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Optimization of two different dispersive liquid–liquid microextraction methods followed by gas chromatography–mass spectrometry determination for polycyclic aromatic hydrocarbons (PAHs) analysis in water

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ABSTRACT

Novel sample preparation methods termed "up-and-down shaker-assisted dispersive liquid – liquid microextraction (UDSA – DLLME)" and "water with low concentration of surfactant in dispersed solvent-assisted emulsion dispersive liquid–liquid microextraction (WLSEME)" coupled with gas chromatography–mass spectrometry (GC–MS) have been developed for the analysis of 11 polycyclic aromatic hydrocarbons (PAHs) in aqueous samples. For UDSA–DLLME, an up-and-down shaker-assisted emulsification was employed. Extraction was complete in 3 min. Only 14 μL of 1-heptanol was required, without a dispersive solvent. Under the optimum conditions, the linear range was 0.08 – 100 μg L^{-1}, and the LODs were in the range 0.022 – 0.060 μg L^{-1}. The enrichment factors (EFs) ranged from 392 to 766. Relative recoveries were between 84% and 113% for river, lake, and field water. In WLSEME, 9 μL of 1-nonanol as extraction solvent and 240 μL of 1 mg L^{-1} Triton X-100 as surfactant were mixed in a microsyringe to form a cloudy emulsified solution, which was then injected into the samples. Compared with other surfactant-assisted emulsion methods, WLSEME uses much less surfactant. The linear range was 0.08 – 100 μg L^{-1}, and the LODs were 0.022 – 0.13 μg L^{-1}. The EFs ranged from 388 to 649. The relative recoveries were 86 – 114% for all three water specimens.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are produced by the burning of plants, volcanic eruption, and as by-products from the burning of fuel, most notably coal. Many PAHs are dangerous to human health, due to their carcinogenicity and mutagenicity. The PAH benzo[*a*]pyrene has been shown to be a potent chemical carcinogen [1]. Thus, the U.S. Environmental Protection Agency (EPA) and the World Health Organization (WHO) have stipulated maximum permissible concentrations of 0.2 μg L^{-1} and 0.7 μg L^{-1}, respectively, for benzo[*a*]pyrene in drinking water [2,3]. It is important to develop a sensitive and simple pre-concentration method for the determination of PAHs in the environment.

Conventional extraction methods such as liquid – liquid extraction (LLE) and solid-phase extraction (SPE) require large volumes of organic solvents and are time-consuming. To address these drawbacks, solid-phase microextraction (SPME) has been developed [4,5]. SPME uses no extraction solvent, but the lifetime of the fiber is limited and it is fragile. Recently, liquid-phase microextraction (LPME) has been introduced for sample preparation. It has been developed as many variants, such as single-drop microextraction (SDME) [6], hollow-fiber-protected liquid-phase microextraction (HF-LPME) [7], and solvent bar microextraction (SBME) [8]. All of these techniques use less organic solvents and have good sensitivity. However, long extraction times are required to reach satisfactory limits of detection (LOD). In 2006, Assadi et al. developed dispersive liquid–liquid microextraction (DLLME) [9]. It represents a rapid and low-cost method. However, it requires toxic halogenated extraction solvents and large amounts of dispersive solvent.

In order to improve DLLME, many kinds of emulsification methods have been applied, such as ultrasound-assisted emulsification microextraction (USAEME) [10], vortex-assisted liquid – liquid microextraction (VALLME) [11], manual shaking and ultrasound-assisted emulsification microextraction (MS-USAEME) [12–14], and a new up-and-down shaker-assisted dispersive liquid – liquid microextraction (UDSA–DLLME) [15,16]. These methods show good

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emulsification performance and afford high extraction efficiencies with small volumes of extraction solvent.

The dispersive solvents used for DLLME are typically methanol, acetone, and acetonitrile, which are not friendly to the environment. In 2010, Wang and co-workers developed surfactant-assisted dispersive liquid–liquid microextraction (SA-DLLME) [17]. In this method, surfactant solution is used in place of an organic solvent as the dispersive medium. The new proposed method, water with low concentration of surfactant in dispersed solvent-assisted emulsion dispersive liquid–liquid microextraction (WLSEME) [18], requires a much smaller amount of surfactant (1.1×10^{-8} M) than other surfactant-assisted emulsification methods (approximately 10^{-5} to 10^{-4} M). The extraction solvent is dispersed in a surfactant solution first, and then injected the mixture into an aqueous sample to extract target compounds. According to the previous data, high enrichment factors were obtained by WLSEME. The extraction completes in a very short time. Therefore, the extraction time is considered as no effect [18].

In the present study, UDSA–DLLME and WLSEME, both combined with an improved solvent collection system for dispersive liquid–liquid microextraction (ISCS-DLLME), have been employed to analyze PAHs in aqueous samples. The goal is to develop sensitive and green methods that are able to solve the common issues, including degradation of analytes, increased solubility of the targets and extraction solvents in aqueous solution, and the use of toxic extraction solvents and large volumes of dispersive solvents in DLLME. The two methods have been optimized and compared with each other and with other assisted emulsification methods such as vortex, ultrasound, and manual shaking with ultrasound assistance.

2. Experiments

2.1. Material and reagents

Acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), fluoranthene (Flt), and benzo[*b*]fluoranthene (BbF) were purchased from Supelco (Bellefonte, PA, USA). Anthracene (Ant), pyrene (Pyr), benzo[*a*]anthracene (BaA), and indeno [1,2,3-cd]pyrene (Ipy) were purchased from Accustandard, Inc. (New Haven, CT, USA). Benzo[*a*]pyrene (BaP) was purchased from Chem Service (West Chester, PA, USA). The above-mentioned PAHs were of analytical grade. 1-Hexanol, 1-heptanol, 1-octanol, 1-nonanol, Tween 60, Tween 80, Triton X-100, and Triton X-114 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol (LC–MS grade) was purchased from J.T. Baker (Phillipsburg, NJ, USA). Acetone (HPLC grade) was obtained from Merck (Darmstadt, Germany). Sodium chloride was obtained from Merck (Darmstadt, Germany). Deionized (D.I.) water was purified on a Milli-Q water system (Millipore, Bedford, MA, USA).

Stock solutions of acenaphthene, benzo[*b*]fluoranthene, and indeno[1,2,3-cd]pyrene were prepared in methanol at a concentration of 200 mg L^{-1}. The other PAHs were diluted in methanol to a concentration of 100 mg L^{-1}. The solutions were stored in a refrigerator at 4 °C. A standard working solution was prepared by diluting the stock solutions to 10 mg L^{-1} of each analyte in methanol. To optimize the conditions, the working solution was diluted with DI water to 10 μg L^{-1}. River water (Hsinchu, Taiwan), lake water (National Tsing Hua University, Hsinchu, Taiwan), and field water samples (Hsinchu, Taiwan) were filtered through a 0.45 μm nylon membrane filter (Millipore, Bedford, MA, USA) prior to analysis and stored at 4 °C.

2.2. Instrumentation

The up-and-down shaker (model FS-6) used was designed in our laboratory and made by Sunway Scientific Corporation (Hsinchu, Taiwan); the holder was customized to hold conical glass tubes. The vortex agitator used was a Vortex genie® 2 mixer model no. G560 (Scientific Industries, USA). An ultrasonic cleaner model no. B5510DTH (Scientific Industries, USA) was also used. Samples were centrifuged with a model no. CN-2200 centrifuge from Hsiantai Machinery Industry (Hsinchu, Taiwan) or a DVC-12060-C00 miVac desktop centrifuge (Stockholm, Sweden). The ISCS system used microtubes designed in-house (15×3 mm^2; inner diameter, 1.8 mm; total volume, 38 μL; Qing-Fa Company, Hsinchu, Taiwan) in separating the aqueous and organic phases..

A Varian (Walnut Creek, CA, USA) CP-3800 gas chromatograph was equipped with a 1079 injector in splitless mode and set at

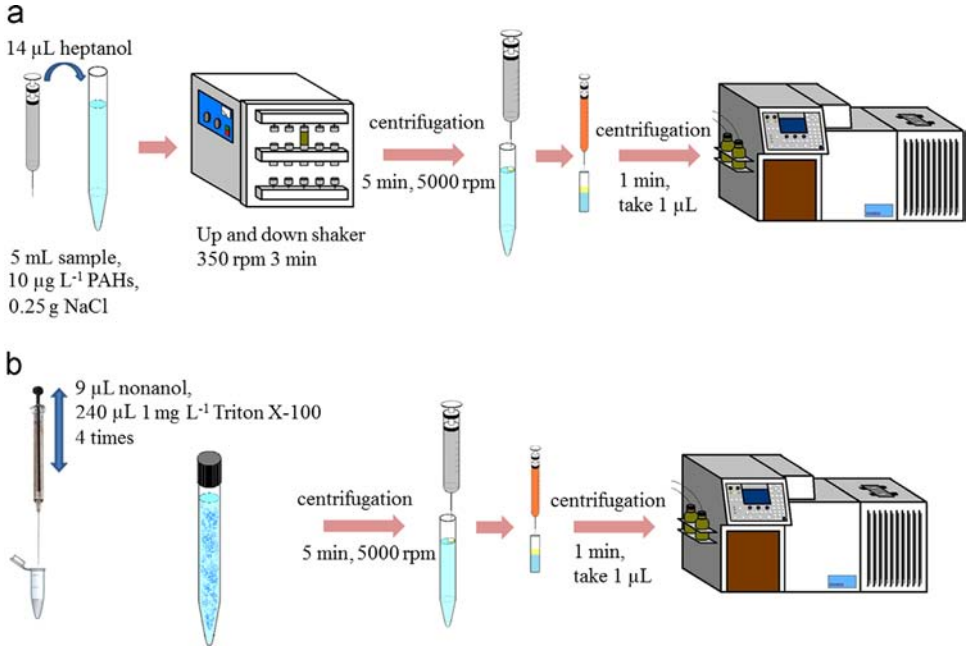

Fig. 1. Diagrammatic sketch: (a) UDSA–DLLME and (b) WLSEME.

270 °C. A DB-5 MS UI (30 m, 0.25 mm I.D., 0.25 μm film thickness) fused silica capillary column from J&W Scientific (Folsom, CA, USA) was used. The carrier gas was helium (purity 99.9995%) at a constant flow rate of 1 mL min^{-1}. The oven temperature was initially held at 90 °C for 1 min, increased to 162 °C at 30 °C min^{-1} and held for 4 min, then raised to 185 °C at 30 °C min^{-1} and held for 5 min, and finally increased to 300 °C at 30 °C min^{-1} and held for 8 min; the total analytical time was thus 26 min. A Varian Saturn 2000 ion-trap GC–MS system was used for unit resolution selected ion storage (uSIS). The main ions selected for quantitative determination were the following: Acy *m*/*z* 152, 153, 151; Ace *m*/*z* 153, 154, 152; Flu *m*/*z* 166, 165, 163; Phe and Ant *m*/*z* 178, 179, 176; Flt and Pyr *m*/*z* 202, 203, 200; BaA *m*/*z* 228, 229, 226; BbF and BaP *m*/*z* 252, 253, 250; and Ipy *m*/*z* 276, 277, 274. The ion-trap mass spectrometer was operated in electron ionization (70 eV) mode; the spectrum was scanned over the range *m*/*z* 100–300 and the source temperature was set at 220 °C.

2.3. Extraction procedure for UDSA–DLLME

As shown in Fig. 1(a), an 8 mL conical-bottomed glass tube was charged with a 5 mL water sample containing 10 μg L^{-1} of PAHs and NaCl (0.25 g) and then 1-heptanol (14 μL) was rapidly injected into the sample solution by means of a Hamilton 25 μL syringe (Reno, NV, USA). The tube was then placed in a tube holder and shaken by the up-and-down shaker for 3 min at a rate of 350 rpm to emulsify the solution and form the cloudy state. After centrifugation at 5000 rpm for 5 min, the organic phase floating on the surface of the solution was collected in a microtube by means of a 10 μL SGE (Australia) microsyringe. The floating phase was transferred to a microtube (15×3 mm^2) by microsyringe. The floating phase was further centrifuged at 12,000*g* for 1 min. The organic phase was easily collected and recovered in the upper portion of the microtube. The whole transference was done quickly and carefully using the gas-tight microsyringe. The floating phase was approximately 2.8 ± 0.1 μL, of which 1 μL was injected into the GC–MS for analysis. Three replicates for each trial where performed.

2.4. Extraction for WLSEME

In Fig. 1(b), 1-nonanol (9 μL; as an extraction solvent) and 1 mg L^{-1} Triton X-100 (240 μL; as a surfactant) were mixed in an eppendorf. Then, a Hamilton 500 μL microsyringe (Reno, NV, USA) was used to withdraw and discharge the mixture back and forth four times in 10 s. A cloudy emulsified solution was formed in the syringe, which was then injected into a 5 mL water sample containing 10 μg L^{-1} of PAHs and NaCl (0.25 g) in an 8 mL conical-bottomed glass tube. The floating phase was transferred to a microtube using a 10 μL SGE (Australia) microsyringe after centrifugation at 5000 rpm for 5 min. The floating phase was transferred to a microtube (15×3 mm^2) by microsyringe. The floating phase was further centrifuged at 12,000 *g* for 1 min. The volume was approximately 3.0 ± 0.1 μL, of which 1 μL was injected into the GC–MS for analysis. Three replicates for each trial where performed.

3. Results and discussion

Experimental parameters for UDSA–DLLME, including the type and volume of extraction solvent, salt addition, and extraction time, were optimized. For WLSEME, the extraction solvent, type of surfactant, volume of extraction solvent, concentration of surfactant solution, volume of surfactant solution, and salt addition were considered.

Enrichment factors (EFs) were calculated as the ratio of the concentrations in the floating phase after extraction to the initial concentrations of analytes in the aqueous solution.

3.1. Optimization of UDSA–DLLME

3.1.1. Type and volume of extraction solvent

The selection of an extraction solvent is crucial for extraction efficiency. An appropriate extraction solvent should have certain properties, including: (a) immiscibility with water, (b) good extraction efficiency for analytes, (c) low toxicity, and (d) good chromatographic performance. On this basis, we examined the use of 1-hexanol, 1-heptanol, 1-octanol, and 1-nonanol as extraction solvents. As the solubilities of these solvents are different, if identical amounts were added, the final volumes floating on water would be different. To avoid a dilution effect, different volumes of extraction solvent were added to obtain a final volume of the organic phase of approximately 3.6 μL. The amounts of the different extraction solvents used were 1-hexanol (33 μL), 1-heptanol (14 μL), 1-octanol (12 μL), and 1-nonanol (10 μL). After centrifugation, 3.9, 3.2, 3.2, and 4.0 μL, respectively, of floating organic phase was collected. 1-Heptanol gave the best extraction efficiency, as shown in Fig. 2(a). Consequently, 1-heptanol was used as extraction solvent in the following experiments.

Different volumes (14, 16, and 18 μL) of 1-heptanol were tested to determine the optimum volume. As the amount of extraction solvent was increased, the volume of the floating phase also increased, which led to dilution of the analytes. The highest EFs were obtained with 14 μL of 1-heptanol. When the volume of the

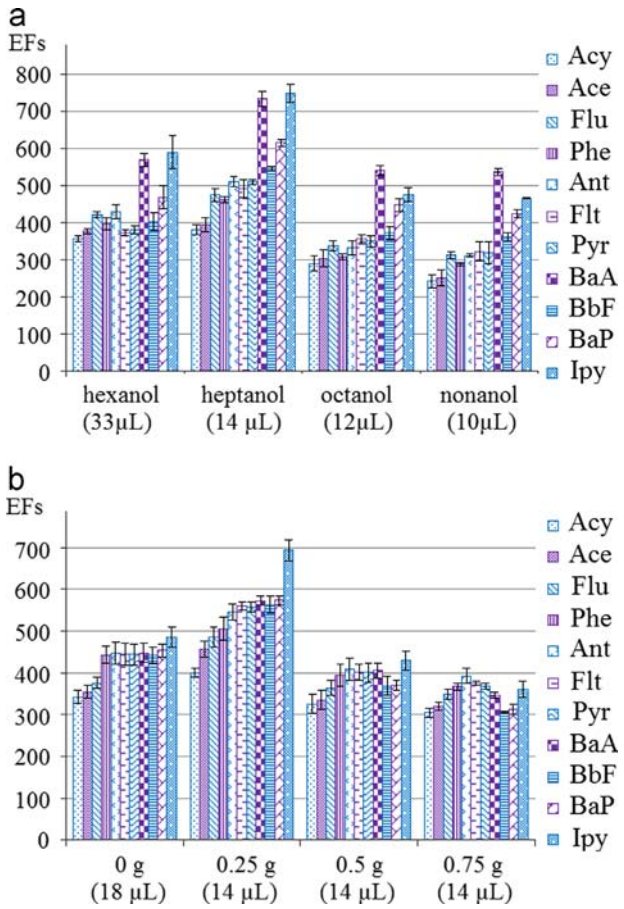

Fig. 2. (a) Effect of type of extraction solvent ($n=3$) using UDSA–DLLME. Extraction conditions: 0.25 g NaCl; shaking 3 min and (b) effect of salt (NaCl) ($n=3$). Extraction conditions: 1-heptanol; shaking 3 min.

extraction solvent was less than 14 μL, the volume of collected organic phase was too small to withdraw into the microsyringe. The results also showed poor precision. Finally, 14 μL was chosen as the amount of extraction solvent.

3.1.2. Salt addition (NaCl)

Addition of a salt increases the ionic strength of the aqueous solution and improves the extraction efficiency owing to the salting-out effect [19]. The effect of salt was studied by adding 0, 0.25, 0.50, and 0.75 g of NaCl to 5 mL water samples. The results are shown in Fig. 2(b); the EFs increased as the amount of salt added was increased from 0 to 0.25 g. However, when the amount of salt added was increased beyond 0.25 g, the EFs decreased because the volume of the floating phase also increased. After centrifugation, the floating organic phases amounted to 3.5 μL, 2.9 μL, 4.7 μL, and 6.0 μL. The results indicated that adding 0.25 g of salt yielded better EFs. Therefore, 0.25 g of NaCl was selected.

3.1.3. Extraction time

In DLLME, fine droplets of organic solvent are generated in the sample solution, allowing easier mass transfer of analytes from the aqueous phase to the extraction solvent. Here, homogeneous fine droplets were generated reproducibly with the aid of the shaker without the need for a dispersive solvent. The impact of shaking time was evaluated over the range 0–5 min. The extraction time is defined as the shaking time. The speed of the up-and-down shaker was 350 rpm (maximum setting). The EFs were significantly enhanced with longer times. As good EFs and good precision were obtained after shaking for 3 min, this was considered as the optimal extraction time.

3.2. Optimization of WLSEME

3.2.1. Extraction solvent

The selection of extraction solvent is an important parameter for the extraction efficiency. The alcohols 1-heptanol, 1-octanol, and 1-nonanol were applied here. To consistently collect 3.1 μL of floating organic phase, different amounts of extraction solvent had to be added, specifically 1-heptanol (16 μL), 1-octanol (13 μL), and 1-nonanol (11 μL), and subsequently mixed with 180 μL Triton X-100 (1 mg L^{-1}) in a microsyringe first before injecting the mixture into a sample solution. The floating phases collected amounted to 3.0 μL, 3.2 μL, and 3.1 μL, respectively. The results are shown in Fig. 3(a) and reveal that in the presence of Triton X-100 the EFs with 1-nonanol were the highest among the three solvents. This may result from less 1-nonanol added initially, which led to higher extraction efficiency. As a result, 1-nonanol was chosen as the extraction solvent for further experiments.

3.2.2. Type of surfactant

For WLSEME, an ultra-low concentration of surfactant solution is required compared with surfactant-assisted DLLME. Selection of an appropriate surfactant is crucial in assisting the formation of organic drops and thereby affects the extraction. A suitable hydrophilic–lipophilic balance (HLB) is the primary criterion for the selection of a surfactant. When the HLB of a surfactant lies between 12 and 16, the surfactant is considered as an appropriate oil-in-water emulsifier. Thus, the nonionic surfactants Tween 60, Tween 80, Triton X-100, and Triton X-114 were chosen for evaluation in the experiments. Triton X-100 showed the best extraction efficiency, and was thus selected as the optimal surfactant (Fig. 3(b)).

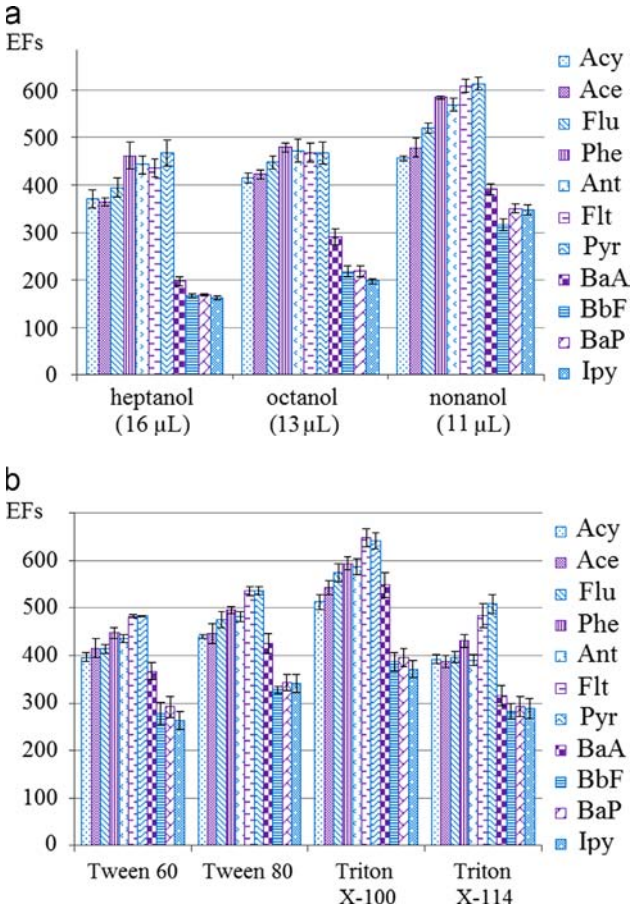

Fig. 3. (a) Effect of type of extraction solvent ($n=3$) using WLSEME. Extraction conditions: 0.25 g NaCl; 180 μL 1 mg L^{-1} Triton X-100 and (b) effect of type of surfactant solvent ($n=3$). Extraction conditions: 11 μL 1-heptanol; 0.25 g NaCl; 180 μL 1 mg L^{-1} Triton X-100.

3.2.3. Volume of extraction solvent

A range of volumes of the extraction solvent (9, 11, 13, and 15 μL) were tested. When the solvent volume was less than 9 μL, the amount of organic phase was insufficient for analysis. As the volume of the extraction solvent was increased, the volume of the floating phase increased accordingly. This resulted in dilution of the extractants. The EFs were maximized when the solvent volume was 9 μL. Hence, 9 μL was selected as the extraction solvent volume.

3.2.4. Concentration of surfactant

The concentration of Triton X-110 solution was varied over the range 0.2–100 mg L^{-1}. As shown in Fig. 4(a), the EFs improved as the concentration of surfactant was increased. This result might have been due to more efficient dispersion of the organic solvent in the aqueous sample, leading to rapid partitioning of analytes between the aqueous phase and extraction solvent. On the basis of eco-awareness and extraction efficiency, the optimal concentration of surfactant in the aqueous solution for further experiments was selected as 1 mg L^{-1}.

3.2.5. Volume of surfactant

When the surfactant volume is sufficient, dispersion may be complete. However, with excess amounts of aqueous surfactant, the extraction efficiency may decrease because the analytes in aqueous solution become more soluble. To study the effect of volume of surfactant in the aqueous solution, different volumes

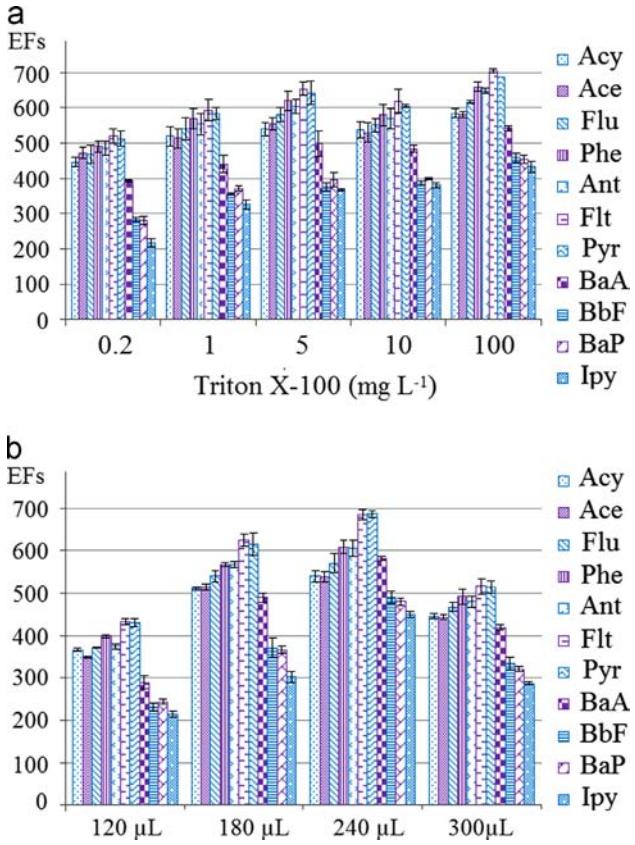

Fig. 4. (a) Effect of concentration of surfactant ($n=3$). Extraction conditions: 9 μL 1-heptanol; 0.25 g NaCl; 180 μL Triton X-100 and (b) effect of volume of surfactant ($n=3$). Extraction conditions: 9 μL 1-heptanol; 0.25 g NaCl; 1 mg L^{-1} Triton X-100.

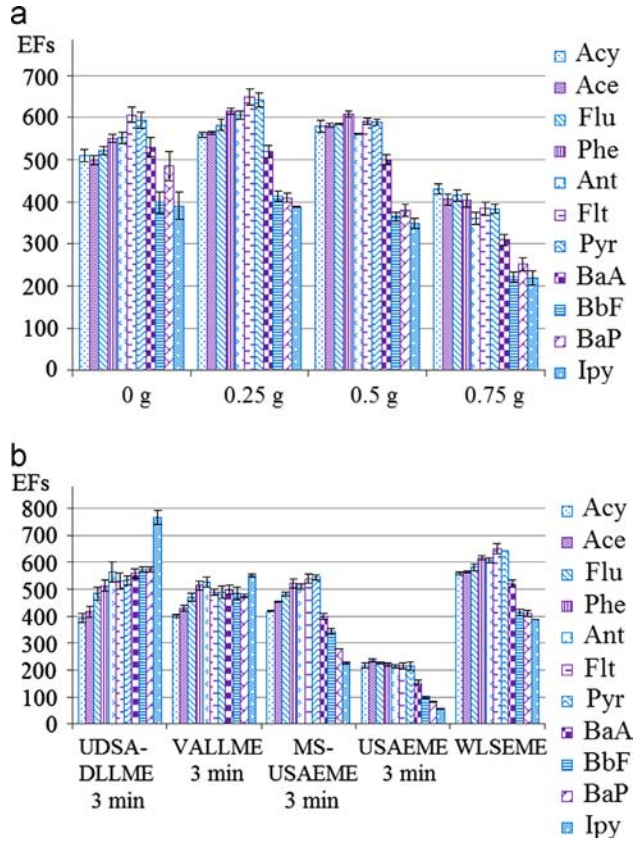

Fig. 5. (a) Salt effect (NaCl) ($n=3$). Extraction conditions: 9 μL 1-heptanol; 240 μL Triton X-100 and (b) comparison with other emulsification methods ($n=3$).

(120, 180, 240, and 300 μL) were tested. Fig. 4(b) shows that the greater the volume of aqueous solution added, the better the obtained emulsion effect. However, beyond 240 μL, the EFs decreased. At such excessively high surfactant volumes, the analytes presumably dissolved in the aqueous solution to a greater extent. Therefore, 240 μL was chosen as the optimal amount of surfactant solution.

3.2.6. Salt addition (NaCl)

The addition of salt enhances the extraction of analytes in standard LLME through the salting-out effect [19]. The experiments on WLSEME were performed by adding various amounts of sodium chloride (0–0.75 g). The results are shown in Fig. 5(a). Above 0.25 g of NaCl, the EFs decreased. This reversal in trend might be due to the increase in the density of the electric double layer to a level that prevents analytes from easily penetrating the organic phase. It may also be due to an increased volume of the floating phase, thereby diluting the extractant and causing lower EFs. Thus, 0.25 g of NaCl was selected as the optimum amount.

3.3. Comparison with other emulsification methods

Having established the optimum conditions for UDSA–DLLME and WLSEME, the performances of the two methods were compared with those of other emulsification methods such as vortex-assisted, ultrasound-assisted, and that of manual shaking before ultrasound-assisted extraction. The optimized conditions (except extraction time) for UDSA−DLLME were applied to the latter three methods. The extraction times were optimized individually, and were fortuitously found to be 3 min in each case. Fig. 5(b) reveals that using UDSA–DLLME and WLSEME yielded higher EFs for the majority of analytes compared to the other emulsification methods. Moreover, WLSEME showed better precision than UDSA–DLLME.

3.4. Quantitative aspects

3.4.1. Quantitative aspects of UDSA–DLLME

Under the optimum conditions, the linearity, EFs, LOD, and precision were evaluated for the extraction performance of UDSA–DLLME, and the results are summarized in Table 1. Calibration curves were constructed for different concentrations ranging from 0.08 to 100 μg L^{-1} in DI water. These calibration curves exhibited coefficients of determination (r^2) ranging from 0.9966 to 0.9999, showing that UDSA−DLLME yielded a good linear relationship. The EFs were between 392 and 766. The LODs are defined as the lowest concentration an analyte can be quantified with a defined confidence level. The calculated LOD for this method is determined by analyzing a low concentration standard seven times, as follows:

$$\mathrm{LOD} = t_{(n-1,1-\alpha=0.99)} \times \sigma$$

σ: the standard deviation and is calculated for the seven replicates.

$t(\ n\text{-}1,\ 1\text{-}\alpha\text{=}.99)$: the students′ t value appropriate for a 99% confidence level and a standard deviation estimate with $n-1$ degrees of freedom.

The calculated LOD=3.143 ($n=7$) x standard deviation. The LOD is to be determined in reagent (blank) water, prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the

Table 1
Linearity, EFs, LOD and relative recoveries (RR) of UDSA–DLLME.

Compound	Linearity ($\mu g\ L^{-1}$)[a]	R^2	EFs[b]	LOD ($\mu g\ L^{-1}$)[c]	RSD (%)[d], Intraday, $n=7$	RSD (%)[e], Interday, $n=6$	Spiked, concentration ($\mu g\ L^{-1}$)	River, RR (%)	Lake, RR (%)	Field, RR (%)
Acy	0.2–100	0.9999	392	0.025	2.3	3.4	0.5	111	110	101
							5	104	96	98
							50	103	106	106
Ace	0.2–100	0.9999	416	0.022	3.2	3.9	0.5	108	109	101
							5	103	94	97
							50	101	103	107
Flu	0.2–100	0.9994	483	0.030	3.1	5.0	0.5	112	106	103
							5	103	94	98
							50	103	108	108
Phe	0.2–100	0.9977	511	0.040	4.9	6.5	0.5	104	100	94
							5	104	94	98
							50	104	108	109
Ant	0.2–100	0.9979	575	0.040	3.3	6.4	0.5	102	101	98
							5	101	96	98
							50	103	106	107
Flt	0.2–100	0.9983	538	0.032	6.0	7.3	0.5	98	106	91
							5	102	94	99
							50	103	108	107
Pyr	0.2–100	0.999	532	0.035	7.8	8.3	0.5	96	97	86
							5	101	94	99
							50	105	110	109
BaA	0.2–100	0.9999	559	0.034	6.3	6.7	0.5	100	98	93
							5	105	95	99
							50	98	97	93
BbF	0.2–100	0.9997	572	0.025	2.4	4.9	0.5	94	88	87
							5	98	96	100
							50	93	92	95
BaP	0.2–100	0.9999	574	0.060	3.4	5.7	0.5	102	103	95
							5	108	98	101
							50	91	89	88
Ipy	0.2–100	0.9966	766	0.060	4.5	9.2	0.5	101	104	100
							5	113	93	95
							50	87	87	84

[a] Water sample spiked with 0.2, 0.5, 2, 5, 10, 50, 100 $\mu g\ L^{-1}$, $n=3$.
[b] Water sample spiked with 10 $\mu g\ L^{-1}$, $n=3$.
[c] Water sample spiked with 0.2 $\mu g\ L^{-1}$, $n=7$.
[d] River water sample spiked with 0.2 $\mu g\ L^{-1}$.
[e] River water sample spiked with 0.2 $\mu g\ L^{-1}$.

estimated method detection limit (Recommend between 1 and 5 times the estimated method detection limit) [20].

The LODs were in the range $0.022-0.060\ \mu g\ L^{-1}$, and were thus compatible with the regulations of the EPA and WHO concerning the quality of water for human consumption. Relative standard deviations (RSDs) ranged from 2.3% to 7.8% for intraday and 3.4% to 9.2% for interday.

3.4.2. Quantitative aspects of WLSEME

The linearity of WLSEME was estimated under the optimum conditions, as shown in Table 2. Calibration curves were constructed for different concentrations ranging from 0.08 to 100 $\mu g\ L^{-1}$ in DI water. The range of coefficients of determination (r^2) was from 0.9966 to 0.9999. The LODs for WLSEME were determined using the same method as that for UDSA–DLLME. They ranged from 0.022 to 0.13 $\mu g\ L^{-1}$, and the EFs were between 388 and 649. The RSDs were from 2.5% to 5.9% for intraday and 3.4% to 9.1% for interday.

3.4.3. Analysis of environmental water samples

Three environmental samples, including river water, lake water, and field water from Hsinchu, Taiwan, were a filtered through a 0.45 μm nylon membrane filter before extraction. No analytes were found in these real water samples. Thus, all specimens were spiked with 5, 50, and 500 $\mu g\ L^{-1}$ of PAHs. The relative recoveries (RR) of the analytes from river water, lake water, and field water were 87–113%, 87–110%, and 84–109%, respectively, by UDSA–DLLME (Table 1). For WLSEME, the RRs of the analytes in the water samples were 87–110%, 86–112%, and 92–114% (Table 2). The RSDs of the RRs

Table 2
Linearity, EFs, LOD and relative recoveries (RR) of WLSEME.

Compound	Linearity ($\mu g\ L^{-1}$)[a]	R^2	EFs[b]	LOD ($\mu g\ L^{-1}$)[c]	RSD (%)[d], Intraday, $n=7$	RSD (%)[e], Interday, $n=6$	Spiked concentration ($\mu g\ L^{-1}$)	River, RR (%)	Lake, RR (%)	Field, RR (%)
Acy	0.2–100	0.9998	559	0.022	3.8	4.0	0.5	110	97	99
							5	108	102	103
							50	95	105	103
Ace	0.2–100	0.9988	564	0.024	3.7	4.4	0.5	107	102	104
							5	100	97	101
							50	94	105	104
Flu	0.2–100	0.9998	582	0.029	4.5	4.6	0.5	110	103	104
							5	107	104	106
							50	93	103	104
Phe	0.2–100	0.9999	615	0.035	4.7	6.4	0.5	103	101	100
							5	103	101	107
							50	94	101	103
Ant	0.2–100	0.9998	606	0.028	2.5	4.3	0.5	99	93	111
							5	102	103	109
							50	95	103	104
Flt	0.2–100	0.9999	649	0.070	4.0	5.7	0.5	100	102	108
							5	98	97	108
							50	92	99	105
Pyr	0.2–100	0.9999	642	0.063	5.9	9.1	0.5	96	104	97
							5	101	103	106
							50	93	100	107
BaA	0.2–100	0.9953	520	0.051	5.0	5.1	0.5	101	89	92
							5	99	100	105
							50	91	105	114
BbF	0.5–100	0.9996	413	0.12	4.8	8.9	0.5	99	100	110
							5	104	111	112
							50	90	91	103
BaP	0.5–100	0.9982	410	0.13	2.7	3.4	0.5	98	96	101
							5	102	112	114
							50	90	92	104
Ipy	0.5–100	0.9966	388	0.13	4.9	8.3	0.5	96	93	97
							5	101	107	113
							50	87	86	99

[a] Water sample spiked with 0.2, 0.5, 0.8, 2, 5, 10, 50, 100 $\mu g\ L^{-1}$, $n=3$.
[b] Water sample spiked with 10 $\mu g\ L^{-1}$, $n=3$.
[c] Water sample spiked with 0.2 $\mu g\ L^{-1}$ for Acy, Ace, Flu, Phe, Ant, Flt, Pyr, BaA and spiked with 0.5 $\mu g\ L^{-1}$ for BbF, BaP, Ipy, $n=7$.
[d] River water sample spiked with 0.5 $\mu g\ L^{-1}$.
[e] River water sample spiked with 0.5 $\mu g\ L^{-1}$.

were less than 10.9%. The RRs of the three real water samples imply that there was no significant interference from matrix in either method. Fig. 6 shows the chromatograms obtained for the analysis of river water extracted by UDSA–DLLME.

3.4.4. Comparison with other extraction methods

Compared to other extraction methods in the literature (Table 3), although the proposed methods are not the most sensitive, they are applicable for evaluating regulations concerning the quality of water stipulated by the EPA [2] and the WHO [3]. With both methods, equilibrium was attained more quickly than with other microextraction techniques [21–23]. In applying these two methods, we used only 9 and 14 μL of alcohols of low toxicity instead of highly toxic toluene or chlorinated solvents [9,21,24]. In DLLME based on solidification of floating organic droplet [25], a solvent of low toxicity was applied to extract halogenated organic compounds. The extraction was complete in few seconds. However, the method was restricted by requiring high melting point extraction solvents.

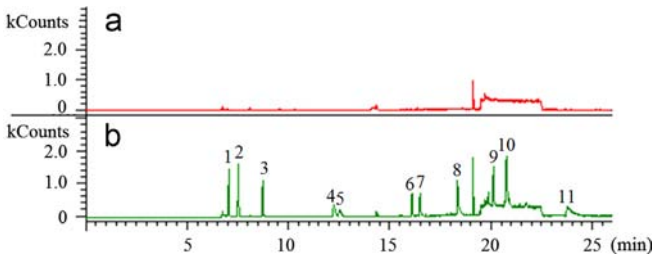

Fig. 6. Chromatograms using UDSA–DLLME. (a) River water sample and (b) river water spiked with 0.5 $\mu g\ L^{-1}$ of each analyte. (1) Acy (2) Ace (3) Flu (4) Phe (5) Ant (6) Flt (7) Pyr (8) BaA (9) BbF (10) BaP and (11) Ipy.

Table 3
Comparison with other extraction methods.

Method	Instrument	PAHs	Extraction solvent	Extraction time (min)	Linear range ($\mu g\ L^{-1}$)	LOD ($\mu g\ L^{-1}$)	Ref.
SPME[a]	GC–MS	16 PAHs	–	45	0.01–10	0.001–0.029	[22]
HSPME[b]	GC-FID	11 PAHs	–	30	0.1–50	0.03–0.3	[23]
HF-LPME[c]	GC–MS	13 PAHs	Toluene	15	10–2000	0.01–0.95	[21]
DLLME[d]	GC-FID	16 PAHs	Carbon tetrachloride	A few seconds	0.02–200	0.007–0.03	[9]
DLLME-SFO[e]	HPLC-VWD	5 PAHs	1-Dodecanol	A few seconds	0.1–500	0.045–1.1	[25]
USAEME[f]	GC-FID	9 PAHs	Toluene	0.5	0.05–100	0.02–0.05	[24]
UDSA–DLLME[g]	GC–MS	11 PAHs	Heptanol	3	0.08–100	0.022–0.060	This work
WLSEME[h]	GC–MS	11 PAHs	Nonanol	A few seconds	0.08–100	0.022–0.13	This work

[a] Solid-phase microextraction.
[b] Headspace solid-phase microextraction.
[c] Hollow-fiber liquid phase microextraction.
[d] Dispersive liquid–liquid microextraction.
[e] Dispersive liquid–liquid microextraction based on solidification of floating organic drop.
[f] Ultrasound-assisted emulsification microextraction.
[g] Up-and-down shaker-assisted dispersive liquid – liquid microextraction.
[h] Water with low concentration of surfactant in dispersed solvent-assisted emulsion dispersive liquid–liquid microextraction.

4. Conclusion

Novel sample preparation methods designated as UDSA – DLLME and WLSEME have been developed for the determination of PAHs in water samples. Combined with ISCS, they require smaller amounts of environmentally friendly alcohols as extraction solvents. WLSEME requires a low concentration of aqueous surfactant (7.1×10^{-8} M), which is a thousand times lower than for other surfactant-assisted emulsion methods. Both methods proved to be rapid and afforded high extraction efficiencies. Comparison with other extraction methods such as SPME and DLLME showed UDSA – DLLME and WLSEME to be viable alternative techniques for sample pre-concentration in terms of performance and speed.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.11.073.

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