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Microscale solid-phase extraction system for explosives

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Abstract

A simple, semi-automated, microcolumn solid-phase extraction (SPE) system is optimized for the extraction, preconcentration and HPLC analysis of seven different explosives and explosive derivatives contaminating seawater, river water and well water samples. The microcolumns were constructed from 1/16 in. O.D. PTFE tubing (1 in.=2.54 cm) packed with 0.5–1.5 mg of SPE material, LiChrolut EN or Porapak R. The extraction system consisted of two syringe pumps and several solenoid valves. Optimal detection limits were realized when the sample water flow-rate was maximally increased within the limits of the pump, 5–10 ml/min (despite exceeding the breakthrough threshold of the SPE microcolumn), and when the eluate volume collected from the column was minimized, <5 µl (despite very low recovery percentages).
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1. Introduction

Nitroaromatic compounds pose an environmental water hazard to natural flora and fauna, as well as agricultural crops and humans [1]. Surface and groundwaters emanating from former military munitions sites are particular areas of environmental concern [2]. In addition, unexploded ordinances (UXOs) left in the ocean following various military exercises are known to release a signature plume containing toxic levels (ng/l to µg/l range) of 2,4,6-trinitrotoluene (TNT) and 2,4-dinitrotoluene (DNT) [3]. The US Environmental Protection Agency (EPA)

has determined, for example, that TNT is toxic at levels above 2 µg/l [4].

We, along with various other groups, have been investigating the development of capillary electrophoresis (CE) microchips capable of detecting aromatic explosives in the ng/l regime to ensure accurate water analysis below toxic levels [5–8]. Our intent is to develop field portable sensors capable of (1) locating UXOs or land mines present in our ocean's harbors for removal and clean-up, and (2) determining the explosives contamination level of surface and groundwaters in close proximity to munition's practice sites. The CE microchip benefits from its portable size, rapid separation times (seconds), and extremely small sample size requirements (nanoliter). The primary disadvantage to the CE microchip for explosives analysis is that, despite various efforts to improve the sensitivity, the limit of

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detection for these devices (e.g., 20 µg/l for TNT [8]) continues to be insufficient for the stringent detection requirements described above.

Solid-phase extraction (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, e.g., seawater. While the current EPA Method 8330 for analysis of explosives relies on the application of a time-consuming and labor-intensive salting out solvent extraction method, Jenkins et al. directly compared and demonstrated the utility of off-line SPE with a poly(styrene–divinylbenzene)-based membrane and a divinylbenzene–vinyl-pyrrolidone copolymer resin-based cartridge (Porapak R) [9]. Bouvier and Oehrle examined offline SPE of aqueous samples using cartridges packed with a specially cleaned resin (Porapak RDX), and subsequent analysis of the acetonitrile eluate by HPLC [10]. This method was time consuming, requiring 70 min per sample for SPE of a 500-ml sample, and wasteful, utilizing only 40 µl of the 5-ml acetonitrile extract for analysis, but reported excellent detection limits, e.g., 0.1 µg/l for TNT. Harvey and Clauss utilized the same SPE material as an on-line trace enrichment system based upon a system comprised of two Rheodyne valves and two HPLC pumps [11]. The on-line transfer of analyte to an HPLC column enabled significant advantages over the previously described method, including the attainment of equivalent detection limits with a factor of 50 less sample (10 vs. 500 ml), and significantly faster analysis times (30 min/sample versus 100 min/sample). Renner et al. utilized a similar on-line enrichment cartridge packed with a divinylbenzene–ethylvinylbenzene SPE material (LiChrolut EN), incorporating a Rheodyne valve and two HPLC pumps with connection to an HPLC column for chromatographic analysis [12]. This system took advantage of thermally assisted desorption to improve detection limits, which for TNT, for example, was 0.07 µg/l for a 50-ml sample that required a longer total analysis time of approximately 90 min (50 min for SPE and 40 min for HPLC).

The primary objective of this work was to develop a potentially 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 by Renner et al. and Harvey and Clauss were not considered viable options due to their stringent power requirements. The SPE apparatus presented here is reasonably lightweight and compact, consisting of two 24-V d.c., 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 (i.e., <2 µl vs. the 40-µl injection used by Bouvier and Oehrle [10]). The separation and detection of seven explosives and explosive derivatives are performed using EPA Method 8330 (HPLC) in order to permit direct comparison of this approach to previously described efforts.

Many applications in the field require only qualitative accuracy in the analysis of explosives contamination, but extremely high sensitivity, e.g., determinations of whether or not the toxicity threshold for groundwater has been surpassed or in the identification and localization of sea mines. This study was designed to investigate whether improvements in speed and sensitivity could be realized when utilizing (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 utilizing microscale columns for SPE, the speed of analysis, extent of waste generation, and size of the eluate plug are all minimized. This study demonstrates the development of a potentially portable SPE system that provides sensitive detection limits in the ng/l regime with acceptable qualitative accuracy (<25% error) in a reasonably short timeframe and in any of three different water matrices examined: seawater, river water and well water.

2. Experimental

2.1. Chemicals

LiChrolut EN and Porapak R (80/100) were

obtained from EM Science and Supelco, respectively. Chem Service, was the supplier of all explosives or explosive degradation reagents examined, each prepared as 1000 mg/l standards in acetonitrile. Explosives fortified seawater was formulated by diluting the following explosive standards from 1000 mg/l down to 5 µg/l in the seawater, river water or well water samples: 1,3,5-trinitrohexahydro-1,3,5-triazine (RDX), 1,3,5-trinitrobenzene (1,3,5-TNB), 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT), *o*-nitrotoluene (*o*-NT), *p*-nitrotoluene (*p*-NT), and *m*-nitrotoluene (*m*-NT).

2.2. Water samples

The seawater, purchased from Sigma, was collected from the Gulf of Mexico and was already pre-filtered and sterilized. The river water was collected from the Potomac River, filtered using a 0.2-µm nylon membrane. The well water samples were collected from Pendleton County in West Virginia, USA and were similarly filtered prior to analysis.

2.3. Microcolumn SPE procedure

Two Kloehn 50300 syringe pumps were used to aspirate and dispense the various solutions and reagents during the SPE procedure (see Fig. 1). The first pump, pump A, was equipped with a five-port discharge rotary valve and a 5-ml glass syringe, both available from Kloehn. The second pump, pump B, was equipped with a four-port discharge rotary valve and a 2.5-ml glass syringe (Kloehn). Two three-port solenoid valves and two, two-port solenoid valves (Bio-Chem Valve) were also utilized to control the movement of reagents. The two, two-port solenoid valves were used in place of a single three-port valve because of their capability for withstanding higher pressures (1400–2000 kPa).

Microscale SPE columns were created by packing the appropriate SPE material (LiChrolut or Porapak) into 1/16 in. O.D.×750 µm I.D. PTFE tubing (1 in.=2.54 cm). Nylon mesh (Cole Parmer) placed over the end of the tubing and held in place by a ferrule was an effective means of containing the SPE material during all experiments. The microcolumns were packed to a column length of 1 cm, beyond
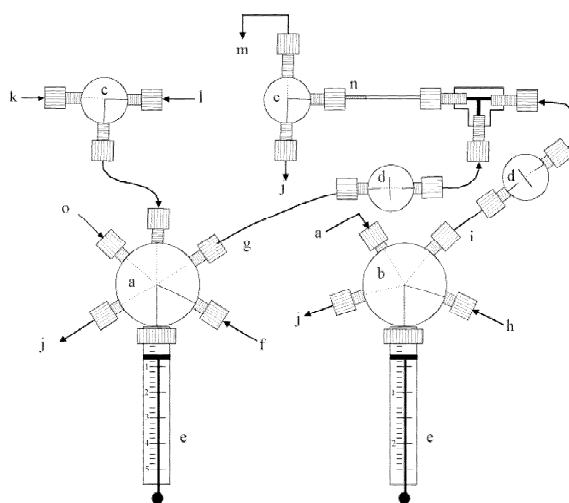

Fig. 1. Diagram of the semi-automated solid-phase extraction system: (a) five-position rotary valve; (b) four-position rotary valve; (c) three-port solenoid valve; (d) two-port solenoid valve; (e) syringe; (f) seawater sample in; (g) seawater sample, distilled water or air out; (h) acetonitrile in; (i) 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.

which the backpressures became too high for the syringe pumps. The SPE microcolumn was inserted into the SPE apparatus as shown in Fig. 1. The packing materials, LiChrolut and Porapak R, were chosen specifically because of previous success obtained in the application of these materials for explosives SPE [9,12]. Although both materials were examined during each stage of optimization, only data pertaining to LiChrolut EN is displayed and discussed. Both materials reported very similar trends in SPE behavior when packed to a column length with comparable backpressures, but the LiChrolut EN SPE microcolumn exhibited better sensitivity, due in part to the significantly enhanced surface area of this material (LiChrolut ~1300 m^2/g and Porapak R ~550 m^2/g). LiChrolut particles also have a higher packing efficiency, and, therefore, a higher extraction efficiency per unit column length.

2.4. HPLC method

A Hewlett-Packard series 1100 HPLC system was used to separate the extracted compounds and inte-

grate 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 μm packing diameter. The mobile phase was methanol–water (50:50, v/v). All water was 18 MΩ ultrapure water from a Millipore system filtered with a 0.22-μm Millipak 40 filter from Millipore. Methanol was filtered with 0.2-μm nylon filter discs. 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 in order to investigate significantly smaller eluate volumes. The absorbance was monitored at 240 nm instead of the recommended 254 nm because of the enhancement in sensitivity observed at this wavelength for the majority of explosives being examined.

3. Results and discussion

3.1. SPE system

In order to meet the requirements of a semi-automated, potentially field portable SPE device for explosives, the system shown in Fig. 1 was designed. The apparatus contains two syringe drives (24 V d.c., 48 W), one that is devoted to pumping the sample (pump A), and the other to pumping the acetonitrile eluent (pump B). Original attempts at utilizing a multichannel peristaltic pump were unsuccessful due to backpressures from these SPE microcolumns which exceeded the limits of the pump (~300 kPa). The syringe drives and various solenoid valves utilized in this design were capable of operating at much higher backpressures, from 1400 to 2000 kPa, although this parameter still placed limitations on the achievable sample flow-rates and SPE microcolumn lengths which could be employed, as will be discussed later.

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 seawater across the SPE microcolumn and out to waste. The total volume and flow-rate of seawater can be carefully controlled by this ap-

paratus. In the second step, the microcolumn and its associated tubing are washed and dried prior to the acetonitrile elution step. Distilled water (2.5 ml at 5.0 ml/min) is first pumped through the micro-column (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 five-port discharge rotary valve in order to protect the PTFE plunger of the syringe from any abrasive salt precipitates. Finally, air is used to dry the PTFE 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 two 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.

3.2. HPLC of explosives

Fig. 2 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. The three peaks appearing near 5 min were always present, and are the result of organic interferences from the seawater. Due to overlap with these impurities, explosive compounds such as tetryl were purposely omitted from this study.

It is important to note that a 2-μl injection loop was intentionally used for all HPLC analyses in place of the EPA Method 8330 recommended injection loop size of 100 μl. This step was taken, at
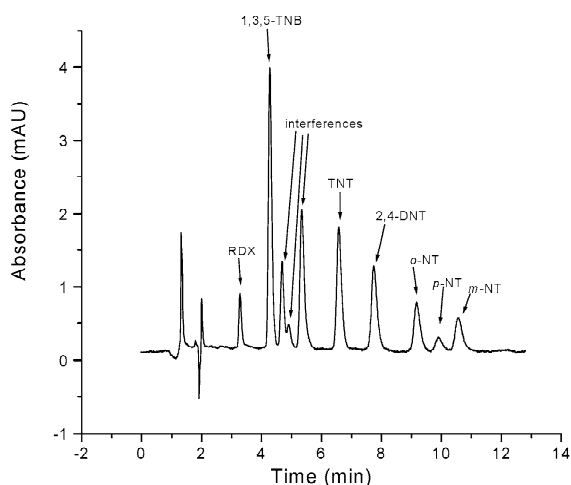
Fig. 2. Chromatogram of seven explosives or explosive derivatives obtained via HPLC with UV absorbance detection. The compounds were extracted from a 5 μg/l fortified seawater solution, using a 1.0-cm Lichrolut column, a seawater pumping rate of 5.0 ml/min, 25.0 ml total seawater volume, and 8.5 μl collected eluate volume.

the sacrifice of overall HPLC detection sensitivity, in order to enable investigations into the concentration enhancement factors achievable in the first few microliters of eluate arising from the SPE microcolumn, a volume of eluate that is more appropriate for introduction to microchip-based sensors that require only nanoliter volume injection sizes. For the purposes of this paper, concentration enhancement is defined as:

$$\text{Concentration enhancement} = \frac{\text{LOD with SPE}}{\text{LOD with no SPE}}$$

where LOD is the limit of detection, as defined by that signal which gives a signal/noise ratio of 3:1. The benefit of utilizing this parameter is for comparison purposes. The concentration enhancement factors discussed here may be correlated with any analytical technique that utilizes these small injection volumes, including the CE microchip, to give an approximation of the detection limits attainable when using this SPE apparatus. Working with a 2-μl injection loop did, however, create some sensitivity issues for HPLC. In the absence of SPE, 1,3,5-TNB is the most sensitively monitored analyte, at approximately 100 μg/l, while the nitrotoluene standards have 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 SPE.

Although all seven explosives and explosive derivatives were examined during each SPE study, for the purposes of simplifying data display and taking into consideration the fact that all seven compounds exhibited the same general trends, only three representative explosive components within the seawater sample are displayed: 1,3,5-TNB, TNT, and *m*-NT. These three explosives were chosen to be representative of the entire range of explosives examined, representing early, mid and late eluting components by HPLC, respectively.

3.3. Run to run reproducibility

The ability to continually reuse an SPE-packed microcolumn is closely tied to the regeneration efforts applied post-extraction. Future applications would benefit greatly from the capability of utilizing a single microcolumn for repeated seawater samples over the course of several days. This need is tempered by the desire to have the most rapid throughput of individual seawater samples by minimizing the time spent regenerating the microcolumn. The SPE protocol was tested to confirm the degree of reproducibility for the microcolumns in the extraction, separation and detection of 5 μg/l TNT in seawater in the presence of six other explosives or explosive derivatives (each at 5 μg/l). The same extraction protocol was repeated 24 consecutive times on each column. The microcolumns were packed in 1/16 in. PTFE tubing to a length of 1.0 cm. Each run consisted of sampling 20 ml of fortified seawater at a flow-rate of 3.0 ml/min through the microcolumn. Approximately 15 μl of acetonitrile were collected as the eluate. For each sample, the peak area for TNT was calculated and compared. The deviation for both SPE packing materials fell within 20% of the average, which was considered acceptable. Achieving this level of reproducibility required the inclusion of distilled water washing steps to eliminate the presence of trace salts, adequate air drying steps to prevent irreproducible extraction by the nonaqueous solvent, acetonitrile, and microcolumn regeneration via acetonitrile washing.

3.4. Eluate volume

The benefit of utilizing the SPE microcolumns described here was that very small eluate volumes (≤ 5 μl) could be collected and analyzed by HPLC. As a result, changes in the concentration enhancement factor as a function of the eluate volume recovered from the microcolumn were studied (see Fig. 3). Utilizing the same SPE conditions detailed above, the concentration of explosives in the eluate was found to drop rapidly as the eluate volume increased from approximately 5 to 20 μl, indicating the importance of minimizing the extractant volume utilized with these microcolumns. This rapid decline in concentration enhancement is followed by a more gradual decrease in explosives concentration for eluate volumes above 20 μl.

While the concentration enhancement for TNT, for example, is excellent, over 500 times for an eluate volume of 4 μl, the recovery percentage is actually quite low under these conditions, at 11% (see Fig. 4). Fig. 4 indicates that for an eluate volume of 100 μl, the recovery plateaus at approximately 80% for TNT, which is indicative of the fact that, under the conditions being utilized for the SPE, there is a ~20% breakthrough of TNT through the microcol-

Fig. 4. Change in recovery percentage observed with increasing eluate volume collected from the Lichrolut (■, 1,3,5-TNB; ○, TNT; ▲, *m*-NT) microcolumn. Conditions utilized for the SPE: 1.0 cm column length, a seawater pumping rate of 3.0 ml/min, and 20.0 ml total seawater volume.

umn during the sampling procedure, and that an eluate volume of 100 μl is sufficient in attaining complete (~100%) recovery of all TNT adsorbed to the SPE microcolumn. In summary, the largest concentration enhancements were observed for the smallest eluent volumes collected (<5 μl), despite the fact that the SPE procedure is operating in a breakthrough regime with regards to the seawater sampling step, and that very low recovery percentages were being realized at these small eluate volumes.

3.5. Seawater flow-rate and total volume sampled

The important realization that extremely high concentration enhancements can be attained in small eluate volumes in spite of operating under conditions that permit breakthrough and have low recovery percentages, led us to further investigate the effect of operating under even faster seawater sampling rates. In order for a broader range of flow-rates to be examined, the column length for this study was reduced to 0.5 cm. The seawater volume (20.0 ml) and initial concentration of explosives (5 μg/l) remained constant. The flow-rates were varied from 1.0 to 10.0 ml/min, with each flow-rate being
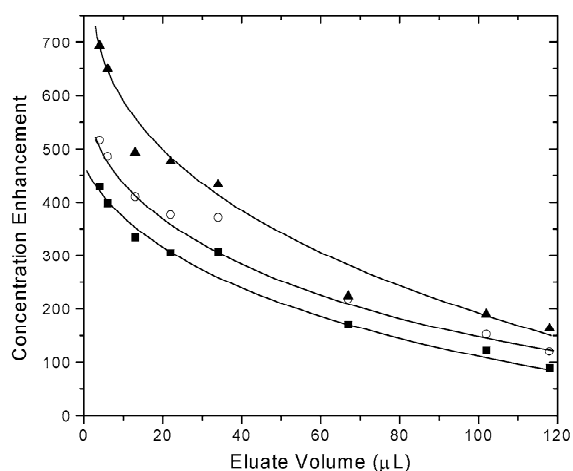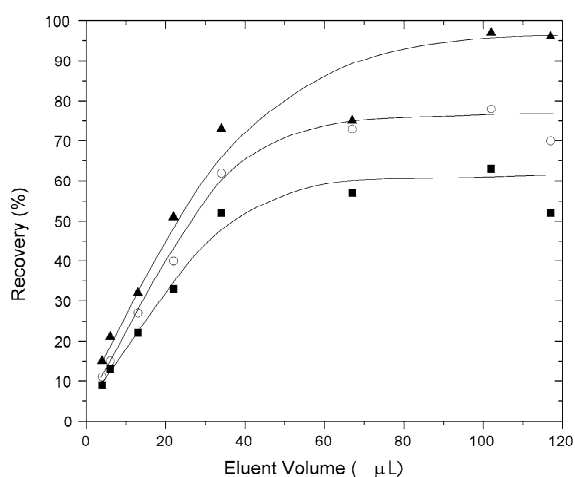

Fig. 3. Change in concentration enhancement observed with increasing eluate volume collected from the Lichrolut (■, 1,3,5-TNB; ○, TNT; ▲, *m*-NT) microcolumn. Conditions utilized for the SPE: 1.0 cm column length, a seawater pumping rate of 3.0 ml/min, and 20.0 ml total seawater volume.

repeated in triplicate, and the average concentration enhancement being plotted.

As expected, the delivery of identical volumes of seawater at slower seawater flow-rates exhibited greater concentration enhancements due to the resultant decrease in breakthrough percentage from the SPE microcolumn (see Fig. 5). Note, however, the gradual decrease in concentration enhancement that is observed as the flow-rate is increased from 1 to 10 ml/min. TNT, for example, reported only a 38% decrease in concentration enhancement despite an increase in sample flow-rate of 10 times (and a commensurate decrease in sampling time of 10 times).

An important point to discern with regards to the application of this SPE unit, is whether, for a given sampling time and an assumed unlimited sample volume, it is more beneficial to operate at a slower sample flow-rate that minimizes or prevents breakthrough, or to increase the sample flow-rate at the expense of increased breakthrough in order to further increase the total mass load adsorbed to the SPE microcolumn. Variations in the total volume of sampled seawater with a constant sample flow-rate were investigated to help clarify this issue (see Fig. 6). The concentration of explosives in the seawater

Fig. 6. Variation of the observed concentration enhancement with increasing total seawater volume sampled for the Lichrolut (■, 1,3,5-TNB; ○, TNT; ▲, *m*-NT) microcolumn. Conditions utilized for the SPE: 1.0 cm column length, a seawater pumping rate of 3.0 ml/min, 10.0–50.0 ml total seawater volume, and 10 μl collected eluate volume.

remained constant at 5 μg/l. The flow-rate was 3.0 ml/min, and the column length was 1.0 cm. The seawater volume was varied from 10 to 50 ml and each volume was done in triplicate, with the average concentration enhancement being plotted. It is certainly intuitive that a larger volume of fortified seawater will provide a more concentrated eluate. The concentration enhancement increased linearly for all seven explosive components as the total sample volume was increased. It is worth noting that 50 ml of fortified seawater provided more than 1500 times concentration enhancement for *m*-NT and 1200 times for TNT. Furthermore, examining the TNT data more closely, increasing the volume sampled by five times (from 10 to 50 ml), gave in increase in the concentration enhancement factor by as much as 4.3 times. This increase far surpasses any decrease realized as a result of increasing breakthrough (i.e., Fig. 5). Working under the conditions of a constant sampling time and assuming an unlimited sample supply, the data summarized in Figs. 4–6 indicate that (1) the sample flow-rate should be increased as much as possible (within the limits of the syringe pumps, themselves, 5 ml/min for a 1-cm column), in order to maximize the mass loading of explosives on the SPE microcolumn; and (2) the eluate volume
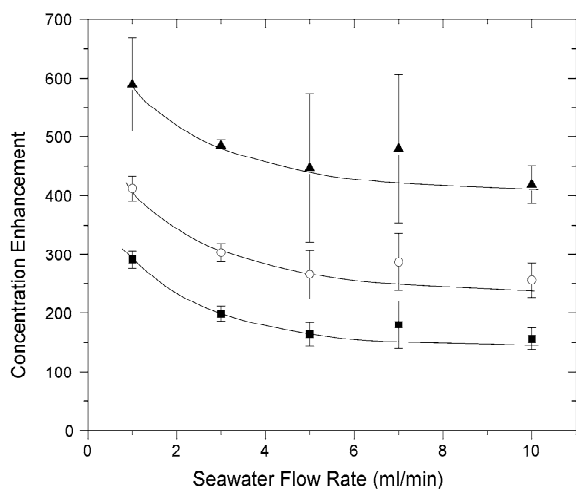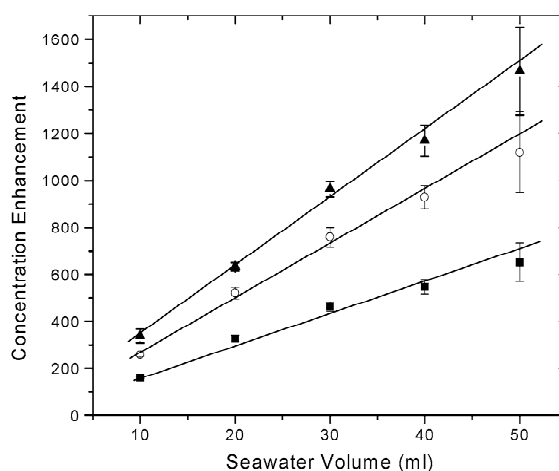

Fig. 5. Effect of seawater flow-rate on the observed concentration enhancement for the Lichrolut (■, 1,3,5-TNB; ○, TNT; ▲, *m*-NT) microcolumn. Conditions utilized for the SPE: 0.5 cm column length, a seawater pumping rate of 1.0–10.0 ml/min, 20.0 ml total seawater volume, and 10 μl collected eluate volume.

collected should be minimized (<5 µl) in order to maximize the explosives concentration enhancement realized.

3.6. Water analysis results

Based upon the previously described studies, 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, 3.0 ml/min sample water flow-rate, 5 µl eluate (acetonitrile) volume collected. Table 1 summarizes the HPLC results obtained for three different water matrices, seawater, river water and well water, contaminated with low µg/l levels of all seven explosives. The pumping time was limited to approximately 7 min (3 ml/min and 20 ml total sample volume), and the system required an additional 8 min (total 15 min sampling time) for the various washing and drying steps described previously.

The SPE method was found to perform comparably well in each of the three different water matrices examined. The detection limit obtained for TNT in seawater of 215 ng/l compares very well with that obtained by Harvey and Clauss [11], for example, whose detection limit was 100 ng/l. It is important to bear in mind that the HPLC detection limits reported here were obtained using a significantly smaller sample injection loop than that utilized by Harvey and Clauss (2 vs. 20 µl). When comparing the actual explosives concentration to the experimen-

tally derived value, the error was less than 25%. The concentration enhancement factors can be seen to range from 200 to as high as 1000 times.

This SPE system is particularly useful for applications desiring large concentration enhancements in a small volume of eluate, e.g., "laboratory-on-a-chip." The microscale SPE system described here, was utilized in combination with a CE microchip system for explosives analysis, lowering its detection limits in the separation of 1,3,5-TNB, TNT, and 2,4,6-trinitrophenyl-*N*-methylnitramine (tetryl) by 240 to more than 1000 times: TNB 0.25 µg/l; TNT 0.34 µg/l; and tetryl 0.19 µg/l [5].

4. Conclusions

A semi-automated, microscale SPE system was optimized for several parameters in the preconcentration of explosives from seawater, river water and well water into acetonitrile. Maximal concentration enhancements were realized when the sample water flow-rate was increased (even beyond the breakthrough threshold of the SPE microcolumn), and when the eluate volume collected from the column was minimized (despite very low recovery percentages). The portability, qualitative accuracy, high concentration enhancement factors, low eluate volume requirements and reasonable speed characteristics of this microscale SPE system make it potentially suitable for a number of different field applications.

Table 1
Quantitative results obtained in the analysis of three different water samples (seawater, river water and well water) contaminated with seven different explosives or explosive derivatives

Explosives	Actual conc. (µg/l)	Calculated concentration (µg/l)			Concentration enhancement factor			Detection limit (ng/l)		
		Seawater	River water	Well water	Sea-water	River water	Well water	Sea-water	River water	Well water
RDX	1.00	0.96 (±0.17)	0.98 (±0.03)	1.12 (±0.05)	271	217	238	275	326	232
TNB	0.75	0.57 (±0.09)	0.65 (±0.01)	0.67 (±0.01)	202	172	180	160	136	113
TNT	0.90	0.83 (±0.02)	0.94 (±0.00)	0.92 (±0.02)	469	435	411	215	173	176
DNT	0.90	0.87 (±0.06)	0.93 (±0.02)	0.95 (±0.05)	486	428	425	325	206	194
o-NT	1.50	1.32 (±0.40)	1.58 (±0.04)	1.61 (±0.29)	801	738	785	790	726	742
p-NT	1.50	1.54 (±0.63)	1.77 (±0.19)	1.71 (±0.29)	977	887	900	1100	1038	1080
m-NT	1.50	1.34 (±0.39)	1.54 (±0.15)	1.63 (±0.25)	754	713	792	930	900	912

Conditions utilized: Lichrolut 1.0 cm column length, a seawater pumping rate of 3.0 ml/min, 20 ml total sample volume, and 5 µl collected eluate volume.

Acknowledgements

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