A novel method for PAHs in aqueous samples based on ultrasound-assisted solidified floating organic drop microextraction

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Abstract. A novel method for determining PAHs in river samples has been developed based on ultrasound-assisted solidified floating organic drop microextraction. The effect of several factors, such as volume of extracting solvent, volume of aqueous sample, sample temperature and extraction time, were evaluated and optimized. Best enrichment efficiency was obtained under the following experimental conditions: 60 μL of 1-undecanol added into a 30 mL sample solution at 40°C, and then extracted for 10 min in an ultrasonic bath. It showed a good performance for determining the PAHs in river samples and will be a promising method for the reliable determination of PAHs at a very low level in river.

1. Introduction

Many trace pollutants in the environment have resulted in serious environment pollution [1, 2]. A lot of analytical methods have been developed for determining these pollutants [3]. In these analytical procedures, sample pretreatment is a critical step for environmental samples and usually consumed most time and cost [4, 5]. Hence, sample preparation methods featured by environment-friendly, high-efficiency, time-saving, user-friendly are called for development. Liquid phase microextraction (LPME) is a very popular pretreatment method that meets all the above demand and has been developed versatile ameliorations [6, 7]. Dispersive liquid liquid microextraction (DLLME) is one excellent amelioration method of LPME which aims to extract trace pollutants efficiently from abundant environmental sample into only a few microliter extraction agent and cost a little time [8]. The extracting liquid is dispersed into many fine droplets by a ternary disperser solvent and makes the environment solution cloudy which makes the pollutants transferring equilibrium between extraction solvent and aqueous sample reached quickly. The extracting liquid is then centrifuged at the bottom of a conical tube and taken out for analysis. However, the taking out operation is so difficult that a professional staff is needed while the ternary dispersive liquid used in the extraction usually means a lot of interference. Another LPME, called solidified floating organic drop microextraction (SFODME) was developed as an improvement [9, 10]. In this method, a droplet of an extracting liquid is floated on the surface of the agitated aqueous sample to perform the extraction. The extracting drop in sample can be solidified at 10-30°C in an ice bath and is allowed to melt for determination. The solidified extracting liquid makes it easy to be taken out. However, in spite of its many advantages, some aspects yet limit its application. For example, the mass transfer area is still limited a lot compared with that of DLLME. A steady drop should be kept on the surface of sample solvent which limits the speed of...
agitating. These all mean a longer extraction time. Therefore, a conjunction of DLLME and SFODME may be a good choice. In recent years, ultrasound-assisted solvent extraction is widely used for organic compound extraction which provides a more efficient contact between the two different phases due to the increased pressure and temperature [11, 12]. Ultrasound can be used as a dispersive way of extracting liquid in DLLME as a replacement of dispersing agent. Several work reported the application of ultrasound assisted SFODME (UA-SFODME) [13-18]. And the superiority of UA-SFODME has been summarized as more high-efficiency, time-saving, user-friendly and needless disperser [19]. Polycyclic aromatic hydrocarbons (PAHs) are of worldwide concern for their potential carcinogenic activity in mammals and ubiquitous existence throughout the environment. In the original SFODME, PAHs have been selected as the target analytes [9]. And the original SFODME method was improved by a mode of conjunction of DLLME and SFODME (DLLME-SFOD) with methanol as the dispersive solvent [20]. However, no work was reported for the application of ultrasound in the extraction of PAHs by SFODME method in spite of great development in UA-SFODME. In this work, the application of UA-SFODME was discussed for enrichment of some PAHs in aqueous samples.

2. Method and materials

2.1. Chemicals and apparatus

Acenaphthene (ACY), anthracene (ANT), Fluoranthene (FLT), Pyrene PYR), Benzanthracene (BaA), Benzopyrene (BaP) were adopted as analytes. 1-undecanol was selected as the extracting solvent for its excellent performance according to the result obtained in reference [9]. Standards of PAHs and 1-undecanol were purchased from Aldrich (Milwaukee, USA). Stock standard solutions of PAHs were prepared in methanol. A fresh standard sample was prepared by spiking certain amount of PAHs stock standard solutions into ultrapure water. All reagents and chemical used are analytical pure or better. A high performance liquid chromatograph with an ultraviolet detector (HPLC-UV) was employed for the detection of PAHs (Dionex, Sunnyvale, USA).

2.2. Extracting procedures

Extraction was performed in the following procedures (figure 1): a few microdrops of 1-undecanol was added in aqueous sample and sonicated for 10 min. Then, the sample was centrifuged and cooled in a crushed ice contained beaker. The solidified solvent drop was transferred into a conical vial where it melted immediately. This phase was diluted to 250 μL with methanol and determined by HPLC-UV. Effects of several factors, such as volume of extracting solvent, volume of aqueous sample, sample temperature and extraction time, were evaluated and optimized.

![Figure 1. Extracting processures of UA-SFODME. 1 ultrasound, 2 centrifugation, 3 solidification, 4 melt and analysis.](image)
3. Results and discussion

3.1. Volume of extracting solvent
The volume of extracting solvent determines the interfacial area between the two liquid phases which dominates the transferring speed of the analytes into microdrop. Different volumes of 1-undecanol (40-140 µL) were examined for optimum extracting solvent volume (figure 2). The analytical signals of PAHs increased by the increasing extracting solvent volume in the range of 40-60 µL, and then decreased in the following examined volumes. It demonstrates that interfacial area dominated the enrichment of analytes when the extracting solvent was below 60 µL and the enrichment was weakened by more extracting solvent.

3.2. Solution temperature
Solution temperature controls the molecular thermodynamic movement of analytes which dominates the moving ability of analytes from the aqueous solution to the organic solution. The effect of sample solution temperature on the extraction efficiency was studied in the range of 20–60 °C (figure 3). The extraction efficiency increased along with increased sample temperature until 40 °C reached. The extraction system was hard to separate the sample and organic solvent when high temperature was adopted. Thus, solution temperature of 40 °C was held in further experiments.

![Figure 2. Effects of extracting solvent volume on extraction efficiency of PAHs.](image-url)
3.3. Ultrasound time

Enough time is needed to guarantee the equilibrium of analytes between the sample and organic solution. A series of experiments were performed for obtaining the optimum extraction time (figure 4). Results showed that the extraction equilibrium can be reached in 15 min which illustrates the high efficiency of ultrasound.

3.4. Sample volume

The extracting solvent/sample volume ratio is one of important factors which dominate the preconcentration factor. The convection efficiency of analytes also may be influenced by sample volume which controls the extraction efficiency. Sample volumes in the range of 15-35 mL were investigated for the optimal extracting efficiency (figure 5). Results show that largest preconcentration factor appears at the sample volume of 30 mL. Hence, 30 mL was chosen as the optimum sample volume in the following experiments.
3.5. Ionic strength
Salting-out effect has been discussed in this work. The enrichment ratio was decreased when the salt strength was increased. It can be explained by the fact that the addition of salt increased the viscosity of sample which slowed down the transport of the analytes to the extraction drop.

3.6. Quality assurance perspective
The performance of analytical method is usually evaluated by parameters such as linearity, precision, accuracy and limit of detection which make sure the suitability of analytical method for its intended use [21,22]. The calibration curves shown in table 1 were obtained under the optimized condition. A good linearity was observed in the range of 10–400.00 µg L$^{-1}$ with regression coefficient ($r$) ranged from 0.9961-0.9989 without internal standard. Relative standard deviations (RSD) without using internal standard at concentration of 20 µg L$^{-1}$ were below 7% indicating a good detection precision of this method. The limit of detection (LODs), based on signal-to-noise ratio (S/N) of 3, ranged from 0.17 to 2.62 µg L$^{-1}$, which is very low by using HPLC-UV. Enrichment factors were measured in the range of 73–592 which may satisfy the detection need of PAHs in many natural water samples. For each set of six samples, a procedural blank and a spiked sample with standards were run to check for the interference and cross-contamination.

3.7. River sample analysis
River sample was collected from a city river and used for evaluating the performance of the present method in the detecting of PAHs in real aqueous samples. The river samples were transported to the laboratory in an amper glass bottles and stored at 4 °C after collection. Before analysis, the samples were filtered by 0.45 µm cellulose acetate filter. ACY, ANT, FLT, PYR, BaA besides BaP, were all detected in river samples. Reliability was checked by spiking the sample at spiking levels of 20 µg L$^{-1}$. The detected concentration and recoveries of the spiked samples were listed in table 2. The recoveries for all analytes were all above 78% which were satisfactory for river water samples and illustrated the reliability of the present work.

Figure 5. Effects of sample volume on extraction efficiency of PAHs.
Table 1. Parameters of the present method for determining PAHs by UA-SFODME

| Species | DLR\(^r\) (µg L\(^{-1}\)) | r     | RSD (%) (n=5) | LOD (µg L\(^{-1}\)) | R\(^o\) (%) (n=5) | EF\(^c\) |
|---------|----------------|-------|--------------|------------------|----------------|---------|
| ACY     | 10.0-400       | 0.9986| 4.3          | 0.22             | 89.5±4.3       | 461     |
| ANT     | 10.0-400       | 0.9989| 5.2          | 0.17             | 94.8±4.7       | 592     |
| FLT     | 10.0-400       | 0.9981| 4.9          | 1.13             | 96.7±3.8       | 470     |
| PYR     | 10.0-400       | 0.9985| 6.1          | 1.35             | 97.3±6.2       | 452     |
| BaA     | 10.0-400       | 0.9978| 5.2          | 1.24             | 92.1±4.1       | 210     |
| BaP     | 10.0-400       | 0.9961| 6.8          | 2.62             | 83.6±5.6       | 73      |

*a,* detector linear range, *b,* recovery rate, *c,* enrichment factor.

3.8. Merits of method and green analytical chemistry perspective

Merits of method for PAHs based on SFOD have been confirmed in reference [9] and [20] compared to other works reported in literatures. The present work was just compared with SFODME and DLLME-SFOD for extraction and determination of PAHs reported in reference [9] and [20] (table 3). The detector linear ranges, enrichment factor, recovery rate, limits of detection in this work are comparable to that of SFODME and DLLME-SFOD. However, the extracting time is substantially shortened while the determining precision revealed by regression coefficient and recovery rate is improved compared with that of SFODME. Furthermore, the operation of the method become very easy due to the needless of a steady drop kept on the centre of sample solvent surface which may be crucial in SFODME. Compared with DLLME-SFOD, the performance of the present work is comparable or better in spite of on-use of disperser which may interfere the determining of PAHs.

Table 2. Results of the determining PAHs in river samples by UA-SFODME.

| Species | Concentration (µg L\(^{-1}\)) | Concentration (µg L\(^{-1}\)) | RSD (%) (n=5) | RSD (%) (n=5) | R\(^b\) (%) (n=5) |
|---------|----------------|----------------|--------------|--------------|----------------|
| ACY     | 12.7           | 29.3           | 5.5          | 4.8          | 89.5±4.3       |
| ANT     | 14.1           | 32.3           | 3.2          | 5.0          | 94.8±4.7       |
| FLT     | 10.3           | 29.3           | 4.7          | 3.9          | 96.7±3.8       |
| PYR     | 6.5            | 25.8           | 3.5          | 6.4          | 97.3±6.2       |
| BaA     | 4.6            | 22.7           | 6.5          | 4.5          | 92.1±4.1       |
| BaP     | n.d. \(^a\)   | 16.7           | -            | 5.7          | 83.6±5.6       |

*a,* not detectable, *b,* recovery rate.

The green analytical chemistry (GAC) of this method was evaluated according to reference [19, 23]. As the same as that summarized in reference [19], the present work fulfills several recommendations of GAC: the high enrichment ability, the very little consumption of extracting solvent, the low toxic used organic solvents and the reduced exposure time for operators are accordance with No.2,7,11,12 principle of GAC, respectively. All in all, the present work is proved to be a potential alternative method for the extraction of PAHs from aqueous solution.

Table 3. Comparison of the present method with SFODME and DLLME-SFOD for extraction and determination of PAHs

| Method       | r             | RSD (%) (n=5) | R\(^a\) (%) (n=5) | Ef\(^b\) | Time (min) | Disperser need | Operation | Ref. |
|--------------|---------------|--------------|------------------|---------|------------|----------------|-----------|------|
| Present      | 0.9961-0.9989 | 4.3-6.8      | 89.5±4.3         | 73-592  | 15         | No             | Easy      | -    |
| SFODME       | 0.9904-0.9999 | 1.1-12.6     | 94.8±4.7         | 594-1940| 35         | No             | Hard      | [9]  |
| DLLME-SFOD  | 0.9980-0.9996 | 2.6-4.3      | 88-110           | 88-118  | 10         | Yes            | Easy      | [20] |

*a,* recovery rate, *b,* enrichment factor.
4. Conclusions
This study has developed a novel UA-SFODME for sensitive determination of PAHs in river samples. For this purpose, the influence of different parameters affecting the extraction efficiency was evaluated. Best enrichment efficiency was obtained under the following experimental conditions: 60 μL of 1-undecanol added into a 30 mL sample solution at 40°C, and then extracted for 10 min in an ultrasonic bath. The present work shortens the extraction time, reduces the operation elaboration compared with traditional SFODME and eliminates the use of disperser agent used in DLLME-SFOD. It will be a promising method for the reliable determination of PAHs at a very low level in river samples.

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