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Application of Solid-Phase Microextraction Method to Determine Bioavailable Fraction of PAH in Hazardous Waste

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Abstract The solid-phase microextraction (SPME) method was developed to determine PAH free dissolved concentration (C_{free}) in field leachates from hazardous waste disposal. SPME technique, involving a 100-μm polydimethylsiloxane (PDMS) fiber coupled to GC–MS was optimized for determination of C_{free}. The following PAH were found in bioavailable form: acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, with C_{free} varying between 2.38 and 62.35 ng/L. Conventional solvent extraction was used for measurement of total concentration (C_{total}) in the same samples, and ranging from 1.26 to 77.56 μg/L. Determining C_{free} of the hydrophobic toxic pollutants could give useful information for risk assessment of the hazardous waste.

Keywords PAH · SPME · GC–MS · Bioavailability · Hazardous wastes

When analyzing complex matrices, such as waste materials, containing organic matter and non-aqueous liquids it is very important to evaluate risks associated with bioavailability of hydrophobic contaminants (Van der Wal et al. 2004; Ter Laak et al. 2006; Witt et al. 2009). Polycyclic aromatic hydrocarbons (PAH) belong to a group of hydrophobic (lipophilic) compounds with a high affinity for any solid matrix. Also, PAH included to priority hazardous substances by the EC (Regulation EC No 166/2006) and listing of the US Environmental Protecting Agency (16 US EPA). As a rule, the bioavailable fraction of contaminant is defined as the freely dissolved concentration (C_{free}). C_{free} is the fraction of PAH, which is not bound to the matrix and bears the most direct relation to bioaccumulation and effects (Kraaij et al. 2003; Enell et al. 2004; Kalbe et al. 2008; Hale et al. 2012). Determination of the bioavailable fraction of PAH could be done by solid-phase microextraction (SPME) method, introduced by Pawliszyn (Pawliszyn 1997). SPME can measure only free or easily extractable/available fraction of the contaminants that is detected and analyzed by sampling with SPME fiber (Witt et al. 2009; Kraaij et al. 2003). The concentration of compound in the fiber coating is directly related to the free concentration in the sample through the partition coefficient (K_{SPME}) of the chemical between water and chemical or by a calibration curve established in water at fixed exposure time of the SPME fiber (Heringa and Hermens 2003). SPME integrates solventless extraction, concentration and sample introduction in a simple process, by using a polymer-coated fibre (Doong et al. 2000; King et al. 2004; Zuazagoitia et al. 2007). Determination of the PAH in the fiber after an extraction can provide information on C_{free} of a compound in the sample (Heringa and Hermens 2003; Witt et al. 2009). Further, the combination of SPME with gas chromatography mass spectrometry (GC–MS) could be applied for the extraction and quantification of PAH in environmental samples (Akkanen and Kukkonen 2003; Vas and Vékey 2004; Hawthorne et al. 2005;

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Ouyang and Pawliszyn 2006; Fernández-González et al. 2007). Even more, SPME was successfully used for estimation of bioavailable fraction of PAH in different contaminated matrices, i.e. wastewater, soil, sediments (Van der Wal et al. 2004; Ter Laak et al. 2006; Witt et al. 2009; Gomes et al. 2009; Cornelissen et al. 2009).

Lack of information about risk associated with bioavailable PAH in hazardous waste disposal in actual environmental conditions has motivated our study. The waste concerned is spent shale, which formed during oil processing from oil shale. The spent shale typically contains a considerable amount of residual organics (10 %–20 %), including hazardous PAH. The content of PAH in the waste can reach 13 mg/kg (Otsa and Tang 2003; Saether et al. 2004; Kirso et al. 2007). The objectives of the present study were: to develop and optimize SPME method to determine PAH C_{free}; use the method received to determine the bioavailable fraction of PAH in deposited material in field conditions; to characterize the role of freely dissolved concentrations of PAH in leachate from real waste disposal.

Materials and Methods

The set of 16 US EPA PAH were subjected for analysis in the present work: naphthalene (NA), acenaphthylene (ACN), acenaphthene (AC), fluorene (FL), phenanthrene (PHE), anthracene (AN), fluoranthene (FA), pyrene (PY), benz[a]anthracene (B[a]A), chrysene (CHR), benzo[b]fluoranthene (B[b]F), benzo[k]fluoranthene (B[k]F), benzo [a]pyrene (B[a]P), indeno[1,2,3 cd]pyrene (IP), dibenz[a,h]-anthracene (D[a,h]A) and benzo[g,h,i]perylene (B[g,h,i]P). The standard mixture of 16 PAH at a concentration of 100 µg/mL in toluene, for internal standards 5-fluoroacenaphththylene, 1-fluoropyrene, 9-fluorobenzo[k]fluoranthene at a concentration of 10 µg/mL in toluene were purchased from Chiron AS (Trondheim, Norway). Solvent *n*-hexane (analytical grade) was obtained from Merck (Darmstadt, Germany). For standard solutions deionized ultra-pure water from a Milli-Q water purification system (Millipore) was used. Polydimethylsiloxane-coated (PDMS) disposable SPME fibers (100 µm film thickness) were purchased from Supelco.

Sampling of field leachate was done on the waste disposal located in Kohtla-Järve (Northeast Estonia) close to oil shale retorting plants of Viru Keemia Grupp. The field leachate samples were collected from spent shale disposal using new type of sampling devices (for more information, see Kirso et al. 2007). The connected to the sampler a Teflon pipe permit leachate collection during different time scales to the glass containers located in the excavated pit. Samples collected were transported to the laboratory for the analysis. General characteristics (pH and total dissolved solids, TDS) of mineral matrix compounds in leachate were measured using a BENCH PC 510 pH/conductivity meter (Eutech Instruments Pte Ltd, Singapore/Oakland Instruments, Vernon Hills, IL, USA).

PDMS-coated fiber was chosen for the extraction of the target analytes investigated in this work, since it provides well-defined absorptive retention, high permeability and good thermal stability (King et al. 2004; Vas and Vékey 2004; Ouyang and Pawliszyn 2006; Witt et al. 2009; Kirso et al. 2011). Prior to use fibers were conditioned in the injection port of a GC for 2 h according to instructions provided by the manufacturer. More than 100 samplings were completed with the same fiber with no lost in the sensitivity. For SPME extractions 40 mL of sample on 40 mL vials capped with PTFE-coated septa were used. Fiber was inserted in a sample using a syringe needle that pierced the septum. The needle was carefully removed and the position of the fibers was adjusted so that fiber remained in the sample water during extraction. Retracting the fiber into the needle through the septum terminated the extraction. The SPME device was transferred immediately to the GC–MS, where analysis was carried out. Each sample was analyzed minimum in triplicate.

Injector temperature, injection depth, desorption time are parameters, which should be optimised when SPME is coupled to GC–MS. The highest operating temperature recommended for the fiber by the supplier is 280°C, and conditioning temperature is 250°C. The injector temperature of 250°C was used for desorption due to highest desorption efficiency and prolonging the lifetime of fiber. On several occasions, some analytes would retain on the SPME fiber after injection in the GC–MS, which is known as carry-over effect. Thus, blank desorptions of the fiber were carried out to ensure contamination-free situation was present both before and during use. If the fiber was exhausted the conditioning of the fiber was performed for 15–30 min. To find hottest part of the GC injector series of experiments were carried out with desorption phenomena at different depths of injector, keeping the other parameters constant. A depth of 2.5 cm has been chosen in all further experiments because it provided maximum desorption efficiency. Thermal desorption time was studied by leaving the fiber in the injector for progressively longer periods of time, after it had been exposed to the same concentration solution with identical absorption parameters. For complete desorption of the compounds the 3 min at 250°C was used. No further improvement was observed when desorption time was increased. Blank desorptions of the fiber were done to avoid contamination during usage.

A sample of spent shale field leachate (pH 7.15, TDS 496 ppm) from oil shale waste disposal was employed to select optimal SPME extraction conditions for determination PAH C_{free}. There are a number of factors that may influence the efficiency of the SPME technique including the fiber exposure time, temperature and agitation during exposure (King et al. 2004; Tang and Isacsson 2008; Kirso et al. 2011). In the present study three extraction temperature levels were examined: 20, 45 and 60°C. The sorption time profiles were studied by monitoring the peak area as a function of exposure time immersing the fiber to the sample during 10, 30 and 60 min. Optimal time is the shortest time to reach equilibrium or an amount high enough to obtain proper quantification (Eriksson et al. 2001; Tang and Isacsson 2008). Previous studies showed, that agitation of the sample during fiber exposure enhances analyte extraction (King et al. 2004; Tang and Isacsson 2008), so magnetic stirring for agitation (800 rpm) was applied in all experiments. In all cases, the SPME device was transferred immediately to the GC–MS, where analysis was carried out using the method described previously. Each analysis was carried out in triplicate.

The linearity of the SPME–GC–MS method was tested in SIM mode by extracting aqueous standards with concentration between 0.001 and 1.0 μg/L under optimized SPME conditions. Calibration solutions were made using standard additions of the PAH mixture to distilled water. The calibration curves show excellent linearity with the correlation coefficient ranging between 0.96 and 0.99 for all standards. The external standards were analyzed under the same conditions as the samples concerned. The relative standard deviation (RSD) averaged 14 % (range 2 %–25 %) for real leachate samples, which includes variation between SPME sampling, manual injections and instrumental analysis. The limit of detection (LOD) and limit of quantification (LOQ) were from 0.1 to 1 ng/L and 1 to 10 ng/L, respectively. As a rule, the higher RSD, LOD and LOQ values were determined for compounds having higher molecular mass. To confirm the applicability of the SPME method to extract PAHs from real water samples, SPME–GC–MS method recovery was obtained from the ratio of spiked real water sample to spiked ultrapure water. Both samples were extracted by SPME according to the optimized method. The relative recovery obtained was observed to be good: 91 %–104 %. Blank tests were done on ultrapure water, which was extracted by SPME according to the optimized method to assess contamination from reagents and materials.

To determine the total concentration (C_{total}) of PAH samples were at first subjected to the conventional solvent extraction. The water sample (~600 mL) was transferred to a 1-L glass separatory funnel and shaked for 5 min with 4 mL of *n*-hexane added, followed by the collection of the n-hexane phase. The extraction step was repeated twice and both solvent extracts were combined. Anhydrous Na_2SO_4 was used to remove residual water from the extracts. The solvent was evaporated under nitrogen, then 1 mL of n-hexane was added and the samples were subjected to final determination by GC–MS analysis. Solvent extraction recovery efficiency was 92 %–100 %.

GC Agilent HP 7890, Palo Alto, CA, USA, with the HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) and Model 5975B mass selective detector was used. Chemstation software Agilent G1701DA GC/MSD was applied for the treatment of data. The GC oven temperature program stated as follows: 60°C for 0.8 min, then 15°C/min to 320 (total run time 20.13 min). The target compounds were quantified in SIM mode, using the molecular ion and one qualifier ion for each compound. Full-scan acquisition was used for compound confirmation. GC was operated in the splitless mode (splitless time–4 min). Helium (99.99 %) was used as a carrier gas.

Results and Discussion

Effect of temperature on the extraction efficiency is presented in Fig. 1. When the temperature was raised up to 45 and 60°C the extracted amount of AC and ACN increased, whereas for FL, PHE, FA and PY no further extraction efficiency improvement was observed (extraction time 30 min). This could be explained by the PAH desorption from the fiber at high temperatures. Extraction temperature 20°C was chosen in this study and was used for further experiments, suggesting the best recovery for the most compounds. Furthermore, these experimental conditions could give more realistic data on PAH bioavailability in environmental matrices. Longer sampling time increased the extraction efficiency of all compounds studied (Fig. 2). Higher ring PAH was not found in free form. It could be explained by that heavier PAH have tendency to bind with organic and mineral (colloid, clay) matter due to very low solubility of these compounds. The 30–min extraction time chosen for further experiments is a compromise between sensitivity and sufficiently short extraction time. Longer extraction time was not studied in order to avoid the excessive extension of the analysis time. The final conditions selected were: injector temperature 250°C, injector depth 2.5 cm, desorption time 3 min, magnetic stirring (800 rpm), and extraction time–30 min at 20°C. SPME can be approached as a partition or equilibrium extraction technique. A non-equilibrium determination SPME was implemented during the experiment. As the extraction conditions chosen are below the equilibrium time, slight changes in variables, such as temperature, time and stirring rate can change the results. Neither complete extraction of analytes nor full equilibrium is necessary; however consistent SPME analysis parameters are critical for accurate results (Heringa and Hermens 2003, Tang and Isacsson 2008). Efforts necessary were made to hold these variables precisely for every sample in order to maintain the comparable results.

Samples of leachate from the disposal were subjected for analysis of PAH C_{total} by conventional solvent extraction and C_{free} by developed SPME method (Table 1). Both

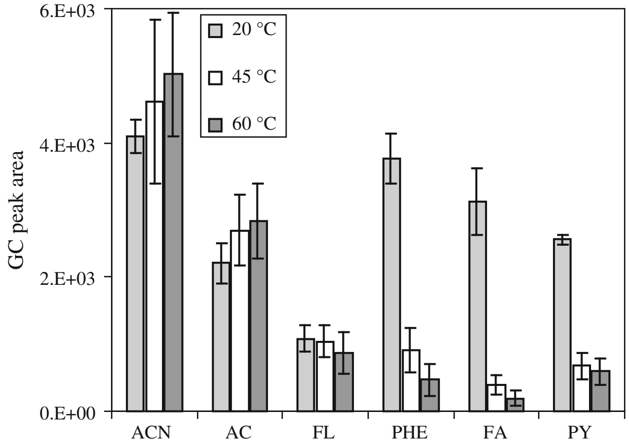

Fig. 1 Effect of the temperature on amount of PAH extracted at 30 min. The vertical lines represent standard deviations

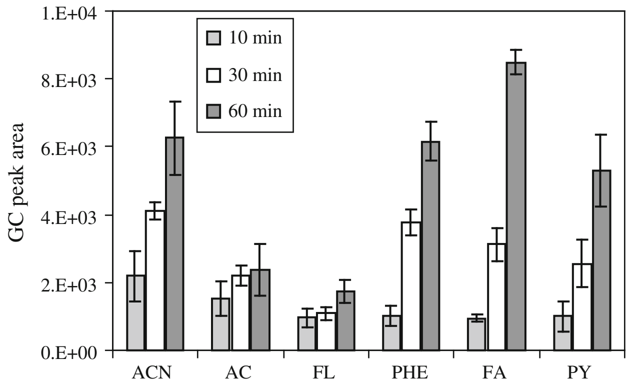

Fig. 2 Effect of the extraction time on amount of PAH extracted at 20°C. The vertical lines represent standard deviations

C_{total} and C_{free} of PAH in field leachate were found to vary with two and three ring PAH predominating. PAH C_{total} ranged from 1.26 to 77.56 µg/L (Table 1). In bioavailable form the following PAH were found: ACN, AC, FL, PHE, AN, FA and PY. PAH C_{free} was found to vary between 2.38 and 62.35 ng/L. It is important to note, that not only low ring PAH compounds presented in Table 1 leached from the disposal, but also five and six ring PAH (B[a]A, B[k]F, B[b]F, B[a]P, B[g,h,i]P) were found in leachates in trace concentration in some samples. Five and six ring PAH were not detected in free dissolved form in current experimental conditions. The absence of heavy PAH in bioavailable form in the leachate samples is indicative of their strong binding to the dissolved or solid matter (Otsa and Tang 2003; Enell et al. 2004; Van der Wal et al. 2004; Ter Laak et al. 2006). Meanwhile, other investigators have also observed, that the fraction of PAH associated with colloids and/or organic matter increases dramatically with number of rings (Kraaij et al. 2003; King et al. 2004; Hawthorne et al. 2005).

The distribution ratio, defined as C_{free}/C_{total} quantifies the difference between relevant for transport C_{total} and for uptake by biota C_{free}. According to obtained results (Table 1) only 0.01 %–2.7 % of C_{total} of studied PAH in the samples comprised for C_{free}. C_{free} is a small fraction of C_{total}, indicating strong association of PAH with organic and mineral (colloid, clay) matter. A study by Cornelissen et al. (2009) determined C_{free} and C_{total} of PAH in leachates from municipal sanitary landfill. These researchers found that 0.1 %–20 % of PAH were freely dissolved, demonstrating that our findings are in close agreement. The heterogeneity of the target material caused not only

Table 1 Total dissolved concentrations (C_{total}, µg/L); freely dissolved concentrations, (C_{free}, ng/L) of PAH in field leachates of spent shale; C_{free}/C_{total}-fraction of bioavailable PAH in percents

Parameters	ACN	AC	FL	PHE	AN	FA	PY
Sample A							
C_{total}	11.12(2.3)[a]	4.02(2.0)	1.26(9.2)	12.17(16)	bc	8.24(3.5)	9.44(7.6)
C_{free}	5.68(15.6)	39.10(5.4)	34.08(6.1)	58.78(3.1)	23.49(0.4)	2.38(0.2)	5.38(24.2)
C_{free}/C_{total}	0.05	0.97	2.70	0.48	–	0.03	0.06
Sample B							
C_{total}	6.35(4.1)	2.81(5.7)	1.35(13.7)	8.21(0.3)[b]		7.87(4.5)	9.34(6.0)
C_{free}	6.46(23.0)	22.73(3.9)	18.29(0.4)	43.8(21.8)	44.35(19.0)	nd	1.2(18.8)
C_{free}/C_{total}	0.10	0.81	1.36	0.53[b]	–	–	0.01
Sample C							
C_{total}	12.16(0.8)	6.30(5.5)	14.05(20.5)	77.56(7.1)	38.04(25.0)	nd	nd
C_{free}	13.05(4.9)	43.16(6.2)	30.44(9.0)	62.35(8.3)	38.78(7.7)	nd	nd
C_{free}/C_{total}	0.09	0.01	0.04	0.12	0.10		

bc below calibration level, *nd* not detected

[a] RSD in percents

[b] PHE + AN

differences in PAH release, but also the differences in general characteristics of spent shale leachates. pH of field leachate in the samples A, B, C were 7.7, 8.4, 12.2, respectively. Electrical conductivity of water is directly related to the concentration of TDS in the water, and the values for the samples A, B, C were 0.25, 0.26, 1.6 ppt, respectively. Differences in distribution of PAH of C_{total} and C_{free}, pH and TDS in leachates from the disposal concerned can be explained by non-homogenous nature of the parent material, caused by different rate of weathering.

To conclude, the optimized SPME technique is appropriate for determination of freely dissolved concentration of PAH in leachates from hazardous waste. SPME technique could be applied for risk assessment of contaminated sites for evaluation of bioavailable fraction of PAH.

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