Continuous-flow microextraction and gas chromatographic–mass spectrometric determination of polycyclic aromatic hydrocarbon compounds in water

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Abstract

A new method of the determination polycyclic aromatic hydrocarbons (PAHs) in water samples was developed by continuous-flow microextraction (CFME) coupled with gas chromatography–mass spectrometry (GC–MS). In this experiment, 15 mL sample solution with no salt-added was flowed at the rate of 1.0 mL min^{−1} through 3 μ L benzene as extraction solvent. Under the optimal extraction conditions, the developed method was found to yield a linear calibration curve in the concentration range from 0.05 to 15 ng mL^{−1}. Furthermore, the accuracy and repeatability of the method were good by calculating from water samples spiked at known concentrations of PAHs, and the recovery of optimal method was satisfactory. The results showed that CFME was an efficient preconcentration method for extraction of PAHs from spiked water samples.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are defined to be composed of two or more fused aromatic rings containing only carbon and hydrogen atoms. They are formed mainly by the incomplete combustion of fossil fuels and coal, petrochemical cracking processing and the degradation of lubricating oils and dyes. PAHs are of wide concern because some of them are classified as probable human carcinogens [1] and show tumorigenic activity and endocrine-disrupting activity in mammals [2]. They exist widely in the atmosphere, soil and water and contact tightly with human health. As high efficient separation tools, GC and HPLC have been used widely for separating all kinds of samples containing complex components. However, the direct determination of extremely trace PAHs by modern

high efficient chromatographic techniques, such as GC–MS [3] and HPLC–UV/FL [4], is still very difficult because the content of PAHs is very low, such as ng level or pg level in water [5]. Therefore, preconcentration techniques are often required. Liquid–liquid extraction (LLE) [4], liquid-phase microextraction (LPME) [6], solid-phase extraction (SPE) [7,8], solid-phase microextraction (SPME) [9–12], liquid membrane extraction (LME) [13,14] and cloud-point extraction (CPE) [15,16] have been developed to separate and concentrate the trace level PAHs in the environmental water.

Traditional LLE was used widely due to its higher potential for physical separation by using mixed solvent and a higher capacity for interfering compounds. However, it was substituted gradually by some rapid, cheap, and convenient pretreatment techniques because of its time-consuming nature and usage of large amounts of expensive high-purity organic solvents. Therefore, many attempts to develop LLE procedures include mainly in two aspects, i.e. miniaturization and automation. LME technique provides the possibility of the automation of LLE. Supported LME [17] is based on a three-phase system with an organic phase sandwiched between two aqueous phases and the

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organic phase is immobilized in a porous hydrophobic membrane. Continuous-flow LME [18] was developed in order to extract polar substances and prolong the life span of the liquid membrane, which was very easy to realize the automation with HPLC. LPME [19], using a microliter drop of organic solvent on the end of a Teflon rod or the needle tip of a microsyringe, was developed as a cheap pretreatment method. It has mainly two modes of static LPME [20] and headspace LPME [21]. Drop of organic solvent in static LPME mode immerses directly in the stirred aqueous solution and in headspace LPME mode it is laid above the aqueous solution. More recently, Liu and Lee [22] introduced a method termed as continuous-flow microextraction (CFME) in order to improve the mass transfer between organic phase and the aqueous solution. Comparing with LPME, the surface of extraction solvent in CFME is refreshed continually when sample solution flows continuously by a pump from the bottom of a virtually immobilized organic drop located on the tip of a microsyringe, which is beneficial to improve extraction efficiency. CFME was developed based on the LLE as a novel sample preparation technique and was fit for the thermodynamic and kinetic equations of LLE:

$$C_{0,\text{eq}} = KC_{\text{aq,eq}} = KC_{\text{aq,ini}}/(1 + KV_0/V_{\text{aq}}) \quad (1)$$

$$\frac{dC_0}{dt} = A_i\beta/V_0(KC_{\text{aq}} - C_0) \quad (2)$$

where $C_{0,\text{eq}}$ is the analyte concentration in the organic phase (solvent drop) at extraction equilibrium; $C_{\text{aq,eq}}$ and $C_{\text{aq,ini}}$ are the equilibrium and initial analyte concentrations in the aqueous phase, respectively; V_0 and V_{aq} are the organic and aqueous phase volumes, respectively; K the distribution coefficient; C_0 and C_{aq} are analyte concentrations in the organic and aqueous phases at time t , respectively; A_i the interfacial area; β the overall mass transfer coefficient with respect to the organic phase. As for the microextraction, the absolute amount of analytes extracted to the solvent during extraction is usually negligible compared with its total amount in solution. Thus, analytes are extracted to the organic solvent as soon as possible and there are high enrichment factor. This method was used to determine the nitroaromatic and chlorobenzenes, and their enrichment factors exceeded 1000-fold. This pretreatment method was also applied to the volatile halohydrocarbons [23] and five commonly used pesticides [24] in water samples. Up to the present, there was no report about application of CFME in analysis of PAH in aqueous solution.

In this work, 16 PAHs in water samples were determined by continuous-flow microextraction coupled with GC–MS. Experimental parameters affecting extraction efficiency, such as extraction solvent type and volume, flow-rate of sample solution, sample volume and salt concentration, were investigated and optimized. Furthermore, a microinfusion pump can substitute for the HPLC pump or peristaltic pump, which can minimize the chance of producing the air bubble, make the organic microdrop more stable and increase the volume of the sample solution. The optimal method was used to determine PAHs in real water samples.

2. Experimental

2.1. Reagents

Reference PAHs (16 compounds, each at 2000 $\mu\text{g mL}^{-1}$) were obtained from Supelco (Walton-on-Thames, UK). The solution was diluted with methanol to prepared 10 $\mu\text{g mL}^{-1}$ the stock standard solution standard solution. All solutions were stored and refrigerated at 4 °C. Then, working standard solution was freshly prepared by diluting the mixed standard solution with distilled water to required concentrations. *n*-Hexane, benzene, toluene, dichloromethane were all of HPLC-grade. *n*-Butanol was of analytical grade. All glasswares were cleaned thoroughly by soaking overnight in diluted HNO_3 and rinsing with ultra-pure water (Millipore, Bedford, MA, USA) before use.

2.2. Apparatus

CFME device was composed of home-made glass chamber (~ 0.2 mL), a microinfusion pump (Zhejiang University Medical instruments Co. Ltd., Zhejiang, China), 20 mL glass syringe (Ningbo He Ping syringe factory, Zhejiang, China) and a 10 μL GC microsyringe (SGE Scientific, Sydney, Australia), see Fig. 1. A minimum length of PTFE tube (i.d. 0.5 mm) was used for all connections.

A Shimadzu GCMS-QP2010 (Shimadzu, Kyoto, Japan) system equipped with a 30 m $\times$ 0.25 mm i.d. fused-silica capillary column with a 0.25 μm Rtx-5 ms coating (5% phenyl: 95% dimethylpolysiloxane). The carrier gas helium was maintained at a constant linear velocity of 50.4 cm min^{-1} . The injector port temperature was set to 270 °C in splitless mode with the splitter activated after 1 min. The column was held at 90 °C for 1 min, increased to 180 °C at the rate of 8 °C min^{-1} , then increased to 280 °C at the rate of 15 °C min^{-1} for 15 min. The mass spectrometer was operated in the electron impact ionization (EI) mode with an ion source temperature of 200 °C and the energy of the electrons was kept at 70 eV. The interface temperature was kept at 260 °C. The target com-

Fig. 1. Schematic diagram of continuous-flow liquid-phase microextraction (CFME).

pounds were quantified in the selected ion monitoring (SIM) mode, using the molecular ion and one qualifier ions for each compound. The molecular ion and one qualifier ions for naphthalene (NaP), acenaphthylene (Acl), acenaphthene (Ace), fluorene (Fle), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flu), pyrene (Pyr), benz[*a*]anthracene (BaA), chrysene (Chr), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), indeno[1,2,3-*cd*]pyrene (IDP), dibenz[*a,h*]anthracene (DBA) and benzo[*ghi*]perylene (BgHiPe) are 128, 127; 152, 150; 154, 153; 166, 139; 178, 176; 178, 176; 202, 200; 202, 101; 228, 226; 228, 226; 252, 126; 252, 250; 252, 250; 276, 138; 278, 276 and 276, 138; respectively.

2.3. Extraction procedures

The aqueous sample was infused and filled in the bulb glass extraction chamber via the connecting PTFE tubing. Then 3 μL benzene, as the extraction reagent, was introduced into the extraction chamber by 10 μL microsyringe, and formed a drop which remained at the tip of the microsyringe. Fifteen milliliters sample solution was flowed continuously through the solvent drop at the rate of 1.0 mL min^{-1} . Finally, the solvent drop was retracted into the microsyringe and directly injected into the GC–MS for the analysis.

3. Results and discussion

3.1. Selection of microdrop solvent

The selection of an appropriate extraction solvent is a key factor for the optimization of CFME process. The extracting solvent needed in this CFMS process should possess a high distribution coefficient for the analyte and good chromatographic behavior and should be immiscible with water. On the basis of this consideration, *n*-hexane, benzene, toluene, dichloromethane and *n*-butanol were chosen and investigated. From the experimental phenomena obtained, it was found that *n*-butanol was very difficult to hang on the tip of the microsyringe. Dichloromethane and toluene were dissolvable in the sample solvent, resulting in the partly loss of the extracting droplet. Fortunately, benzene and *n*-hexane provided the effective extraction abilities for the studied analytes. The results can be seen in Fig. 2(a) and the extraction ability of benzene was greater than that of *n*-hexane, especially for the analyte containing the benzene ring more than four rings in its structure. Therefore, benzene was chosen and used in the further steps.

3.2. Microdrop volume

The effect of drop volume of the extracting solvent was studied in the range of 1–3.5 μL and the result was shown in Fig. 2(b). The peak areas of all the analytes increased with the augment of drop volume. When the volume exceeded 3.5 μL , the drop was not be able to hold on the tip of microsyringe. So 3 μL was selected and used in the following experiments.

Fig. 2. (a) Influence of solvent type on extraction performance (3 μL extraction solvent; sample: $12 \text{ mL } 5 \mu\text{g L}^{-1}$ spiked PAHs in aqueous solution; flow rate: 1.0 mL min^{-1}). (b) Effect of drop volume on the peak area of PAHs (extraction solvent: benzene; sample: $6 \text{ mL } 5 \mu\text{g L}^{-1}$ spiked PAHs in aqueous solution; flow rate: 1.0 mL min^{-1}).

3.3. Flow rate of sample solution

Extraction efficiency is normally controlled by the distribution coefficient of the analyte between the aqueous phase and organic phase, which is affected by the thickness of the interfacial layer surrounding the microdrop. The interfacial layer can be varied with the change of the flow rate of sample solution [19]. Therefore, the flow rate of the sample solution in the CFME system needs to be evaluated. The flow rate in the range of 0.5–1.5 mL min^{-1} was considered. It was found that the flow rate of the sample in CFME system played a very significant effect for the extraction ability of the studied PAHs, especially for the low molecular weight analytes. To describe these results, the hydrophobicity and distribution abilities of each analyte should be considered. The obtained results were shown in Fig. 3. At low flow rate (0.5 mL min^{-1}), the CFME showed low extraction ability. It was due to the low flow rate that provided the wider interfacial layer. The wider interfacial layer might inhibit the distribution of the analyte dissolved in aqueous phase to the organic phase. In addition, at the flow rate higher than 1.0 mL min^{-1} , again the extraction ability of the CFME system for the PAHs was decreased. These results might be due to the fact that the extraction equilibrium could not be reached under the very high flow rate condition. Fortunately, the flow rate ranging from 0.8 to

Fig. 3. Effect of flow rate on the peak area of PAHs (extraction solvent: benzene; drop volume: 3 μL ; sample: 6 mL $5 \mu\text{g L}^{-1}$ spiked PAHs in aqueous solution).

1.0 mL/min provided the suitable extraction CFME system for PAHs. To compromise between the short sample extraction step and high extraction ability for all analytes, 1.0 mL/min was eventually chosen and used in the further steps.

3.4. Sample volume

In general, the higher enrichment factor could be obtained when higher sample volume is loaded. Importantly, the sample volume passing through the CFME system may seriously affect the loss of the extracting solvent, which would lower the extraction efficiency and lead to the poor sensitivity and precision. Therefore, the sample volume ranging from 5 to 25 mL was studied and the relationship between sample volume and volume loss of extraction solvent was shown in Fig. 4. The volume loss is equal to the difference between 3 μL (initial volume) and the remaining volume of droplet when it is retracted into the microsyringe. When sample solution was 15 mL, the volume loss was just 0.1 μL . In this condition, there was the best extrac-

Fig. 4. The relationship between sample volume and the loss of extraction solvent (extraction solvent: benzene; drop volume: 3 μL ; sample: $5 \mu\text{g L}^{-1}$ spiked PAHs in aqueous solution; flow rate: 1.0 mL min^{-1}).

Fig. 5. Effect of ion strength on the extraction performance (extraction solvent: benzene; drop volume: 3 μL ; sample: 15 mL $5 \mu\text{g L}^{-1}$ spiked PAHs in aqueous solution; flow rate: 1.0 mL min^{-1}).

tion efficiency. So 15 mL of sample volume would be the best choice.

3.5. Ionic strength

Generally, the addition of salt to the water causes a decrease in solubility of the organic compounds in water and increases the distribution coefficient, which usually used to enhance the extraction efficiency. Therefore, the influence of salt addition up to 20% (v/w) on CFME was discussed and the result was shown in Fig. 5. Extraction efficiency for the NaP, Ace and Flu had improved after adding some salt. However, for most PAHs, extraction efficiency had been decreased. It was assumed that apart from the salting-out effect, the presence of salt had changed the physical properties of the extraction film, which reduced the diffusion rates of the analytes into the drop. For the aim of contemporary analysis of 16 PAHs, no salt was added in the following experiments.

3.6. Evaluation of the method performance

Under the optimum extraction condition, the chromatogram of 16 PAHs in spiked water at concentration of $0.5 \mu\text{g L}^{-1}$ via CFME was shown in Fig. 6 and some characters of the proposed CFME method such as linear ranges, enrichment factor, correlation coefficients, detection limits and reproducibility were investigated and listed in Table 1. Calibration curves were drawn

Fig. 6. Chromatogram of 16 PAHs in spiked water at concentration of $0.5 \mu\text{g L}^{-1}$ via CFME under the optimum extraction condition. Peaks: (1) NaP; (2) Acl; (3) Ace; (4) Fle; (5) Phe; (6) Ant; (7) Flu; (8) Pyr; (9) BaA; (10) Chr; (11) BbF; (12) BkF; (13) BaP; (14) IDP; (15) DBA; (16) BghiPe.

Table 1

Enrichment factor, correlation coefficients, linearity, limit of detections and reproducibility for the proposed CFME method of PAHs

PAHs	Enrichment factor ^a	Correlation coefficient (r^2)	Linear range ($\mu\text{g L}^{-1}$)	LOD ^b ($\mu\text{g L}^{-1}$)	R.S.D. (%)
NaP	83	0.9990	0.05–15	0.001	8.9
Acl	87	0.9972	0.05–15	0.004	8.7
Ace	79	0.9970	0.05–15	0.003	9.6
Fle	82	0.9948	0.05–15	0.001	9.6
Phe	75	0.9949	0.05–15	0.005	8.4
Ant	78	0.9944	0.05–15	0.01	10
Flu	66	0.9934	0.05–15	0.002	8.4
Pyr	67	0.9941	0.05–15	0.002	8.9
BaA	31	0.9941	0.05–15	0.002	8.9
Chr	18	0.9948	0.05–15	0.002	6.7
BbF	28	0.9947	0.1–15	0.006	8.4
BkF	20	0.9234	0.1–2.0	0.006	8.4
BaP	13	0.9366	0.1–2.0	0.01	8.7
IDP	9	0.9352	0.1–2.0	0.007	25
DBA	3	0.8090	0.1–2.0	0.01	15
BghiPe	2	0.8860	0.1–2.0	0.006	11

^a Enrichment factor was calculated as the ratio of the final concentration of the analyte in the microdrop and its concentration in the original solution.

^b LOD: limit of detection (S/N = 3).

using 6 spiking levels of PAHs in the concentration range of $0.1\text{--}2\text{ }\mu\text{g L}^{-1}$ with respect to BkF, BaP, IDP, DBA and BghiPe, and using 11 spiking levels of PAHs in $0.05\text{--}15\text{ }\mu\text{g L}^{-1}$ for the rest of analytes. As can be seen, higher enrichment factor and good linearity were obtained for less than four rings PAHs with the correlation coefficient (r^2) ranging from 0.9948 to 0.9990. The precision of this method was calculated according to the continuous extraction of five aqueous solutions spiked at $1\text{ }\mu\text{g L}^{-1}$. For the less than four rings PAHs, their relative standard deviations (R.S.D.) were less than 10%.

Table 2

Recovery of CFME of tapwater sample spiked with PAHs at different concentration levels

PAHs	$0.5\text{ }\mu\text{g L}^{-1}$		$1.0\text{ }\mu\text{g L}^{-1}$		$5.0\text{ }\mu\text{g L}^{-1}$	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
NaP	107	7.0	93	9.8	101	0.1
Acl	101	0.8	96	10	98	3.3
Ace	102	0.5	96	10	101	3.8
Fle	108	1.6	95	9.2	102	0.8
Phe	103	9.2	94	8.8	101	2.0
Ant	92	5.5	90	12	99	2.0
Flu	95	0.8	97	9.0	98	0.4
Pyr	94	2.4	99	12	98	0.1
BaA	89	8.5	87	7.1	105	5.6
Chr	91	10	90	11	108	9.2
BbF	94	12	93	9.2	106	7.4
BkF	92	1.8	91	4.9	— ^a	— ^a
BaP	97	10	81	2.8	— ^a	— ^a
IDP	70	20	93	18	— ^a	— ^a
DBA	107	15	115	4.9	— ^a	— ^a
BghiPe	125	18	101	15	— ^a	— ^a

^a The compound was detected but quantification was not possible because the concentration exceeded the linear range.

Finally, the application of this developed method to the real samples was investigated for different spiked concentration levels. Although the method had the low detection limits, no analytes were detected in the blank tap water. Thus, the proposed method was evaluated by the relative recovery in tap water samples spiked with standard mixtures at three concentration levels at 0.5 , 1 and $5\text{ }\mu\text{g L}^{-1}$, respectively. The relative recovery is usually employed instead of absolute recovery, as used in exhaustive extraction procedures [25]. Table 2 shows the results of the three replicates of each spiked water samples obtained by the proposed method. For BkF, BaP, IDP, DBA and BghiPe, their recoveries were not given because water samples spiked with standard mixtures at the concentration of $5\text{ }\mu\text{g L}^{-1}$ were not in their linear range. The result demonstrated that the proposed method was a reliable technique for the determination of trace PAHs in water sample.

4. Conclusions

The developed method, combination of continuous-flow liquid-phase microextraction with gas chromatographic–mass spectrometric towards analysis of trace polycyclic aromatic hydrocarbon compounds in water samples was reported for the first time. The method has favorable extraction effect and higher enrichment factor, especially to the less than four rings PAHs. The reliable, rapid and convenient method was applied successfully to determine PAHs in the water sample. Further investigations can focus on the appropriate new extraction solvent to extract more than four rings PAHs to improve the sensitivity and precision of the method.

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