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Journal of Chromatography A, 1066 (2005) 177–187
JOURNAL OF CHROMATOGRAPHY A
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Use of solid-phase microextraction/gas chromatography–electron capture detection for the determination of energetic chemicals in marine samples

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Received 15 December 2004; received in revised form 13 January 2005; accepted 20 January 2005

Abstract

Gas chromatography with electron capture detection (GC–ECD) is a highly explosive–sensitive analytical technique. However, its application to the analysis of sediment extracts is hampered by the presence of numerous endogenous interferences. In the present study, solid-phase microextraction (SPME) was used both as a purification technique for sediment extracts and as an extraction technique for water samples prior to analysis by GC–ECD. SPME/GC–ECD coupling was optimized and applied to the trace analysis of nine explosives including nitroaromatics and RDX in real seawater and marine sediment samples. Addition of a high concentration of salt (30%, w/v) in the aqueous medium and use of a carbowax/divinylbenzene (CW/DVB) coating led to optimal extraction efficiencies. Method detection limits (MDLs) ranged from 0.05 to 0.81 μg/L in water and from 1 to 9 μg/kg in dry sediment. Except for RDX, spike recoveries in seawater were satisfactory (89–147%) when samples were fortified at 2 μg/L of each analyte. Spike recoveries from dry sediment fortified at 10 μg/kg of each analyte gave lower recoveries but these could also be due to degradation in the matrix. With a smaller volume of aqueous sample required compared to solid-phase extraction (SPE), SPME is an attractive method for the analysis of limited volumes of sediment pore-water. Moreover, the use of SPME eliminated interferences present in sediment extracts thus allowing the detection of the target analytes that were otherwise difficult to detect by direct injection.

Keywords: Water analysis; Sediment analysis; Explosives; Solid-phase microextraction; GC–ECD

1. Introduction

Military training and wartime activities, including dumping of ammunition and sinking of warships have resulted in the undersea deposition of large amounts of unexploded ordnances (UXO). Since most explosives are toxic [1,2], their potential leakage from UXO and the subsequent contamination of various bodies of water are presently a subject of concern to several federal agencies including the Canadian and U.S. Navy. Due to the lack of effective tools to survey underwater areas and map the location of undersea UXO, the detection of the latter by both physical and chemical means is of prime importance. In a marine environment where natural attenuation (biodegradation, photolysis and hydrolysis) of explosives occurs and therefore leads to trace levels, sensitive analytical methods that are able to suppress matrix effects are required.

Water and soil samples collected at military installations are generally analyzed by U.S. Environmental Protection Agency SW-846 Method 8330 [3]. This method involves the extraction of water samples by either salting-out or solid-phase extraction (SPE) [4], the extraction of solid by sonication with acetonitrile, and the analysis of the acetonitrile extract using high-performance liquid chromatography with an ultraviolet detector (HPLC-UV). An alternative gas chromatography method involving an electron capture detector (GC–ECD) has also been developed to complement the SW-

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doi:10.1016/j.chroma.2005.01.049

846 Method 8330 [5–8]. The advantages of the GC–ECD method include lower detection limits and improved chromatographic resolution [5].

SPE is a robust method for extracting explosives from water [4,9–12]. However, the numerous steps that SPE method involves including conditioning, retention, rinse and elution make the technique a lengthy and time-consuming technique. Moreover, in the case of marine samples where the volume of sediments and consequently the volume of pore water may be limited, application of SPE, which generally requires large volumes of water (∼500 mL), may not be possible. An extraction technique that could be applied to smaller volumes of water would thus be profitable. As for the solid fraction of sediments, extraction by sonication with acetonitrile often gives rise to organic-rich extracts that interfere with explosives during GC analysis. A purification technique that allows analyzing traces of explosives in an extract that contains numerous interferences would therefore be beneficial.

Solid-phase microextraction (SPME) that was developed by Pawliszyn is a rapid, simple, sensitive and solvent-free extraction technique [13,14]. Compared with SPE, SPME uses a miniature cylindrical coated fused-silica fiber that allows rapid mass transfers during the adsorption and desorption processes, and therefore requires smaller volumes of samples. SPME extraction has been applied in combination with GC/MS for the determination of TNT and the amino metabolites in seawater [15]. It has also been used by Darrach et al. [16] to purify a water/solvent extract from marine sediment collected near an UXO before applying reversal electron attachment detection (READ) or GC/MS for TNT and DNT analysis, respectively. Furton et al. investigated the use of SPME/GC–ECD and SPME/HPLC-UV for the recovery of explosives from aqueous solutions and demonstrated that both couplings could be used for detecting traces of explosives [17].

The aim of this study was to develop a reproducible method to quantify energetic chemicals (nitroaromatics and nitramines) in seawater and marine sediments while using commercially available and commonly used instrumentation. We used SPME as both a purification technique for sediment extracts and an extraction technique for water samples, and coupled it with GC–ECD, a highly explosive–sensitive detection technique. The method was optimized and accuracy, precision and limits of detection were determined. The applicability of the method to natural samples was evaluated with natural water and sediment samples from Hawaii.

2. Experimental section

2.1. Chemicals

The target analytes were in the form of an acetonitrile solution (8095 calibration mix A) purchased from Restek Corp (Bellefonte, PA). Mix A contained 2-amino-4,6-dinitrotoluene (2-ADNT); 4-amino-2,6-dinitrotoluene (4-ADNT); 1,3-dinitrobenzene (1,3-DNB); 2,4-dinitrotoluene (2,4-DNT); 2,6-dinitrotoluene (2,6-DNT); 1,3,5-trinitro-1,3,5-triazine (RDX); 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX); tetryl; 1,3,5-trinitrobenzene (TNB); 2,4,6-trinitrotoluene (TNT); each at a concentration of 1 mg/mL. 3,4-Dinitrotoluene (3,4-DNT), which was used as an internal standard, was purchased as 1 mg/mL solution in methanol (8330 internal standard) from Restek Corp. (Bellefonte, PA). The solvent used, acetonitrile, was of HPLC grade (Fisher, Nepean, Ont.). Deionized water was obtained with a Milli-Q^{UV} plus (Millipore) system.

2.2. Sediments and seawater sampling

Four areas located offshore near the Hawaiian Islands were selected for collection of marine sediment and water samples. Samples UXO-1 and UXO-3 were collected from WWII-ERA UXO disposal site, sample UXO-5 was collected at a subsurface detonation site and sample REF-7 came from a reference site with no ordnance field nearby. At each labeled site, water was collected about 0.5 m below the surface, in polyethylene 4 L bottles, and samples were immediately transferred into 1 L amber glass bottles containing 1.5 g of sodium bisulphate for acidification. A 4 L sample was also collected in the reference site and stored without acidification. Sediment samples were scooped into 4 L plastic cores. At the end of the 6.5 h campaign, all samples were immediately placed on ice in a commercial cooler and processed for shipping. Upon arrival at BRI-CNRC (Montreal, Que.), samples were immediately stored at 4 °C, and analyzed 3 days later.

Samples were identified as follows: the above site names were used followed by letters “w” or “s” for water or sediment samples, respectively (for instance UXO-1w and UXO-1s correspond to water and sediment samples, respectively, taken at the first site visited). The non-acidified water sample from the reference site was denoted REF-7wna where “na” stands for non-acidified.

2.3. Solid-phase microextraction

Water samples were extracted by immersing a fused-silica fiber coated with the sorbent phase of interest (Supelco) in the aqueous solution (35 mL) that was stirred continuously at room temperature and 990 rpm with a Variomag magnetic stirrer (ColeParmer Instrument, Anjou, Que.). Three different fibers were tested for their ability to extract explosives: a 65 μm film of carbowax/divinylbenzene (CW/DVB); a 65 μm film of polydimethylsiloxane/divinylbenzene (PDMS/DVB); and an 85 μm film of polyacrylate (PA). The three fibers were conditioned in a GC injector port prior to use, according to the

manufacturer's recommendations. Optimization of desorption temperature, concentration of NaCl, and adsorption time will be described herein.

2.4. Solid-phase extraction

For comparison with the SPME technique, water samples were also extracted using solid-phase extraction with a Porapak Rdx Sep-Pak cartridge (500 mg) (Waters, Mississauga, Ont.) as described in the U.S. EPA SW-846 Method 3535A [4]. The cartridge was conditioned with 15 mL of acetonitrile followed by 30 mL of deionized water as recommended by the manufacturer. The aqueous sample (500 mL) was passed through the cartridge at a rate of 10 mL/min. After letting the cartridge dry under reduced pressure, potentially adsorbed contaminants were eluted with 5 mL of acetonitrile. The resulting concentrate was analyzed by GC–ECD (see method below).

2.5. Analysis of sediment samples

Sediment samples were extracted by sonication with acetonitrile, as described in the U.S. EPA SW-846 Method 8330 [3], but using a higher solid-to-liquid ratio. A dry sediment sample (15 g) was weighted, sonicated with 20 mL of acetonitrile at 20 °C for 18 h and centrifuged. The resulting yellow–green extract was analyzed by GC–ECD as described below, and by HPLC using U.S. EPA SW-846 Method 8330 [3] for comparative purposes. Another fraction of the extract (10 mL) was placed in a glass vial, and solvent was evaporated at room temperature under N_2 stream. After desiccation was complete, 35 mL of water and 10.5 g of NaCl were added to each sample, and the sample was sonicated for 2 h. The aqueous solution was then extracted by SPME using the optimized extraction method described herein and analyzed by GC–ECD.

2.6. GC–ECD system

Analyses were carried out on a Hewlett Packard 6890 gas chromatograph coupled to an electron capture detector (ECD) (Agilent Technologies, Wilmington, DE). Separation was performed on a capillary column Rtx-TNT from Restek (6 m × 0.53 mm × 1.50 μm). The GC oven was initially held at 100 °C for 2 min, raised to 200 °C at a rate of 10 °C/min, then to 240 °C at a rate of 20 °C/min, and hold at 240 °C for 1 min. The carrier gas was helium at 15 mL/min. The make up gas for ECD detector was nitrogen (15 mL/min). The detector temperature was maintained at 250 °C. Samples were either injected directly from an acetonitrile solution (2 μL) or from the SPME fiber using a deactivated glass liner for universal packed column inlet (170 μL internal volume). Injector temperature was kept at 225 °C (see below for optimization).

3. Results and discussion

3.1. Optimization of SPME/GC–ECD analysis

3.1.1. Selection of desorption temperature

When using SPME/GC, the desorption temperature can affect drastically the recovery of the analyte from the fiber. Temperature should be high enough to allow fast and quantitative desorption without decomposing the thermolabile chemicals. Three desorption temperatures were investigated (200, 225 and 250 °C) for each of the three fibers, CW/DVB, PDMS/DVB and PA, using the conditions indicated in Fig. 1. Explosives from mix A (each at 10 μg/L) were extracted from an aqueous solution by immersing the fiber for 30 min. The latter was then placed in the GC injector and allowed to desorb for 5 min at the required temperature. As seen in Fig. 1, the temperature effect on analyte desorption depended on the fiber used, but for most explosives, increasing desorption temperature up to 250 °C decreased the chromatographic response. A probable explanation for this is that explosives are by nature thermally unstable. The continuously declining response observed with PA coating upon increasing temperature from 200 to 250 °C may also suggest the occurrence of some reactions between the polyacrylate groups and the nitro-containing energetic chemicals. An optimal temperature of 225 °C was thus selected as the best compromise between a sufficient response and a limited degradation of analytes. Under these conditions, HMX led to very low and irreproducible values, mainly due to its very low vapor pressure (3.33×10^{-14} mmHg at 25 °C, as compared to more than 4.00×10^{-9} mmHg for the other tested explosives) and/or to its low affinity for organic phases such as the SPME fiber ($\text{Log}\, K_{ow} = 0.13$ as compared to ≥ 0.87 for other tested explosives) [18]. Its analysis by SPME/GC–ECD was therefore abandoned, as it has been done previously by Furton et al. [17].

3.1.2. Effect of NaCl concentration on analyte extraction

The addition of salt can reduce the solubility of some analytes thereby favoring their extraction by the fiber. In particular, it was shown for explosives that addition of up to 30% (w/v) of NaCl had positive effects on SPME extraction [17,19]. Because the present study was initiated to analyze marine samples, the effect of NaCl on explosives extraction had to be well understood. Four salt (NaCl) concentrations (0, 0.10, 0.20 and 0.30 g/mL corresponding to 0, 10, 20 and 30% (w/v), respectively) were thus tested, using the three fibers mentioned above and a desorption temperature of 225 °C. Fibers were rinsed before placement into the injection port to avoid accumulation of salt in the syringe needle and/or in the liner. Detailed experimental conditions and results are given in Fig. 2. The addition of salt did not significantly affect the chromatographic response of 1,3-DNB, TNB, RDX and tetryl, but it enhanced the GC response of 4-ADNT, 2-ADNT, 2,4-DNT and 2,6-DNT. As for TNT, addition of NaCl enhanced the peak areas with PDMS/DVB and PA fibers, but

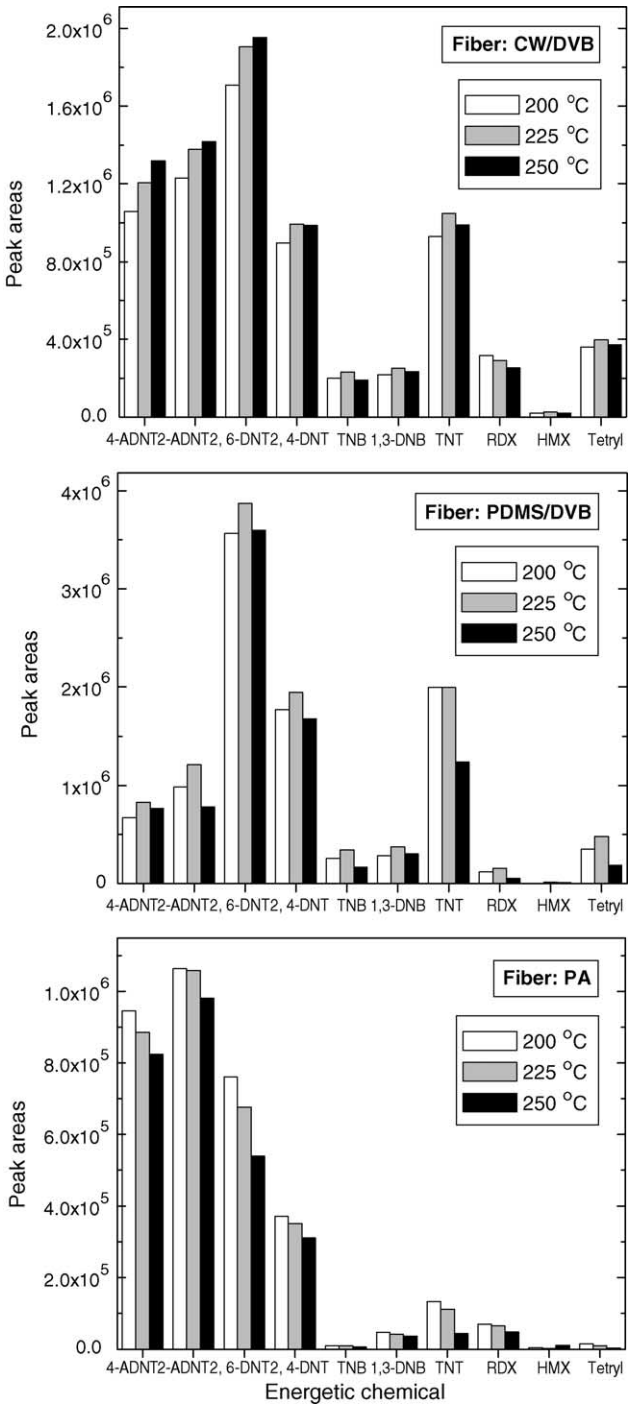

Fig. 1. Effect of desorption temperature on the analysis of explosives by SPME/GC–ECD. Extraction vials contained water (35 mL) and energetic compounds (0.35 μg in 35 μL of acetonitrile). Fiber was immersed for 30 min in the solution stirred at 990 rpm.

decreased them with CW/DVB fiber. The reason why dissimilar behaviors were observed with different fibers for TNT is unclear.

Given the extraction enhancement caused by the addition of salt for several analytes, sodium chloride solutions (30%, w/v) were used throughout the present study

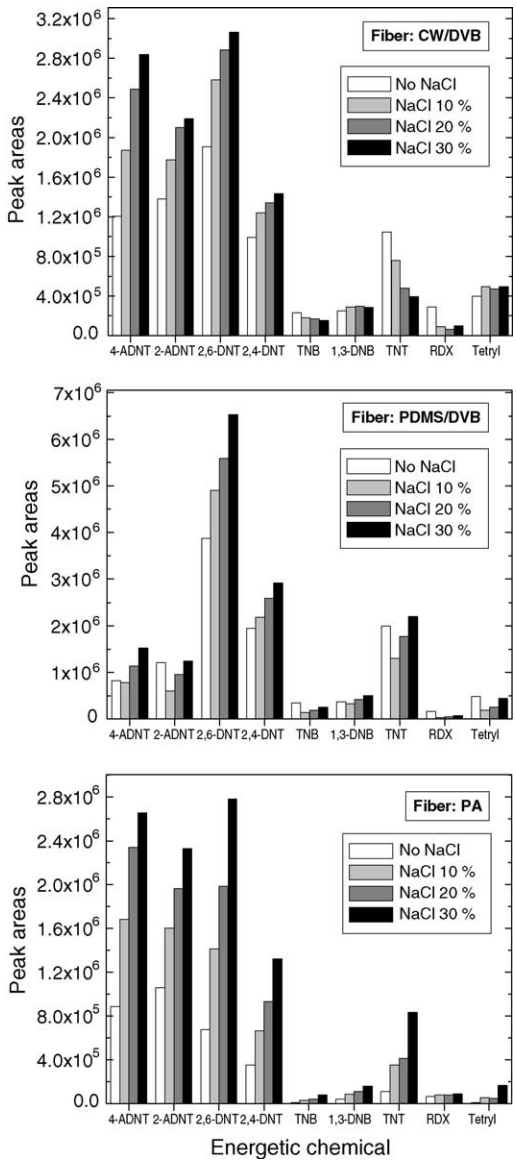

Fig. 2. Effect of medium salinity on the extraction of energetic compounds by SPME/GC–ECD. Extraction vials contained various amounts of NaCl (0, 10, 20 or 30%, w/v), water (35 mL), energetic compounds (0.35 μg in 35 μL of acetonitrile). Fiber was immersed for 30 min in the solution stirred at 990 rpm.

to ensure maximal extraction of most of the tested explosives.

3.1.3. Effect of adsorption time

The amount of analyte adsorbed by the SPME fiber is a function of the distribution constant between the fiber and the solution, the thickness of the adsorbing phase, and the analyte's diffusion coefficient [10]. Given that all of these parameters differ from one analyte to the other and from one fiber to the other, the equilibration time should be measured for each analyte/fiber couple. Adsorption profiles were determined as a function of time for the three fibers, CW/DVB, PDMS/DVB and PA, using the conditions given in Fig. 3. The profiles were

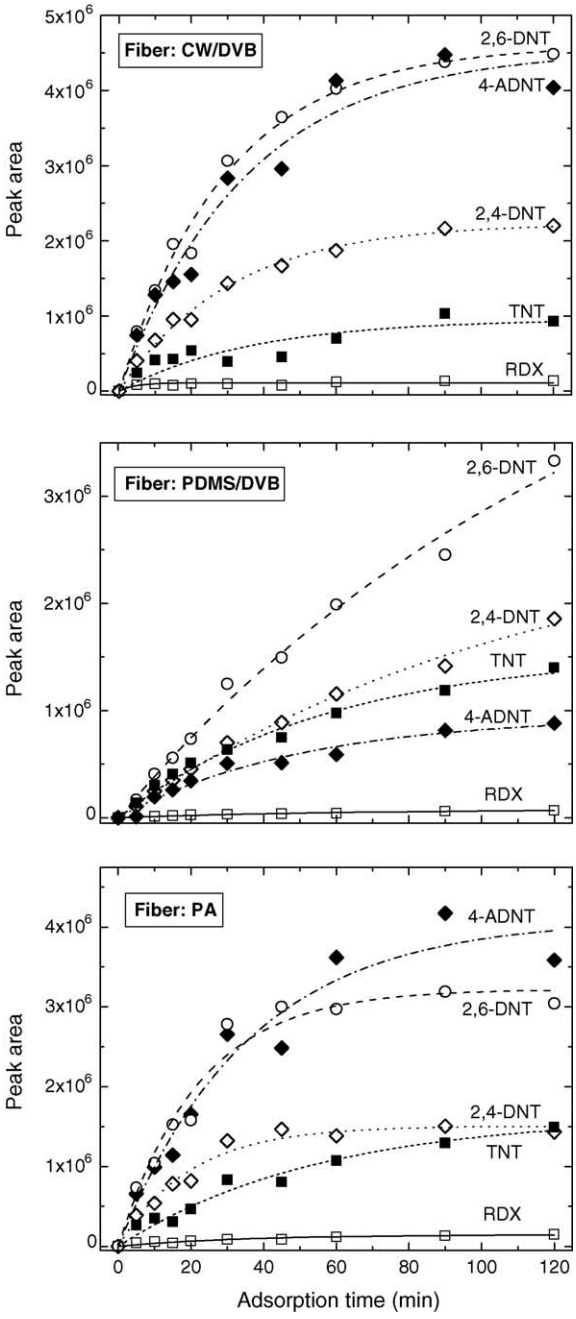

Fig. 3. Adsorption time profiles for RDX (□); TNT (■); 2,4-DNT (◊); 2,6-DNT (○) and 4-ADNT (♦) by SPME/GC–ECD using CW/DVB, PDMS/DVB and PA fibers. Extraction vials contained NaCl (30%, w/v), water (35 mL) and energetic compounds (0.35 μg for CW/DVB and PA fibers, or 0.07 μg for PDMS/DVB, in 350 μL of acetonitrile). Fiber was immersed for various periods of time in the solution stirred at 990 rpm. The desorption temperature was 225 °C.

only represented for five analytes (4-ADNT; 2,4-DNT; 2,6-DNT; TNT; RDX) to avoid curves overlapping. Compound 2-ADNT, which is not represented, behaved similarly to 4-ADNT, whereas tetryl, 1,3-DNB and TNB behaved in the same way as RDX. Analysis of a 10 μg/L solution of mix A with the PDMS/DVB fiber led to an overloaded signal after 30 min of adsorption, so that a 2 μg/L solution was used with this fiber. As for CW/DVB and PA coatings, 10 μg/L solutions were used. As shown in Fig. 3, around 1 h was necessary to reach the equilibrium when using CW/DVB and PA coatings, while more than 2 h were necessary when using PDMS/DVB coating. These equilibrium times were approximately twice longer than those observed by SPME/HPLC with equivalent fibers [19], although the present explosives concentrations (2 or 10 μg/L) were lower than those analyzed by SPME/HPLC (100 μg/L). It is possible that while enhancing the capacity of sorption, the conditioning of GC fibers also lengthened the diffusion path of each analyte. PDMS/DVB coating required longer time for the distribution of analyte between the solid phase and the solution to reach equilibrium (Fig. 3). A similar trend had been observed when applying SPME/HPLC [19]. We previously attributed the slow adsorption of analyte to the longer diffusion path caused by the presence of a porous polymer such as DVB. The faster equilibria reached in the present study with CW/DVB coating suggest that the low polarity of PDMS may have also been partially responsible for the slow adsorption processes.

3.1.4. Selection of SPME fiber

Three different SPME fiber coatings were evaluated for their ability to extract explosives from aqueous solutions. As seen in Fig. 1, the extraction efficiency of the fibers depended on the analyte considered. The PDMS/DVB coating was highly efficient for extracting DNTs and TNT, but was less efficient for the other explosives. In contrast, the polar CW/DVB coating exhibited an extraction efficiency that was inferior to that of PDMS/DVB for DNTs and TNT but that was more homogeneous between analytes. The PA fiber was less efficient than CW/DVB and PDMS/DVB fibers for all analytes. On the basis of the adsorption kinetics, PDMS fiber required 2 h to reach adsorption equilibrium when 1 h was sufficient for CW/DVB and PA and coatings (Fig. 3). Using a 2 h adsorption time would make the technique less advantageous. CW/DVB was thus the only fiber used hereafter to evaluate the performances of the SPME/GC–ECD technique for analyzing various types of explosives in ocean samples.

3.1.5. Effect of acetonitrile on analyte recovery

Energetic chemicals were introduced in all standard solutions dissolved in acetonitrile. By increasing the solubility of the analyte in the aqueous solution, acetonitrile could act negatively on the extraction efficiency of the method. To determine whether both samples and standards should be prepared with a constant volume of acetonitrile, or whether all samples should be prepared free of acetonitrile, the effect of the solvent was evaluated. Samples each containing NaCl (30%, w/v), analytes (each at 2 μg/L), and acetonitrile (0, 35, 350 or 700 μL, corresponding to 0, 0.1, 1 or 2% (v/v), respectively) were prepared and analyzed using the CW/DVB fiber (Fig. 4). No trend, whether positive or negative, could be drawn from the experimental data and in some cases recovery was even enhanced in the presence of acetonitrile. However, with the aim to conduct all extractions under simi-

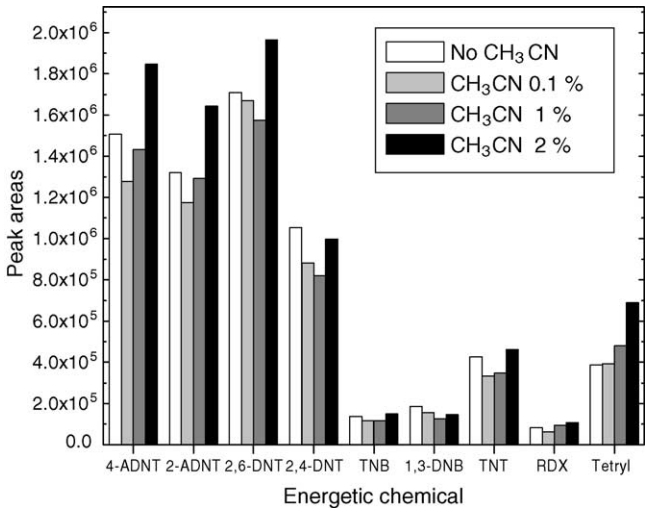

Fig. 4. Effect of the presence of acetonitrile on the extraction of energetic compounds by SPME/GC–ECD. Extraction vials contained water (35 mL), NaCl (30%, w/v), energetic compounds (0.07 μg of each) and acetonitrile (0, 0.1, 1 or 2%, v/v). CW/DVB fiber was immersed for 1 h in the solution stirred at 990 rpm.

lar conditions, acetonitrile (1%, v/v) was added to all samples henceforth.

3.2. Evaluation of SPME/GC–ECD method performance

3.2.1. Preparation of SPME calibration curves

Calibration curves were prepared for the nine analytes spiked at 0.05, 0.1, 0.5, 1, 2, 3, 4 and 5 μg/L using CW/DVB fiber. All standards were analyzed in duplicate using 3,4-DNT as internal standard. The linearity ranges, equation parameters and correlation coefficients resulting from linear regression are given in Table 1. Typical SPME/GC–ECD chromatograms are presented in Fig. 5. While RDX calibration curve was poorly linear ($r^2 = 0.9015$), the curves of the eight other energetic chemicals were well represented by linear equations, as demonstrated by the correlation coefficients (Table 1). Standard curves performed with the same fiber 5 months later showed a slope around 60% lower than the initial ones. The observed drop of response could be due to a deterioration of the fiber over time or to a loss in the response of the GC–ECD system. Calibration should therefore be often repeated to verify the response level of the SPME/GC–ECD system.

3.2.2. Accuracy, repeatability and detection limits for SPME

The method detection limits (MDLs) were calculated for the nine analytes according to published guidelines [20], as three times the standard deviation for a measurement value not higher than 10 times the MDL. On the basis of these guidelines, the method quantification limits (MQL) can also be estimated as 10 times the standard deviation. The accuracy (% recovery) and precision (% RSD) of the SPME/GC–ECD method were evaluated for each analyte by analyzing deionized water spiked with a standard solution (concentration in the spiked solution: 2 μg/L for RDX, 1,3-DNB and TNB; 0.25 μg/L for other analytes) seven times, on different days, and quantifying it using linear calibration curves. The results for the detection limits, precision and accuracy of quantification are given in Table 2. Depending on the analytes, detection limits ranged between 0.05 and 0.81 μg/L, in good agreement with the detection limits measured by Furton et al. [17] using a similar technique. The precision, as determined by the relative standard deviation, ranged from 5 to 23%. When comparing the measured concentrations of all analytes to the nominal concentrations in the check standard, recoveries ranging from 78 to 139% were obtained (Table 2).

3.2.3. Comparison of SPME and SPE for the analysis of aqueous samples

Since the SPE method is commonly used for routine analysis of explosives in water, its accuracy, precision and detection limits were also determined and compared to that of SPME using detection by GC–ECD (Table 2). Data obtained with the conventional SPE/HPLC technique [19] were also included in Table 2 for comparison.

The precision of both SPME/GC–ECD and SPE/GC–ECD techniques was found to be more or less in the same range (5% < RSD < 23% for SPME/GC–ECD; 4% < RSD < 28%

Table 1
Analysis of calibration standards[a] by SPME/GC–ECD using CW/DVB coating

Analyte	Linearity range (μg/L)	Linear equation[b]	Correlation coefficient[c] (r^2)
4-ADNT	0.1–5	$y = 4.2942 \times 10^5\ (\pm 8431)\,x$	0.9940 ($n = 7$)
2-ADNT	0.05–5	$y = 5.3644 \times 10^5\ (\pm 6366)\,x$	0.9978 ($n = 8$)
2,6-DNT	0.1–5	$y = 6.5756 \times 10^5\ (\pm 2.039 \times 10^4)\,x$	0.9873 ($n = 7$)
2,4-DNT	0.1–5	$y = 3.069 \times 10^5\ (\pm 2135)\,x$	0.9993 ($n = 7$)
TNB	0.5–5	$y = 3.3537 \times 10^4\ (\pm 515)\,x$	0.9972 ($n = 6$)
1,3-DNB	0.5–5	$y = 5.0084 \times 10^4\ (\pm 1774)\,x$	0.9822 ($n = 6$)
TNT	0.5–5	$y = 2.0154 \times 10^5\ (\pm 5733)\,x$	0.9900 ($n = 6$)
RDX	0.5–5	$y = 2.5272 \times 10^4\ (\pm 2188)\,x$	0.9050 ($n = 6$)
Tetryl	0.1–5	$y = 1.3175 \times 10^5\ (\pm 2113)\,x$	0.9960 ($n = 7$)

[a] All standards contained 30% (w/v) NaCl and 1% (v/v) acetonitrile in distilled water.
[b] y is the measured peak area and x is the concentration of energetic chemical in μg/L. Errors are given between brackets.
[c] Determined from the linear regression analysis of six to eight (n) standards, using Microcal™ Origin 6.0 software.

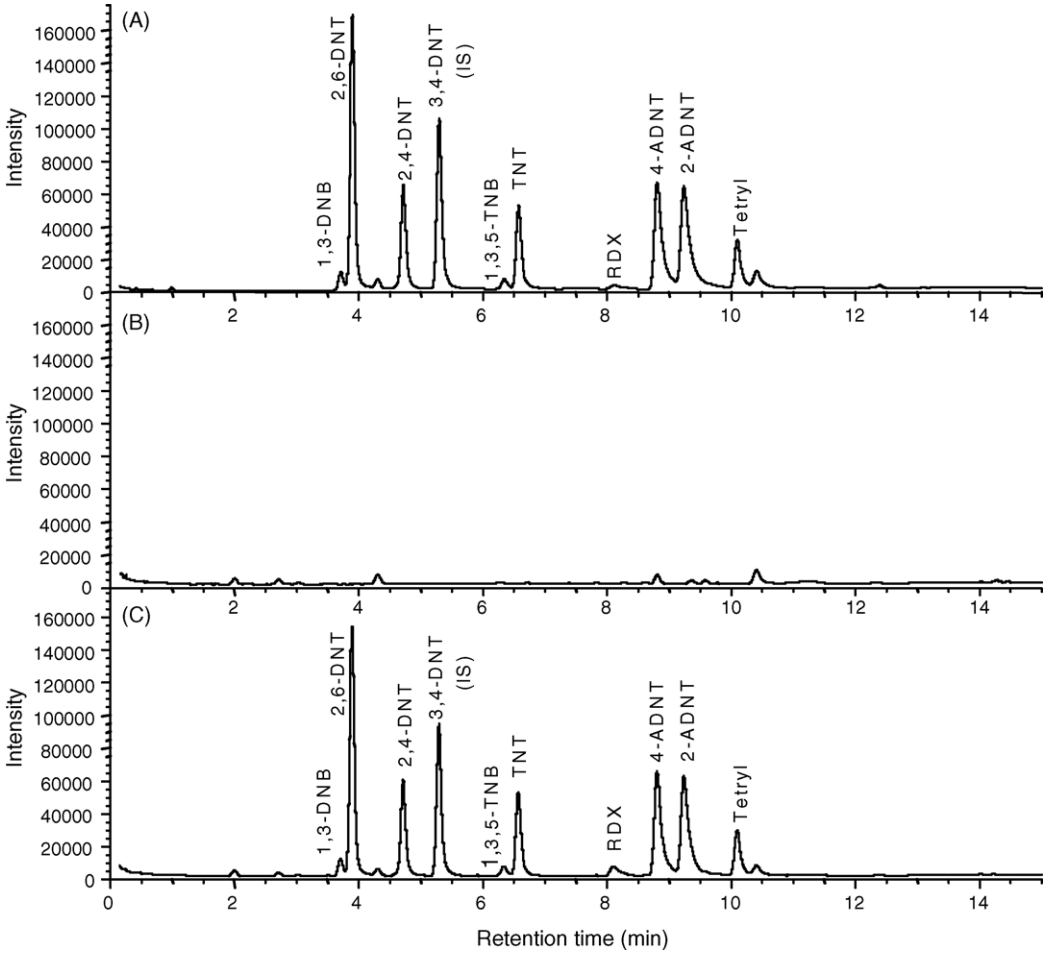

Fig. 5. Typical SPME/GC–ECD chromatograms of (A) a standard solution of a mixture of explosives, each at 2 μg/L; (B) an ocean sample (REF-7wna) from Hawaii; and (C) sample REF-7wna spiked with explosives each at 2 μg/L. Samples (350 μL of acetonitrile, 30% (w/v) of NaCl and 35 mL water) were stirred at 990 rpm and extracted for 30 min using a CW/DVB fiber. (IS) internal standard: 3,4-DNT.

for SPE/GC–ECD). This precision was significantly poorer than that of SPE/HPLC technique (3% < RSD < 13%), suggesting that the detection technique was a factor affecting the precision of the analysis. Except for RDX, 1,3-DNB and TNB, which were less efficiently extracted by SPME fiber and hence less efficiently detected, both SPME/GC–ECD and SPE/GC–ECD led to similar levels of detection limits (0.05–0.25 μg/L, depending on the analyte). These values

Table 2
Comparison of detection limits, precision (% RSD) and accuracy (% recovery) for analysis of aqueous samples by SPME and SPE

Analyte	SPME/GC–ECD (using CW/DVB)			SPE/GC–ECD			SPE/HPLC		
	MDL[a] (μg/L)	% RSD[b] at MQL	% Recovery[c]	MDL[a] (μg/L)	% RSD[b] at MQL	% Recovery[c]	MDL[a] (μg/L)	% RSD[b] at MQL	% Recovery[c]
4-ADNT	0.14	13.3	139.4	0.11	17.5	87.1	0.27	9.8	91.4
2-ADNT	0.13	19.0	92.3	0.06	10.3	73.5	ND[d]	ND[d]	ND[d]
2,6-DNT	0.05	6.4	104.4	0.04	4.5	105.5	ND[d]	ND[d]	ND[d]
2,4-DNT	0.06	6.6	122.9	0.04	5.1	106.9	0.14	4.6	97.6
TNB	0.62	9.4	111.4	0.16	24.1	87.4	0.10	13.12	99.7
1,3-DNB	0.27	4.5	102.0	0.07	8.6	105.1	0.03	4.4	100.3
TNT	0.18	22.3	105.3	0.08	10.9	96.7	0.12	4.8	84.1
RDX	0.81	10.0	135.1	0.12	19.9	78.7	0.29	10.0	97.0
Tetryl	0.13	21.8	78.3	0.28	28.0	135.4	0.15	6.7	75.9

[a] Method detection limits were calculated using the equation, MDL = 3σ, where σ is the standard deviation of seven measurements of low-concentration spikes.
[b] Percent relative standard deviation was based on seven replicate analyses at concentrations not exceeding 10 times the MDL.
[c] Percent recoveries were based on seven replicate analyses at concentrations not exceeding 10 times the MDL.
[d] Not determined due to co-elution of 2-ADNT with 4-ADNT and 2,4-DNT with 2,6-DNT.

Table 3
SPME and SPE recoveries (%) of explosives by GE–ECD in artificially contaminated ocean water samples (REF-7wna) in the presence and absence of $NaHSO_4$[a]

	4-ADNT	2-ADNT	2,6-DNT	2,4-DNT	TNB	1,3-DNB	TNT	RDX	Tetryl
Spike, mix A: 0.25 μg/L									
SPME									
pH 8.0	Inter.[c]	65	75	50	ND[b]	ND	129	ND	134
pH 2.0	Inter.[c]	ND	99	126	ND	ND	ND	ND	ND
SPE									
pH 8.0	92	61	102	101	127	97	119	98	201
pH 2.0	67	50	98	86	132	76	122	91	235
Spike, mix A: 2 μg/L									
SPME, t = 0 d									
pH 8.0	96	89	103	108	147	118	117	332	122
pH 2.0	21	32	107	118	132	110	133	410	89
SPME, t = 10 d (4 °C)									
pH 8.0	78	85	102	111	142	113	141	212	65
pH 2.0	9	1	113	135	177	127	159	155	86

[a] Duplicate experiments. SPME samples contained 350 μL of acetonitrile and 10.5 g of NaCl in addition to the analytes.
[b] ND: not detected.
[c] Interference.

are in the same order of magnitude as the detection limits measured by SPE/HPLC (Table 2).

The SPME method has the advantage of being organic solvent-free and more rapid than SPE. For example, total SPME analysis including adsorption and analysis did not exceed 80 min as opposed to approximately 6 h (including time needed for cartridge conditioning, adsorption, elution and analysis) for the SPE method. In addition to the time factor, collecting sediments in a marine field where UXOs are still present requires numerous precautionary measures including use of specialized equipment and restriction of the amount of sample. A technique like SPME that utilizes smaller volumes of samples than SPE thus becomes very attractive.

3.3. Application of SPME/GC–ECD to real samples

3.3.1. Aqueous samples

SPE and SPME were compared for their efficiencies to analyze ocean water samples (UXO-1w, UXO-3w, UXO-5w and UXO-7w) using GC–ECD detection. Whether SPME or SPE was applied as the extraction method prior to GC–ECD analysis, none of the 10 explosives used in the present study was detected in any of the four ocean water samples. Sample UXO-7wna (pH 8.0) was thus fortified with 8.75 or 70 μL of a 1 mg/L acetonitrile solution of the 10 explosives to yield 0.25 or 2 μg/L of each energetic chemical in the samples. In order to check the effect of pH on explosives extractability, similar samples were prepared but with 1.5 g/L of $NaHSO_4$ (pH 2). The results for the quantification of energetic chemicals in the spiked natural samples using either SPME/GC–ECD or SPE/GC–ECD are given in Table 3. As mentioned above, HMX was not analyzed in the solutions. RDX, TNB and 1,3-DNB could not be detected by SPME/GC–ECD when their concentration did not exceed 0.25 μg/L. In the non-acidified sample fortified at 2 μg/L all nine explosives could be detected (Fig. 5C) and percent recoveries [(measured concentration/actual concentration) × 100], except that of RDX, ranged from 89 to 147%, implying a reasonably good agreement between nominal and measured concentrations (Table 3). RDX on the other hand led to a much higher recovery (332%). Since analysis of REF-7wna did not show any interference with RDX (Fig. 5B), the source of overestimation was most likely analytical. This could be explained by the fact that the level of spike (2 μg/L) was lower than the method quantification limit for this compound (MQL = 10σ = 3.33 MDL = 2.7 μg/L).

Acidification of the medium had a drastic effect on the detection of most of the explosives at 0.25 μg/L: only two energetic chemicals (2,6-DNT and 2,4-DNT) were detected in the solution at pH 2 when five (2-ADNT, 2,6-DNT, 2,4-DNT, TNT and tetryl) had been detected at pH 8 (Table 3). A previous study involving SPME/HPLC for the analysis of explosives demonstrated that protonation of amines under acid conditions prevented their extraction by SPME [19], which explains that 2-ADNT was not observed here. As for tetryl and TNT, their absence at pH 2 is likely related to their higher detection limits (Table 2) coupled to an increased background noise when performing the extraction under acid conditions. These findings indicate that neutral or slightly alkaline conditions are favorable for detecting traces of energetic chemicals by SPME/GC–ECD. Samples that have been maintained under acid conditions to limit decomposition (hydrolysis, biotransformation) should thus be also analyzed after neutralization.

In contrast to SPME, all nine explosives, present at 0.25 μg/L, could be detected by GC–ECD following SPE extraction. The concentration of tetryl measured represented twice the nominal concentration however the level of spiking (0.25 μg/L) was below the method quantification limit for this compound (MQL = 3.33 MDL = 0.93 μg/L).

Table 4
Analysis of explosives in field sediment samples: direct GC–ECD and HPLC vs. SPME/GC–ECD

Sample	2,4-DNT (μg/kg)			2,6-DNT (μg/kg)		
	Desiccation[a] SPME	Direct Inj. (GC–ECD)	Direct Inj.[b] (HPLC)	Desiccation[a] SPME	Direct Inj. (GC–ECD)	Direct Inj.[b] (HPLC)
UXO-1s (I)[c]	497	813	675	25	39	–
UXO-1s (II)[c]	ND[d]	ND	ND	ND	ND	–
UXO-3s (I)	1398	1856	1997	113	123	–
UXO-3s (II)	ND	ND	ND	ND	ND	–
UXO-5s (I)	ND	ND	ND	ND	ND	–
UXO-5s (II)	ND	ND	ND	ND	ND	–
REF-7s (I)	ND	ND	ND	ND	ND	–
REF-7s (II)	ND	ND	ND	ND	ND	–

[a] Ten milliliters of extract was evaporated to dryness and redissolved in 35 mL of water containing 10.5 g of NaCl.
[b] 2,4-DNT and 2,6-DNT were not separated using U.S. EPA Method 8330. Signal was quantified using 2,4-DNT response factor.
[c] Numbers I and II correspond to two different subsamples.
[d] ND: not detected.

In order to evaluate the potential loss of explosives during the time period separating sampling and analysis, the non-acidified and acidified samples each containing 2 μg/L of each of the 10 energetic chemicals were stored for 10 days at 4 °C and analyzed again by SPME/GC–ECD (Table 3). Most of the analytes did not show significant variation in concentration, except for tetryl, which is known to undergo hydrolysis in water [21] and consequently degraded at pH 8.0. Results suggested that if any of the chemicals, ADNTs, DNTs, 1,3-DNB, TNB and TNT, were present at a concentration ≥2 μg/L in the ocean samples, they should have been detected by the method described herein. Overall, although slightly less sensitive than SPE method, SPME gave satisfactory results when used to quantify explosives in ocean water.

3.3.2. Sediment samples

Four sediment samples, UXO-1s and UXO-3s (disposal site), UXO-5s (subsurface detonation site) and REF-7s (reference site) were each extracted in duplicate with acetonitrile. Sonication of the four sediment samples led to greenish-yellow extracts, which were analyzed directly by HPLC and by GC–ECD. A fraction of each extract was also evaporated to dryness and redissolved in 35 mL of NaCl aqueous solution (30%, w/v) for subsequent SPME/GC–ECD analysis (Table 4). Samples UXO-1s and UXO-3s showed the presence of 2,4- and 2,6-DNT at concentrations that varied between duplicates, thus suggesting a heterogeneous distribution of explosives, in agreement with the heterogeneous nature of the samples. Several GC chromatograms showed peaks at retention times that matched those of TNT, TNB, RDX and 2-ADNT but extensive LC/MS analysis of the extracts concentrated 10 times did not confirm the presence of these compounds.

Direct analysis of extracts from samples UXO-1s and UXO-3s by GC–ECD led to values comparable to the HPLC ones (Table 4). Walsh and Ranney observed that GC concentrations of TNT and 2,4-DNT in soils were found to be higher than the HPLC measurements [7]. It should be noted here that 2,4-DNT and 2,6-DNT were not separable by HPLC applying the U.S. EPA Method 8330. GC–ECD, with its higher chromatographic resolution, allowed quantifying each chemical separately.

Evaporation of the above acetonitrile extracts to dryness and redissolution in water followed by SPME/GC–ECD gave lower results as compared to direct analysis by GC–ECD. DNTs being among the most volatile compounds considered [18], the lower concentrations observed for 2,4- and 2,6-DNT by SPME/GC–ECD could result from a loss occurring during the evaporation step required before applying SPME. Moreover, a potential competitive distribution of DNTs between the fiber and the organic matter extracted from the sediment that was deposited when the acetonitrile extract was evaporated to dryness could also be responsible for the lower recoveries observed for the two chemicals.

To evaluate the applicability of the SPME/GC–ECD method to the analyses of low concentrations of explosives in sediments, sample REF-7s was spiked with 10 μg/kg of each analyte of the standard mix A by adding the required volume of an acetonitrile solution. Spiked samples were allowed to stand for 2 h under the fume hood before adding acetonitrile for extraction. After sonication with acetonitrile, the resulting extract was analyzed by GC–ECD either directly or after desiccation, resuspension of the residue in water, and extraction by SPME. Direct GC analysis of the extract led to the appearance of several large peaks that interfered with the detection and quantification of most explosives (Fig. 6). Moreover, repeated injections led to a significant degradation in the GC peak shapes of energetic chemicals.

Applying SPME after evaporating and resuspending the extract in water improved significantly the detection of the energetic chemicals (Fig. 7). MDLs were determined for SPME/GC–ECD by extracting seven times the sediment REF-7s fortified at 10 μg/kg (Table 5), and were found to be between 1 and 9 μg/kg, which is generally higher than the MDLs previously reported for direct analysis of soil extracts by GC–ECD [8]. However, the organic interferences present

Table 5
Recoveries and method detection limits (μg/kg) of explosives in spiked sediment SPME/GC–ECD[a]

	4-ADNT	2-ADNT	2,6-DNT	2,4-DNT	TNB	1,3-DNB	TNT	RDX	Tetryl
% Recovery data for spiked REF-7s (mix A: 10 μg/kg)									
Direct injection	Inter.[b]	Inter.	107 ± 10	Inter.	52 ± 21	Inter.	Inter.	Inter.	70 ± 10
Desiccation SPME	30 ± 4	Inter.	79 ± 11	95 ± 16	151 ± 14	64 ± 20	103 ± 28	Inter.	27 ± 10
MDL (μg/kg) (mix A: 10 μg/kg)									
Desiccation SPME	1.2	Inter.	3.3	4.8	4.2	6.0	8.4	Inter.	3

[a] Fifteen gram sediment extracted with 20 mL acetonitrile; 10 mL of extract was desiccated and redissolved in 35 mL of water containing 10.5 g of NaCl. Seven replicates were conducted.
[b] Interference.

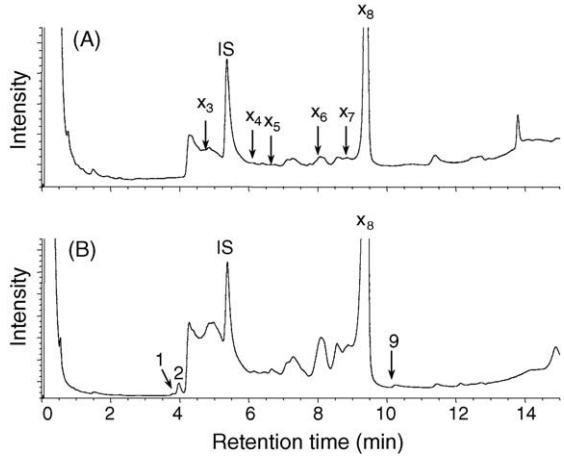

Fig. 6. GC–ECD chromatograms of the extracts of (A) non-spiked and (B) spiked (mix A: 10 μg/kg) REF-7s sediment. Sediment (15 g) was extracted with acetonitrile (20 mL). (1) 1,3-DNB; (2) 2,6-DNT; (9) tetryl; (X_3) interference with 2,4-DNT; (X_4) interference with 1,3,5-TNB; (X_5) interference with TNT; (X_6) interference with RDX; (X_7) interference with 4-ADNT; (X_8) interference with 2-ADNT. (IS) internal standard: 3,4-DNT.

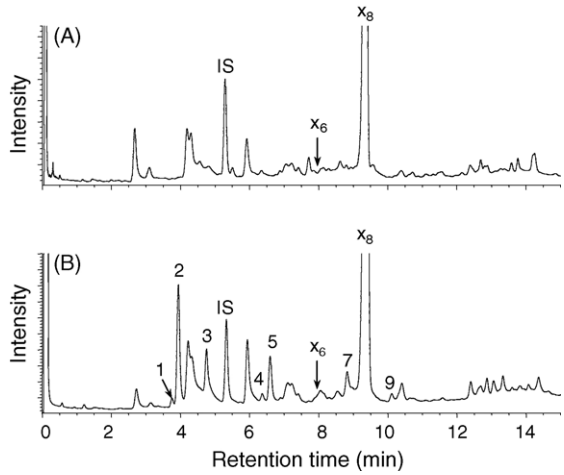

Fig. 7. SPME/GC–ECD chromatograms of the extracts of (A) non-spiked and (B) spiked (mix A: 10 μg/kg) REF-7s sediment. Sediment (15 g) was extracted with acetonitrile (20 mL). Extract (10 mL) was desiccated and resuspended in 35 mL of water containing CH_3CN (350 μL) and NaCl (30%, w/v). (1) 1,3-DNB; (2) 2,6-DNT; (3) 2,4-DNT; (4) 1,3,5-TNB; (5) TNT; (7) 4-ADNT; (9) tetryl; (X_6) interference with RDX; (X_8) interference with 2-ADNT. (IS) internal standard: 3,4-DNT.

in sediment REF-7s were larger than what is commonly obtained in soils so that most of the analyzed explosives could not be observed directly by GC–ECD at 10 μg/kg.

SPME/GC–ECD recoveries from the 10 μg/kg spiked sediment ranged between 27 and 151% (Table 5), with tetryl and 4-ADNT showing the poorest recoveries. Tetryl hydrolysis [21], and irreversible binding of aminodinitrotoluenes to the organic matter [22] of sediments might have contributed to their loss. The excessive recovery of TNB (151%) is likely due to the interference that can be seen in the non-spiked sediment REF-7s (Fig. 7A).

4. Conclusion

An SPME/GC–ECD method was optimized for the determination of explosives in marine water and sediment samples. Addition of a high concentration of salt (30%, w/v) in the aqueous medium and use of a carbowax/divinylbenzene coating led to optimal extraction efficiencies. When applying SPME/GC–ECD to the analysis of real marine samples, MDLs ranged from 0.05 to 0.81 μg/L in water and from 1 to 9 μg/kg in dry sediment. Except for RDX, spike recoveries in seawater were satisfactory (89–147%) but poorer analytes recoveries were obtained for sediment, which was possibly due to degradation/irreversible binding of the chemicals rather than to the accuracy of the analytical method. With a smaller volume of aqueous sample required compared to solid-phase extraction, SPME appeared as an attractive method for the analysis of limited volumes of sediment pore-water. Moreover, the use of SPME eliminated interferences present in sediment extracts (Figs. 6 and 7) allowing detection of the target analytes that were not detected by direct injection of the extracts.

Acknowledgements

The authors gratefully acknowledge Drs. M.T. Montgomery and C.L. Osburn from the Naval Research Laboratory, Washington, DC, and LT M. Runkle and crew of EOD MU3 DET MIDPAC for marine sample collection. Louise Paquet and Stéphane Deschamps are thanked for their technical assistance. Funding for this research was provided by the Of-

fice of Naval Research (ONR, US Navy) (Grant N00014-03-1-0269) and the U.S. DoD/DoE/EPA Strategic Environmental Research and Development Program (SERDP CP-1431).

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