ELSEVIER

Journal of Chromatography A, 723 (1996) 259–271

JOURNAL OF CHROMATOGRAPHY A

Optimization of microwave-assisted solvent extraction of polycyclic aromatic hydrocarbons in marine sediments using a microwave extraction system with high-performance liquid chromatography–fluorescence detection and gas chromatography–mass spectrometry

Kok Kay Chee, Ming Keong Wong*, Hian Kee Lee

Department of Chemistry, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511, Singapore

Received 10 March 1995; revised 31 July 1995; accepted 11 August 1995

Abstract

In the present study, a microwave-assisted solvent extraction (MASE) technique using a microwave extraction system (MES) has been developed for the extraction of polycyclic aromatic hydrocarbons (PAHs) from marine sediment. The optimum MASE conditions can be obtained by performing the mixed-level orthogonal array design (OAD) procedure. A comparison between the Soxhlet extraction method and the MASE technique showed that although both techniques gave comparable results on certified reference materials (CRM) HS-4 and HS-6, the MASE technique allows the use of less solvent and is also time-saving and cost-effective without affecting its extraction efficiency. The optimum MASE technique was coupled to two analytical techniques: high-performance liquid chromatography (HPLC) with both ultraviolet (UV) and fluorescence detectors and gas chromatography–mass spectrometry (GC–MS) for the qualitative and quantitative screening of PAHs in CRM and "real world" samples. Recoveries of PAHs from two CRMs were all above 73.3%. The concentration of PAHs in marine sediment collected from primary industrial areas was between 0.03 and 0.35 μg/g on a dry weight basis.

Keywords: Microwave-assisted solvent extraction; Microwave extraction system; Marine sediments; Sediments; Mixed-level orthogonal array design; Polycyclic aromatic hydrocarbons

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are serious and ubiquitous environmental pollutants because of their high carcinogenicity and mutagenicity [1,2]. The extraction of organic pollutants, e.g., PAHs, from marine sediments can be achieved with a number of established methods. The most conventional one is Soxhlet extraction. This sample preparation technique requires 12–24 h. Moreover, the high consumption of hazardous and toxic organic solvents is another disadvantage of this technique. Normally, several clean-up procedures must be used in order to remove the co-extractives, e.g., aliphatic hydrocarbons and polar compounds, before the final analysis. Thus, it is both costly and time-consuming.

Recently, a new microwave-assisted solvent extraction (MASE) method has been reported as a sample preparation technique for various solid samples. Ganzler and Salgo [3,4] were the first to report

*Corresponding author.

0378-4347/96/$15.00
SSDI 0021-9673(95)00882-9

the use of microwave energy to irradiate solid matrices such as soil, seeds, foods and feeds in the presence of solvents with high dipole moments. The system was able to extract polar organic compounds more efficiently than conventional Soxhlet extraction. Furthermore, the desorption process during the irradiation does not cause breakdown of the organic compounds of interest, thus further enhancing the extraction efficiency of this method. Through microwave heating, solvent temperatures above their normal boiling points are obtainable within closed vessels. The extraction of organochlorine pesticides, polycyclic aromatic hydrocarbons, phenols and base/neutral compounds from sediments and soils using a domestic microwave oven or a modified microwave digestion system (MDS) has been previously described [5,6]. The microwave-assisted solvent extraction technique has also been extended to the extraction of essential oils and natural products from different biological materials such as plants and fish tissues, and stabilizers from polyolefins [7–9]. This method is able to reduce the sample preparation time to less than 30 min and solvent consumption volumes to under 50 ml as compared with the many hours and hundreds of milliliters of organic solvents needed for conventional techniques. Several control and safety features, e.g., temperature and pressure control systems, solvent detector, integrated exhaust systems and closed vessel technology allow easier and safer operation, and also better extraction efficiency with little sample loss.

PAHs are commonly analyzed by capillary gas chromatography with flame ionisation detection (FID) or mass spectrometric detection (GC–MS) [10–15] or by high-performance liquid chromatography (HPLC) with ultraviolet (UV) and/or fluorescence detection [16–18]. GC–MS and HPLC–fluorescence detection tend to be the more reliable techniques for the determination of PAHs due to their sensitivity and selectivity. GC–MS can be used to confirm the presence of PAHs in real samples. As for HPLC, it is ideally suited for the determination of sixteen United States Environmental Protection Agency (US EPA)-priority PAHs. Good separation and also an optimal wavelength program for both UV and fluorescence detection of individual PAHs can be developed with HPLC [19–21].

In order to optimize the conditions for the MASE technique, we have employed the orthogonal array design (OAD) procedure. The theory and methodology of OAD as a chemometric method for the optimization of analytical procedures have been described in detail elsewhere [22–28]. After the OAD procedure has been conducted, analysis of variance (ANOVA) is used for estimating the factor effects. In the case of mixed-level OAD, the use of HPLC determination of PAHs as a practical example to demonstrate its applicability in handling discrete main variables and also variables in different level settings has been described [28]. The advantages of using a mixed-level OAD over the two-level designs have also been mentioned earlier [28]. Thus, the mixed-level OAD procedure with OA_{16} $(4^1 \times 2^{12})$ matrix was used to optimize the following variables for MASE technique: extracting solvent, extraction temperature, duration of extraction and volume of extracting solvent.

It is the aim of the present paper to develop a MASE technique using MES for the extraction of PAHs from marine sediment, and show its advantages over the classical Soxhlet extraction: higher extraction efficiency, faster sample preparation time and lower solvent consumption. The use of the mixed-level OAD procedure for the optimization of the MASE conditions was also demonstrated in this work. Finally, the optimized MASE technique was applied to coastal marine sediment sampled from Tuas Bay and Jurong Bay (primary industrial areas in Singapore) for the determination of the level of PAHs in these areas.

2. Experimental

2.1. Reagents and instrumentation

All pesticide-grade and HPLC-grade organic solvents used were obtained from Fischer Scientific (Fair Lawn, NJ, USA). The sixteen polycyclic aromatic hydrocarbons (PAHs) studied were naphthalene, acenaphthalene, acenaphthylene, fluorene, phenanthrene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene, benzo[*a*]pyrene, dibenzo[*a,h*]anthracene, benzo[*ghi*]perylene and indeno[1,2,3-*cd*]-pyrene. They were purchased from Ultra-Scientific

(North Kingston, RI, USA) and were of analytical grade. A standard solution mixture of sixteen PAHs was prepared in benzene–dichloromethane (1:1, v/v) containing a nominal concentration of 2000 mg/dm^3 for each component. The standard solution was further diluted with toluene or methanol to 1 mg/dm^3 as working standard solution. Semi-volatile internal standards mixture containing acenaphthene-d10, chrysene-d12, 1,4-dichlorobenzene-d4, naphthalene-d8, perylene-d12, phenanthrene-d10 was obtained from Ultra-Scientific. A 1000 mg/dm^3 stock solution was prepared in dichloromethane and diluted to 50 mg/dm^3 as the working solution with dichloromethane–toluene (1:1, v/v).

Two certified reference materials (HS-4 and HS-6) for PAHs in marine sediment collected from four harbours in Nova Scotia were used in this study. They were obtained from the National Research Council of Canada, Atlantic Research Laboratory (Halifax, Canada). Both standards were prepared by a process of freeze-drying, sieving through a 125-μm sieve, homogenizing in a cement mixer and then subsampling into 200-g portions.

The microwave-assisted solvent extraction was carried out using a CEM MES-1000 microwave extraction system (CEM, Matthews, NC, USA) equipped with a solvent detector, a safety feature of the MES-1000. It is a tin oxide semiconductor gas sensor designed for detecting vapours of organic solvents, i.e., C–H compounds which may be present in the system cavity. The MES-1000 is able to extract concurrently twelve solid samples in Teflon-lined extraction vessels under identical extraction conditions (temperature and pressure). The temperature control system consists of a microwave-inert fiber optic temperature probe with phosphor as the temperature sensor positioned at the end of the probe. Phosphor is a material which emits fluorescent light after excitation by an optical energy source. An inboard pressure control system is installed for monitoring and controlling pressure conditions inside the extraction vessels.

For HPLC determination of PAHs, a Waters (Milford, MA, USA) powerline system consisting of the Waters 600E systems controller, Waters 486 tunable absorbance detector, Waters 470 scanning fluorescence detector, and Waters 700 satellite WISP autosampler was employed. The Maxima 825/Baseline 815 powerline HPLC software was used to control the HPLC system. The HPLC conditions employed for the effective separation of sixteen PAHs were described in a previous paper [28]. For UV detection, the wavelength was set at 254 nm. For quantitation of PAHs in sediment samples, a nine-step fluorescence program was developed to optimize the fluorescence detection for all individual PAHs except for acenaphthylene, which does not show any fluorescence response (Table 1). Thus, UV detection was used for the quantitation of acenaphthylene. The excitation (E_x) and emission (E_m) wavelengths in the fluorescence program were selected from previous publications [19–21] and then further optimized by comparing the relative fluorescent intensity for individual PAHs under a different combination of E_x/E_m wavelengths.

For GC analysis of PAHs, a Hewlett-Packard (Palo Alto, CA, USA) HP 5890 series II gas chromatograph equipped with a MS detector was used. The GC–MS was operating in the selective-ion monitoring (SIM) mode, and a macro programmed with nine acquisition groups was allocated for SIM of all the PAHs' quantitation ions. The separation of PAHs was conducted with a Hewlett-Packard HP-5 column (30 m×0.25 mm I.D., and film phase thickness of 0.25 μm). Helium gas was used as the carrier. The line source temperature, injector port temperature and transfer line temperature were held isothermally at 200°C, 280°C and 300°C, respectively. The initial oven temperature was held for 12 min at 50°C and increased linearly at a rate of 10°C/min to 280°C, with the final temperature held for 10 min.

2.2. Optimization strategy for MASE

The conditions for the MASE technique should be optimized before carrying out any comparative study with the classical methods or running MASE technique for routine analysis. The four variables selected for optimization of MASE conditions were: (i) different types of extracting solvents (factor A); (ii) extraction temperature (factor B); (iii) duration of extraction (factor C); and (iv) volume of extracting solvent (factor D). The different types of extracting solvents being used in this optimization include: dichloromethane (I), acetone–hexane (1:1, v/v, II),

Table 1
Fluorescence program for the detection of sixteen priority PAHs

PAHs	Peak No.	Retention time (min)	Program time (min)	Fluorescence (nm) (excitation/emission)
Naphthalene	1	4.5	0	218/357
Acenaphthylene	2	5.0[a]		
Acenaphthene	3	5.8	4.9	280/340
Fluorene	4	6.0		
Phenanthrene	5	6.5	6.2	250/375
Anthracene	6	7.0	6.8	234/440
Fluoranthene	7	8.2	8.0	270/410
Pyrene	8	9.0		
Benzo[*a*]anthracene	9	9.4	9.1	286/405
Chrysene	10	10.6	9.9	265/385
Benzo[*b*]fluoranthene	11	13.2	11.3	290/420
Benzo[*k*]fluoranthene	12	13.5		
Benzo[*a*]pyrene	13	14.5		
Dibenzo[*a,h*]anthracene	14	15.2		
Benzo[*ghi*]perylene	15	17.1	16.0	287/412
Indeno[1,2,3-*cd*]pyrene	16	17.5	17.2	293/498

[a] UV detection.

acetone–petroleum ether (1:1, v/v, III) and methanol–toluene (9:1, v/v, IV). The level setting values of the main variables (A, B, C and D) used in mixed-level OAD are displayed in Table 2. One factor not considered was the power setting of the microwave heating. The power setting of the microwave heating was proportional to the number of samples to be extracted during one extraction run. Basically, the power is maintained at 50%. Because one four-level and five two-level variables were to be considered, the OA_{16} $(4^1 \times 2^{12})$ matrix was employed to assign the variables considered, and for the following two-variable interactions which might occur. Previous experience with MASE and intuition were necessary to handle two-variable interactions. Two of the two-variable interactions to be considered were A×B (interaction between different types of extracting solvent and extraction temperature) and A×D (interaction between different types of extracting solvents and volume of extracting solvents). The assignment of the main-variable and two-variable interactions and their levels has been previously described [28].

Average recovery (AR) was calculated from the sum of percentage recoveries of sixteen PAHs found in CRM HS-6. It can be used as a response function because it can take into consideration the effect of changes in the variables on the extraction efficiency

Table 2
Assignment of the variables and the arrangement of the experiment runs using an OA_{16} $(4^1 \times 2^{12})$ matrix

Column No.												
1	2	3	4	5	6	7	8	9	10	11	12	13
A	B	$(A\times B)_1$ **$(C\times D)$**[a]	$(A\times B)_2$	$(A\times B)_3$	C	$(A\times C)_1$ **$(B\times D)$**	$(A\times C)_2$	$(A\times C)_3$ **$(A\times D)_1$**	B×C	D	$(A\times D)_2$	$(A\times D)_3$
I	115				15					30		
II	135				5					45		
III												
IV												

A=Different types of extracting solvents; B=extraction temperature (°C); C=extraction time (min); D=volume of solvent (ml).
[a] The interactions in bold can be neglected according to experience.

of this technique. However, one important point to note is that this response function is not a continuous response. In other words, no quadratic polynomial representing the response surface can be established.

2.3. Sample preparation

For every sample in each batch extracted by MASE technique with MES, 5 g of sediment samples were accurately weighed out and quantitatively transferred into the Teflon-lined extraction vessel. After adding the respective volume of the extracting solvents (Table 2 and Table 3), an equilibration of 10 min was allowed before extraction. The rupture membrane within the reference extraction vessel was renewed before each batch of extraction. During optimization of the MASE conditions, the extraction conditions of each pre-designed experimental trial (a total of 16) were set according to the mixed-level OAD procedure given in Table 2 and Table 3. After extraction, all extraction vessels were cooled down to room temperature before opening. This was to prevent any organic solvent fumes being detected by the solvent detector within the instrument cavity, causing a shut-down. After a series of filtration, washing and centrifugation, and then preconcentration by nitrogen blowdown, a 2-ml extract was finally ready for instrumental analysis. In the case of HPLC analysis, solvent exchange of the extract to methanol is necessary. A volume of 20 μl of the extract was injected for HPLC analysis. In the case of GC–MS analysis, solvent exchange of the extract to toluene is carried out whenever possible, especially during the optimization exercise. The extract was concentrated down to less than 2 ml by nitrogen blowdown, and followed by the addition of a small volume of internal standards solution to make-up the extract volume to 2 ml.

For samples using Soxhlet extraction, 5-g samples were carefully weighed and quantitatively transferred into a pre-rinsed (using dichloromethane) extraction thimble and extracted with 300 ml dichloromethane for 16 h in a Soxhlet apparatus. The sample treatment procedure was similar to that mentioned above for MASE samples.

"Real world" sediment samples were collected

Table 3
The OA_{16} ($4^1 \times 2^{12}$) matrix with the experimental results

Expt. No.	Column No.													Average recovery
	1	2	3	4	5	6	7	8	9	10	11	12	13	
1	1	1	1	1	1	1	1	1	1	1	1	1	1	85.0
2	1	1	1	1	1	2	2	2	2	2	2	2	2	73.2
3	1	2	2	2	2	1	1	1	1	2	2	2	2	69.5
4	1	2	2	2	2	2	2	2	2	1	1	1	1	78.1
5	2	1	1	2	2	1	1	2	2	1	1	2	2	78.0
6	2	1	1	2	2	2	2	1	1	2	2	1	1	88.9
7	2	2	2	1	1	1	1	2	2	2	2	1	1	75.4
8	2	2	2	1	1	2	2	1	1	1	1	2	2	79.2
9	3	1	2	1	2	1	2	1	2	1	2	1	2	68.9
10	3	1	2	1	2	2	1	2	1	2	1	2	1	77.0
11	3	2	1	2	1	1	2	1	2	2	1	2	1	65.0
12	3	2	1	2	1	2	1	2	1	1	2	1	2	59.2
13	4	1	2	2	1	1	2	2	1	1	2	2	1	60.6
14	4	1	2	2	1	2	1	1	2	2	1	1	2	64.0
15	4	2	1	1	2	1	2	2	1	2	1	1	2	74.8
16	4	2	1	1	2	2	1	1	2	1	2	2	1	65.0
	Average responses													
r_1	76.5	74.5	73.6	74.8	70.2	72.2	71.6	73.2	74.3	71.8	75.1	74.3	74.4	
r_2	80.4	70.8	71.6	70.4	75.0	73.1	73.6	72.0	71.0	73.5	70.1	70.9	70.9	
r_3	67.5													
r_4	66.1													

from locations ca. 1 km from the coast of primary industrial areas (Jurong and Tuas bays) in Singapore. The wet sediment samples were air-dried at 25°C for 72 h and then homogenized to give regular size sediment particulates. The sediment samples were subjected to MASE, and then the extracted samples

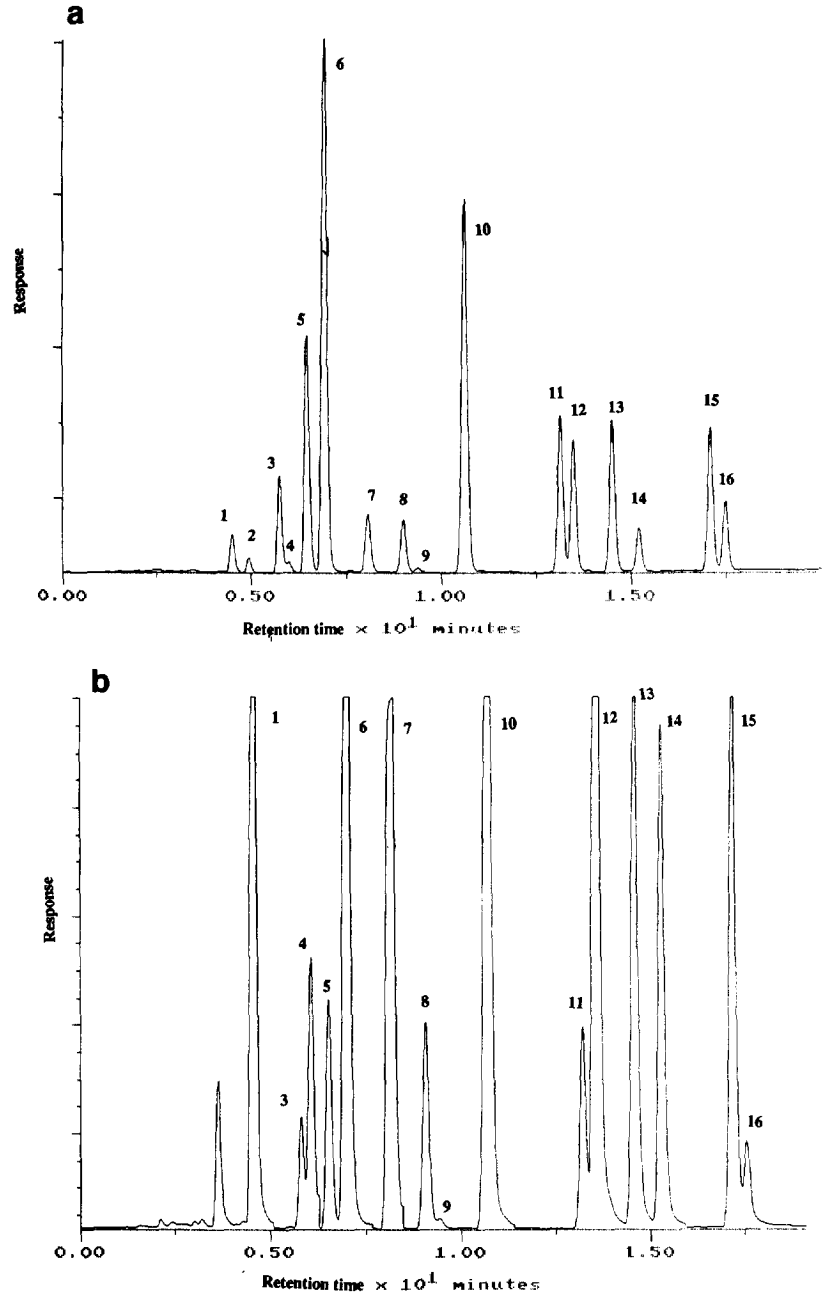

Fig. 1. (a) HPLC–UV chromatogram of standard mixture of sixteen PAHs in methanol. (b) HPLC–fluorescence detection chromatogram of standard mixture of sixteen PAHs in methanol using the optimal fluorescence program given in Table 1. The order of elution of PAHs and their respective retention times are given in Table 1.

were sent for HPLC–UV–fluorescence detection or GC–MS analysis for the determination of PAHs. The results are presented in Table 7.

3. Results and discussion

HPLC chromatograms for the chromatographic determination of a standard mixture of sixteen PAHs using UV and fluorescence detection are given in Fig. 1a and Fig. 1b, respectively. Using the optimized HPLC conditions, the whole chromatographic run was completed within 20 min. The order of elution of sixteen PAHs and their respective retention times are provided in Table 1. Fluorescence detection is more sensitive than UV detection by at least two to three orders of magnitude. Among the sixteen PAHs, only acenaphthylene does not display any fluorescence. Thus, using the optimal fluorescence program, the fluorescence abundance of all individual PAHs can be used for the quantitation of PAHs in sediment samples. The program also takes into account the relative sensitivity of each PAH, the probable occurrence and abundance of each PAH in environmental samples, and finally the ease of operating the program within the elution profiles of all PAHs without affecting the resolution and the baseline during the run. However, in practice, owing to the presence of some PAHs in high concentrations (phenanthrene, fluoranthene, pyrene, and chrysene) in sediment samples, their sensitivities are deliberately detuned to prevent overloading of signals by using less optimized E_x and E_m wavelengths.

During the GC–MS analysis, all 21 PAHs including the 5 internal standards can be well-resolved within 45 min. Naphthalene-d8 was eluted first at about 17.9 min, while benzo[*ghi*]perylene was eluted last at about 43.2 min. The same GC program was used throughout the whole analysis. The effect of solvent on the responses of the hydrocarbons has been well-addressed [29–33]. Grob and Grob [29–31] identified the effect of solvent as one of the important factors for hydrocarbons during splitless injection mode and on-column injection. The theoretical basis of the solvent effect has also been provided by Jennings et al. [32]. The effect of solvent on the responses of late-eluting PAHs has been further demonstrated during the optimization of GC parameters for sixteen top-priority PAHs [33]. The responses of late-eluting PAHs can be increased by using xylene as the solvent system instead of benzene [33]. In order to reduce the effect of solvent on the responses of PAHs, standard solutions can be prepared in the same solvent system as the final extract during quantitative analysis. In our present work, toluene was used as the solvent system for the final extract, as the solvent is suitable for PAH analysis.

After implementing the sixteen experimental trials which were pre-designed according to the OA_{16} ($4^1\times2^{12}$) matrix, the corresponding AR results for each experimental trial were calculated and then tabulated in Table 3. The average of the responses (r_1, r_2, r_3 and r_4) for each factor at different levels were also calculated and given in Table 3.

Based on methods described in previous work [22,23,28] the results of the sums of squares for different variables and so-called interaction columns were calculated and are given in Table 4. Two-variable interactions assigned to columns 7, 8, 9 and

Table 4
ANOVA table including percent contribution for the percentage recovery in the OA_{16} ($4^1\times2^{12}$) matrix

Source	SS	DF	MS	F^a	Significance
Extracting solvent (A)	557.2	3	185.7	11.4	$P<0.05$**
Extraction temperature (B)	88.4	1	88.4	5.4	$P<0.1$*
Extraction time (C)	4.8	1	4.8	0.3	
Vol. of solvent (D)	57.8	1	57.8	3.5	
A×B	255.2	3	85.1	5.2	$P<0.1$*
A×D	134.6	2	67.3	4.1	
Error (columns 7–10)	65.0	4	16.3		
Total	1163.0	15			

SS=sum of squares; DF=degrees of freedom; MS=mean squares.
[a] The critical F value is 7.71 (**$P<0.01$) and 4.54 (*$P<0.1$).

Table 5
Four-by-two table for the analysis of the A×B interaction

	Average recovery			
	A_1	A_2	A_3	A_4
B_1	79.1	83.5	75.0	62.3
B_2	73.8	77.3	60.1	69.9

10 could be treated as dummies and used for estimating the error variance. Consequently, the ANOVA tables were constructed (Table 4). From Table 4, it can be seen that factor A (extracting solvent), factor B (extraction temperature), and the two-variable interaction A×B (interaction between extracting solvent and extraction temperature) are statistically significant ($P<0.1$), whereas no statistical differences are observed for any other variable or interaction considered ($P<0.1$). Minor differences are observed for the following variables: factor A is significant at $P<0.05$, while both factor B and A×B are significant at $P<0.1$. However, care must be taken during the interpretation of significance (F ratio) in the mixed-level OAD. This is because the level of significance obtained from the calculation of the F ratio reflects just the relative magnitudes of the incremental effects of variables. For example, factor A has three incremental effects, adding up to the total effect of A, whereas factor B has one incremental effect. From the total effect of A and B, it is clear that the effect of A is larger than that of B. Thus, the magnitude of the significance (F ratio) should not be mistaken as an indication of the overall effect [28].

Coming back to compare the r_1, r_2, r_3 and r_4 for factor A, and r_1 and r_2 for factor B, it is clear that the optimum levels are A_2 and B_1. However, because the interaction A×B is statistically significant ($P<0.1$), the choice of the optimum experimental conditions for factors A and B must depend on their interaction, which can be evaluated by using a 4×2 table. The method of construction of a 4×2 table is the same as that for the 2×2 table described in detail elsewhere [22]. The 4×2 table constructed (Table 5 was shown in Table 10 in Ref. [28], from which it was clear that for AR, the combination of A_2 and B_1 would result in the maximum response.

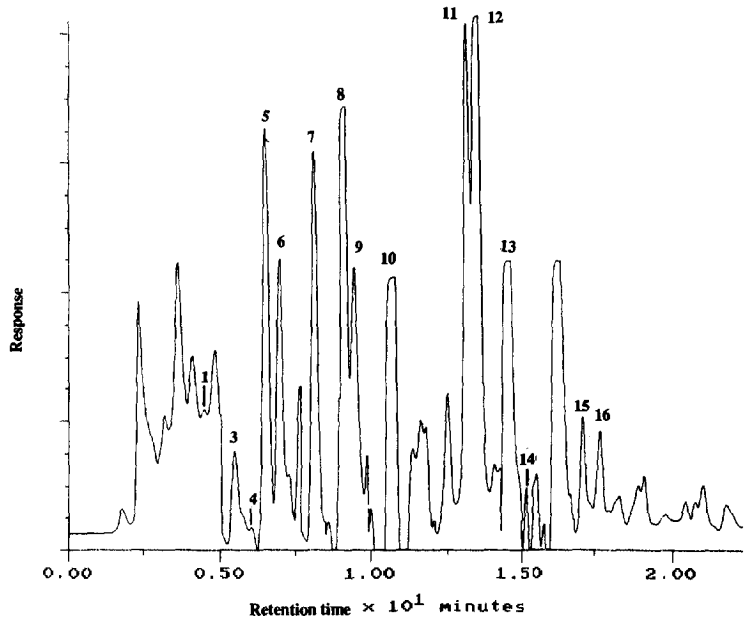

Fig. 2. HPLC–fluorescence detection chromatogram of PAHs in sediment for CRM HS-4 using the optimal fluorescence program given in Table 1. The assignment of peaks is as follows: 1=naphthalene; 3=acenaphthalene; 4=fluorene; 5=phenanthrene; 6=anthracene; 7=fluoranthene; 8=pyrene; 9=benzo[*a*]anthracene; 10=chrysene; 11=benzo[*b*]fluoranthene; 12=benzo[*k*]fluoranthene; 13=benzo[*a*]pyrene; 14=dibenzo[*a,h*]anthracene; 15=benzo[*ghi*]perylene; 16=indeno[1,2,3-*cd*]pyrene. Peak 2, acenaphthylene, is quantitated using UV detection.

Indeed, the combination of A_1 and B_1 should also be included as it gave comparable results. Thus, the optimum MASE conditions for the extraction of PAHs from sediment samples were as follows: 30 ml of acetone–hexane (1:1, v/v), an optimum microwave heating temperature of 115°C, and a 5-min duration of microwave heating. The microwave power was not optimized as it depends on the number of samples to be extracted in one run. The solvent temperature was able to reach the pre-set microwave temperature within 3–5 min of microwave heating. However, in practice, dichloromethane was used for the extraction of PAHs in sediment samples and then solvent exchange to methanol. Methanol is a more suitable solvent for the HPLC analysis as it improves the resolution during the chromatographic separation. During GC–MS analysis of PAH extract, hexane–acetone (1:1, v/v) was used as the solvent system for extractions. During optimization, it was noticed that both solvents gave comparable recoveries of PAHs from CRM HS-6. The above optimized MASE conditions were used for the extraction of PAHs in CRM HS-4 and HS-6 and analyzed by HPLC using the optimal fluorescence program. The HPLC chromatograms for CRM HS-4 and HS-6 are provided in Fig. 2 and Fig. 3, respectively. An important point to note when employing the OAD procedure is that although some parameters (duration of extraction and volume of solvent) may not be statistically significant during this optimization exercise, it does not mean that they are not important for the recoveries of PAHs during MASE. The selection of levels for each parameter may be too close or too far apart in magnitude to be statistically significant at all. Thus, it should be emphasized that adequate judgement and pre-experience are necessary in identifying appropriate factors and their levels, and this is normally a case-by-case situation.

The two analytical methods being employed in this study for the analysis of PAHs in marine sediments were also compared in terms of reproducibility of analytical results. The relationships between the concentrations of PAHs in CRMs (HS-4

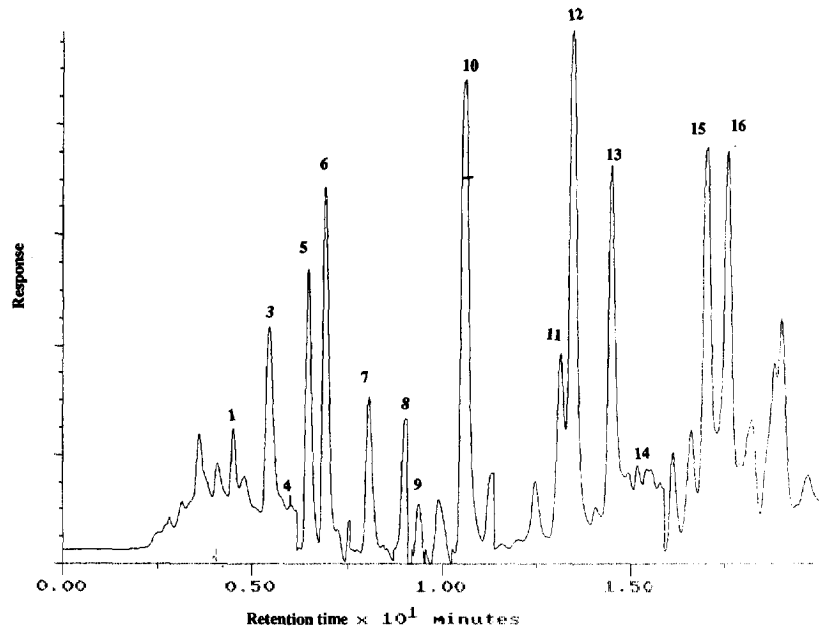

Fig. 3. HPLC–fluorescence detection chromatogram of PAHs in sediment for CRM HS-6 using the optimal fluorescence program given in Table 1. The assignment of peaks is as follows: 1=naphthalene; 3=acenaphthalene; 4=fluorene; 5=phenanthrene; 6=anthracene; 7=fluoranthene; 8=pyrene; 9=benzo[*a*]anthracene; 10=chrysene; 11=benzo[*b*]fluoranthene; 12=benzo[*k*]fluoranthene; 13=benzo[*a*]pyrene; 14=Dibenzo[*a,h*]anthracene; 15=benzo[*ghi*]perylene; 16=indeno[1,2,3-*cd*]pyrene. Peak 2, acenaphthylene, is quantitated using UV detection.

and HS-6) as determined by HPLC–fluorescence detection and those measured by GC–MS analysis during six consecutive analyses were evaluated by linear regression. The recoveries for some of the selected PAHs (fluorene, fluoranthene, pyrene, chrysene, benzo[*a*]pyrene, and benzo[*ghi*]perylene) in CRM HS-4 obtained were highly correlated (r^2= 0.98) (Fig. 4a). A similar correlation (r^2=0.98) was also found for the recoveries of PAHs in CRM HS-6 (Fig. 4b) using these two analytical methods during six consecutive analyses. The figures showed that analytical results for the concentration of PAHs in two CRMs as analyzed by these methods are reproducible. Thus, both HPLC–fluorescence detection and GC–MS analytical methods are suitable for the qualitative and quantitative screening of PAHs in sediment samples.

The results for the comparative study between the classical Soxhlet extraction and MASE methods with HPLC analysis are provided in Table 6. The recoveries of PAHs from CRM HS-4 for Soxhlet extraction were in the range of 66.0% to 111.1%, while the MASE technique gave recoveries of 73.3% to 95.7% (Table 6). In the case of CRM HS-6, Soxhlet extraction gave 69.4–100.0% recovery, while MASE gave 73.5–136.8% recovery (Table 6). The recovery results in Table 6 show that both extraction techniques were comparable in terms of efficiency concerning the recoveries of PAHs from CRMs. However, MASE using MES is able to extract twelve samples in one run in less than 30 min as compared with 16 h for one sample in Soxhlet extraction. MASE using MES is able to raise the temperature of extracting solvents above their normal

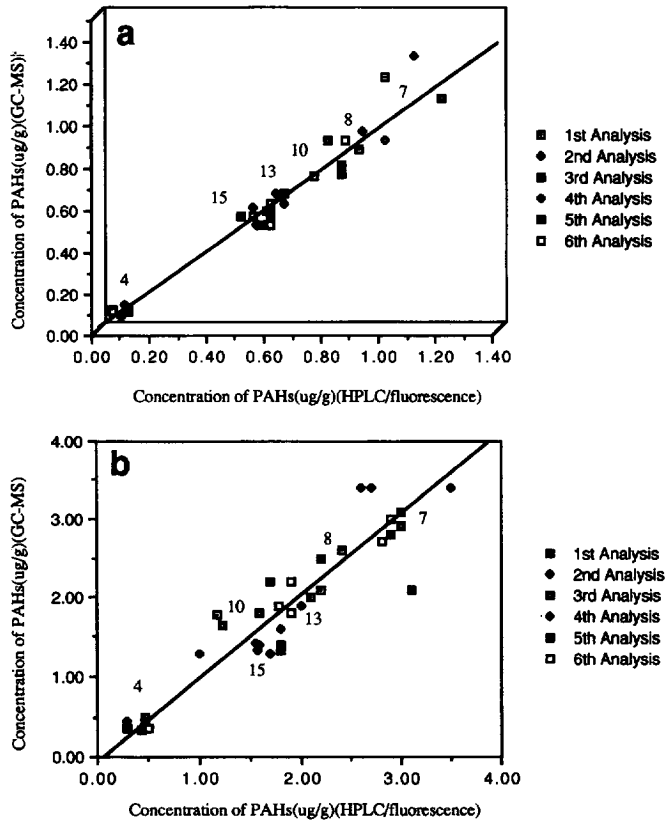

Fig. 4. (a) Correlation between recovery results of PAHs of varying concentrations in CRM HS-4 using HPLC–fluorescence detection and GC–MS analysis. (b). Correlation between recovery results of PAHs of varying concentrations in CRM HS-6 using HPLC–fluorescence detection and GC–MS analysis. The assignment of peaks for both figures are as follows: 4=fluorene; 7=fluoranthene; 8=pyrene; 10=chrysene; 13=benzo[*a*]pyrene; 15=benzo[*ghi*]perylene.

Table 6
Comparison between Soxhlet extraction (SOX) and microwave-assisted solvent extraction (MES) techniques using PAHs in marine sediments, CRM HS-4 and HS-6

PAH	HS-4 (% recovery)[a]		HS-6 (% recovery)[a]	
	SOX	MES	SOX	MES
Naphthalene	73.3	80.0	74.4	96.1
Acenaphthylene	106.7	80.0	89.5	136.8
Acenaphthene	66.7	73.3	73.9	78.3
Fluorene	73.3	73.3	89.1	94.7
Phenanthrene	85.3	88.2	85.0	101.9
Anthracene	100.0	85.7	82.7	87.6
Fluoranthene	78.4	88.0	81.6	85.6
Pyrene	96.8	95.7	97.2	84.4
Benzo[*a*]anthracene	66.0	84.9	100.0	96.5
Chrysene	98.5	93.8	88.6	73.5
Benzo[*b*]fluoranthene	101.4	92.9	84.6	100.1
Benzo[*k*]fluoranthene	111.1	75.0	81.7	86.4
Benzo[*a*]pyrene	92.9	94.3	90.0	105.0
Indeno[1,2,3-*cd*]pyrene	98.0	92.2	96.4	78.9
Dibenzo[*a,h*] anthracene	75.0	91.7	69.4	79.6
Benzo[*ghi*]perylene	82.4	86.3	88.8	84.3

[a] Average of two determinations.

boiling points using microwave irradiation, thus reducing the duration of extraction significantly. The solvent consumption per sample for MASE is only one-tenth of that in the classic Soxhlet extraction. In MASE, sample preparation time and clean-up problems can be sufficiently reduced. Thus, MASE using MES is more advantageous than the classical method as it is rapid, convenient, time- and cost-saving, has a higher extraction efficiency and is also well-suited for routine analysis.

The optimized MASE conditions were employed for the extraction of PAHs from coastal marine sediment samples obtained near primary industrial areas, and the results are given in Table 7. Only two of the sixteen PAHs being analysed were not present in the sediment. Eight PAHs (acenaphthylene, acenaphthalene, fluorene, fluoranthene, pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and benzo[*a*]pyrene) were consistently found in all the samples analysed. The individual concentrations of fourteen PAHs ranged from 0.03 to 0.35 μg/g. PAHs are nearly ubiquitous organic pollutants of marine environments, even in regions remote from industrial activities. Generally, their primary anthropogenic source in the marine environment is from combustion of fossil fuels from industries, burning of organic matter as in solid waste incinerators, activities from refinery and petroleum industries, and oil spills from tankers and other water transports. However, open solid waste burning in Singapore has

Table 7
Level of PAHs (μg/g) [a] in Singapore coastal sediment samples extracted with the microwave-assisted solvent extraction technique using the microwave extraction system

PAHs	Location[b]			
	JWF1	JWF2	TB1	TB3
Napthalene	ND	ND	ND	ND
Acenaphthylene	0.17	0.15	0.17	0.17
Acenaphthalene	0.11	0.07	0.07	0.07
Fluorene	0.17	0.17	0.22	0.17
Phenanthrene	ND	ND	0.08	ND
Anthracene	ND	ND	0.06	ND
Fluoranthene	0.24	0.17	0.20	0.20
Pyrene	0.24	0.18	0.20	0.21
Benzo[*a*]anthracene	ND	ND	ND	ND
Chrysene	ND	0.08	0.11	ND
Benzo[*b*]fluoranthene	0.35	0.31	0.35	0.34
Benzo[*k*]fluoranthene	0.29	0.23	0.27	0.27
Benzo[*a*]pyrene	0.22	0.19	0.24	0.20
Indeno[1,2,3-*cd*]pyrene	0.03	ND	ND	0.01
Dibenzo[*a,h*]anthracene	ND	ND	ND	0.15
Benzo[*ghi*]perylene	0.04	ND	0.03	ND

[a] Average of two determinations (dry weight basis).
[b] JWF=Jurong Bay; TB=Tuas Bay.
ND=not detected (<0.02 μg/g).

been tightly controlled by the Ministry of Environment. Atmospheric input could be another source of PAH contamination in the marine environment. Since PAHs are low in solubility and hydrophobic in nature, they may be greatly enriched in the inorganic and organic air particles during forest fires or other combustion processes and may be transported throughout the ecosystem by atmospheric agents. Global water movement may have an increasing effect on the accumulation of PAHs in the sediment. The persistence of sedimentary PAH upon deposition may result in greater accumulation. Further investigations have to be carried out in order to determine the main contributors responsible for the release of PAHs into the environment. Basically, the measured PAH content of CMS samples collected from the primary industrial areas (Tuas and Jurong) in Singapore can be considered moderately low. Evidently, contamination of the local marine environment by PAHs is not a serious problem.

4. Conclusions

In summary, a MASE technique using MES has been developed which is well-suited for the routine analysis of PAHs in environmental samples. Based on the amount of sample preparation procedures and cost of operation that have to be carried out prior to instrument analysis, it has many advantages over Soxhlet extraction in terms of faster extraction time, greater sample throughput, and low quantity of solvent used. The capability of a mixed-level OAD procedure for the optimization of MASE conditions has also been demonstrated in this work. The optimized MASE conditions can be used to achieve better recovery results for PAHs in marine sediment. Hence, the technique is more versatile and flexible than conventional techniques. Based on the correlation results, both analytical methods (HPLC–fluorescence detection and GC–MS) are suitable for the instrument analysis of PAHs in marine sediment. Recoveries of PAHs from two CRMs using MASE with MES were all above 73.3%. Based on the optimized MASE conditions, the level of PAHs in "real world" sediment samples collected near primary industrial areas in Singapore was found to be between 0.03 and 0.35 μg/g.

Acknowledgments

Kok Kay Chee wishes to thank the National University of Singapore for providing a research scholarship. The authors express their gratitude towards the Government of Canada for grant assistance under the ASEAN–Canada Cooperative Programme on Marine Science (CPMS) Phase II project.

References

[1] M.L. Lee, M.V. Novotny and K.D. Bartle, Analytical Chemistry of Polycyclic Compounds, Academic Press, New York, 1982, p. 462.
[2] G. Grimmer (Editor), Environmental Carcinogenens; Polycyclic Aromatic Hydrocarbons, CRC Press, Boca Raton, FL, 1983.
[3] K. Ganzler and A. Salgo, Z. Lebensm. Unters. Forsch., 184 (1987) 274.
[4] K. Ganzler and A. Salgo, J. Chromatogr., 371 (1986) 299.
[5] F.E. Onuska and K.A. Terry, Chromatographia, 36 (1993) 191.
[6] V. Lopez-Avilla, R. Young and W.F. Beckert, Anal. Chem., 66 (1994) 1097.
[7] J.R.J. Paré, et al. U.S. Patent 5 002 784, 1991.
[8] J.R.J. Paré, Eur. Pat. Appl. EP 485668 Al, 1992, Chem. Abstr., 117 (1992) 15743y.
[9] W. Freitag and O. John, Angew. Makromol. Chem., 175 (1990) 181.
[10] G.A. Eiceman and K. Lindley, Int. J. Environ. Anal. Chem., 32 (1988) 29.
[11] I.D. Brindle and X.F. Li, J. Chromatogr., 498 (1990) 11.
[12] G. Castello and T.C. Gerbino, J. Chromatogr., 642 (1993) 351.
[13] W. Auer and H. Malissa, Anal. Chim. Acta, 237 (1990) 451.
[14] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller and J. Pawliszyn, Anal. Chem., 65 (1993) 338.
[15] C. Ostman, A. Bemgard and A. Colmsjo, J. High Resolut. Chromatogr., 15 (1992) 437.
[16] H. Zobel and F. Ruppel, LaborPraxis, 3 (1993) 30.
[17] C. Grosse-Rhode, H.C. Kicinksi and A. Kettrup, J. Liq. Chromatogr., 13 (1990) 3415.
[18] J.D. Smith, J. Bagg and I. Wrigely, Water Res., 25 (1991) 1145.
[19] P.J. Marriott, P.D. Carpenter, P.H. Brady, M.J. McCormick, A.J. Griffiths, T.S.G. Hatvani and S.G. Rasdell, J. Liq. Chromatogr., 15 (1993) 3229.
[20] M.W. Dong, J.X. Duggan and S. Stefanou, LC·GC, 11 (1993) 802.
[21] E.R. Brouwer, A.N.J. Hermans, H. Lingeman and U.A.T. Brinkman, J. Chromatogr. A, 669 (1994) 45.
[22] W.G. Lan, M.K. Wong, N. Chen and Y.M. Sin, Analyst, 119 (1994) 1659.

[23] W.G. Lan, M.K. Wong, N. Chen and Y.M. Sin, Analyst, 119 (1994) 1669.
[24] H.B. Wan, W.G. Lan, M.K. Wong and C.Y. Mok, Anal. Chim. Acta, 289 (1994) 371.
[25] H.B. Wan, W.G. Lan, M.K. Wong, C.Y. Mok and Y.H. Poh, J. Chromatogr. A, 677 (1994) 255.
[26] W.G. Lan, M.K. Wong, N. Chen and Y.M. Sin, Talanta, 41 (1994) 1917.
[27] W.G. Lan, K.K. Chee, M.K. Wong and Y.M. Sin, Analyst, 120 (1995) 273.
[28] W.G. Lan, K.K. Chee, M.K. Wong, H.K. Lee and Y.M. Sin, Analyst, 120 (1995) 281.
[29] K. Grob and K. Grob, Jr., J. Chromatogr. Sci, 7 (1969) 584.
[30] K. Grob and K. Grob, Jr., J. Chromatogr., 94 (1974) 53.
[31] K. Grob and K. Grob, Jr., J. High Resolut. Chromatogr. Chromatogr. Commun., 1 (1978) 57.
[32] W.G. Jennings, R.R. Freemann and T.A. Rooney, J. High Resolut. Chromatogr. Chromatogr. Commun., 1 (1978) 275.
[33] W.R. Trevelin, L.H. Vidal, M.D. Landgraf, I.C.E. Silva and M.O.O. Rezende, Anal. Chim. Acta, 268 (1992) 67.