

Ultrasound-Assisted Dispersive Liquid–Liquid Microextraction Combined with Low Solvent Consumption for Determination of Polycyclic Aromatic Hydrocarbons in Seawater by GC–MS

Xingliang Song · Jinhua Li · Chunyang Liao ·
Lingxin Chen

Received: 9 August 2010 / Revised: 13 April 2011 / Accepted: 27 April 2011 / Published online: 15 May 2011
© Springer-Verlag 2011

Abstract Ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME) with low solvent consumption was demonstrated for gas chromatography–mass spectrometry (GC–MS) determination of 16 typical polycyclic aromatic hydrocarbons (PAHs) in seawater samples. Factors affecting the extraction process, such as extraction and dispersive solvent, phase ratio, temperature, extraction and centrifugation time, were investigated thoroughly and optimized. The linear range was 20–2,000 ng L⁻¹ except for acenaphthylene (Acy) at 10–2,000 ng L⁻¹ and phenanthrene (Phe), fluoranthene (Flu) and pyrene (Py) all at 5–2,000 ng L⁻¹. Enrichment factors (EFs) ranging from 722 to 8,133 were obtained, achieving limits of detection at 1.0–10.0 ng L⁻¹. The method attained good precision (relative standard deviation, RSD) from 3.4 to 14.2% for spiked 50 ng L⁻¹ individual PAHs standards. Method recoveries were in the range 87–124% and 70–127% for spiked samples from simulated seawater and beach seawater, respectively. The proposed USA-DLLME helped to obtain about 1.1–10 times higher EFs in a minimum amount of solvent and in less time than traditional DLLME.

Keywords Polycyclic aromatic hydrocarbons · Ultrasound-assisted dispersive liquid–liquid microextraction · Seawater samples · Gas chromatography–mass spectrometry

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are well recognized as being among the most carcinogenic, mutagenic and toxic contaminants [1, 2] existing widespread throughout the environment. Sixteen kinds of PAHs have been listed by the United States Environmental Protection Agency (USEPA) as priority pollutants [3]. PAHs are also hydrophobic compounds with very low water solubility [4] and, therefore, they often occur at typically low levels in complicated and polluted aquatic environment [5] and tend to adsorb rapidly on suspended material or sediment [6]. Thus, it is highly necessary and crucial to develop proper sample pretreatment methods for recovering sufficient target PAHs from seawater samples.

Sample cleanup and enrichment procedures play an important role in the determination of PAHs in environmental samples because of matrix complexity and the low concentration of these analytes [7]. The extraction of PAHs from environmental water samples is usually performed using conventional liquid–liquid extraction (LLE) [6, 8] or solid-phase extraction (SPE) [9, 10]. However, these methods require large amounts of organic solvents that are often poisonous and hazardous, and the procedure is time consuming and tedious [11]. Efforts have focused on the miniaturization of the SPE or LLE extraction procedure by greatly reducing the amount of organic solvent required, leading to the development of solid-phase microextraction (SPME) [12, 13] or liquid-phase microextraction (LPME) [14]. Several

X. Song
Department of Chemistry, Linyi University,
276005 Linyi, China
e-mail: xlsongvv@163.com

X. Song · J. Li · C. Liao · L. Chen (✉)
Key Laboratory of Coastal Zone Environmental Processes,
Yantai Institute of Coastal Zone Research,
Chinese Academy of Sciences, Yantai,
Shandong Province 264003, China
e-mail: lxchen@yic.ac.cn

different types of LPME have been developed, such as headspace LPME [15], hollow fiber LPME (HF-LPME) [16], solvent bar LPME (SB-LPME) [17], and dynamic LPME [18], continuous-flow LPME (CF-LPME) [19], liquid-liquid-liquid microextraction (LLLME) [20], single drop microextraction (SDME) [21], and dispersive liquid-liquid microextraction (DLLME) [22]. This last method, DLLME, not only gives good extraction efficiencies but also reduces extraction time and organic solvent consumption [23]. Nevertheless, some drawbacks, such as droplet instability and relatively low precision are often reported [24]. A new DLLME based on solidification of floating organic droplets (DLLME-SFO) was developed for the determination of five PAHs in water samples [25]. Ultrasound-assisted (USA) emulsification-microextraction was performed for the determination of PAHs in water samples by checking the variables that affect their performances [26, 27].

In this work, we report the successful development of USA-DLLME coupled with gas chromatography-mass spectrometry (GC-MS) for concurrent cleanup of seawater and concentration of PAHs at trace levels. The application of sonication accelerated the mass-transfer process between two immiscible phases with DLLME, which led to a rapid increment in the extraction efficiency in a minimum amount of solvent and time. Key parameters affecting extraction efficiency of trace level PAHs in aqueous solutions were systematically evaluated. The proposed method was validated and applied successfully to the determination of different PAHs in seawater samples. Comparison with conventional DLLME was also discussed.

Experimental

Reagents and Chemicals

Stock standard solutions containing 16 kinds of congeners of PAHs (nominated by USEPA) at $500 \mu\text{g L}^{-1}$ (in dichloromethane) were purchased from Supelco (Bellefonte, PA). Their chemical structures and physicochemical property parameters are listed in Table 1. Chromatographic grade chlorobenzene, tetrachloroethylene, chloroform and methanol were all obtained from Merck (Darmstadt, Germany). These solvents were all distilled at least four times and used as extraction solvents. Analytical grade diethyl ether (DE), *tert*-butyl methyl ether (TBME) and acetone as dispersive solvents were all obtained from Shanghai Chemical Reagent (Shanghai, China). Analytical grade NaCl, NaBr, NaHCO₃, and MgSO₄·7H₂O were all purchased from Tianjin Chemical Research Institute (Tianjin, China). Doubly distilled water for preparing all aqueous solutions was produced by a Milli-Q Ultrapure water system (Millipore, Bedford, MA).

Equipment and Working Conditions

A Shimadzu GCMS-QP 2010 coupled with a split/splitless injector system was used for separation and determination of PAHs; a quadrupole mass spectrometer was operated in the electron ionization mode at 70 eV and mass spectra were acquired with a selected ion monitoring (SIM) mode. Separation was carried out on a Rtx-5MS capillary column (30 m × 0.25 mm) with a 0.25 μm stationary film thickness, 95% methyl-5% phenyl copolymer column (Shimadzu, Tokyo, Japan). The oven temperature was programmed as follows: initial 70 °C held for 2 min, increased to 90 °C (held for 4 min), afterwards increased to 290 °C at a rate of 10 °C min⁻¹, and eventually held for 20 min. The total time for one GC run was 44 min. The injector temperature was set at 300 °C, and the injection was performed in a splitless mode for 1 min. Helium (purity 99.999%) was employed as carrier gas at a flow rate was 1.0 mL min⁻¹.

A 40 kHz and 600 W US-Bath with temperature control (Lab analytical grade, KQ-300DB, Kunshan Ultrasonic Instruments, Jiangsu, China) was used for assisting the dispersion process of the micro-extraction technique. A Flying Pigeon Centrifuge (model Anke GL-12B, Shanghai Anting Scientific Instrument Factory, Shanghai, China) was used for centrifuging. Microphotographs were obtained with an Olympus XI-71 Inverted Microscope (Olympus, Optical, Tokyo, Japan). The volume of extraction phase was measured using a 250 μL syringe (SGE Analytical Science, Austin, TX). The injection volume was 1.0 μL into the GC-MS using a 10 μL syringe. Peak identification was based on the base peak and the base ions were selected as a quantitative ion (Table 1 [28–30]).

Preparation of Standards and Samples

A fresh standard solution containing the 16 kinds of PAHs was prepared at concentration levels of $100 \mu\text{g L}^{-1}$ by diluting the stock solution of $500 \mu\text{g L}^{-1}$ in dichloromethane using methanol, in which dichloromethane is soluble. Further dilutions were made in artificial seawater for 250 mL each spiked solutions, to yield the daily standard working solutions of different concentrations. All the prepared solutions were stored at 4 °C for use.

Artificial seawater comprised 24.7 g NaCl, 13.0 g MgCl₂·6H₂O, 9.0 g Na₂SO₄·10H₂O and 954 g H₂O per kg solution [31]. Surface seawater samples were collected free of air bubbles in amber glass containers from Zhanqiao Pier Beach, Shilaoren Beach and Zhanshan Beach (Qingdao, China). Once in the laboratory, seawater samples were filtrated through a 0.45 μm PTFE syringe filter membrane (Phenomenex, Torrance, CA) and analyzed within 24 h.

Table 1 Gas chromatography-mass spectrometry (GC-MS) parameters and physicochemical properties of the analyzed polycyclic aromatic hydrocarbons (PAHs)

PAHs	Structure	<i>t</i> ^a (min)	Quantitative ion (<i>m/z</i>)	b.p. (°C) ^b	log <i>K</i> _{ow} ^c [28–30]
Naphthalene (Na)		6.66	128	218	3.3
Acenaphthylene (Acy)		10.34	152	270	4.0
Acenaphthene (Ace)		10.82	154	279	3.92
Fluorene (Fl)		12.07	166	294	4.18
Phenanthrene (Phe)		14.72	178	340	4.45
Anthracene (Ant)		14.88	178	340	4.46
Fluoranthene (Flu)		19.96	202	383	5.16
Pyrene (Py)		21.08	202	404	5.18
Benz[a]anthracene (BaA)		27.93	228	435	5.91
Chrysene (Chr)		28.15	228	448	5.86
Benzo(b)fluoranthene (BbF)		34.00	252	481	6.32
Benzo[k]fluoranthene (BkF)		34.14	252	481	6.45
Benzo[a]pyrene (BaP)		35.63	252	496	6.04
Indeno[1,2,3-cd]pyrene (IPy)		42.45	276	534	6.58
Dibenz[a,h]anthracene (DahA)		42.78	278	535	6.86
Benzo[ghi]perylene (BghiP)		44.29	276	542	6.58

^a Retention times of PAHs^b Boiling point^c Octanol/water partition coefficient

USA-DLLME Procedure

A 6 mL seawater sample was placed in a 10 mL glass-centrifuge tube. Then, 0.05 mL 6.15 mol L⁻¹ NaCl and 80 µL mixture of tetrachloroethylene-diethyl ether (3:7, v/v) was added to the tube. The resulting mixture was

immersed into an ultrasonic bath for 5 min at 35 ± 2 °C. The resulting solution was centrifuged at 8,000 rpm for 5 min and then 1 µL aliquot of the tetrachloroethylene phase was removed from the conic bottom of the centrifuge tube and injected into GC-MS system for analysis. All samples were analyzed soon after preparation. The USA-

DLLME procedure is similar to that reported [32] with minor modifications.

Enrichment Factor and Extraction Recovery in USA-DLLME

The enrichment factor (EF) and extraction recovery (ER) in USA-DLLME have been defined previously by Rezaee et al. [22]. EF is the ratio of the analyte concentration in the sedimented phase (C_{sed}) to the initial concentration of analyte within the sample (C_0):

$$\text{EF} = \frac{C_{\text{sed}}}{C_0} \quad (1)$$

ER is defined as the percentage of the analyte amount extracted in the sedimented phase (n_{sed}) to the total analyte amount (n_0):

$$\text{ER} = \frac{n_{\text{sed}}}{n_0} \times 100 = \frac{C_{\text{sed}} \times V_{\text{sed}}}{C_0 \times V_{\text{aq}}} \times 100 \quad (2)$$

where, V_{sed} and V_{aq} are the volumes of sedimented phase and aqueous solution, respectively.

Results and Discussion

USA-DLLME Condition Optimization

It is well known that the extraction efficiency of USA-DLLME can be affected by various factors [33], such as types of extraction solvent, volume of extraction solvent, extraction time and temperature as well as centrifugation time. The optimization was performed for high ERs and high EFs. Samples of 6 mL of aqueous solutions containing 16 kinds of PAHs at $2 \mu\text{g L}^{-1}$ of each congener were used to investigate extraction conditions and the corresponding peak area was used to evaluate the extraction efficiency of the proposed USA-DLLME method.

Selection of Extraction Solvent

The selection of an appropriate extraction solvent is the most predominant contributing factor of the proposed method, since its physicochemical properties can account for the dispersion phenomenon and affect the extraction efficiency [32]. In the selection of extraction solvent, several factors should be considered: (1) higher density than water, (2) low solubility in water, (3) good chromatographic behavior, and (4) high extraction capability of interested compounds. Thereby, considering practical purposes [22], the following solvents were selected: chloroform (1.48 g mL^{-1}), chlorobenzene (1.10 g mL^{-1}) and tetrachloroethylene (1.63 g mL^{-1}). A series of sample solutions

were studied. The sedimented phase formed was $2.5 \mu\text{L}$ measured using a $10 \mu\text{L}$ syringe, during sonication for 5 min. To achieve similar volumes of the sedimented phase, 30, 20, and $20 \mu\text{L}$ chloroform, chlorobenzene, and tetrachloroethylene were added, respectively. The resulting organic-phase volume was measured by using a $250 \mu\text{L}$ glass syringe, and then $1 \mu\text{L}$ aliquot of this phase in the $10 \mu\text{L}$ syringe was injected for GC-MS analysis. Comparison of the peak responses obtained with the different extraction solvents (Fig. 1a) showed that tetrachloroethylene was the most effective extraction solvent. Tetrachloroethylene ($\epsilon = 2.2$) possesses relatively weaker polarity and higher solubility, therefore exhibiting higher enrichment efficiency in comparison with chlorobenzene ($\epsilon = 5.6$) and chloroform ($\epsilon = 5.2$). So, tetrachloroethylene was selected as the extraction solvent for further studies.

Disperser solvent was added to further improve the extraction efficiency of tetrachloroethylene, which was selected based on its miscibility in the organic phase and aqueous phase. Accordingly, acetone, diethyl ether, and TBME were used as disperser solvents, added to tetrachloroethylene at a ratio of 1:1, respectively. Then, $100 \mu\text{L}$ of those organic mixtures was added to the aqueous solution. Figure 1b shows that the addition of diethyl ether helped to attain the highest response for all 16 PAHs. Acetone was miscible with water so it would dissolve immediately after adding the mixed solvent to the water since the volume of disperser solvent used was too small, and the tetrachloroethylene droplets would then coagulate together, thereby leading to a relatively lower GC-MS response (Fig. 1b). To achieve good tetrachloroethylene dispersal in the sample solution, a large amount (0.5–1.0 mL) of acetone was required for the conventional DLLME [34–37]. The lower amount of diethyl ether in the tetrachloroethylene favored the dispersion of droplets, due to the lower solubility of diethyl ether in water than that in acetone, so the relative GC-MS response of PAHs using diethyl ether as a disperser solvent would be better than that using acetone (Fig. 1b). The presence of TBME in tetrachloroethylene also favored the dispersion of droplets; however, it also markedly improved the water solubility, even for the high lipophilic PAHs, which caused decreased extraction efficiency. Therefore, diethyl ether was chosen as the optimum disperser solvent.

The best ratio between diethyl ether and tetrachloroethylene was then tested. Different percentages of diethyl ether were first mixed together, and then $100 \mu\text{L}$ of those organic mixtures was added to the aqueous solution. As shown in Fig. 1c, the relative response of each PAH increases with the ratio increasing of diethyl ether ranging from 40 to 70%, which probably resulted from the corresponding decrease in volume of the sedimented tetrachloroethylene. When a higher volume ratio of DE was used,

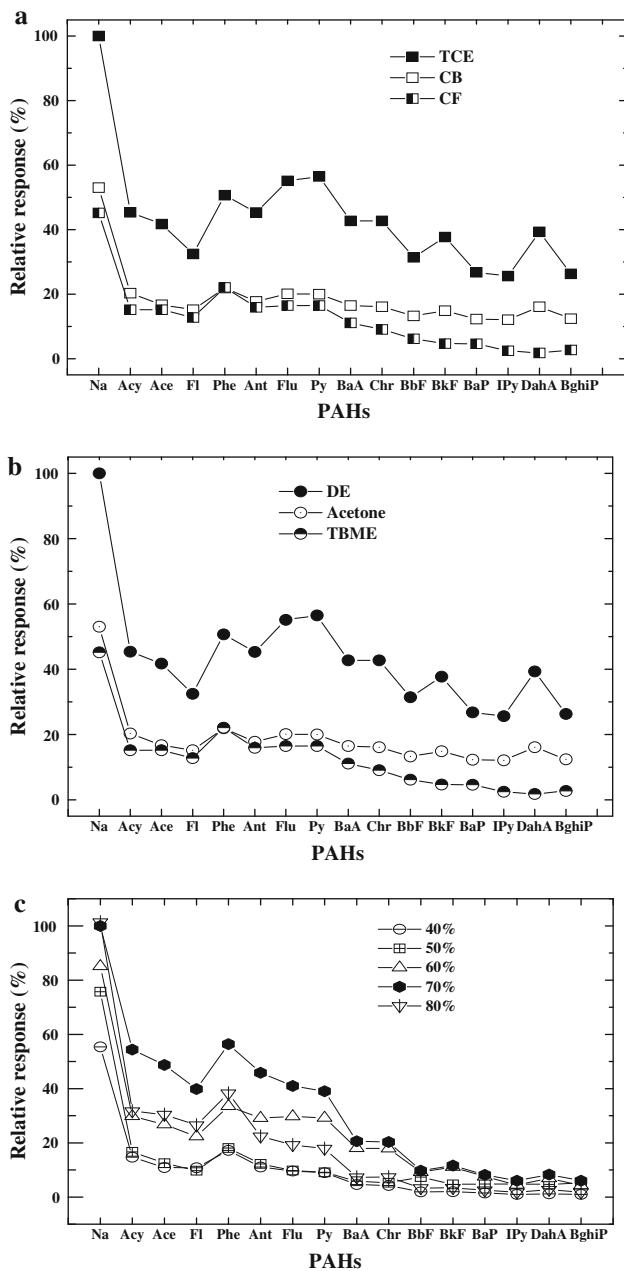


Fig. 1 Effects on gas chromatography-mass spectrometry (GC-MS) relative responses for polycyclic aromatic hydrocarbons (PAHs) of **a** extraction solvent type, **b** disperser solvent type, and **c** volume ratio of diethyl ether (DE) in mixture with tetrachloroethylene (TCE). Other extraction conditions: sample volume, 6 mL; extraction time, 5 min; centrifugation time, 3 min; sonication time, 5 min; extraction temperature, 35 °C; each of PAHs, 2 µg L⁻¹. CB chlorobenzene, CF chloroform, TBME *tert*-butyl methyl ether

up to 80%, the tiny droplets of tetrachloroethylene were dispersed extremely well in the sample solution during sonication, and the solubility of PAHs in water greatly increased, so that the extraction efficiency decreased (Fig. 1c). Thus, according to the response of all the PAHs, a mixed solvent containing 70% diethyl ether was selected

for subsequent experiments, namely the optimum volume ratio between diethyl ether and tetrachloroethylene was 7:3.

Effect of Extraction Solvent Volume

The optimal volume of the extraction solvent was also investigated. The volume of extraction solvent to be added was studied by using a mixture (tetrachloroethylene: diethyl ether, 3:7, v/v) volume within 80–110 µL, since mixed solvent volumes less than 80 µL were completely dissolved in the aqueous bulk, and were insufficient to quantitatively extract PAHs. At 80 µL, the highest relative response of the PAHs was achieved. Higher volumes reported lower relative responses due to a dilution effect of the PAHs into the resulting organic phase. Therefore, 80 µL mixture, namely 24 µL tetrachloroethylene extractant was selected for subsequent studies.

Effect of Extraction Temperature

Temperature is a critical factor that can influence markedly the extraction efficiency of PAHs from water samples. The effects result mainly from the two factors of mass transfer rates and partition coefficients [38]. The extraction efficiency would either increase or decrease depending on the dominating factor. The effect of temperature in the extraction procedures was investigated within 20–55 °C. The water solubility of PAHs diminished at lower temperatures (<35 °C), and showed lower relative response values, which indicated that the mass transfer rate was the predominant factor [39, 40]. With the increase in temperature, extraction recoveries for all the PAHs increased, with the maximum value being obtained at 35 °C, while at higher temperatures the relative response decreased markedly. Under these conditions, partition coefficients might play a predominant role [39, 40]. Especially at 55 °C, it was not possible to achieve a homogeneous dispersion because of the complete dissolution of the tetrachloroethylene into the aqueous bulk. Hence, 35 °C was finally selected for extraction.

Effect of Extraction and Centrifugation Time

The time of both extraction and centrifugation is also a crucial parameter that can affect extraction. In this method, extraction time is defined as the interval of time elapsed between the beginning of sample injection of solvent (mixture of disperser and extraction solvent) and the end of the sonication stage. The effect of extraction time (0–7 min) was tested under constant experimental conditions. Figure 2 shows the relative response of 16 PAHs versus extraction time. It was observed that by increasing

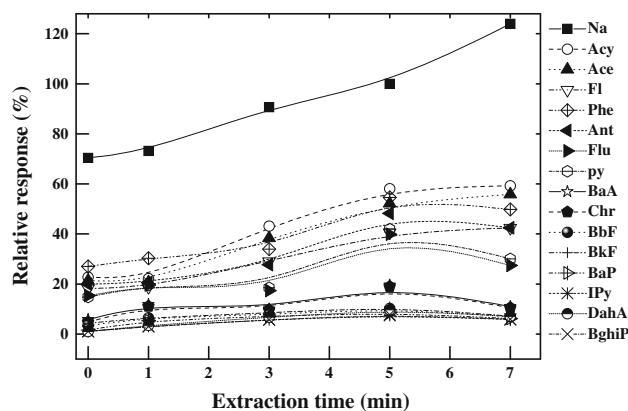


Fig. 2 Effect of extraction time on relative response for PAHs determined by ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME)-GC-MS. Extraction conditions: extraction solvent, tetrachloroethylene; disperser solvent, diethyl ether; volume ratio of diethyl ether in mixture with tetrachloroethylene, 70%; mixed solvent volume, 80 μ L. Other extraction conditions as described in Fig. 1

the extraction time, the relative response increased gradually, reaching a maximum value after 5 min, and no significant variation was given when the extraction time exceeded 5 min, except Na. Therefore, 5 min extraction time was chosen as working conditions for further studies. This is indicative of the very large surface area between extraction solvent and aqueous sample; this time-independent extraction is the most important advantage of DLLME.

The influence of centrifugation time on extraction efficiency should also be considered. Different centrifugation times, ranging from 1 to 5 min at 8,000 rpm, were assayed. Extraction recovery increased with longer centrifugation times, and 5 min was selected as the centrifugation time

necessary and sufficient to get a satisfactory biphasic system and obtain better extraction efficiency.

Additionally, sonication and vigorous stirring were compared as dispersion-assistance. By stirring the solution vigorously for 5 min, the obtained relative responses of PAHs were found to be lower than following sonication. Sonication stirring produced smaller droplets of organic solvent in the aqueous bulk than vigorous stirring, as shown in Fig. 3. During ultrasonic irradiation, implosion bubbles were generated due to the cavitation phenomenon, which produces intensive shock waves in the surrounding liquid and high-velocity liquid jets. Such microjets can cause droplet disruption in the vicinity of collapsing bubbles and thus, improve dispersion by generating a smaller droplet size of the dispersed phase right after disruption. This leads to a rapid increase in the extraction efficiency of the USA-DLLME in a short period of time [40, 41]. Thus, the sonication time for subsequent analysis was set at 5 min.

Effect of Ionic Strength

The influence of ionic strength on the USA-DLLME efficiency should be examined. For this purpose, different amounts of NaCl were added, in the range of 0–0.3 mL of 6.15 mol L^{-1} , under constant experimental conditions for testing. Figure 4 shows the relative responses of PAHs versus the addition of NaCl. Obviously, the extraction efficiency first increased upon increasing the volume of NaCl from 0 to 0.05 mL, but decreased from 0.05 to 0.2 mL, after which it remained constant. This may be because the solubility of analytes decreased with increasing salt concentration [32], but at a high concentration, microextraction was inhibited due to the increasing sedimented

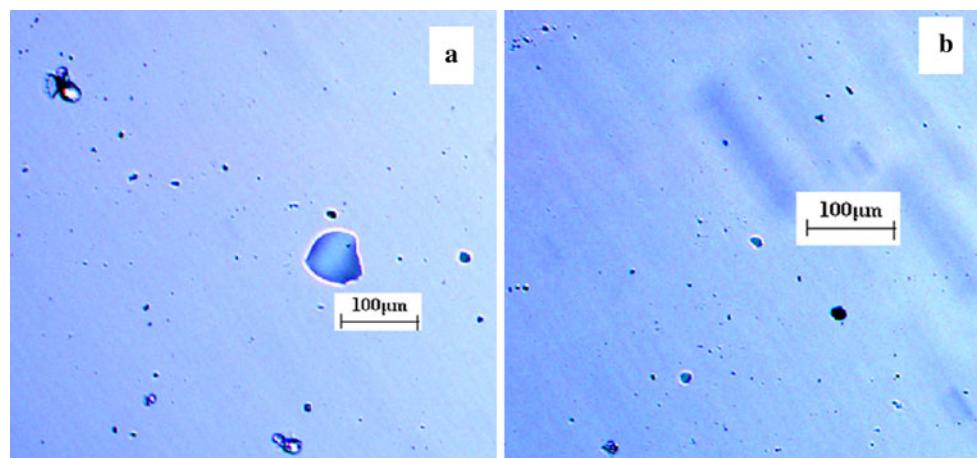


Fig. 3 Microphotographs of tetrachloroethylene droplets attained by **a** vigorously stirring, and **b** sonication stirring. Extraction time was 5 min and the other extraction conditions as described in Fig. 2

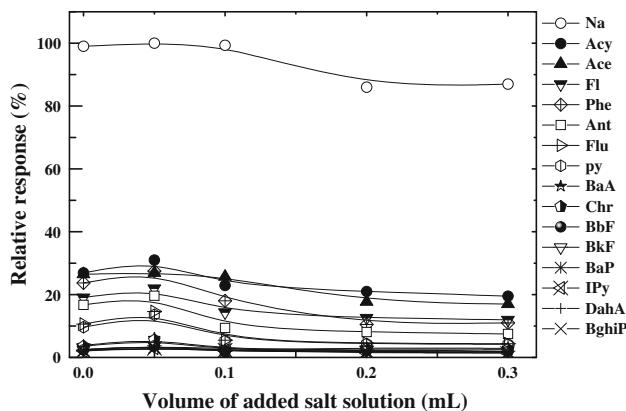


Fig. 4 Ionic strength effect on the relative response for PAHs determined by USA-DLLME-GC-MS. Other extraction conditions were as described in Fig. 3

organic phase volume and dilution effect [42]. Therefore, 0.05 mL 6.15 mol L⁻¹ NaCl was added in further experiments.

Analytical Performance

The analytical performance of the proposed USA-DLLME-GC-MS method was evaluated in terms of repeatability, linearity, correlation coefficient, detection limit and enrichment factors under optimized experimental conditions, as summarized in Table 2. The linear calibration of targeted PAHs was examined in the range of 20–2,000 ng L⁻¹, except for Acy (10–2,000 ng L⁻¹), Phe, Flu and Py (5–2,000 ng L⁻¹). Good linear behavior was seen, with coefficients of correlation (*r*) ranging from 0.979 to 0.998. The limits of detection (LODs) values of the analytes for the preconcentration of 6 mL sample volume, which were calculated as three times signal to noise ratio (S/N = 3), ranged from 1.0 to 10.0 ng L⁻¹ for synthetic seawater. The EFs of PAHs in the range of 762–8,133 were obtained. The reproducibility in peak responses was investigated on five replicate runs with the relative standard deviations (RSD%) ranging from 3.4 to 14.2% for all the 16 PAHs.

It is worth noting that the response factor of Phe and Ant in MS is the same (Table 1), while the presented analytical performance is different, e.g., different linear range (Phe: 5–2,000 ng L⁻¹; Ant: 20–2,000 ng L⁻¹) and different detection limit (Phe: 1.4 ng L⁻¹; Ant: 1.0 ng L⁻¹) (Table 2). These are understandable and acceptable results. The performance difference might be attributed to the fact that the linear relationship was established by using artificial seawater samples but not standards, which involved many interference factors, possibly leading to different linear ranges and detection limits between Phe and Ant. In addition, different linear ranges between Phe and Ant might be due to the different enrichment factors (EFs)

Table 2 Ultrasound-assisted dispersive liquid–liquid microextraction (USA-DLLME)-GC-MS analytical performance

PAHs	RSD (%) ^a	EF	Linear range (ng L ⁻¹)	<i>r</i>	LOD (ng L ⁻¹)
Na	12.6	722	20–2,000	0.995	8.9
Acy	9.7	2,439	10–2,000	0.996	2.3
Ace	3.4	2,170	20–2,000	0.998	9.5
Fl	4.9	5,804	20–2,000	0.987	5.9
Phe	4.1	8,133	5–2,000	0.986	1.4
Ant	11.5	5,091	20–2,000	0.982	1.0
Flu	7.5	6,433	5–2,000	0.991	2.1
Py	10.5	6,033	5–2,000	0.994	1.6
BaA	8.4	4,178	20–2,000	0.990	6.3
Chr	13.1	4,844	20–2,000	0.988	6.5
BbF	5.5	3,445	20–2,000	0.979	9.9
BkF	14.2	1,577	20–2,000	0.994	9.2
BaP	4.1	862	20–2,000	0.980	10.0
IPy	9.4	1,423	20–2,000	0.981	8.8
DahA	10.7	1,705	20–2,000	0.976	5.9
BghiP	12.5	1,463	20–2,000	0.993	6.6

Confidence interval 95%, *n* = 5

^a Spiked 50 ng L⁻¹ individual PAHs

caused by the great difference in solubility (Phe: 1.0–1.3 mg L⁻¹; Ant: 0.05–0.07 mg L⁻¹), namely 8,133 and 5,091 for Phe and Ant, respectively (Table 2). Different linear ranges between Phe and Ant are also reported [22], i.e., Phe within 0.02–200 µg L⁻¹ and Ant within 0.02–100 µg L⁻¹. Excitingly, the proposed EFs were far greater than those reported [22] (941 and 954 for Phe and Ant, respectively), which might indicate the much greater dispersive capability of our improved USA-DLLME by using a different dispersive solvent with a small volume.

Application to Real Samples

The USA-DLLME-GC-MS method was applied for the determination of 16 kinds of priority PAHs in seawater samples and immediately analyzed as described above, to demonstrate the applicability and reliability of the proposed trace enrichment method for environmental purposes. To estimate the matrix effect of seawater samples, all the samples were spiked with the 16 standards of individual PAH at 0.3 µg L⁻¹ to calculate the recovery of the targeted compounds. The sample results and the recovery study were performed in triplicate (Table 3). Recoveries for spiked simulated seawater were ≥87% and they were also satisfactory (≥71%) for the natural seawaters. Trace levels of PAHs, such as the endogenous contents of IPy, DahA and BghiP were detected at 0.08, 0.05 and 0.08 µg L⁻¹, respectively, in the Seawater of Zhanqiao

Table 3 Recoveries of PAHs determined by USA-DLLME-GC-MS in different seawater samples^a

PAHs	Simulated seawater		Seawater (Zhanqiao Pier Beach)		Seawater (Shilaoren Beach)		Seawater (Zhanshan Beach)	
	0.3 µg L ⁻¹ spiked		Level found (µg L ⁻¹)	0.3 µg L ⁻¹ spiked	Level found (µg L ⁻¹)	0.3 µg L ⁻¹ spiked	Level found (µg L ⁻¹)	0.3 µg L ⁻¹ spiked
	Found (\bar{x})	Recovery (%)	Found (\bar{x})	Recovery (%)	Found (\bar{x})	Recovery (%)	Found (\bar{x})	Recovery (%)
Na	0.33	120 ± 15	0.86	0.31	104 ± 12.7 ^a	1.11	0.32	106 ± 12.8
Acy	0.35	117 ± 10	0.23	0.35	118 ± 6.6	ND ^b	0.33	111 ± 13.3
Ace	0.37	124 ± 4.8	0.23	0.32	107 ± 3.1	ND	0.37	122 ± 8.5
Fl	0.29	96 ± 12	0.33	0.27	90 ± 6.1	ND	0.26	88 ± 15
Phe	0.29	107 ± 13	0.65	0.27	89 ± 8.3	ND	0.29	95 ± 11
Ant	0.28	95 ± 10	0.02	0.28	93 ± 5.9	ND	0.22	73 ± 11
Flu	0.30	99 ± 9.4	0.31	0.27	90 ± 16.4	ND	0.29	96 ± 6
Py	0.29	96 ± 7.8	0.26	0.24	81 ± 3.4	ND	0.27	91 ± 7.3
BaA	0.35	117 ± 3.1	0.08	0.32	105 ± 7.8	ND	0.34	112 ± 11.9
Chr	0.36	120 ± 2.2	0.19	0.29	97 ± 6.2	ND	0.32	107 ± 11.6
BbF	0.27	89 ± 10.8	ND	0.23	77 ± 8.1	ND	0.24	79 ± 9.5
BkF	0.29	95 ± 8	ND	0.23	78 ± 12	0.08	0.27	90 ± 3.7
BaP	0.27	91 ± 13	ND	0.26	85 ± 2.8	0.06	0.21	70 ± 13.7
IPy	0.28	92 ± 10.2	0.08	0.26	88 ± 6.5	ND	0.24	80 ± 8.2
DahA	0.29	96 ± 7	0.05	0.26	87 ± 9.8	ND	0.21	71 ± 6.5
BghiP	0.26	87 ± 12	0.08	0.21	71 ± 14.2	0.12	0.23	77 ± 7.1
							0.13	0.26
								85 ± 8

Extraction conditions are as follows: sample volume, 6 mL; 80 µL mixture of diethyl ether and tetrachloroethylene (7:3, v/v); 5 min extraction time; 5 min centrifugation time; sonication time, 5 min; extraction temperature, 35 °C

^a Recovery (%) = [(total amount of detected-amount of endogenous PAHs)/amount of added] × 100; data are expressed as the mean ± SD determined from triplicate independent experiments

^b Not detected

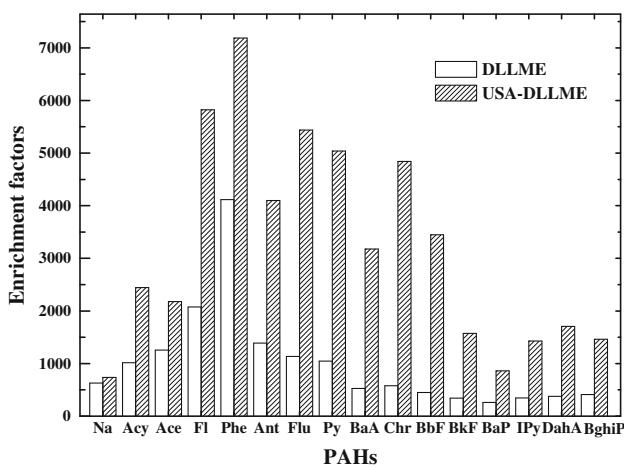


Fig. 5 Comparison of enrichment factors (EFs) between USA-DLLME and DLLME. Extraction conditions: sample volume, 6 mL; extraction solvent, tetrachloroethylene; disperser solvent, diethyl ether; volume ratio of diethyl ether in mixture with tetrachloroethylene, 70%; the mixed solvent volume, 80 µL; centrifugation time, 3 min; sonication time, 5 min; extraction temperature, 35 °C; each of PAHs, 2 µg L⁻¹. Except 0.5 mL acetone containing 6 µL of tetrachloroethylene for DLLME without sonication, and 80 µL of the mixture solvents (diethyl ether: tetrachloroethylene = 7:3, v/v) USA-DLLME with 5 min sonication time

Pier Beach. The results also indicated that matrix effects could be reduced by virtue of the USA-DLLME procedure. It was also observed that BbF, BkF and BaP were not detected in the analyzed seawater samples of Zhanqiao Pier Beach.

Comparison between USA-DLLME and DLLME

The performance of the proposed USA-DLLME method in PAHs microextraction and determination from water samples was compared with the corresponding performance of conventional DLLME with reference to enrichment factors. It can be observed that the extraction efficiency of USA-DLLME was markedly higher than that of conventional DLLME for PAH determination (Fig. 5). Disperse solvent improved the dispersion of extraction solvent in a sample solution; however, the large amounts of solvents required for conventional DLLME would also worsen extraction efficiency. Therefore, the USA-DLLME technique, in which the sonication of a little dispersive solvent assisting in optimizing dispersal and favoring extraction, resulting in better extraction performance, becomes more competitive. Moreover, USA-DLLME does

not involve any labor intensive or time-consuming steps. It is demonstrated to be a simple, rapid, inexpensive, easy to use and environmentally friendly technique.

Conclusions

In this work, a USA-DLLME procedure was developed as an efficient microextraction technique based on the emulsion phenomenon induced by sonication, which was applied satisfactorily to the trace level determination of PAHs in real seawater samples combined with GC–MS. Under optimized working conditions, high enrichment factors were obtained for the targeted PAHs over a wide linear range, thereby reaching lower detection limits with acceptable precision. Additionally, because of the low organic solvent consumption, the presented USA-DLLME proved an inexpensive and environmentally friendly technique. The robustness was proved when the recovery study was carried out over several real samples. Also, it appears to be a time-saving technique, especially for laboratories performing analysis of a large number of samples with a rapid reporting time. The developed USA-DLLME technique thus shows great potential to determine trace levels of persistent organic pollutants (POPs) in environmental matrices in routine analysis, and will contribute greatly to improving sample throughput in this analytical method in future.

Acknowledgments This work was supported by the Department of Science and Technology of Shandong Province of China (2008GG20005005, 2010GSF10222), the Natural Science Foundation of China (20907039), the Natural Science Foundation of Shandong Province of China (Y2007B38), Yantai Research and Development Program (2007323) and the 100 Talents Program of the Chinese Academy of Sciences.

References

- Kennish MJ (2002) Environmental threats and environmental future of estuaries. *Environ Conserv* 29:78–107. doi:[10.1017/S0376892902000061](https://doi.org/10.1017/S0376892902000061)
- Nemr AE, Abd-Allah AMA (2003) Chemosphere 52:1711–1716. doi:[10.1016/S0045-6535\(03\)00300-X](https://doi.org/10.1016/S0045-6535(03)00300-X)
- Yan J, Wang L, Fu PP, Yu H (2004) Mutat Res 557(1):99–108. doi:[10.1016/j.mrgentox.2003.10.004](https://doi.org/10.1016/j.mrgentox.2003.10.004)
- Zander M (1986) Book review: spectral atlas of polycyclic aromatic compounds. In: Karcher W, Fordham RJ, Dubois JJ, Claude PGJM, Lighthart JAM (eds) *Angewandte Chemie International Edition* in English, 25:379–380. doi:[10.1002/anie.198603791](https://doi.org/10.1002/anie.198603791)
- Kursheva AV, Litvinenko IV, Petrova VI, Galishev MA (2009) Oceanology 49:655–662. doi:[10.1134/S0001437009050063](https://doi.org/10.1134/S0001437009050063)
- Puig D, Barcelo D (1996) Trends Anal Chem 15:362–375. doi:[10.1016/0165-9936\(96\)00057-X](https://doi.org/10.1016/0165-9936(96)00057-X)
- Zhao XN, Fu LY, Hu J, Li JW, Wang HL, Huang CJ, Wang XD (2009) Chromatographia 69:1385–1389. doi:[10.1365/s10337-009-1099-7](https://doi.org/10.1365/s10337-009-1099-7)
- Yasuhara A, Shiraishi H, Nishikawa M, Yamamoto T, Uehiro T, Nakasugi O, Okumura T, Kenmotsu K, Fukui H, Nagase M, Ono Y, Kawagoshi Y, Baba K, Noma Y (1997) J Chromatogr A 774:321–332. doi:[10.1016/S0021-9673\(97\)00078-2](https://doi.org/10.1016/S0021-9673(97)00078-2)
- Murillo-Tovar MA, Amador-Munoz O, Villalobos-Pietrini R, Marriott PJ (2010) Chromatographia 72:913–921. doi:[10.1365/s10337-010-1738-z](https://doi.org/10.1365/s10337-010-1738-z)
- Ma JP, Xiao RH, Li JH, Yu JB, Zhang YQ, Chen LX (2010) J Chromatogr A 1217:5462–5469. doi:[10.1016/j.chroma.2010.06.060](https://doi.org/10.1016/j.chroma.2010.06.060)
- Nicholson BC, Bursill DB, Couche DJ (1985) J Chromatogr A 325:221–230. doi:[10.1016/S0021-9673\(00\)96022-9](https://doi.org/10.1016/S0021-9673(00)96022-9)
- Bagheri H, Salemi A (2004) Chromatographia 59:501–505. doi:[10.1365/s10337-004-0226-8](https://doi.org/10.1365/s10337-004-0226-8)
- Hutchinson JP, Setkova L, Pawliszyn J (2007) J Chromatogr A 1149:127–137. doi:[10.1016/j.chroma.2007.02.117](https://doi.org/10.1016/j.chroma.2007.02.117)
- Jeannot MA, Cantwell FF (1996) Anal Chem 68:2236–2240. doi:[10.1021/ac960042z](https://doi.org/10.1021/ac960042z)
- Tankeviciute A, Kazlauskas R, Vickackaite V (2001) Analyst 126:1674–1677. doi:[10.1039/B103493F](https://doi.org/10.1039/B103493F)
- Pedersen-Bjergaard S, Rasmussen KE (2004) Trends Anal Chem 23:1–10. doi:[10.1016/j.jchromb.2004.08.034](https://doi.org/10.1016/j.jchromb.2004.08.034)
- Jiang XM, Lee HK (2004) Anal Chem 76:5591–5596. doi:[10.1021/ac040069f](https://doi.org/10.1021/ac040069f)
- Wu JM, Ee KH, Lee HK (2005) J Chromatogr A 1082:121–127. doi:[10.1016/j.chroma.2005.05.077](https://doi.org/10.1016/j.chroma.2005.05.077)
- Li Y, Zhang T, Liang P (2005) Anal Chim Acta 536:245–249. doi:[10.1016/j.aca.2004.12.033](https://doi.org/10.1016/j.aca.2004.12.033)
- Jiang XM, Basheer C, Zhang J, Lee HK (2005) J Chromatogr A 1087:289–294. doi:[10.1016/j.chroma.2005.06.010](https://doi.org/10.1016/j.chroma.2005.06.010)
- Ahmadi F, Assadi Y, Hosseini MRM, Rezaee M (2006) J Chromatogr A 1101:307–312. doi:[10.1016/j.chroma.2005.11.017](https://doi.org/10.1016/j.chroma.2005.11.017)
- Rezaee M, Assadi Y, Hosseini MRM, Aghaei E, Ahmadi F, Berijani S (2006) J Chromatogr A 1116:1–9. doi:[10.1016/j.chroma.2006.03.007](https://doi.org/10.1016/j.chroma.2006.03.007)
- Farajzadeh MA, Bahram M, Jafary F, Bamorowat M (2011) Chromatographia 73:393–401. doi:[10.1007/s10337-010-1895-0](https://doi.org/10.1007/s10337-010-1895-0)
- Xu L, Basheer C, Lee HK (2007) J Chromatogr A 1152:184–192. doi:[10.1016/j.chroma.2006.10.073](https://doi.org/10.1016/j.chroma.2006.10.073)
- Xu H, Ding ZQ, Lv LL, Song DD, Feng YQ (2009) Anal Chim Acta 636:28–33. doi:[10.1016/j.aca.2009.01.028](https://doi.org/10.1016/j.aca.2009.01.028)
- Ozcan S, Tor A, Aydin ME (2010) Anal Chim Acta 665(2):193–199. doi:[10.1016/j.aca.2010.03.047](https://doi.org/10.1016/j.aca.2010.03.047)
- Saleh A, Yamini Y, Faraji M, Rezaee M, Ghambanian M (2009) J Chromatogr A 1216(39):6673–6679. doi:[10.1016/j.chroma.2009.08.001](https://doi.org/10.1016/j.chroma.2009.08.001)
- Mackay D, Shiu WY, Ma KC, Lee SC (2006) *Handbook of physical–chemical properties and environmental fate for organic chemicals*, 2nd edn. Lewis, Boca Raton
- Crunkilton RL, de Vita WM (1997) Chemosphere 35(7):1447–1463. doi:[10.1016/S0045-6535\(97\)00217-8](https://doi.org/10.1016/S0045-6535(97)00217-8)
- Manoli E, Samara C (1999) Trends Anal Chem 18:417–428. doi:[10.1016/S0165-9936\(99\)00111-9](https://doi.org/10.1016/S0165-9936(99)00111-9)
- Zhang ZB, Liu LS (1989) *Marine physical chemistry*. Science, Beijing
- Fontana AR, Wuilloud RG, Martínez LD, Altamirano JC (2009) J Chromatogr A 1216:147–153. doi:[10.1016/j.chroma.2008.11.034](https://doi.org/10.1016/j.chroma.2008.11.034)
- Regueiro J, Llompart M, Psillakis E, Monteagudo JCG, Jares CG (2009) Talanta 79:1387–1397. doi:[10.1016/j.talanta.2009.06.015](https://doi.org/10.1016/j.talanta.2009.06.015)
- Farajzadeh MA, Bahram M, Jönsson JÅ (2007) Anal Chim Acta 591:69–79. doi:[10.1016/j.aca.2007.03.040](https://doi.org/10.1016/j.aca.2007.03.040)
- Farajzadeh MA, Bahram M, Mehr BG, Jönsson JÅ (2008) Talanta 75:832–840. doi:[10.1016/j.talanta.2007.12.035](https://doi.org/10.1016/j.talanta.2007.12.035)
- Liu Y, Zhao E, Zhu W, Gao H, Zhou Z (2009) J Chromatogr A 1216:885–891. doi:[10.1016/j.chroma.2008.11.076](https://doi.org/10.1016/j.chroma.2008.11.076)

37. Xiong CM, Ruan JL, Cai YL, Tang Y (2009) J Pharm Biomed Anal 49(2):572–578. doi:[10.1016/j.jpba.2008.11.036](https://doi.org/10.1016/j.jpba.2008.11.036)
38. Kuramochi H, Maeda K, Kawamoto K (2007) Chemosphere 67:1858–1865. doi:[10.1016/j.chemosphere.2006.05.076](https://doi.org/10.1016/j.chemosphere.2006.05.076)
39. Berijani S, Assadi Y, Anbia M, Milani HMR, Aghaei E (2006) J Chromatogr A 1123:1–9. doi:[10.1016/j.chroma.2006.05.010](https://doi.org/10.1016/j.chroma.2006.05.010)
40. Luque de Castro MD, Priego-Capote F (2007) Talanta 72:321–334. doi:[10.1016/j.talanta.2006.11.013](https://doi.org/10.1016/j.talanta.2006.11.013)
41. Luque de Castro MD, Priego-Capote F (2006) Analytical applications of ultrasound. Elsevier, Amsterdam
42. Wu YL, Dai LP, Cheng J, Guo F, Li YK (2010) Chromatographia 72:695–699. doi:[10.1365/s10337-010-1719-20009-5893/10/10](https://doi.org/10.1365/s10337-010-1719-20009-5893/10/10)