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Improved ultrasonic extraction procedure for the determination of polycyclic aromatic hydrocarbons in sediments

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Abstract

The aim of this work was to optimize an ultrasonic extraction procedure for the determination of polycyclic aromatic hydrocarbons (PAHs) in sediments and to compare it with the reflux procedure using methanolic potassium hydroxide. Sample extracts were purified with a miniaturized silica gel chromatographic column and analyzed by gas chromatography–mass spectrometry (GC–MS). Ultrasonication using *n*-hexane–acetone (1:1, v/v) solvent mixture on dried homogenized marine sediment gave better precision (smaller relative standard deviation (RSD) values) and comparable quantities of individual PAH's compared to the reflux procedure. Ultrasonication with the *n*-hexane–acetone (1:1, v/v) mixture, utilizing four 15 min extraction cycles, was found to be sufficient for extracting PAHs from wet sediments. The optimized ultrasonic extraction procedure extracted aliphatic and aromatic hydrocarbons from the National Institute of Standards and Technology SRM 1941a with recoveries greater than 90%. The major advantages of ultrasonication compared to the reflux method are the lower extraction times, simplicity of the apparatus and extraction procedure. The optimized ultrasonication procedure has been used in our laboratory to extract hydrocarbons from naturally wet sediments from rivers, and coastal and marine areas.

Keywords: Ultrasonic extraction; Polycyclic aromatic hydrocarbons; Chromatographic column

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are recognized as potent carcinogens and numerous studies have shown that they are ubiquitous contaminants in a wide variety of matrices such as air, food, fly ash, soil, sediments, water, crude oil and petrochemicals [1–4]. There are a wide variety of solvent extraction techniques commonly used for extracting hydrocarbons from soils and sediments. Traditional extraction procedures include Soxhlet [5–8], ultrasonication [6,9–11], mechanical shaking [12,13], reflux with methanolic KOH [8,14], and steam distillation [15]. Modern techniques include supercritical fluid extraction (SFE) [16–20], pressurized liquid extraction (PLE; Dionex trade name ASE for accelerated solvent extraction) [21–27], microwave assisted extraction (MAE) [28–30] and focused microwave assisted extraction (FMAE) [31–32]. Each technique has its own merits and the choice of extraction depends on several factors including capital cost, operating cost, simplicity of operation, amount of organic solvent required, sample throughput and the availability of a standardized method [33].

Various studies have been conducted to compare traditional procedures with modern techniques of extraction [5,12,22,23,26,28,29,31,33–37]. Soxhlet extraction is the recommended method by the US Environmental Protection Agency (EPA) for extracting semi-volatile and non-volatile organics from solid matrices. Soxhlet extraction has been the standard and preferred method since it is an easily standardized technique with high recoveries, compared to matrix-dependent techniques such as MAE, PLE and particularly

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SFE [5,23,33]. However, Soxhlet extraction is laborious, requires a large amount of solvent and can degrade thermally labile compounds [5].

In comparison, ultrasonication is an efficient technique, when compared to reflux methods for extracting trace organics from soils and sediments. For example, studies have shown that ultrasonic extraction yields comparable [6,38] or even greater quantities [10,37,39–43] of hydrocarbons than other techniques of extraction, although ultrasonication gave lower recoveries in other studies [7,12,42,44]. The reproducibility obtained with ultrasonic extraction was higher [37,43] or lower [12,45,46] than those from Soxhlet extraction. Optimization of the ultrasonic extraction parameters, including solvent or solvent composition, extraction time, sample load, and water content are therefore required for more efficient and reproducible extractions [12].

Various studies have been reported on the efficiency of ultrasonication for organic analytes in sediments and soils utilizing different solvents. Grimalt et al. [6] reported higher efficiencies with methylene chloride–methanol solvent as compared to *n*-hexane and chloroform for extracting aromatic hydrocarbons in a freeze-dried marine sediment. No significant improvement was observed beyond six extraction cycles, when using a solvent/sediment ratio of 4 (v/w). However, when using a solvent/sediment ratio of 8 (v/w), the extraction was essentially accomplished in three extraction cycles. In comparison, Babíc et al. [47] reported that acetone gave the highest recovery rate for spiked pesticide soil samples compared with other solvents such as diethyl ether, chloroform, *n*-hexane, benzene, acetonitrile and dichloromethane. For a 10 g sample, the best recovery of pesticides was obtained with acetone (a single extraction) for 15 min. Longer ultrasonic extraction time caused a decrease in pesticides recovered probably due to degradation. Sun et al. [10] reported on the extraction efficiencies of various solvent media utilizing ultrasonication and solid phase extraction clean-up for the US EPA 16 priority pollutant PAHs in soils. Acetone was found to be most efficient solvent for extracting PAHs in the soil sample investigated. The order of extraction efficiencies of PAHs by the solvents used were as follows: acetone > methanol > dichloromethane ≈ acetonitrile > 2-propanol > cyclohexane.

In addition to improved recoveries obtained using polar solvents such as acetone [47] or methanol [6], ultrasonication using these solvents circumvents the need for sample drying prior to extraction [36,39,48]. Both oven-dried [8,12] and freeze-dried samples [12] have been shown to reduce the recoveries of hydrocarbons. Losses of hydrocarbons up to 16% were observed, as a consequence of oven drying [8] of samples at 45 °C. Use of high temperatures in Soxhlet extraction [43] also results in losses of hydrocarbons attributable to volatilization and/or oxidation of highly volatile and thermally labile species.

Ultrasonication offers several advantages that make it an ideal method for analyzing a large number of samples. These include high extraction efficiency, lower equipment costs and ease of operation, little or no sample preparation (e.g. wet sediments), lower extraction temperatures and the ability to process batches of samples makes ultrasonic extraction an ideal method for laboratories analyzing large numbers of samples.

The aim of this work was to optimize an ultrasonic procedure for extracting hydrocarbons in marine sediments and to compare it with the standard reflux procedure (IOC, 1982) [14] used in our laboratory. For the optimization of the ultrasonic extraction procedure, the homogenized sediment samples were first analyzed for the EPA 16 PAHs priority pollutants. The accuracy and precision of the optimized ultrasonic extraction procedure was then determined by comparison with values of pristane, PAHs and selected aliphatic hydrocarbons obtained by extraction, and those values reported from the Standard Reference Material, SRM 1941a (marine sediment from the National Institute of Standards and Technology (NIST), Gaithersberg, MD, USA) [49]. Reduction of cost and time of petroleum hydrocarbon extraction from wet sediments, using environmentally friendly solvents was also intended.

2. Experimental

2.1. Reagents and materials

All solvents used (acetone, *n*-hexane, dichloromethane and methanol) were of analytical reagent grade (>99%) and supplied by Fisher Scientific, NJ, USA. Silica gel used for miniaturized column chromatography was 70–230 mesh (Sigma Aldrich, St Louis, MO, USA) and was activated by heating at 180 °C for 12 h. The silica gel was then cooled and stored in a desiccator. Anhydrous sodium sulfate, copper filings and KOH pellets were supplied by BDH (Poole, UK). Copper filings were activated by a 7 M HCL solution and consecutively rinsed with distilled water, acetone and *n*-hexane respectively, prior to use.

A standard mixture of the EPA 16 priority PAHs (2000 μg/ml, dichloromethane/benzene): naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), benzo[*a*]anthracene (B[*a*]A), chrysene (Chr), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), indeno[123-*cd*]pyrene (I[*c*,*d*]P), dibenzo[*a*,*h*]anthracene (DB[*a*,*h*]A) and benzo[*ghi*]perylene (B[*g*,*h*,*i*]P) was obtained from Supelco, Bellefonte, PA, USA. Appropriate working dilutions of the standard solution with dichloromethane were made. Aliphatic hydrocarbons (*n*-C12, *n*-C14, *n*-C16, *n*-C17, *n*-C18, *n*-C22 and *n*-C24) as well as internal standards ([$^2H_8$]naphthalene, [$^2H_{12}$]chrysene, [$^2H_{12}$]perylene and 5α-androstane) were also obtained from Supelco. A Standard Reference Material (SRM 1941a) was obtained from NIST.

2.2. Miniaturized chromatographic column purification of extracts

The miniaturized chromatographic column consisted of a pasteur pipette (0.5 i.d. × 10 cm) fitted at its base with a plug of glass wool. Activated copper filings, filled to a height of 1 cm, were placed at the base of the column to remove elemental sulfur. A silica gel slurry was made up in *n*-hexane and filled under gravity into the column to a height of 5 cm prior to use. Extracts (not more than 1.0 ml and washes) were loaded into the column after which a small amount of anhydrous silica gel (0.5 cm in height) was applied to the top of the column to prevent disturbance by the eluting solvent. The chromatographic column was eluted under gravity (flow-rate of approximately 2 drop/s) with 5 ml of *n*-hexane to remove the aliphatic fraction, and 10 ml of *n*-hexane–dichloromethane (10:1, v/v) mixture, to provide the aromatic fraction. The eluates were concentrated to a few μl before gas chromatographic–mass spectrometric analysis (GC–MS). Spiking of the miniaturized chromatographic column was done with the US EPA 16 priority PAHs and recoveries obtained for all PAHs were greater than 95%.

2.3. Sample collection and preparation of sediment material

A dried, homogenized sediment was used in the optimization experiments. This was necessary such that the extraction efficiency of different procedures could be reasonably compared, since homogenization reduces the variability of analyte content between sub-samples [6]. The variability due to a heterogenous matrix could be larger than the difference between methods of extraction such that statistical significance between methods cannot be determined using such matrix. The sediment sample was collected from the Gulf of Paria (GOP), Trinidad, in a subtidal area that was previously studied [50] and known to contain PAHs. Three grab samples were collected by means of a *n*-hexane-rinsed 0.04 m^2 van Veen grab sampler (Kahlsico International Corp., USA). Approximately 15 kg of wet sediment was placed in a *n*-hexane-rinsed aluminum bag for transport to the laboratory. The sediment sample was placed on a metal tray and dried at 40 °C for 72 h in an oven. The dried material was crushed and sieved to obtain the <125 μm fraction. This sediment fraction was stored in a clean (aluminum stoppered) glass jar until analysis. Aliquots of 15 g sediment sample was accurately weighed out and used for each extraction procedure. The moisture content of the sub samples was determined before each experiment and found to be <2%.

2.4. GC–MS analysis conditions

The sample extracts were analyzed on a HP 6890 Gas Chromatograph, equipped with an HP 6890 Mass Selective Detector (Hewlett-Packard Ltd., USA). A 30 m × 0.25 mm i.d. with a 0.25 μm film thickness HP 5% phenylmethyl siloxane capillary column was used for all analyses. The temperature program used was as follows: initial column temperature of 90 °C (held for 1 min), 9 °C/min to 150 °C (held for 2 min), 6 °C/min to 220 °C, 7 °C/min to 260 °C, 2.5 °C/min to 280 °C, then finally 6 °C/min to 300 °C (held for 10 min). Helium was used as carrier gas at constant flow-rate of 1.2 ml/min. The split/splitless injector was set at 250 °C and operated in the splitless mode (purge delay 1 min, purge flow 5 ml/min). Splitless injection (1 μl) was performed by an HP 6890 automatic injector (Hewlett-Packard Ltd., USA). The temperature of the ion source and mass spectrometer transfer line was maintained at 180 and 290 °C, respectively. Data was acquired in the full scan mode (electron impact: 70 eV: 2500 V) applying a mass range of *m*/*z* 45–550 with scan time of 0.6 s throughout this study. The full scan mode is particularly useful for archival purposes, when investigating environmental samples so that the presence of different compounds, other than target compounds could be verified later. However, it is advisable to operate in the SIM mode when limited compounds of interest are being investigated, since it is more sensitive than the scan mode and better precision would be obtained. The analytes in the samples were identified by matching the retention time of each compound with the retention times in the calibration standards and mass spectral library (NBS 56K). PAH and aliphatic hydrocarbon quantitation was achieved using a five-point calibration plot (containing 10, 5, 2.5, 1 and 0.5 μg/ml standard mixture) utilizing the internal standards ($[^2H_8]$naphthalene, $[^2H_{12}]$chrysene, $[^2H_{12}]$perylene and 5α-androstane). Linear regression was used with correlation coefficients between 0.9998 and 0.9980. Limits of detection (LOD) and limits of quantification (LOQ) were estimated as the lowest concentration of analytes having clear discerned peaks with signal to noise ratios (S/N) of 3 and 10, respectively. A spiked standard sample was used in which serial dilution of the resultant extract were made at the final lowest concentration, to give the desired S/N ratios for estimating LOD and LOQ values. LOD were approximately 1 μg/kg/PAH and 2 μg/kg/aliphatic whereas LOQ were approximately 3 μg/kg/PAH and 6 μg/kg/aliphatic. Data was acquired and processed with Chemstation software (Hewlett-Packard Ltd., USA).

2.5. Extraction

2.5.1. Methanolic KOH extraction

This procedure was done according to the method outlined in the IOC Manuals and Guides No. 11 (IOC, 1982) [14]. Extractions were performed in triplicate and included a method blank. Homogenized sediment (15 g) was accurately weighed out in a 250 ml round bottom flask, 100 ml of 0.5 M methanolic KOH was added and sample refluxed for 12 h. The sample was then filtered through Whatman #40 filter paper, under vacuum in a buchner funnel. The filtrate was extracted with *n*-hexane (50 ml × 3) in a separatory funnel and the combined filtrate evaporated on a rotary evaporator

Table 1
Extraction procedures used for extraction of PAHs from homogenized sediment

Procedure code	Sediment condition	Solvents (total volume—100 ml)	Time (min × number of cycles)	Notes about extraction cycle interval[a]
Reflux method	Dry[b]	Methanolic KOH	–	–
A	Dry	*n*-Hexane–methanolic KOH (1:1, v/v)	30 × 2	50 ml of solvent mixture added and sonicated for 30 min, solution removed and step repeated for each cycle
B	Dry	*n*-Hexane–acetone–methanolic KOH (1:1:1, v/v)	30 × 2	Same as procedure A, but with different solvent
C	Dry	*n*-Hexane–acetone (1:1, v/v)	30 × 2	Same as procedure A, but with different solvent
D	Dry	*n*-Hexane–acetone (1:1, v/v)	15 × 2	Same as procedure C, but at 15 min intervals
E	Dry	*n*-Hexane–acetone (1:1, v/v)	10 × 3	Same as procedure D, but at 10 min. intervals using 33.3 ml of solvent for each cycle
F	Wet[c]	*n*-Hexane–acetone (1:1, v/v)	10 × 3	Same as procedure E, but using wet sediment
G	Wet	*n*-Hexane–acetone (1:1, v/v)	10 × 3	16.7 ml of acetone added first, sonicated for 5 min before addition of 16.7 ml *n*-hexane and sonicated for further 5 min, solution removed and step repeated for each cycle
H	Wet	*n*-Hexane–acetone (1:1, v/v)	7.5 × 4	Same as procedure G, but using 12.5 ml of each solvent and sonication for 3.75 min intervals for each cycle
I	Wet	*n*-Hexane–acetone (1:1, v/v)	6 × 5	Same as procedure G, but using 10 ml of each solvent and sonication for 3 min intervals for each cycle

[a] Before each extraction cycle, contents of flask allowed to settle for approximately 3 min.
[b] Approximately 2% moisture.
[c] Approximately 40% moisture, distilled water added to sediment samples and left overnight for 8 h prior to extraction.

(Büchi R-114) at 40 °C to near dryness (≥1 ml). Extracts were separated into aliphatic and aromatic fractions by use of the miniaturized chromatographic column and analyzed by GC–MS for PAHs.

2.5.2. Optimization of ultrasonic extraction

Differences or similarities of extraction procedures were compared based on the relative amounts of PAHs extracted from the sediment. Details of each procedure are shown in Table 1. Ultrasonic extraction procedures labeled A–C utilizing different solvent mixtures were initially performed and compared with the reflux procedure. Ultrasonication procedures D and E (Table 1) were performed to investigate both the time and the number of extraction cycles. Procedures F and G were done to determine the effect of (1) water and (2) the sequence of first adding acetone to the media on the extraction efficiency of the solvent mixture. Procedures H and I were performed on the wet sediments to determine the effect of increased extraction cycles on the extraction efficiency of the solvent mixture.

All sediment/solvent combinations used were ultrasonicated in an ultrasonic bath (frequency 50–60 Hz, Bransonic 2200, Connecticut, USA) at 28 °C (room temperature). The extraction medium was added and ultrasonicated, after which the solution from the settled mixture was decanted or withdrawn using a dropping pipette. The combined extracts obtained from the ultrasonic procedures were first filtered to remove sediment particles by use of a *n*-hexane rinsed dropping pipette fitted at base with glass wool, and then evaporated to a small volume (≥1 ml) on a rotary evaporator. For the ultrasonic procedures involving the wet sediments a further liquid–liquid extraction stage was necessary to obtain the final organic extract. The combined aqueous solvent extracts were first rotary evaporated to remove primarily the acetone solvent. The organic extracts was then partitioned into *n*-hexane solvent via a separatory funnel, dried using a small amount of anhydrous sodium sulfate and then concentrated by rotary evaporation. Removal of the acetone solvent from the aqueous extracts was essential for the formation of the immiscible solvent layers to allow partitioning of the organic extracts into the *n*-hexane solvent. The final organic extract was then separated into aliphatic and aromatic fractions by use of the miniaturized silica gel chromatographic column and analyzed by GC–MS for PAHs.

2.5.3. Ultrasonic extraction of Standard Reference Material (SRM1941a)

The accuracy and precision of the optimized ultrasonication method was determined by comparison with certified values from the NIST Standard Reference Material (SRM 1941a). Extraction of the sediment SRM1941a was done according to the optimized ultrasonic procedure G described in Section 2.5.2. with details shown in Table 1. Distilled water was added to the sediment samples to obtain moisture content of approximately 40%. Three replicates were analyzed and included a method blank.

3. Results and discussion

The results for the quantities of PAHs extracted by the different procedures are shown in Table 2. Statistical significance between extraction procedures was determined using

Table 2
Quantities of PAHs (μg/kg dry weight) extracted as mean and RSD for the different procedures ($n = 3$)

	Dry sediment						Wet sediment			
	Methanolic KOH	A	B	C	D	E	F	G	H	I
Phe	14.8 (16.4)	10.4 (33.0)	9.7 (21.9)	12.1 (3.6)	11.5 (8.2)	11.0 (6.9)	9.2 (8.0)	11.6 (7.2)	11.1 (3.0)	8.6 (5.2)
Ant	7.0 (13.1)	6.9 (6.4)	6.4 (3.9)	7.1 (2.9)	6.7 (9.7)	7.4 (10.0)	7.1 (4.1)	7.2 (7.5)	7.4 (6.1)	6.9 (1.9)
Flt	23.3 (20.2)	19.4 (20.5)	21.8 (32.5)	24.5 (4.7)	20.8 (10.4)	21.0 (7.1)	19.1 (3.6)	23.6 (5.6)	25.4 (4.3)	21.9 (2.7)
Pyr	21.3 (18.8)	17.6 (20.4)	17.5 (14.9)	21.3 (5.3)	18.3 (10.1)	18.2 (5.8)	17.2 (2.8)	19.7 (4.4)	20.1 (3.1)	19.6 (2.4)
B[*a*]A	22.7 (20.9)	17.1 (15.6)	19.9 (25.5)	22.5 (7.1)	20.8 (8.3)	21.2 (9.0)	18.8 (5.0)	22.5 (2.4)	23.6 (6.2)	19.4 (3.2)
Chr	19.3 (28.7)	13.0 (23.6)	15.4 (35.8)	19.8 (4.7)	16.8 (9.9)	18.0 (9.2)	15.2 (5.4)	19.4 (9.7)	20.4 (3.5)	14.8 (5.5)
B[*b*]F	32.2 (17.4)	23.9 (13.7)	26.4 (20.2	31.9 (3.4)	29.6 (6.6)	31.2 (9.3)	28.3 (5.5)	30.2 (5.3)	32.6 (4.9)	27.3 (5.1)
B[*k*]F	22.5 (19.1)	17.1 (15.5)	18.5 (23.9)	21.8 (2.4)	19.7 (7.0)	21.6 (6.8)	20.4 (4.5)	21.6 (3.6)	22.4 (4.6)	18.7 (4.6)
B[*a*]P	36.0 (16.5)	27.8 (9.8)	31.9 (16.2)	31.6 (6.2)	33.0 (5.4)	35.9 (6.6)	32.4 (8.1)	33.3 (3.0)	37.7 (4.2)	32.7 (3.7)
I[*c*,*d*]P	54.4 (6.8)	49.3 (4.8)	51.8 (7.2)	54.1 (3.5)	53.6 (2.9)	56.1 (4.1)	54.2 (2.9)	54.7 (3.2)	57.6 (2.3)	53.4 (1.8)
DB[*a*,*h*]A	39.8 (2.9)	37.7 (1.1)	38.3 (3.4)	35.7 (3.3)	38.9 (1.5)	39.4 (2.1)	38.8 (0.5)	39.5 (6.7)	39.2 (2.2)	38.8 (0.8)
B[*g*,*h*,*i*]P	27.4 (11.1)	22.8 (9.1)	24.5 (11.5)	26.8 (8.4)	26.5 (4.2)	28.4 (6.8)	26.3 (5.0)	26.4 (1.5)	28.8 (3.5)	25.9 (3.2)
Total PAHS (average RSD)	320.6 (16.0)	262.9 (14.5)	282.0 (18.1)	309.1 (4.6)	296.1 (7.0)	309.3 (7.0)	286.8 (4.6)	309.7 (5.0)	326.2 (4.0)	287.7 (3.3)

See Table 1 for details for each extraction procedures. Average RSD for each extraction procedures are given in brackets.

the two-tailed unpaired Student *t*-test (at the 95% confidence interval) on the values of the individual PAHs extracted from three replicate sediment samples. In each case, the level of significance was determined and when this value was greater than 0.05, the null hypothesis was accepted. The accuracy of the optimized ultrasonication method was determined by comparison of certified values from the NIST Standard Reference Material (SRM 1941a).

3.1. Comparison of ultrasonic extraction procedures with methanolic KOH reflux method

Analysis of the dried homogenized sediment yielded 12 of the US EPA's 16 priority PAHs. Fig. 1 shows the GC–MS chromatogram of the contaminated sediment after ultrasonic extraction with the *n*-hexane–acetone solvent and clean-up with the miniaturized silica gel column. Ultrasonic extraction

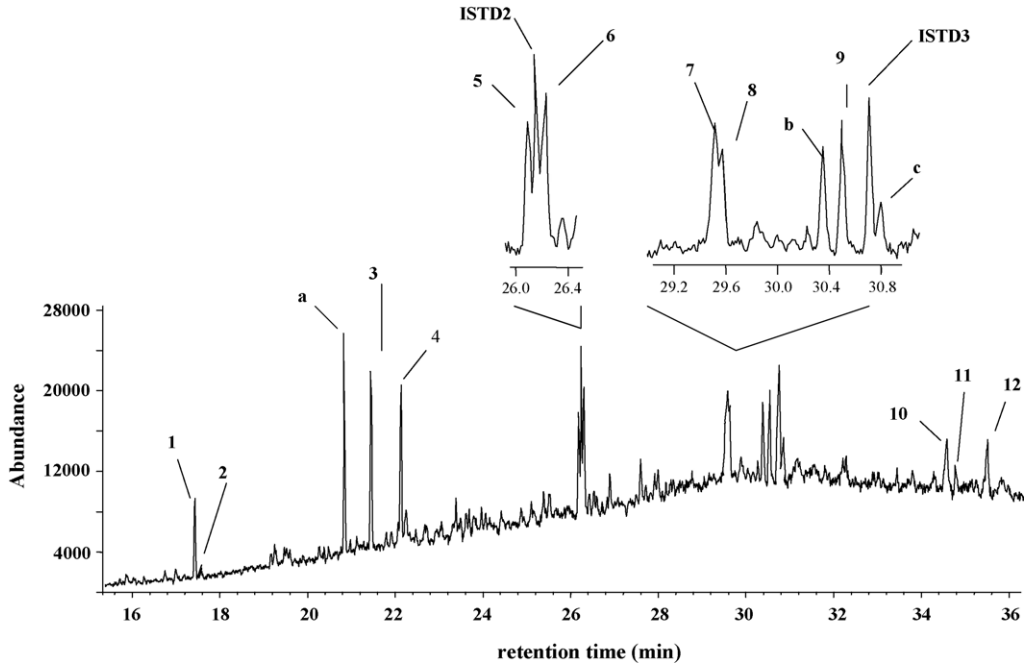

Fig. 1. GC–MS total ion chromatogram of contaminated sediment showing 12 of the EPA 16 priority PAHs. Sediment ultrasonicated according to procedure C with *n*-hexane–acetone (1:1, v/v) solvent and extract cleaned-up with miniaturized silica gel column. 1, Phe; 2, Ant; 3, Flt; 4, Pyr; 5, B[*a*]A: ISTD2, Internal standard, chrysene-d_{12}; 6, Chr; 7, B[*b*]F; 8, B[*k*]F; 9, B[*a*]P; ISTD3, Internal standard, perylene-d_{12}; 10, I[*c*,*d*]P; 11, DB[*a*,*h*]A; 12, B[*g*,*h*,*i*]P; a, 5α-androstane (internal standard for quantification of aliphatics); b, B[*e*]P; c, perylene. Analyte peaks are enlarged for clarity as shown above.

of the sediment using the *n*-hexane–acetone solvent mixture (procedure C) extracted comparable quantities of PAHs compared to the methanolic KOH reflux method.

Statistical evaluation utilizing the unpaired Student *t*-test indicated no significant differences (p values >0.050) between the individual PAHs analyte values ($n = 3$) extracted by ultrasonication procedure C and the methanolic KOH reflux method. However, there were slight differences in the PAH profiles extracted by the two methods. Lower amounts of PAHs, that consisted of five and six molecular rings such as B[*k*]F, B[*a*]P, DB[*a*,*h*]A and B[*g*,*h*,*i*]P, were extracted by ultrasonication procedure C as compared to the methanolic KOH extraction. The reverse was true for the three and four molecular ring PAHs such as Ant, Flt, and Chr. This may be attributed to the loss of the more volatile three and four molecular ring PAHs or the greater extraction efficiency of the more tightly bound five and six molecular ring PAHs by the methanolic KOH extraction as compared to C. Comparison of the precision of the procedures, by analysis of the relative standard deviation (RSD) of the PAHs extracted, showed that ultrasonication procedure C was more precise than the methanolic KOH extraction as well as the other two ultrasonication procedures. The average RSD values of ultrasonication procedure C were lower than the reflux method and ultrasonication procedures A and B (Table 2). The lower RSD values obtained in ultrasonication procedure C probably resulted from a greater degree of homogenization of the sediment material in the acetone solvent, when compared to the reflux procedure and the other two ultrasonic procedures using methanolic KOH.

The differences in the quantities of PAHs extracted as well as the RSDs obtained for the reflux and ultrasonic procedures may result from the mode of extraction involving the effect of temperature and solvent used. Lower quantities of PAHs were obtained by ultrasonication procedures A and B, which utilized the solvent mixtures *n*-hexane–methanolic KOH and *n*-hexane–methanolic KOH–acetone, respectively, compared with the reflux method. The differences in quantities of PAHs extracted could be largely due to the effect on temperature on the mode of extraction utilizing the methanolic KOH solvent. The higher temperature from the reflux method compared with the ultrasonic procedures A and B done at room temperature (28 °C), probably facilitated the release of greater quantities PAHs into the extracting solvent by the breakdown the organic matrix of the sediment. Greater quantities of PAHs extracted would also result from the increased solubility of analytes in the solvent at higher temperatures. In this study, the effect of temperature on ultrasonic extraction utilizing different solvents such as methanolic KOH was not done, and should be considered in further studies.

Comparison between the extraction efficiency of ultrasonication procedure A with procedures B and C (done at room temperature) indicated that greater quantities of PAHs were obtained when the ultrasonication media contained acetone. However, when considering the large RSD (up to 10%) statistical analyses indicated that the differences between the extraction procedures were not significant (p values >0.05). It could be argued that ultrasonication at room temperature using acetone solvent, compared with methnolic KOH solvent, can penetrate the pores of a sediment matrix to a greater extent and provide a more efficient contact between the sediment particles and itself as the extracting solvent thus resulting in higher quantities of PAHs being extracted.

3.2. Optimization of ultrasonic extraction procedure

Ultrasonication procedure C utilizing the *n*-hexane–acetone mixture was chosen to be further optimized since it gave the greatest recovery of PAHs compared to the other two ultrasonication procedures.

3.2.1. Influence of time and number of extraction cycles on dry sediment

For the determination of PAHs, an ultrasonic extraction time of 30 min has been used [10,12,36]. From the extractions performed in the present study, reducing the overall time of ultrasonic extraction from 60 to 30 min (Table 2) showed no significant differences on the quantities of PAHs extracted in ultrasonication procedures C and D (both utilizing *n*-hexane–acetone mixture with two extraction cycles). No significant differences ($p > 0.05$) were also obtained on the quantities of PAHs extracted when the number of extraction cycles was increased from two (procedures D) to three (procedures E). Although the differences in quantities of PAHs extracted were not statistically significant, higher quantities of PAHs were obtained (with the exception of Phe and Pyr) in ultrasonication procedures E when compared with ultrasonic procedures D (Table 2).

3.2.2. Influence of water in the sediment

The presence of water in sediment samples has been shown in previous studies [6,36,39] to reduce the extraction efficiency of PAHs. However, improvement in the extraction efficiency of wet samples depends on the choice of extraction method and the optimization of the various steps of extraction. Budzinski et al. [31] have shown that the quantity of water is of primary importance among other parameters utilized in MAE for extracting PAHs in sediments. Heemken et al. [36] have reported that ultrasonication extraction is not influenced by the moisture of the sample, if a suitable solvent such as acetone or 2-propanol is chosen.

In this study, wet homogenized sediment (40% water content) was used since the optimized ultrasonication method would be utilized for extracting naturally wet sediments. Ultrasonication of the wet homogenized sediment was compared with the dried homogenized sediment using an *n*-hexane–acetone mixture. Lower quantities of PAHs were extracted in ultrasonication procedure F (wet sediment) compared with ultrasonication procedure E (dry sediment) (Table 2). However, statistical analyses indicated that the differences between the extraction procedures were not significant (p values >0.05). Observed was clumping of the wet

sediment particles that could have decrease the surface area for equilibrium partitioning of analytes between the sediment particles and solvent mixture.

3.2.3. Influence of acetone on the wet sediment

To prevent clumping of the wet sediment, acetone was initially added and the flask contents ultrasonicated for 5 min after which *n*-hexane was added (extraction procedure G). This sequence of initially adding acetone resulted in relatively higher quantities of PAHs extracted from the sediment compared to ultrasonication procedure F. The increase in extraction efficiency of the PAHs was significant (p values <0.05) for the three and four molecular ring PAHs such as Phe, Flt, Pyr, B[*a*]A and Chr but not significant for the five and six molecular rings such as B[*b*]F, B[*k*]F, B[*a*]P, I[*c*,*d*]P, DB[*a*,*h*]A and B[*g*,*h*,*i*]P.

The *n*-hexane–acetone solvent mixture has been used in other studies to extract quantitative amounts of PAHs in matrices such as soil, sediment and plant material [10,26,31,36,51]. Use of hydrophobic extracting solvents which are immiscible with water can reduce the extraction efficiency of PAHs in naturally wet sediments. However, this problem can be overcome by using acetone as a solvent. Acetone can penetrate the pores of a wet matrix thereby reducing the surface area for equilibrium partitioning of analytes between the sediment particles and itself as the solvent. Since acetone can reduce the moisture content of a sample during successive extractions and it is miscible in both water and non-polar solvents (such as *n*-hexane), it can be used with hydrophobic solvents to extract organics in wet sediments. Compared with chlorinated solvents, acetone is environmentally friendly and its use in ultrasonic extraction procedures can eliminate tedious sample preparation steps (such as air-drying, freeze-drying or use of anhydrous sodium sulfate) when extracting naturally wet samples for organic analytes.

3.2.4. Influence of number of extraction cycles on wet sediment

Previous studies on ultrasonic extractions of either wet or dry soil/sediments utilized a single extraction cycle [10,12] whereas in other studies [6,11,36] repeated extractions were done on the matrix using fresh solvent. In the present study, increasing the number of extraction cycles from three (extraction G) to four (extraction H) (with addition of acetone to prevent clumping) led to an increase in the quantities of PAHs extracted with the exception of Phe and DB[*a*,*h*]A (Table 2). However statistical analyses indicated that the differences between the quantities of PAHs extracted in the procedures were not significant (p values >0.05). Extraction I showed a decrease in extraction efficiency on further increasing the

Table 3
Recovery of aliphatic and aromatic hydrocarbons calculated from comparison with Standard Reference Material (SRM1941a)

Compound	Certified and non-certified values SRM1941a concentration (μg/kg dry weight)	RSD (%)	Ultrasonication ($n = 3$) concentration (μg/kg dry weight)	RSD (%)	Ultrasonication % recovery
Aromatic hydrocarbons (certified values)					
Nap	1010 ± 140	13.9	763 ± 27	3.5	75.5
Acy	37 ± 14	37.8	29 ± 2	6.9	78.4
Ace	41 ± 10	24.4	31 ± 2	6.5	75.6
Flu	97.3 ± 8.6	8.8	80 ± 5	6.3	82.2
Phe	489 ± 23	4.7	430 ± 21	4.9	87.9
Ant	184 ± 14	7.6	170 ± 10	5.9	92.4
Flt	981 ± 78	8.0	919 ± 40	4.4	93.7
Pyr	811 ± 24	3.0	757 ± 35	4.6	93.3
B[*a*]A	427 ± 25	5.9	409 ± 21	5.1	95.8
Chr[a]	380 ± 24	6.3	404 ± 17	4.2	106.3
B[*b*]F	740 ± 110	14.9	682 ± 28	4.1	92.2
B[*k*]F	361 ± 18	5.0	349 ± 24	6.9	96.7
B[*a*]P	628 ± 52	8.3	593 ± 27	4.6	94.4
I[*c*,*d*]P	501 ± 72	14.4	465 ± 21	4.5	92.8
DB[*a*,*h*]A[b]	73.9 ± 9.7	13.1	88 ± 3	3.4	119.1
B[*g*,*h*,*i*]P	525 ± 67	12.8	601 ± 25	4.2	114.5
Aliphatic hydrocarbons (non-certified values)[c]					
n-C_{12}	164 ± 10	6.1	74 ± 5	6.8	45.1
n-C_{14}	264 ± 35	13.3	153 ± 10	6.5	58.0
n-C_{16}	147 ± 19	12.9	135 ± 20	14.8	91.8
n-C_{17}	269 ± 38	14.1	307 ± 18	5.9	114.1
Pristane	61 ± 25	41.0	62 ± 5	8.1	101.6
n-C_{18}	151 ± 15	9.9	158 ± 20	12.7	104.6
n-C_{22}	128 ± 11	8.6	120 ± 6	5.0	93.8
n-C_{24}	168 ± 16	9.5	180 ± 13	7.2	107.1

[a] Concentration is sum of chrysene and triphenylene.
[b] Concentration is sum of dibenz[*a*,*h*] anthracene and dibenz[*a*,*c*]anthracene.
[c] The non-certified values have not been confirmed by an independent analytical technique as required for certification. The non-certified concentrations can be useful for comparison with results obtained using a similar procedure.

number of extraction steps, probably as a result of losses of hydrocarbons due to volatilization from the process (Table 2).

3.3. Recovery and precision of the ultrasonic extraction procedure as determined by analysis of Standard Reference Material (SRM1941a)

Table 3 presents the results of the recoveries of aromatic and aliphatic hydrocarbons from the Standard Reference Material (SRM 1941a) using the optimized ultrasonication extraction procedure H. Distilled water was added to the Standard Reference Material to obtain moisture content of approximately 40% prior to ultrasonic extraction. Satisfactory recovery values were obtained for most PAHs, which were greater than 90%. Lower recovery values were obtained for the more volatile two and three molecular ring PAHs such as Nap, Acy, Ace, Flu and Phe. Similar results were obtained from other studies, which utilized rotary evaporation and a gentle stream of nitrogen for concentration of extracts before analysis [52]. The recoveries obtained for DB[*a*,*h*]A and B[*g*,*h*,*i*]P were above 110% and could be attributed to the overestimation of the chromatographic peaks by co-eluting compounds for those PAHs.

The precision of the procedure was relatively good, since the RSD of the PAHs extracted was less than 5%, with the exception of Acy, Ace, B[*a*]A and DB[*a*,*h*]A which had RSDs of less than 10%. Good recoveries were also obtained for the aliphatic hydrocarbons and the isoprenoid pristane, which were also greater than 90% with the exception of the more volatile *n*-C12 and *n*-C14, which had low recoveries of 45% and 58%, respectively. Although the values of the aliphatics and pristane in the Standard Reference Material (SRM 1941a) are noncertified values, which have not been confirmed by an independent analytical technique, comparison was made since the procedure of analysis was similarly done, i.e. GC/MS and compatible column used [49]. The precision of the ultrasonic procedure of extraction for the aliphatic hydrocarbons and pristane was also good, since the RSD were less than 15%.

3.4. Procedure comparison by general parameters

Similar volumes of solvents were used for the ultrasonication and the methanolic KOH reflux procedures. Both procedures can extract organics from naturally wet sediments without the use of chemical drying agents such as anhydrous sodium sulfate during the extracting stage. The extraction time of ultrasonication (30 min) was considerably much lower than the reflux procedure (12 h) thus facilitating a higher throughput of batches of extracted samples per day. The extraction stage of the reflux procedure (to obtain the organic extract) was more tedious than the ultrasonication

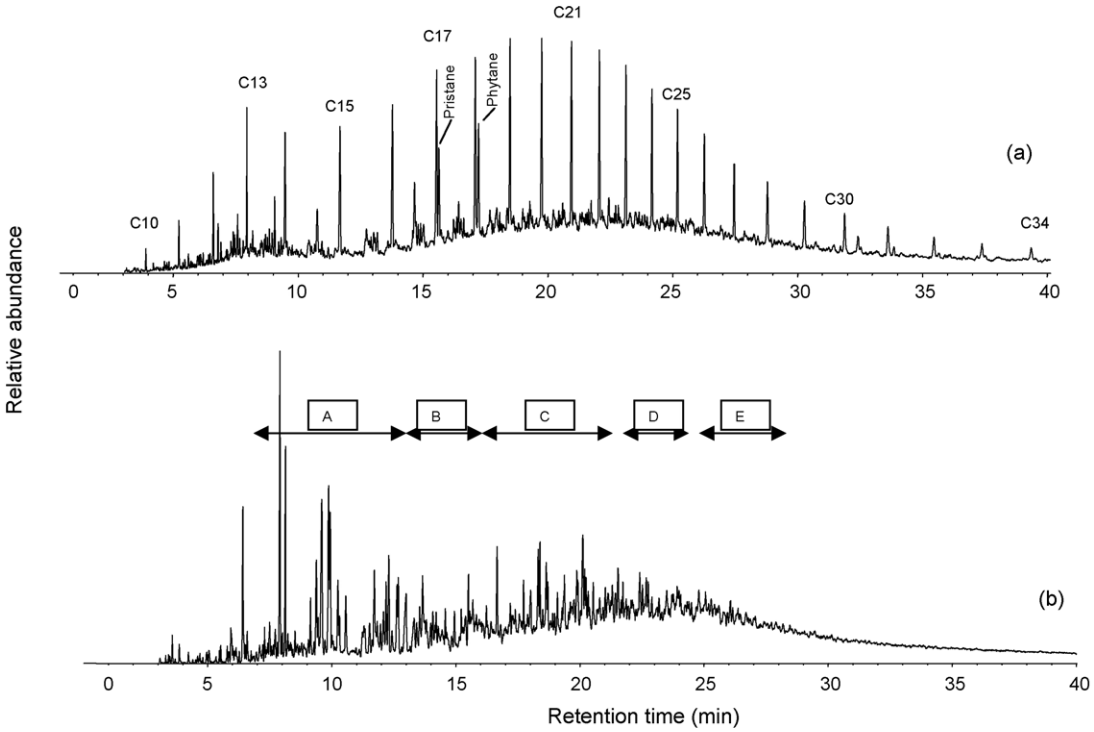

Fig. 2. GC–MS total ion chromatogram of soil sample contaminated with bunker C fuel oil. Extraction by ultrasonication with acetone/hexane (1:1, v/v) solvent and extract cleaned-up with miniaturized silica gel column. (a) Aliphatic fraction showing resolved peaks of *n*-decane (C10) to *n*-tetratriacontane (C34), and isoprenoid hydrocarbons, pristane and phytane. (b) Aromatic fraction consisting of; (A) methylated napthalene, (B) methylated fluorene, (C) methylated phenanthracene/anthracene, (D) methylated pyrene and (E) methylated chrysene hydrocarbons. The intensity of peaks is expressed as relative abundance for each chromatogram.

procedure since it involves filtration of the sediment material and a further liquid–liquid extraction step, which utilize additional glassware, apparatus and solvents. Although a liquid–liquid extraction step is included in the ultrasonication procedure for wet sediments, it was not required for dry sediments. The overall cost of the ultrasonic extraction procedure was lower than the reflux procedure since it utilize low cost apparatus and glassware.

3.5. Application of procedure for environmental samples

The optimized ultrasonication procedure has been used in our laboratory to extract hydrocarbons from naturally wet sediments from rivers, and coastal and marine areas in the GOP, Trinidad. The procedure has extracted levels of total PAHs ranging from 50 to 2500 ug/kg dry weight in soil and marine sediments. The method of extraction was also used for chemical fingerprinting and source determination of hydrocarbons in soils and sediments. For this purpose the method was able to extract a wide range of hydrocarbons which is a critical factor when to aid in the fingerprinting process. The complete separation of the saturated and unsaturated hydrocarbons by use of the miniaturized silica gel chromatographic column was also useful. Fig. 2 shows the GC–MS chromatogram of the aliphatic and aromatic fractions of a soil sample contaminated with bunker C fuel oil in which the extraction of the hydrocarbons was obtained by ultrasonication and clean-up of extracts by the miniaturized silica gel chromatographic column.

4. Conclusions

This study has shown that ultrasonic extraction of a dried homogenized sediment, using the *n*-hexane–acetone solvent mixture, extracted comparable quantities of individual PAHs compared to the standard reflux procedure which utilizes methanolic KOH. Extraction with the *n*-hexane–acetone mixture yielded higher amounts of individual PAHs than the ultrasonic procedures, which utilizes *n*-hexane–methanolic KOH and *n*-hexane–acetone–methanolic KOH solvent mixture. Statistical evaluation utilizing the unpaired Student *t*-test indicated that the differences between the methods of extraction were not significant (p values >0.050). Ultrasonic extraction using the acetone/hexane mixture was shown to be more precise as a result of smaller RSD values than those from reflux or ultrasonic extraction methods using the methanolic KOH solvent mixtures.

The presence of water decreased the extraction efficiency of ultrasonication using the *n*-hexane–acetone mixture. The sequence of adding acetone followed by ultrasonication for a few minutes had a significant effect on increasing the extraction efficiency of the *n*-hexane–acetone mixture by preventing initial clumping of the wet sediment. Four extraction cycles were found to be sufficient for extraction PAHs in the wet sediment, whereas five extraction cycles were found to decrease the extraction efficiency of the *n*-hexane–acetone. The optimized ultrasonic extraction procedure was found to extract pristane and aliphatic and aromatic hydrocarbons from the SRM 1941a with recoveries greater than 90% for most analytes. The method has been used in our laboratory to extract hydrocarbons from naturally wet sediments from rivers, and coastal and marine areas. The wide range of hydrocarbons extracted and the complete separation of the aliphatic and aromatic hydrocarbons obtained by the optimized method, was found to be very useful in the application of fingerprinting and source determination of oil contaminated samples. The major advantage of ultrasonication is the much lower extraction time and the elimination of an additional clean-up stage involving additional glassware and apparatus. This method with excellent extraction efficiency, precision and recovery of hydrocarbons combined with little sample preparation and use of low cost apparatus makes it an ideal technique for laboratories engaged in analyzing a large number of sediment samples.

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