were observed at more negative potentials.

The separation of neutral (nitroaromatic) compounds using MEKC requires an addition of a surfactant, SDS. The influence of the SDS concentration on the separation of DNB and TNT was assessed (Figure 4). Increasing the surfactant concentrations from 5 to 25 mM results in significantly higher resolution of the peaks; however, the sensitivity is compromised at the SDS concentration above 15 mM.

Figure 5 demonstrates the utility of the microchip CE/electrochemical system for analyzing a mixture of common nitroaromatic explosives. The microchip explosive analysis was performed with a borate buffer (15 mM, pH 8.7) containing 25 mM SDS. The electropherogram of Figure 5 indicates the rapid separation and detection of five explosive compounds (DNB, 2,4-DNT, 2,6-DNT, 4-NT, and TNT) with a total time of around 3 minutes at a separation potential of +1500 V. Despite the relatively negative potential (-0.70 V) essential for reducing the nitro moiety, the thick-film electrochemical detector displays a low background noise and sharp peaks for these 10- to 20-mg/L concentrations. Such favorable signal-to-noise characteristics result in low detection limits ranging from 0.6 mg/L (for DNB and TNT) to 2.0 mg/L (for 4-NT; S/N = 3). Such detectability and speed comply with various onsite security and environmental needs.

5.1.3.New Electrode Materials for CE/Electrochemical Detection of Explosives

A new electrode was fabricated using chemically vapor-deposited boron-doped diamond (BDD) film band (0.3 x 6.0 mm). The BDD electrode was integrated with a CE microchip and used for the end-column amperometric detection of explosives. The attractive

performance of the diamond-electrode microchip detector has been demonstrated by comparing it with a commonly used thick-film carbon detector (Figure 6, Figure 7). The diamond electrode offers enhanced sensitivity, lower noise levels, more stable baseline, and sharper peaks for the detection of nitroaromatic explosives. The favorable signal-to-background characteristics of the BDD-based CE detector are coupled with the greatly improved resistance to surface fouling and the greater isolation from high separation voltages. The enhanced stability is indicated from a relative standard deviation (RSD) of 0.8% for 60 repetitive measurements of 5 ppm 2,4,6-trinitrotoluene (vs. RSD of 10.8% at the thick-film carbon electrode). Highly linear response was obtained for the explosives 1,3-dinitrobenzene and 2,4-dinitrotoluene over the 200- to 1000-ppb range, with detection limits of 70 and 110 ppb, respectively (Figure 8). Factors influencing the performance of the BDD detector were assessed and optimized. The attractive properties of BDD make it a very promising material for electrochemical detection in CE microchip systems.

5.1.4. CE-Microchip Based on Polymer Substrate for CE Detection of Explosives

One important issue for the microchip device is how to reduce the costs of the microchip and the microfabrication. Glass chip is relatively time-consuming for microfabrication. Hence, we fabricated the polymer microchip to replace the glass chip. CE separation with electrochemical detection using polymer microchip has been tested for the determination of nitroaromatic explosives. Three compounds, including TNT, 1,3,5 trinitrobenzene (TNB), and 4-amino-2,6-DNT, were successfully separated within 150 seconds (Figure 9). Reproducibility of the signals for 14 repeated injections is 4.7, 3.8, and 4.4 for TNB, TNT, and 4-amino-2,6-DNT, respectively. These results indicate that polymer CE microchip is promising for separation and detection of the explosives. Since the polymer CE microchips are relatively inexpensive, these devices can be used in field monitoring and can be quickly replaced at low cost.

5.2. Microscale Solid Phase Extraction and Nonaqueous Microchip Detection of Explosives

5.2.1. Portable, Microscale Solid-Phase Extraction System for Explosives

Surface and groundwater emanating from former military munitions sites are particular areas of environmental concern. The EPA has determined, for example, that TNT is toxic at levels above 2 μg/L [27].

The CE microchip developed in our laboratories benefits from its portable size, rapid separation times (in seconds), and extremely small sample-size requirements (in nL to μL). The primary disadvantage of the CE microchip for explosives analysis is that, despite various efforts to improve the sensitivity, the detection limits for these devices (e.g., 70 μg/L for TNT) continues to be insufficient for the stringent detection requirements described above.

SPE techniques have been successfully applied to the preconcentration of aromatic explosives, resulting in two to three orders of magnitude improvements in detection limits in addition to the elimination of troublesome sample matrices. While the current EPA Method 8330 for analysis of explosives relies on the application of a salting out solvent-extraction

method that is time consuming and labor intensive, efforts have been focused on better alternatives. Jenkins et al. [28] have directly compared and demonstrated the utility of off-line SPE with a poly(styrene-divinylbenzene)-based membrane and a divinylbenzene-vinylpyrrolidone copolymer resin-based cartridge (Porapak R). Bouvier and Oehrle [29] examined offline SPE of aqueous samples using cartridges packed with a specially cleaned resin (Porapak RDX), and they subsequently analyzed the acetonitrile eluate by HPLC. This method, although has excellent detection limits (e.g., 0.1 μg/L for TNT), was time consuming, requiring 70 minutes per sample for SPE of a 500 mL sample, and wasteful, using only 40 μL of the 5-mL acetonitrile extract for analysis.

The primary objective of this task was to develop a field portable, SPE system for future application in the sensitive detection of explosives in remote locations, with particular emphasis being placed on its applicability to CE microchip-based devices. The HPLC pump-based SPE systems described above were not considered viable options due to their stringent power requirements. The SPE apparatus discussed here is reasonably lightweight and compact, consisting of two 12-V DC, 48-W syringe drives, a set of four solenoid switching valves, and an SPE microcolumn. Secondary objectives pursued in the optimization of this SPE unit were the maximization of sensitivity for explosives analysis while miniaturizing the eluate volume to more closely meet the volume requirements of CE microchips (<2 μL). The separation and detection of seven explosives and explosive derivatives are performed using EPA Method 8330 (HPLC) to permit direct comparison of this approach to previously described efforts.

This study was designed to examine whether improvements in speed and sensitivity could be realized when using 1) sample volumes and flow rates that exceeded the breakthrough threshold of the SPE microcolumn and 2) very small eluate volumes that had inherently low recovery percentages. By using microscale columns for SPE, the speed of analysis, the extent of waste generation, and the size of the eluent plug are all minimized.

To meet the requirements of a semi-automated, field-portable SPE device for explosives, the system shown in Figure 10 was designed. The apparatus contains two 12-DC (48-W) syringe drives, one that is devoted to pumping the sample (Pump A) and the other to pumping the acetonitrile eluate (Pump B). Two Kloehn Ltd. 50300 syringe pumps were used to aspirate and dispense the various solutions and reagents during the SPE procedure (see Figure 10). The first pump, Pump A, was equipped with a 5-port discharge rotary valve and a 5-mL glass syringe, both available from Kloehn. The second pump, Pump B, was equipped with a 4-port discharge rotary valve and a 2.5-mL glass syringe (Kloehn). Two 3-port solenoid valves and two 2-port solenoid valves (Bio-Chem Valve) were also used to control the movement of reagents. The two 2-port solenoid valves were used in place of a single 3-port valve because of their capability for withstanding higher pressures (1400 to 2000 kPa).

Microscale SPE columns were created by packing the appropriate SPE material (Lichrolut or Porapak) into 1/16-in. OD, 750-micron ID Teflon tubing. Nylon mesh (Cole Parmer) placed over the end of the tubing and held in place with a ferrule was effective in containing the SPE material during all experiments. The SPE microcolumn was inserted into the SPE apparatus as shown in Figure10. Figure 11 shows the photo of the portable microscale SPE system.

A Hewlett-Packard series 1100 HPLC was used to separate the extracted compounds and integrate the resolved peaks. Most of the recommended chromatographic conditions of EPA Method 8330 were followed. A Supelco LC-18 column was used with dimensions 25 cm × 4.6 mm and 5-μL packing diameter. The mobile phase was 50/50 (v/v) methanol/water. The differences between EPA method 8330 and the method used in this study were the injection loop volume and the UV absorbance wavelength. Method 8330 recommends a 100-μL injection loop, but we used a 2-μL loop to investigate significantly smaller eluent volumes.

The SPE procedure consists of four basic phases, each of which is controlled via a computer interface supplied by Kloehn. The first phase consists of pumping a water sample across the SPE microcolumn and out to waste. The total volume and flow rate of water sample can be carefully controlled by this apparatus. In the second step, the microcolumn and its associated tubing are washed and dried before the acetonitrile elution step. Distilled water (2.5 mL at 5.0 mL/min) is first pumped through the microcolumn (Pump A) to waste to eliminate the presence of any salts in the line that may be detrimental to the HPLC analysis. A 1% acetic acid wash (5.0 mL at 30 mL/min) is pumped directly to waste by the 5-port discharge rotary valve to protect the Teflon plunger of the syringe from any abrasive salt precipitates. Finally, air is used to dry the Teflon tubing and microcolumn in preparation for the acetonitrile elution step. Trapped water drastically reduces the extraction efficiency of these explosives. Air from Pump A (5.0 mL) is pushed through the microcolumn, followed by 2 cycles of air from Pump B (5.0 mL). An Upchurch Scientific "T" connector provides the common junction between Pump A, Pump B, and the SPE microcolumn. The third step is the elution of any adsorbed explosives in acetonitrile. Acetonitrile was slowly pumped by Pump B (~300 μL/min) and collected in a small glass vial (typically, 10 μL). Lastly, the column is washed with 1.5 mL of acetonitrile to waste and dried with 2.5 mL of air (Pump B). The column is not completely dry as a result of this final air push. After this final step, the column is immediately ready for the next extraction sequence.

Figure 12 displays a typical chromatogram obtained in this study following the microscale solid-phase extraction of explosives from seawater into acetonitrile. In all cases, the seawater was spiked to contain seven explosives or explosive derivatives, each of which was completely resolved and easily quantitated. The explosive components cover a wide range of polarity, from RDX to *m*-NT. In the absence of SPE, 1,3,5-TNB is the most sensitively monitored analyte, at approximately 100 μg/L, while the nitrotoluene standards had detection limits from 1 to 2 mg/L. In general, an explosives-fortified seawater solution spiked to contain 5 μg/L of each component could be easily quantitated by HPLC following microcolumn solid-phase extraction.

The optimized conditions derived for microcolumn SPE can be summarized as follows: Lichrolut packing material, 1.0-cm column length, 300-μL/min eluent (acetonitrile) flow rate, 5 μL eluent (acetonitrile) volume collected. Table 1 summarizes the concentration enhancements observed for all seven explosives when using the optimized conditions and while minimizing the total time allotted per sample. The pumping time was limited to 7 minutes for 20-mL total sample volume. The concentration enhancement factors can be seen to range from 200 to as high as 900 times.

Table 1. Concentration Enhancements for Well Water Obtained for Seven Explosives or Explosive Derivatives Using a Lichrolut 1.0-cm Column Length, a Well-Water Pumping Rate of 3.0 mL/min, 20-mL Total Sample Volume, and 5-μL Collected Eluent Volume

Explosives	Actual Conc. (ppb, μg/L)	Concentration Enhancement Factor	Detection Limit (ppb)
RDX	1.00	238	0.23
1,3,5-TNB	0.75	180	0.11
TNT	0.90	411	0.18
2,4-DNT	0.90	425	0.19
o-NT	1.50	785	0.74
p-NT	1.50	900	1.08
m-NT	1.50	792	0.91

5.2.2. CE- Separation of Nonionic Compounds in Organic-Aqueous Solutions

The CE microchip separation of explosive compounds in nonaqueous electrolyte (acetonitrile/methonal 87.5/12.5 [v/v], 2.5-mM NaOH, 1-mM SDS) has been demonstrated by our collaborator at the Naval Research Laboratory [30]. To demonstrate the compatibility of nonaqueous CE microchip separation with SPE, trace levels of explosives dissolved in seawater were preconcentrated via *ex situ* SPE and analyzed on the CE microchip. The benefit of using acetonitrile is due to its amenability to both CE and SPE. By using nonaqueous electrolyte in the presence of low concentrations of surfactants, selective detection and separation of three trinitroaromatic explosives from a mixture of 13 explosives or explosive derivatives in less than 20 seconds via microchip CE has been achieved. The compatibility of this system to SPE techniques was demonstrated with trinitroaromatic explosives being detected at levels as low as 0.19 to 0.34 ppb in seawater. The detector used for CE microchip is a UV-Vis spectrophotometer (Hitachi U-3000), which is still bench-scale and is not easy to integrate into a hand-held device as an electrochemical detector. In addition, the spectrophotometric detection of explosives needs a derivation reaction in nonaqueous phase, which causes technical difficulty for the on-line coupling of a SPE/nonaqueous microchip CE with spectrophotometric detection.

On-line integration of SPE with microchip CE with organic-aqueous electrolytes is a more practical approach. MEKC has been demonstrated in our laboratories as a very effective technique for separation of explosives in microchip CE. To be compatible with on-line SPE, a microchip CE needs to be operated at organic-aqueous electrolytes. It is generally acknowledged that micelles are not stable in mixtures of water and organic solvents containing a high percentage of the latter [31]. Despite this, the separation of neutral analytes has been reported in SDS solutions containing up to 50% acetonitrile [32]. Seoaniak used bile salt micelles for MEKC [33]. These were more stable than conventional SDS micelles in the presence of higher percentages of organic solvents.

Another approach for the separation of neutral compounds in CE with organic-aqueous electrolytes is to add an appropriate ionic surfactant or other large ionic molecule to the electrolyte [34]. When added to the background electrolyte, the ionic surfactant or large ionic molecule can interact with neutral analytes to form an ionic association complex even in the presence of a relatively high concentration of an organic solvent. The type and concentration of the organic solvent is a major variable in adjusting conditions for the best separation of neutral analytes by CE in organic-aqueous solution. For example,neutral organic compounds have been separated in acetonitrile-water by use of a large quaternary ammonium salt (Q^+), such as tetrahexylammonium bromide, as the ionic additive [35]. The separation of 16 nonionic aromatic organic compounds have been achieved using an electrolyte of 50 mM tetraheptylammonium bromide, 8 mM borate, 42% v/v acetonitrile at pH 9.4. Other examples include the use of dioctyl sulfosuccinate as the ionic additive [36].

5.2.3. Electrochemical Detection of Explosives in Organic-Aqueous Solution

To verify the compatibility of organic-aqueous electrolytes for electrochemical detection of explosives, voltammetric behaviors of TNT in acetonitrile and acetonitrile/water solution were studied with cyclic voltammetry (CV). Figure 13A shows the cyclic voltammograms of TNT in 0.01 M tetramethylammonium perchlorate in acetonitrile. Two reduction peaks of TNT appear at -0.85V and -1.0 V, respectively. The peak currents increase with increasing concentration of TNT. The effect of water levels (aqueous borate buffer) on voltammetric behaviors of TNT in acetonitrile was shown in Figure 13B. It can be seen that 30 to 50% buffered water in acetonitrile gives reasonable voltammetric behavior. These results indicated that the electrochemical detection of TNT in organic-aqueous solutions is feasible and compatible with SPE-CE in organic-aqueous solutions.

5.2.4. On-Line Coupling of SPE with Microchip CE

Despite the strong impetus in the development of microfluidic chip devices, “world-to-chip” interfacing still appears to be a weak point and much work remains to be done if such devices are to be used routinely under “real-life” conditions, particularly when dealing with CE separation of complicated environmental samples.

CE-on-chip offers a fast separation capability. However, for analysis of real samples, labor- and time-intensive manual sample pretreatment is necessary. Sample pretreatment and handling by the SPE system directly coupled to the CE-on-chip unit will eliminate the need for manual sample handling and thus will greatly increase the sample throughput and analysis sensitivity. One of the issues that must be addressed for successful coupling of the SPE and microchip CE is the small injection volume (nL range) required in the microchip CE. To date, the development of such an interface has not been reported and would be of substantial interest.

A SPE/microchip-CE system includes three parts: the SPE part, the CE part, and a specially designed interface. The SPE part provides automated sample preconcentration. The CE separation technique is characterized by high resolution, short analysis time, and multiple analyte capability.

A prototype device that is designed to interface the SPE system and microchip CE is shown in Figure14. The detector used for this initial testing is an optical detector (UV-Vis). The on-line introduction of multiple explosive samples was separated by air plugs. The dimension of sample flow channel was designed to be much larger than that of the separation channel. While the sample plug passes along the separation channel, a small fraction of the sample is electrokinetically introduced into the separation channel. During the entire sequence, an HV is applied between the Pt anode and Pt cathode positioned as shown in Figure 14. The introduction of samples into the CE system by electrokinetic injection elegantly solves the problem with the small injection volumes required for the CE. Moreover, it permits multiple injections into the separation channel while continuously applying the HV between the two Pt electrodes. The initial testing indicates that on-line sample introduction provides excellent reproducibility between plug to plug (as shown in Figure 15). The results are promising for further development of a microanalytical system based on on-line coupling of SPE/microchip CE for monitoring explosives.

5.2.5. On-Chip SPE/Microchip CE

Another approach for the integrating SPE with microchip-CE is *in situ*/on-chip micro-SPE. Direct incorporation of SPE methods onto a microchip has been demonstrated to be viable by Harrison and co-workers [37]. They used two weirs within a sample channel for trapping C18 coated silica beads on a microchip for subsequent extraction studies of dye BODIPY from water into acetonitrile. In this work, we have used two methods for packing micro-SPE column inside a separation channel (Figure 16). The first method is using the hydrogel positioned C-18 coated silica beads. A porous hydrogel plug, poly(methacrylate), was created by the UV crosslinking approach inside the microchannel (Figure 17). The second method for *in situ*/on-chip SPE is using C-18 coated paramagnetic beads (Figure 18). The paramagnetic beads were held inside the microchannel via a magnet. All transport of the beads (packing and removal) was accomplished using electro-osmotic forces. The proof-of-principle of the two *in situ*/on-chip micro-SPE methods has been successfully demonstrated for preconcentration and elution of dye.

6. CONCLUSION

We have successfully fabricated CE-microchips on both glass and polymer substrates and integrated the CE-microchips with an electrochemical detector. We have systematically optimized the separation and detection processes and demonstrated the analytical performance for fast separation and detection of explosive mixtures (<2 minutes). Electrochemical detectors have already proven to be well suited for microchip CE systems. Our microchip systems are particularly attractive because of their high sensitivity and selectivity, inherent miniaturization, and portability, and low cost.

We have also developed and optimized an automated microscale SPE system for preconcentration of explosives from well water into acetonitrile. The minimized eluted volume (2 to 5 μL) is compatible with the microchip CE. Large concentration enhancements (200 to 100 times) have been realized. Characteristics, such as portability, good detection

accuracy, high concentration enhancement factors, low eluate volume requirements, and fast analysis, make the microscale system potentially suitable for field applications.

One prototype interface device was designed and fabricated for an automated on-line SPE/microchip-CE system. The initial testing demonstrates that the on-line sample introduction provides excellent reproducibility between plug to plug. The results are promising for further development of a microanalytical system based on the on-line coupling of the SPE/microchip CE for monitoring explosives.

The preliminary study of *in situ*/on-chip SPE has also begun. Two methods for packing a micro-SPE column inside a separation channels have been developed. The first method used hydrogel-positioned C-18 coated silica beads. The second method used C-18 coated paramagnetic beads. The proof-of-principle of two *in situ*/on-chip micro-SPE methods has been successfully demonstrated.

In summary, the proof-of-principle results from this SERDP Exploratory Development (SEED) project indicate that the microanalytical system based on the integration of an on-line or *in situ*/on-chip SPE with CE-microchip/ECD has a great potential to meet the need for onsite groundwater monitoring of explosives. It is therefore worth further investment to fully develop and deploy this technology.

Acronyms and Abbreviations

BDD	Boron-Doped Diamond
CE	Capillary Electrophoresis
CEC	Capillary Electrochromatography
CV	Cyclic Voltammetry
DNB	1,3-Dinitrobenzene
DNT	2,6 Dinitrotoluene
DOD	U.S. Department of Defense
ECD	Electrochemical Detector
EPA	U. S. Environmental Protection Agency
ERDC	Engineering Research and Development Center
ESTCP	Environmental Security Technology Certification Program
HDV	Hydrodynamic Voltammogram
HPLC	high-performance liquid chromatography
HV	High Voltage
Hz	hertz
LIF	Laser-Induced Fluorescence

MEKC	Micellar Electrokinetic Chromatography
NMSU	New Mexico State University
NRL	Naval Research Laboratory
NT	Nitrotoluene
PMMA	Polymethylmethacrylate
PNNL	Pacific Northwest National Laboratory
ppb	parts per billion
ppm	parts per million
RDX	Royal Demolition Explosive (Hexahydro-1,3,5-trinitro-1,3,5-triazine)
RSD	Relative Standard Deviation
SDS	sodium dodecyl sulfate
SEED	SERDP Exploratory Development
SERDP	Strategic Environmental Research and Development Program
SPE	Solid Phase Extraction
TAS	Total Analytical Systems
TNB	1,3,5 Trinitrobenzene
TNT	2,4,6 Trinitrotoluene
UV-Vis	ultraviolet-visible
V	volts

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Appendix A: Figures

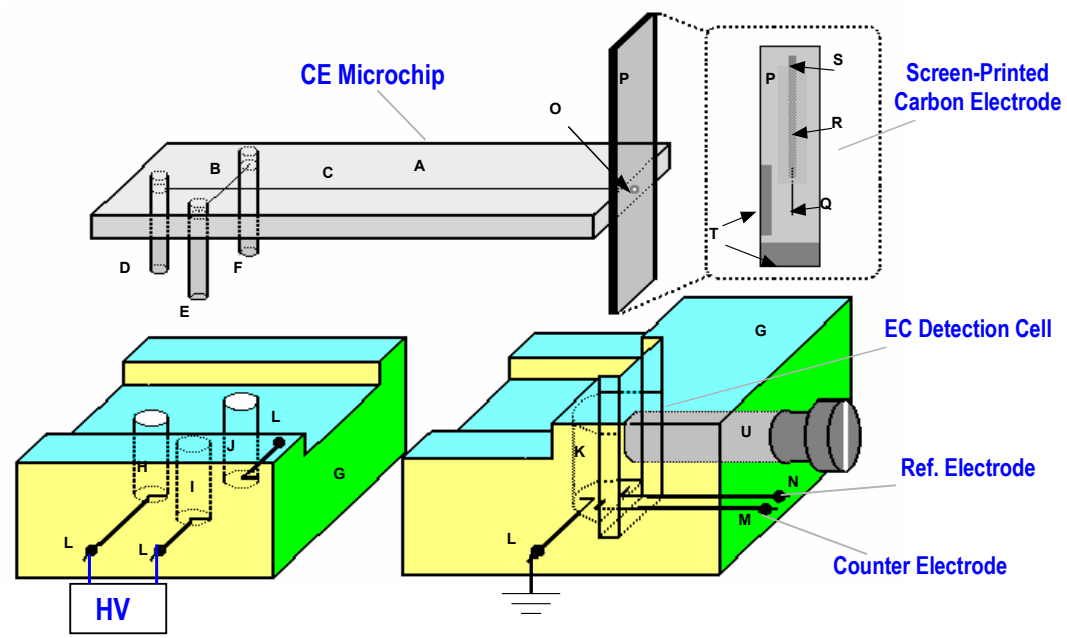

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Figure 1. Capillary Electrophoretic System with Electrochemical Detection. (A) Glass microchip, (B) injection channel, (C) separation channel, (D) pipet tip for buffer reservoir, (E) pipet tip for sample reservoir, (F) pipet tip for reservoir not used, (G) Plexiglass body, (H) buffer reservoir, (I) sample reservoir, (J) blocked (unused) reservoir, (K) detection reservoir, (L) high-voltage power electrodes, (M) counter electrode, (N) reference electrode, (O) channel outlet, (P) screen-printed working-electrode strip, (Q) screen-printed working electrode, (R) insulator, (S) silver ink contact, (T) tape (spacer), (U) plastic screw. For clarity, the chip, its holder, and the screen-printed electrode strip are separated, and dimensions are not in scale.

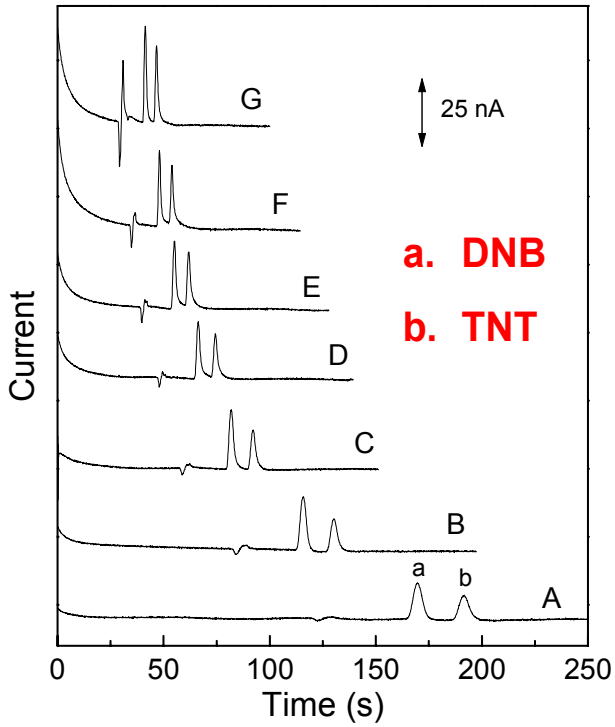

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Figure 2. Influence of the Separation Voltage on the CE Separation of Explosive Mixture of DNB(a) and TNT (b). Separation performed using (A) +1000 V, (B) +1500 V, (C) +2000 V, (D) +2500 V, (E) +3000, (F) +3500, and (F) +4000. Conditions: 15 mM borate buffer (pH 9.2, 15 mM SDS); injection voltage: +1 kV; injection time, 2 seconds; detection potential, –0.70 V (B) (vs. Ag/AgCl).

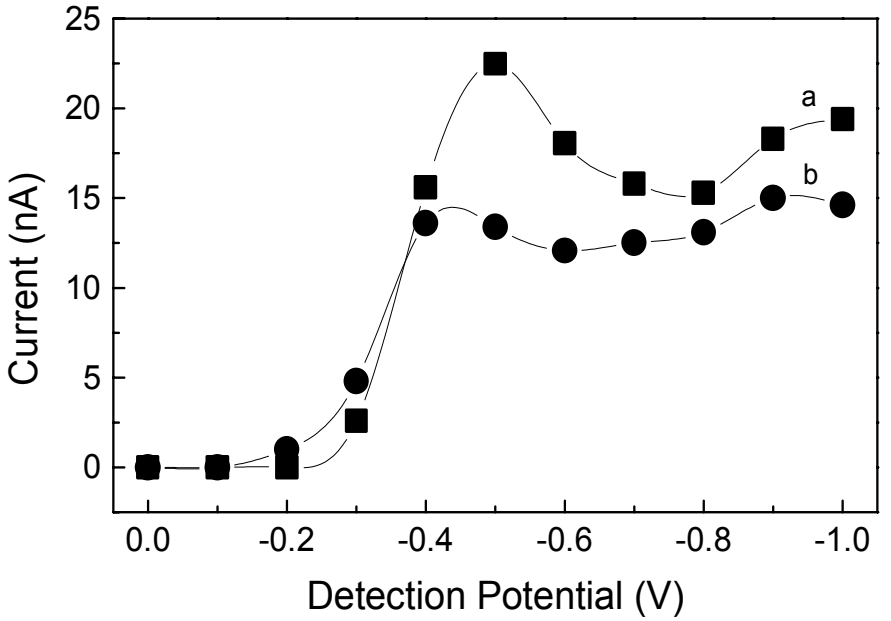

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Figure 3. Hydrodynamic Voltammograms for DNB (a) and TNT (b). Separation Potential, +1500 V. Other conditions, as in Figure 2.

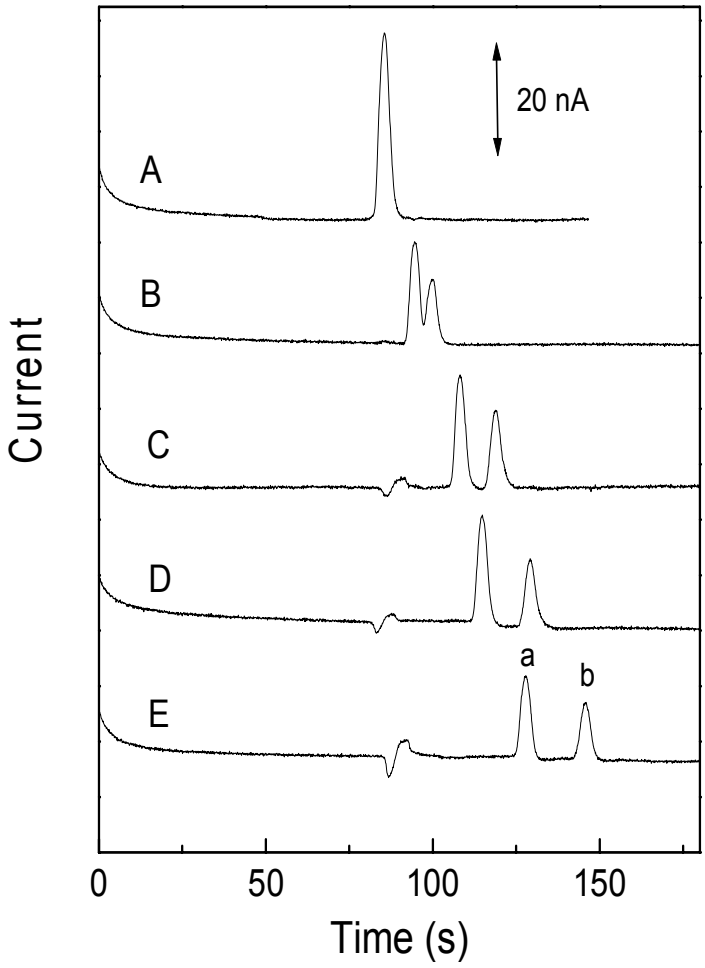

Figure 4. Effect of SDS Concentration on the Separation of DNB (a) and TNT (b). SDS concentration: (A) 5 mM, (B), 10 mM, (C) 15 mM, (D) 20 mM, and (E) 25 mM. Other conditions as Figure 3.

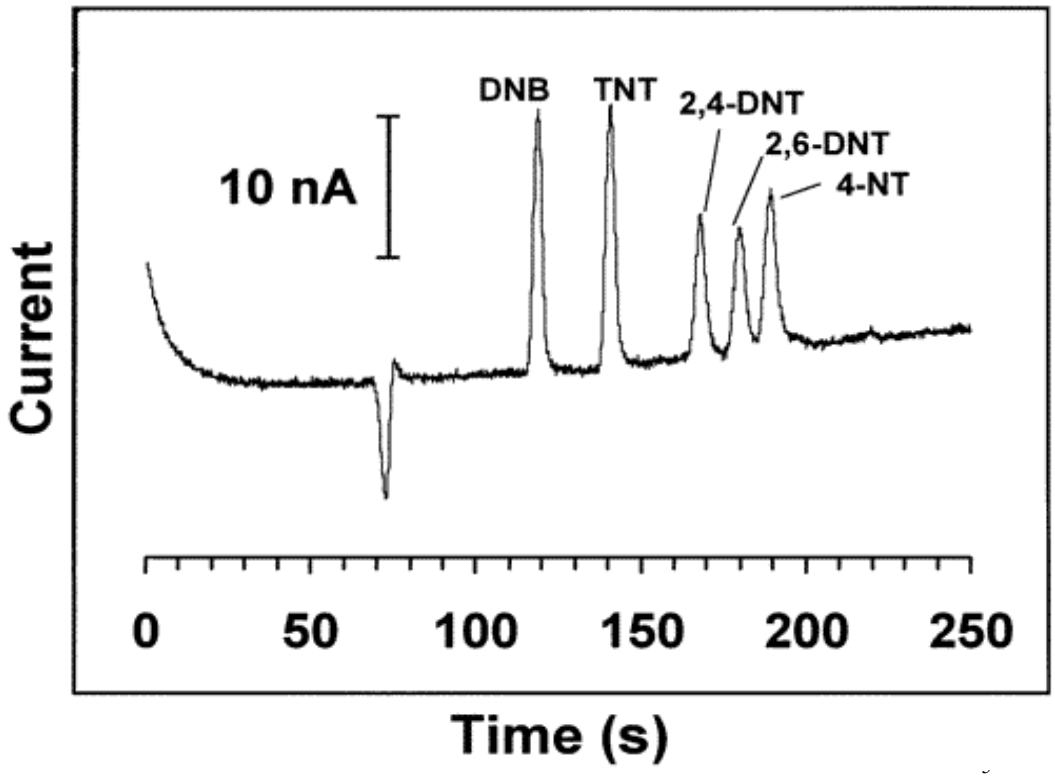

Figure 5. Electropherogram for 10 ppm DNB; 2,4-DNT; 2,6-DNT; and TNT and 20 ppm 4-NT. Conditions: electrophoresis medium, borate buffer (15 mM, pH 8.7) containing 25 mM SDS; separation at +1500 V; sample injection at +1500 V for 3 seconds; detection at -0.70 V using a 60 μm spacing between the detector strip and the channel outlet.

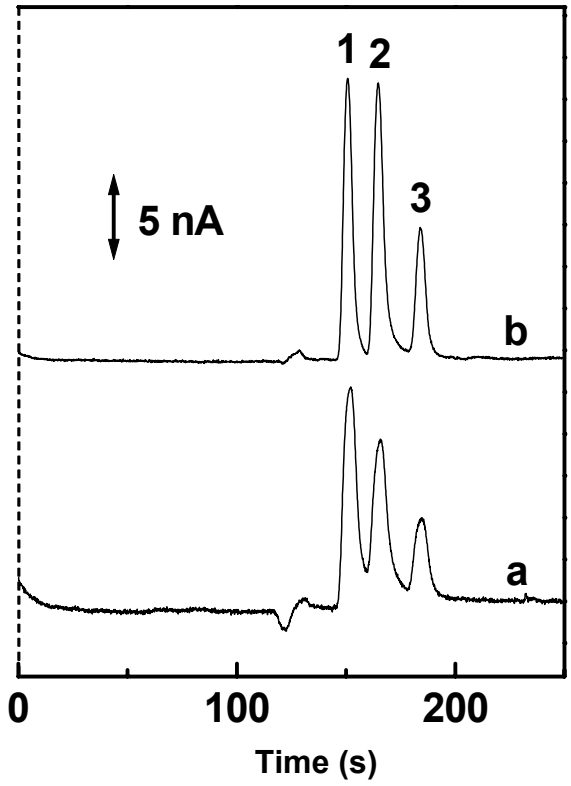

Figure 6. Use of Diamond electrode (b) vs. Screen-Printed Carbon Electrode (a) for Microchip CE Detection of Explosives. Conditions: 15 ppm DNB (1), TNT (2), and DNT (3); 15 mM borate buffer (pH 9.2, 15 mM SDS; separation voltage, + 1 kV; injection voltage, + 1kV; injection time, 2 seconds; detection potential, -0.70 V.

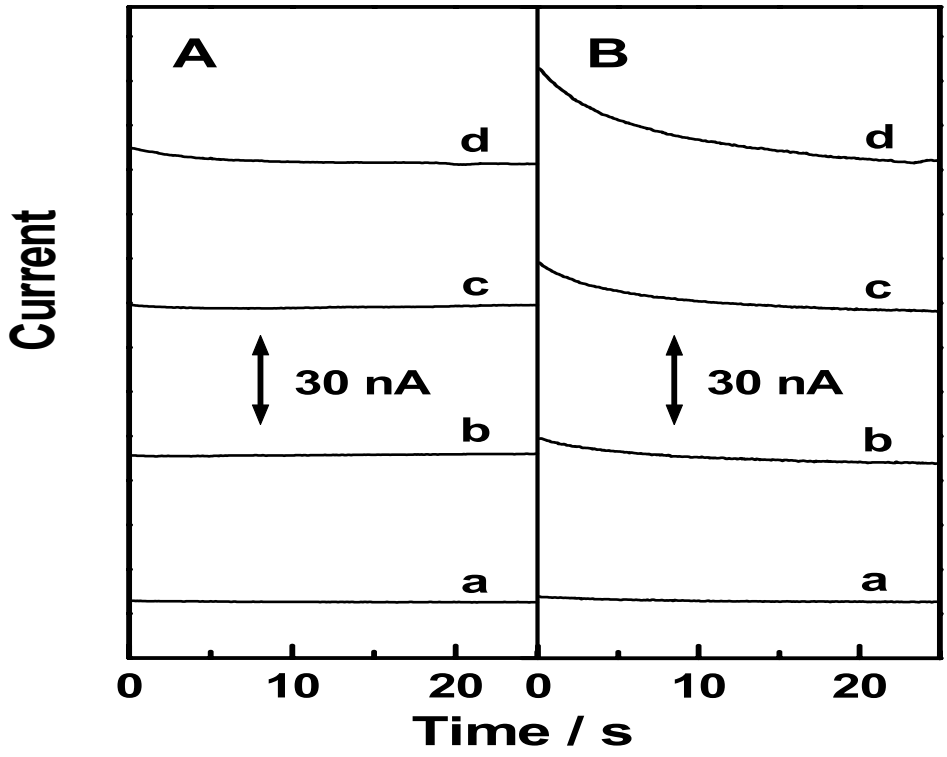

Figure 7. Baseline Stability of Diamond Electrode (A) vs. Screen-Printed Carbon Electrode (B) at Different Separation Voltages. Separation voltages: (a) + 1 kV; (b) + 2 kV; (c) + 3 kV; (d) +4 kV.

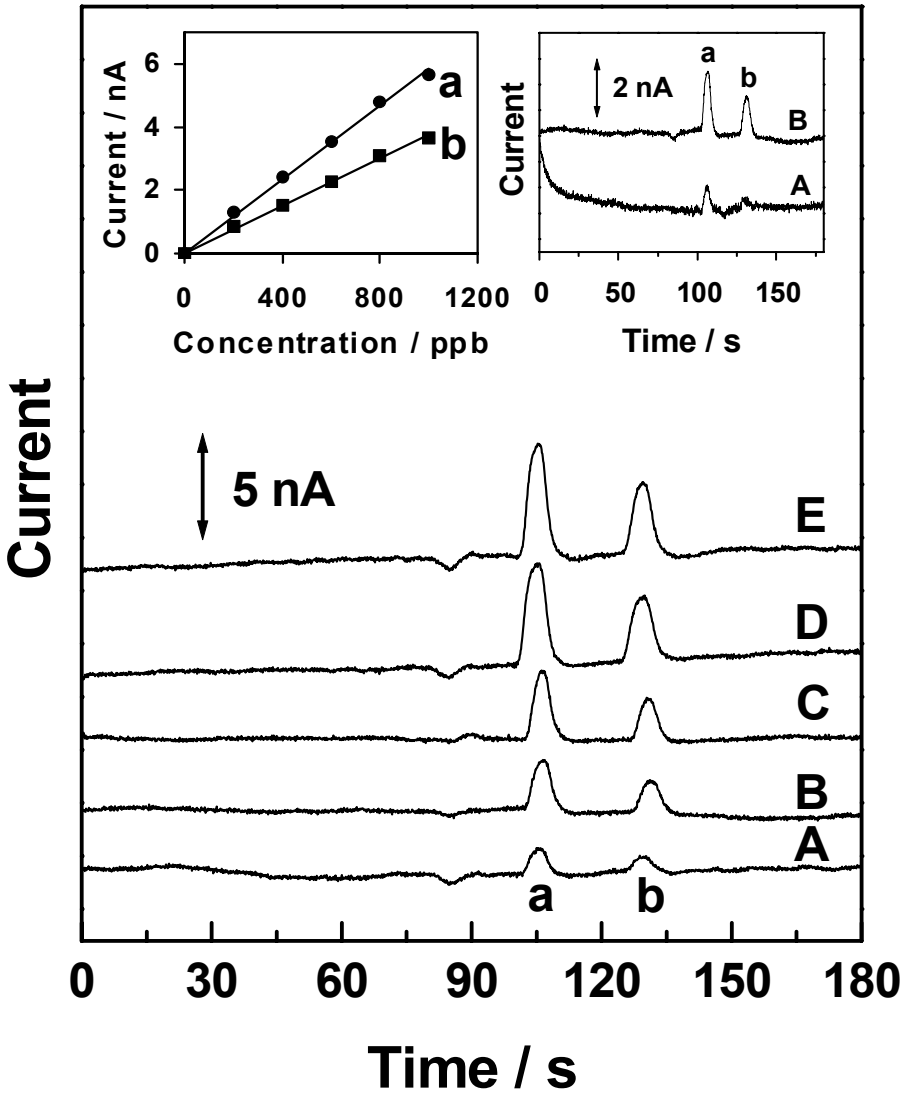

Figure 8. Calibration Data for Mixtures Containing Increasing Levels of DNB (a) and DNT (b) in 200 ppb increments (A–E) at diamond electrode.

Insets: resulting calibration plots and electropherograms for a mixture containing 400 ppb (a) DNB and (b) DNT at the screen-printed carbon (A) and diamond (B) detector electrodes. Sep. voltage, +1500V. Other Conditions as Figure 6.

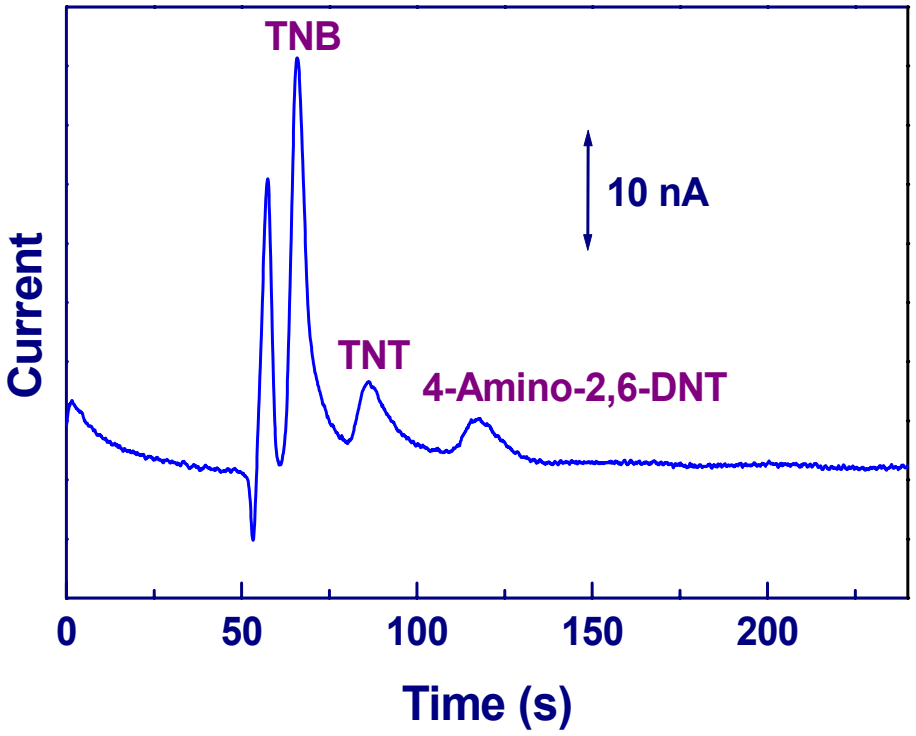

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Figure 9. Polymer (PMMA) Microchip for CE-Separation of Explosives. Conditions as Figure 6. Working electrode: screen-printed carbon electrode.

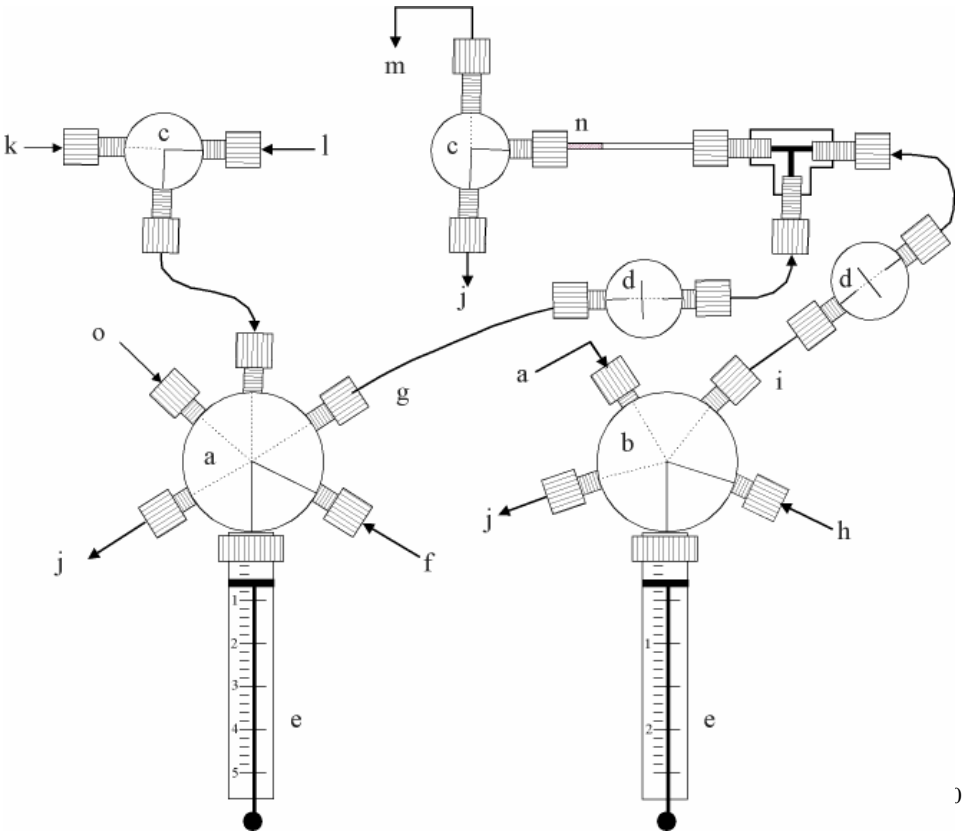

Figure 10. Diagram of the Semi-Automated Solid-Phase Extraction System: a) five-position rotary valve; b) four-position rotary valve; c) three-port solenoid valve; d) two-port solenoid valve; e) syringe; f) water sample in; g) water sample, distilled water or air out; h) acetonitrile in; I) acetonitrile or air out; j) waste; k) distilled water in; l) air in ; m) concentrated acetonitrile sample; n) microcolumn SPE; o) 1% acetic acid wash solution in.

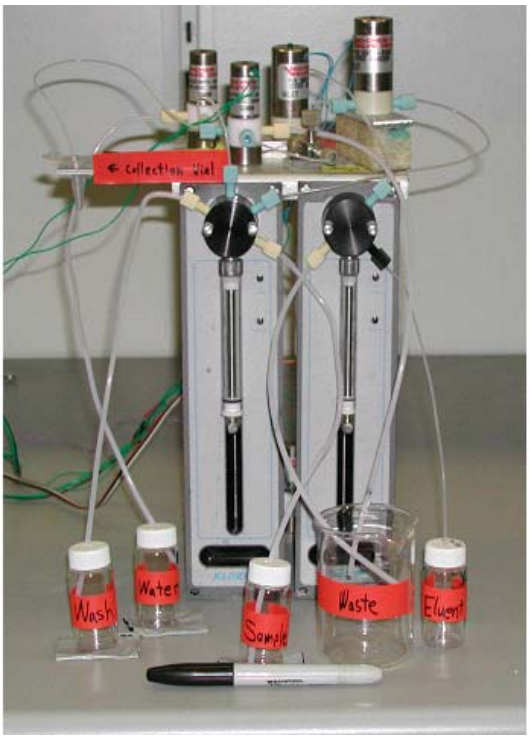

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Figure 11. Photo of the Portable Microscale Solid-Phase Extraction System

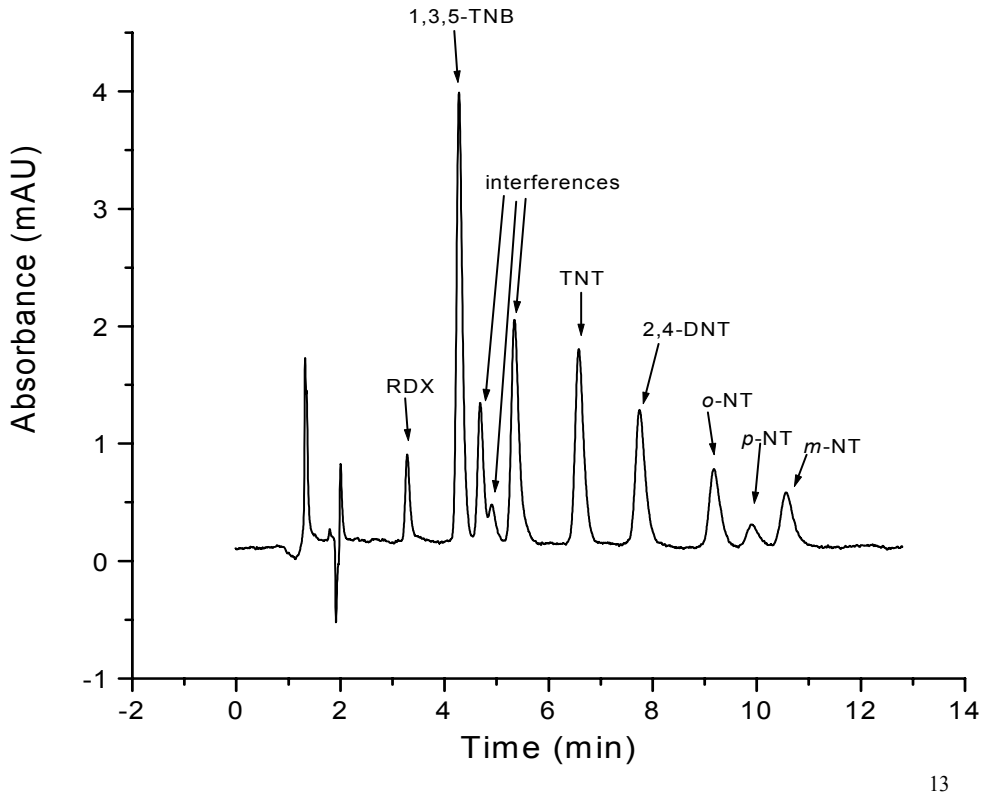


Figure 12. Chromatogram of Seven Explosives or Explosive Derivatives Obtained via HPLC with UV Absorbance Detection. The compounds were extracted from a 5 µg/L fortified seawater solution, using a 1.0 cm Lichrolut column, a seawater pumping rate of 5.0 mL/min, 25.0 mL total seawater volume, and 8.5 µL collected eluent volume.

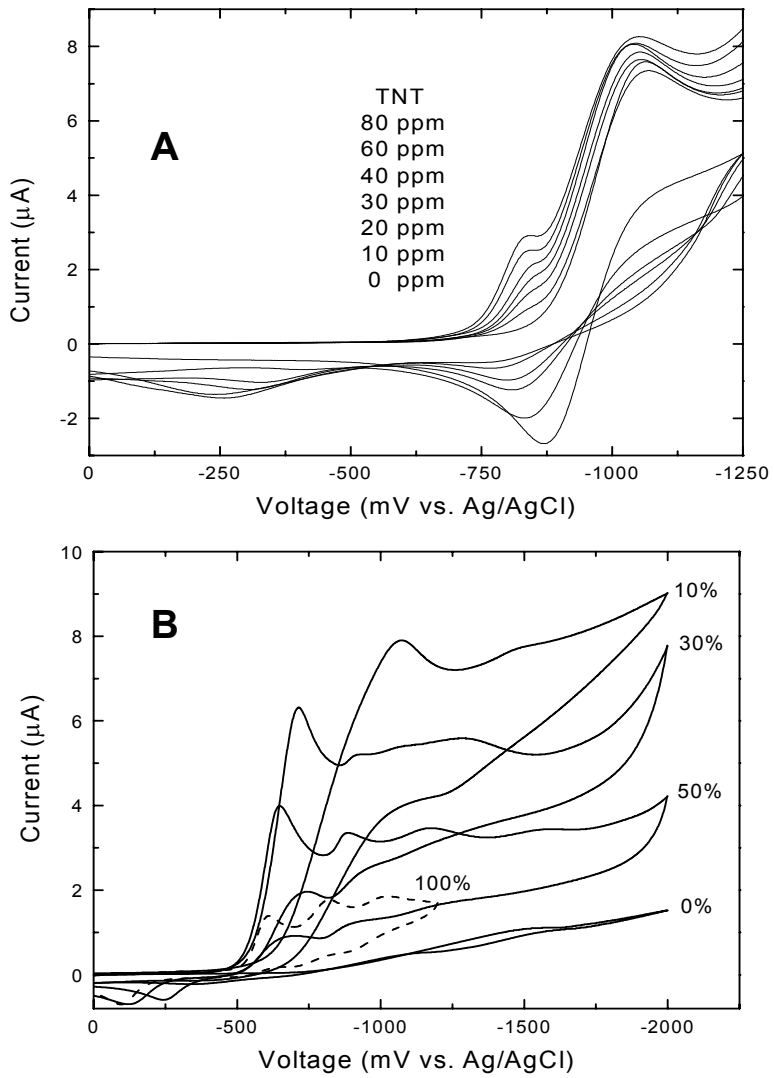


Figure 13. (A) Cyclic Voltammograms of TNT in 0.01 M Tetramethyl-Ammonium Perchlorate in Acetonitrile.

(B) Cyclic Voltammograms of 30 ppm TNT in Acetonitrile with Increasing Levels of Aqueous Borate Buffer.

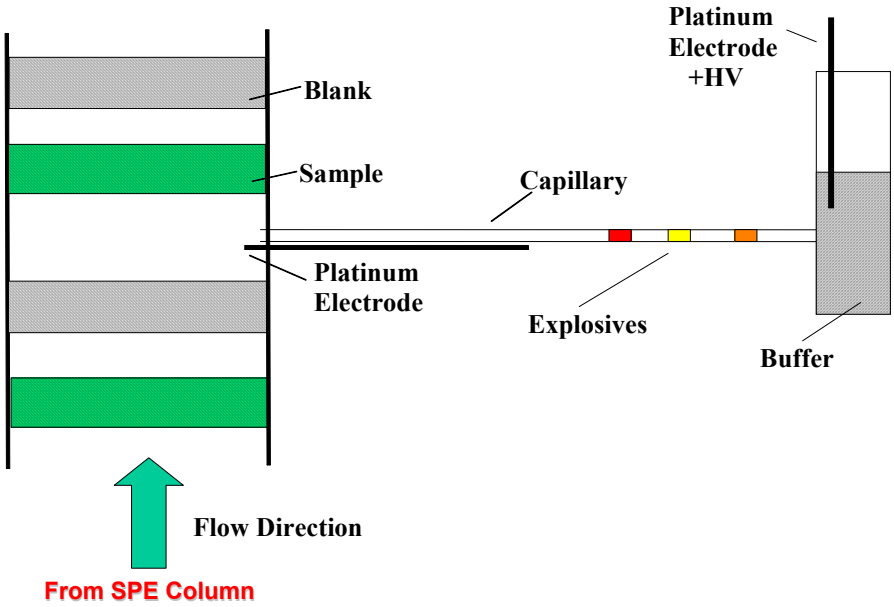


Figure 14. Interface with Flow-through Sampling Channel for On-Line Coupling of SPE with CE

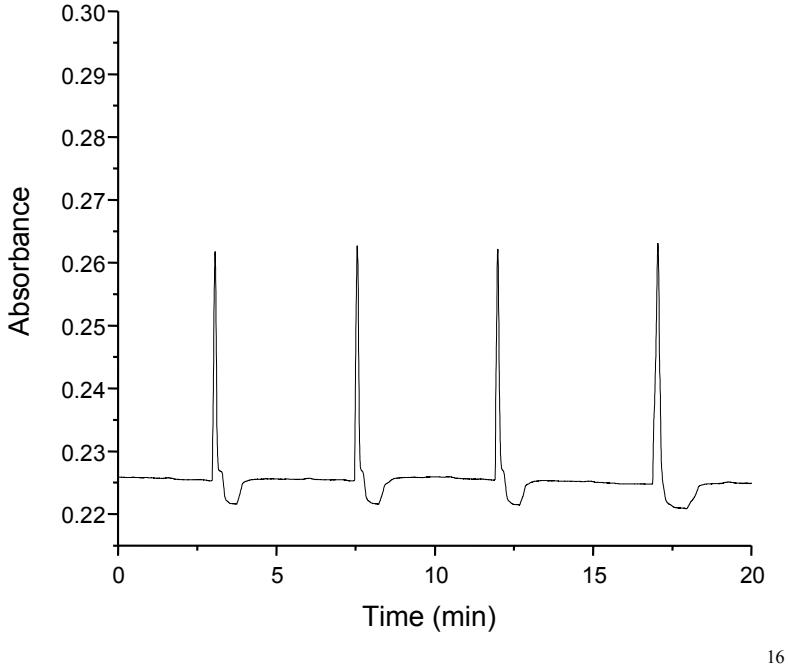


Figure 15. Reproducibility of Repeat Injection with Flow-Through Sampling

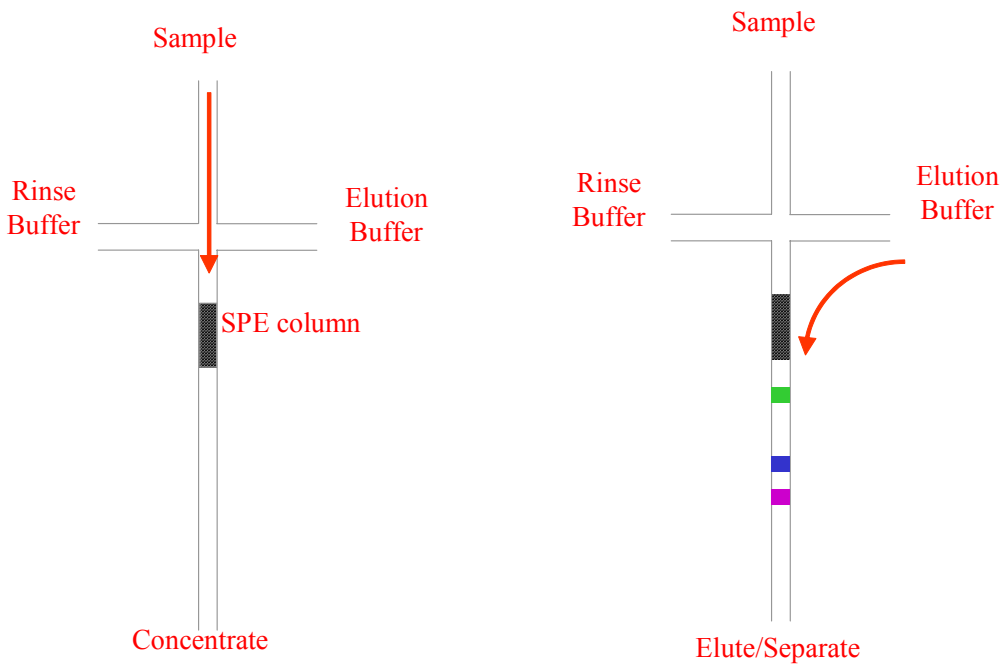
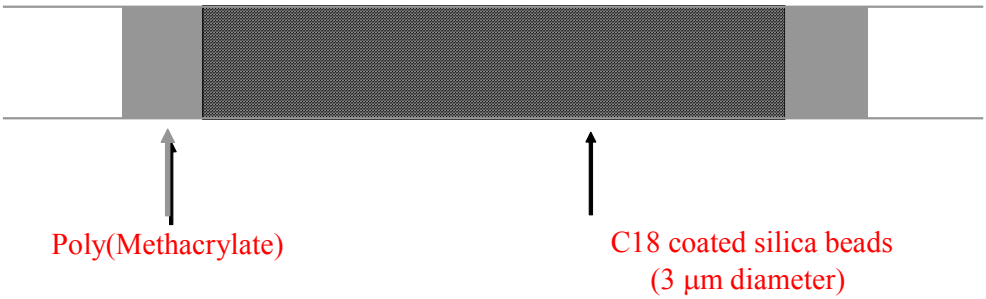


Figure 16. CE Microchip with *In Situ*/On-Chip SPE Column



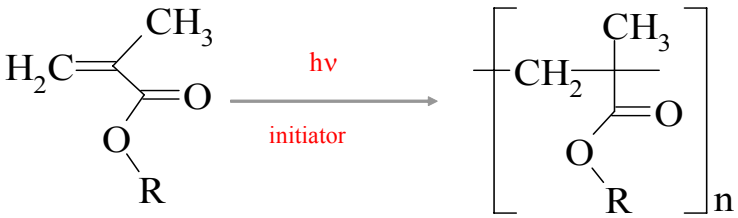


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Figure 17. Fabrication of Porous Hydogel Plugs with UV Crosslinking for On-Chip SPE

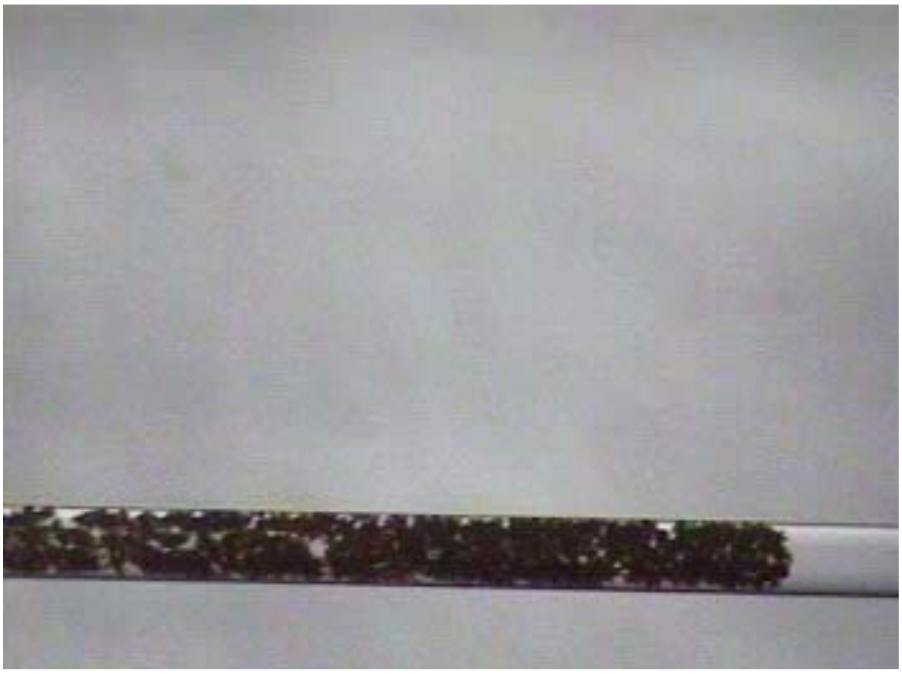

Figure 18. Photo of SPE Column Packed with C18 Coated Paramagnetic Beads
Magnet: 3/4 inch diameter, 0.1875 inch thick, NdFeB (27/30 mix)
Beads: 25 mg/mL C18 coated 1.5 μm magnetic beads in 50% water/50% ethanol
Packing Solution: ~ 0.2 - 0.4 μg/mL beads in ethanol

Appendix B: Publications and Presentations:

1. Lin, Y. 2003. "Microfluidics/Nanoengineered Electrochemical Sensors for Environmental and Health Monitoring." Presented by Yuehe Lin (Invited Speaker) at BioMEMS 2003, San Jose, CA on June 17, 2003.
2. Lin Y. 2002. "Microdevice Based on Integration of Capillary Electrophoresis Microchips with Electrochemical Detector for Monitoring of Explosives." Presented by Yuehe Lin at Partners in Environmental Technology Technical Symposium, sponsored by SERDP and ESTCP, Washington DC, DC on December 4, 2002.
3. Lin, Y., Wang, J.; Pumera, M.; Chatrathi, M. P.; Escarpa, A.; Musameh, M.; Collins, G.; Mulchandani, A. "Microchip for Fast Screening and Detailed Identification of Nitroaromatic Explosives and Organophosphates Compounds." (April, 2002). 223rd American Chemical Society National Meeting in Orlando, FL.
4. Wang, J.; Pumera, M.; Collins, G. E.; Mulchandani, A.; Lin, Y.; Olsen, K. "Single-Channel Microchip for Fast Screening and Detailed Identification of Nitroaromatic Explosives and Organophosphate Nerve Agents." *Anal. Chem.* 74, 1187-1191, 2002.

Presented by Yuehe Lin (Invited Speaker) at BioMEMS 2003, San Jose, CA on June 17, 2003.

Microfluidics/Electrochemical Sensors for Environmental and Health Monitoring

Yuehe Lin, Ph.D., Senior Research Scientist
Pacific Northwest National Laboratory

Abstract

This talk will provide an overview of recent work on microfluidics/electrochemical sensors at Pacific Northwest National Laboratory. A microfluidic platform was fabricated based on a multi-layer lamination method. Fluidic microchannels were produced by sandwiching laser-machined adhesive-backed polyimide gaskets between layers of the device. Individual components, such as microfabricated piezoelectrically actuated pumps and a microelectrochemical cell were designed and fabricated into plug-in modules which can be readily plugged into the microfluidic platform. Nanoengineered electrochemical sensors based on carbon nanotubes and functionalized nanoporous silica thin-films have been developed for enhanced selectivity and sensitivity.

Presented by Yuehe Lin at Partners in Environmental Technology Technical Symposium, sponsored by SERDP and ESTCP, Washington DC, DC on December 4, 2002.

Microdevice Based on Integration of Capillary Electrophoresis Microchips with Electrochemical Detector for Monitoring of Explosives

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Abstract

The objective of this project is to develop a portable analytical system based on the on-line/on-chip coupling of a miniaturized sample processing system with a microfabricated capillary electrophoresis/electrochemical detector for fast separation/detection of explosives and their degradation products in groundwater. Such a system has the potential to provide reliable, cost-effective characterization of groundwater contamination at DoD sites that are undergoing closure and remediation.

A capillary electrophoresis (CE) microdevice, based on the combination of microfabricated separation chips and thick-film electrochemical detector strips, was developed. The microdevice consists of a planar screen-printed carbon line electrode mounted perpendicular to the now direction. Such coupling obviates the need for permanent attachment of the detector, to allow easy and fast replacement of the working electrode. Variables influencing the separation efficiency and amperometric response, including the channel-electrode spacing, separation voltage, or detection potential, are assessed and optimized. The versatility, simplicity, and low-cost advantages of the design are coupled to an attractive performance, with submicromolar detection limits, and good precision. Applicability for assays of mixtures of nitroaromatic explosives has been demonstrated. On-line coupling of preconcentration/microchip separation for explosives will also be presented.

Presented by Yuehe Lin at the 223rd American Chemical Society National Meeting in Orlando, FL. April, 2002

Microchip for Fast Screening and Detailed Identification of Nitroaromatic Explosives and Organophosphate Compounds

Yuehe Lin

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Alberto Escarpa, Mustafa Musameh

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Abstract

A single-channel chip-based analytical microsystem which allows rapid flow-injection measurements of the total content of organic-explosive or nerve-agent compounds, as well as detailed micellar chromatographic identification of the individual ones is described. The protocol involves repetitive rapid flow-injection (screening) assays - for providing a timely warning and alarm - and switching to the separation (fingerprint identification) mode only when harmful compounds are detected. While micellar electrokinetic chromatography (MEKC), in the presence of sodium dodecyl sulfate (SDS), is used for separating the neutral nitroaromatic-explosive and nerve-agent compounds, an operation without SDS leads to high-speed measurements of the 'total' explosives or nerve-agents content. Switching between the 'flow-injection' and 'separation' modes is accomplished by rapidly exchanging the SDS-free and SDS-containing buffers in the separation channel. Amperometric detection was used for monitoring the separation. Key factors influencing the sample throughput, resolution, and sensitivity have been assessed and optimized. Assays rates of ca. 360 and 30 per hour can thus be realized for the 'total' screening and 'individual' measurements, respectively. Ultimately, such development will lead to the creation of a field-deployable microanalyzer, and will enable transporting the forensic laboratory to the sample source. On-line coupling of preconcentration/microchip separation for explosives will also be presented.