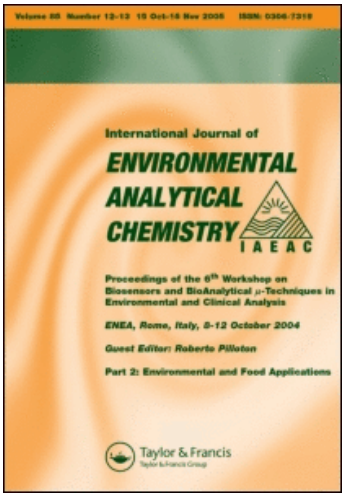

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

A Comparison of Ultrasonic Extraction and Soxhlet Extraction of Polycyclic Aromatic Hydrocarbons from Sediments and Air Particulate Material

C. H. Marvin[a]; L. Allan[a]; B. E. McCarry[a]; D. W. Bryant[b]
[a] Department Of Chemistry, McMaster University, Hamilton, Ontario, Canada [b] Department Of Biochemistry, McMaster University, Hamilton, Ontario, Canada

To cite this Article Marvin, C. H. , Allan, L. , McCarry, B. E. and Bryant, D. W.(1992) 'A Comparison of Ultrasonic Extraction and Soxhlet Extraction of Polycyclic Aromatic Hydrocarbons from Sediments and Air Particulate Material', International Journal of Environmental Analytical Chemistry, 49: 4, 221 — 230
To link to this Article: DOI: 10.1080/03067319208027573
URL: http://dx.doi.org/10.1080/03067319208027573

Intern. J. Environ. Anal. Chem., Vol. 49, pp 221-230
Reprints available directly from the publisher
Photocopying permitted by license only

A COMPARISON OF ULTRASONIC EXTRACTION AND SOXHLET EXTRACTION OF POLYCYCLIC AROMATIC HYDROCARBONS FROM SEDIMENTS AND AIR PARTICULATE MATERIAL

C.H. MARVIN*, L. ALLAN and B.E. McCARRY

Department Of Chemistry, McMaster University, Hamilton, Ontario, Canada, L8S 4M1

D.W. BRYANT

Department Of Biochemistry, McMaster University, Hamilton, Ontario, Canada, L8S 4M1

(Received, 28 January 1992; in final form, 23 March 1992)

The extraction of polycyclic aromatic hydrocarbons (PAH) from sediments and from an urban dust standard reference material (SRM 1649) was compared using two methods; ultrasonication and Soxhlet extraction. Sample weights ranging from 0.5 g to 5 g were extracted using ultrasonication. The yield of organic material from sediment samples using ultrasonication with two solvents was 2.53 ± 0.10% while the Soxhlet method yielded 2.41 ± 0.14% of the initial sample weight. Sequential ultrasonic extraction with two solvents was much more rapid (45 minutes) than Soxhlet extraction (two days) and resulted in equal extraction efficiency. The levels of PAH extracted by ultrasonication from the urban dust standard reference material varied by no more than 15% from the certified values.

KEY WORDS : Ultrasonic extraction, polycyclic aromatic hydrocarbons.

INTRODUCTION

The extraction of organics from solid samples using ultrasonication has been documented[1–4], but Soxhlet extraction[5–8], or the newer technique of supercritical fluid extraction (SFE)[9–12] have been the favoured methods. In some comparison studies, the ultrasonic extraction method has proven to be equally efficient, or more efficient than, Soxhlet extraction[13–17]. The major advantages of ultrasonic extraction are a) the reproducibility of the technique[15,17],

*Author to whom correspondence should be addressed.

b) the applicability of the method to a range of sample sizes, c) the dramatic reduction in time needed to perform highly efficient extractions[3,15], and d) efficient extraction of polar organic compounds.

We have developed a bioassay-directed fractionation scheme which has used sequential Soxhlet extraction with dichloromethane and methanol to ensure that, in addition to the non-polar compounds, the polar organic compounds have been extracted. Since polar organic compounds account for a substantial proportion of the mutagenic activity of some environmental samples, and the Soxhlet method required two days to complete, we have investigated the efficiency of ultrasonic extraction.

In this study, we have compared the efficiencies of the ultrasonication and the Soxhlet methods in the extraction of the organic compounds from equal weights of Lake Ontario bottom sediments and from an urban dust Standard Reference Material (SRM 1649). Samples were extracted in bulk, and the extracts were submitted to clean-up and fractionation steps in order to isolate the low to intermediate molecular weight polycyclic aromatic hydrocarbons (PAH) for determination. The levels of specific PAH extracted by the two methods were compared, and the levels of PAH found in the SRM 1649 were compared with the certified values.

EXPERIMENTAL SECTION

Gases

High purity helium, nitrogen, air, and hydrogen were obtained from Canadian Liquid Air Ltd. (Toronto, Ontario, Canada).

Solvents

Dichloromethane and acetonitrile were of HPLC grade from Fisher Scientific (Fairlawn, NJ). Water was prepared in the laboratory using a Milli-Q purification system (Waters Associates, Millford, MA). Reagent grade benzene and chloroform (Caledon Laboratories, Georgetown, Ontario), acetone (J.T. Baker Inc., Phillipsburg, NJ), and toluene (Fisher Scientific), were distilled in glass before use. Hexane was of HPLC grade from BDH Inc. (Toronto, Ontario).

Instrumentation

Ultrasonic extractions were performed using a 300 watt Fisher Sonic Dismembrator Model 300 with a 3/4 inch diameter titanium tip (Fisher Scientific, Fairlawn, NJ) at maximum power. Reverse and normal phase HPLC were performed on a Hewlett-Packard Model 1090 liquid chromatograph with a built-in diode array detector. The instrument was also equipped with a Hewlett-Packard Chemstation data system (Hewlett-Packard Co., Mississauga,

Ontario), and a Kratos FS 950 fluorometer (Kratos Inc., Westwood, NJ). A Beckman Model 110A HPLC pump equipped with a Beckman Model 153 UV detector (Beckman Instruments, Fullerton, CA) was used in the Sephadex LH-20 clean-up procedure. A Buchi R110 rotavapor (Brinkmann Instruments, Rexdale, Ontario) was used for reduction of organic solvent volumes. Gas chromatography was performed on a Hewlett-Packard model 5890 equipped with an on-column injector, a flame ionization detector, and a model 3390A integrator.

Materials

The sediment samples used in this study were kindly provided by Wynn Booth and Mike Fox of the National Water Research Institute, Canada Center for Inland Waters, Burlington, Ontario. Standard Reference Material 1649, urban dust/organics, was obtained from the National Institute of Standards and Technology, (NIST, Washington, DC). Neutral alumina (Brockman activity 1, 80–200 mesh) was obtained from Fisher Scientific (Fairlawn, NJ) and was activated by heating in an oven at 170°C for 48 hrs. Sephadex LH-20 gel was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Teflon filters (0.5 micron) were obtained from Millipore Corp. (Millford, MA).

PROCEDURE

Sediment samples were dried, prior to extraction, in a vacuum type dessicator over $CaCl_2$ (Drierite).

Soxhlet extraction

Samples were Soxhlet extracted using 350 ml of dichloromethane for a period of 24 hrs followed by extraction with 350 ml of methanol for 24 hrs. Cycle times were approximately 12 minutes for dichloromethane, and 25 minutes for methanol.

Ultrasonic extraction

Samples (in a range from 0.5 g to 5.0 g) were suspended in 50 ml of dichloromehane in a glass beaker. Eight consecutive ultrasonic pulses, each of 15 seconds duration, were applied at full power. The probe tip was situated approximately 1 cm from the bottom of the beaker. Solvent heating was minimized by maintaining intervals of one minute between sonication cycles and by immersing the beaker in an ice bath. The suspension was filtered through a 0.5 micron Teflon filter, using a Millipore filtration system (Millipore Corp., Millford, MA) and a vacuum aspirator. The sediment was reextracted with 50 ml of fresh dichloromethane. The procedure was repeated a third time with 50 ml of methanol. Sediment-free extracts were pooled to form a single extract.

The extracts prepared using either method were reduced to approximately 10 ml by rotary

evaporation at reduced pressure. Six grams of alumina was added to each extract and the residual organic solvent evaporated under reduced pressure. The alumina containing the adsorbed sample was then poured on the top of 12 g of alumina contained in a 1 cm × 30 cm glass column. Aliphatics present in the sample were eluted with hexane (120 ml). The fraction containing the polycyclic aromatic compounds (PAC) was eluted by sequential addition of 100 ml of benzene, followed by 140 ml of chloroform/ethanol (99:1 v/v). Polar compounds were eluted from the alumina by sequential addition of 100 ml of methanol and 100 ml of methanol/water (3:1 v/v). The solvents were allowed to pass through the column by gravity.

The PAC-containing fraction was evaporated to dryness using rotary evaporation followed by a nitrogen blow-down step. The residue was reconstituted in 0.10 ml of dichloromethane and 0.40 ml of Sephadex LH-20 mobile phase (hexane/methanol/dichloromethane (6:4:3 v/v)). The sample (0.5 ml) was then injected onto a 4 cm × 30 cm column packed with Sephadex LH-20 gel (flow rate, 3 ml/min) to remove the remaining aliphatics from the PAC-containing fraction. All material eluting prior to naphthalene was rejected as aliphatic containing components. This was confirmed by GC analysis (as described later).

The sediment sample aromatic fraction from the Sephadex LH-20 clean-up step was evaporated to dryness and reconstituted in a 0.10 ml mixture of equal volumes of dichloromethane and hexane. Further fractionation of the sediment extract was accomplished using a Whatman Partasil PAC semi-preparative normal phase HPLC column (0.94 × 25 cm). Using the gradient elution program (as described later), the low to intermediate weight PAH eluted between 7.5 min and 24.0 min. This fraction was designated as fraction "P" for reference purposes. Analysis of fraction P by reverse phase HPLC allowed the determination of PAH in the molecular weight range of naphthalene (128) to benzo[b]chrysene (278). The SRM 1649 sample was fractionated using the alumina and Sepahadex LH-20 gel procedures, after which the sample was reconstituted in acetone or toluene and analysed by gas chromatography with flame ionization detection (GC-FID) for PAH quantitation.

HPLC operating conditions

The HPLC operating conditions were as follows: diode array UV absorption at a wavelength range from 211 nm to 400 nm; fluorescence excitation at 365 nm with emission cutoff filter of 418 nm; column temperature, 40°C. Compound identification was based upon retention time comparison with standards, and UV spectra (211 nm to 400 nm) comparison with library spectra.

Reverse phase HPLC was performed using a Brownlee precolumn (Brownlee Labs, Santa Clara, CA, 1.5 cm × 4.6 mm i.d.), and two 10 micron Vydac Reverse Phase analytical columns (Separations Group, Hesperia, CA, 25 cm × 4.6 mm i.d.) in series. A 20 microliter sample loop and a mobile phase flow rate of 1.0 ml/min was used in conjunction with the following linear gradient elution program (elapsed time, composition of the mobile phase): initial, 60% acetonitrile and 40% water; 30 minutes, 100% acetonitrile; 60 minutes, 100% acetonitrile.

Normal phase HPLC was performed using an amino precolumn (Brownlee Labs, Santa Clara, CA, 1.5 cm × 4.6 mm i.d.), and a 10 micron Whatman Partasil M9 PAC semi-preparative

column (Whatman, Clifton, NJ, 25 cm × 9.4 mm i.d.). A 100 microliter sample loop and a mobile phase flow rate of 4.2 ml/min was used in conjunction with the following linear gradient elution program (elapsed time, composition of the mobile phase): initial, 100% hexane; 5 min, 100% hexane; 10 min, 99% hexane and 1% dichloromethane; 15 min, 95% hexane and 5% dichloromethane; 40 min, 100% dichloromethane.

GC operating conditions

The GC operating conditions were as follows: detector temperature, 300°C; 1 μl injection volume; helium carrier gas flow rate, 25 cm/sec. The following temperature program was used: 100°C to 150°C at 10°C/min; 150°C to 290°C at 3°C/min; final time at 290°C, 10 min. Analysis was performed using a 30 m × 0.25 mm i.d. DB-5 column with a 0.25 micron stationary phase film coating (J and W Scientific, Folsom, CA). Compound identification was based upon retention time comparison with standards and was confirmed by GC-MS. An internal standard (2-methylanthracene) method was used for quantitation. Relative weight responses were calculated for each of the analytes and linearity of detector response was confirmed over three orders of magnitude in concentration.

Safety considerations

Normal laboratory safety procedures were followed when handling the PAH standards and the urban dust and Hamilton Harbour sediment samples. Standards and samples were handled with latex gloves and manipulated in a fumehood when possible.

RESULTS AND DISCUSSION

The sample preparation scheme outlined in the experimental section was designed for the fractionation of complex environmental samples for the purpose of identifying potentially hazardous compounds based on their mutagenic activity. This method is suitable for extracts prepared from airborne particulate material, particulate material isolated from aquatic samples, sediment, and other environmental samples.

The methodology focuses on PAC by the sequential removal of organic acids and aliphatic compounds. The residual material containing a majority of the aromatic compounds was fractionated to yield several compound classes. These compound classes include the nitro-PAH, keto-PAH, and quinones. An extraction procedure that is efficient in the extraction of both polar and non-polar organic compounds is required. The PAH represent only a small number of compounds with respect to the total extract, but were selected as model compounds as they are frequently targeted as priority pollutants.

The Sephadex LH-20 column separates the aromatics from the aliphatics, which elute first. The mobile phase used in the Sephadex step results in adsorption interactions between the solutes and stationary phase; these interactions predominate over the size exclusion mechanism usually employed when using the Sephadex LH-20 gel.

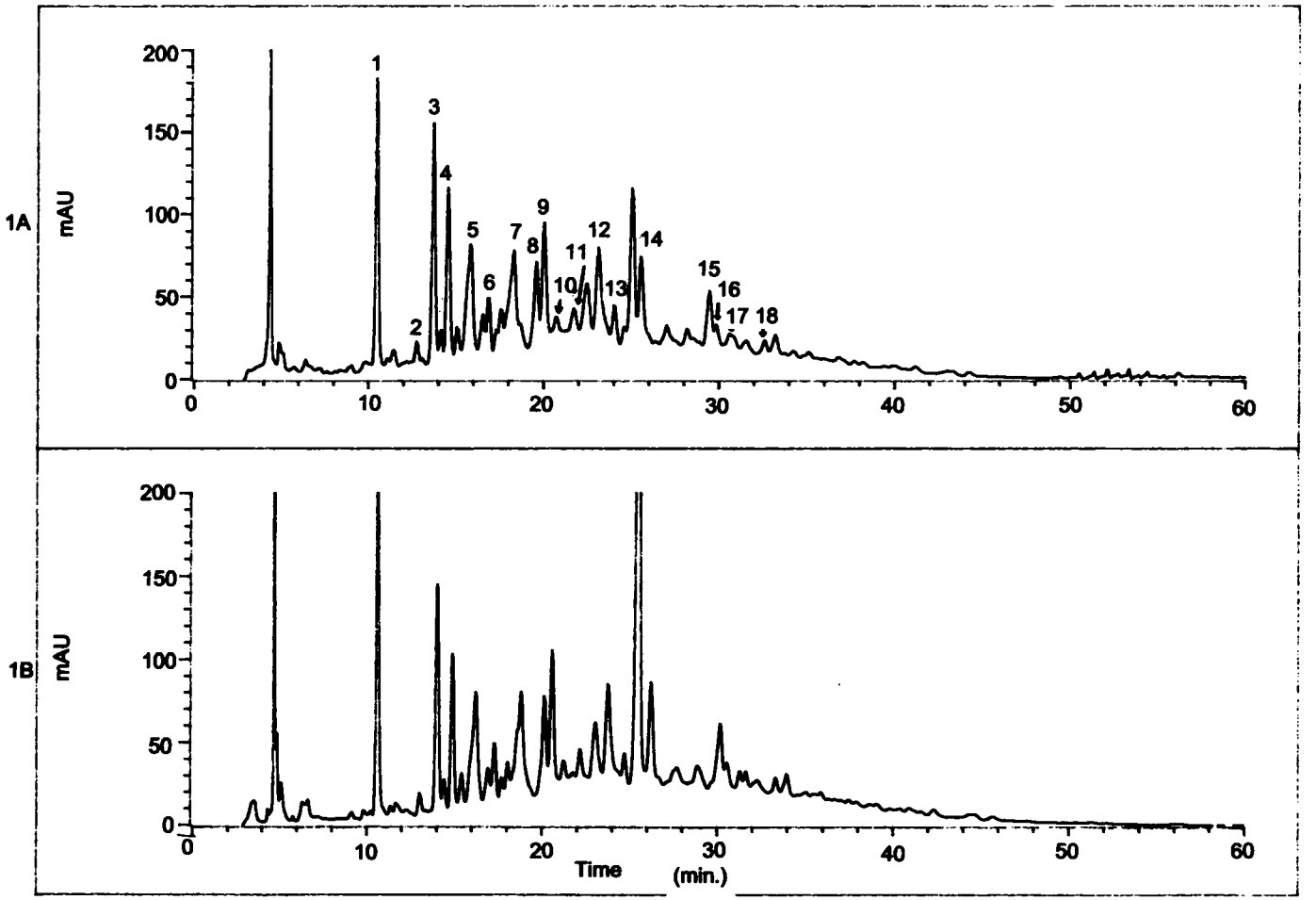

Figure 1 Reverse phase HPLC of the crude extracts prepared from equal weights of sediment using Soxhlet extraction (1A) and ultrasonic extraction (1B). These samples have not been subjected to the fractionation scheme. The peaks are numbered to correspond with compounds listed in Table 1. The injection was equivalent to 5.66 mg of sediment sample. The analytes were detected at 254 nm.

Table 1 Common PAH found in Hamilton Harbour sediment samples. The peaks are numbered as to correspond to those in the figures.

Compound
1. Naphthalene
2. Fluorene
3. Phenanthrene
4. Anthracene
5. Fluoranthene
6. Pyrene
7. Benzo[a]fluorene
8. Benz[a]anthracene
9. Chrysene
10. Benzo[b]fluorene
11. Benzo[b]naphtho [2,1-cd]thiophene
12. Benzo[b]fluoranthene
13. Benzo[k]fluoranthene
14. Benzo[a]pyrene
15. Indeno[1,2,3-cd]pyrene, Benzo[ghi]perylene
16. Benzo[b]chrysene
17. Picene
18. Anthanthrene

The sediment samples had been taken from one of the most heavily polluted areas of the Hamilton Harbour on Lake Ontario. Figure 1 shows a comparison of the reverse phase HPLC chromatograms obtained from the injection of the crude bulk extracts of 5 g sediment samples prepared by Soxhlet extraction (1A), and ultrasonic extraction (1B), with no fractionation. The high levels of PAH apparant in these extracts is a result of extensive coal tar contamination. Table 1 is a list of PAH in the sediment sample extracts. Figure 2 shows a comparison of the reverse phase HPLC chromatograms of the PAH-containing fractions (fraction P) prepared using the fractionation method. By employing a t test, no statistical differences in the individual compound peak areas in the two chromatograms were found. It should be noted that concentrations of approximately 95 μg/g of naphthalene (peak 1, Figure 1) were determined in the crude bulk extract prior to subjecting the samples to the fractionation scheme. During the subsequent preparative procedures, the volatile naphthalene was lost (see Figure 2). Phenanthrene and anthracene are both adequately retained in the sample.

The total amount of organic material extracted by ultrasonication equalled 2.53 ± 0.10% (three samples) of the initial sediment sample weight while the Soxhlet extract equalled 2.41 ± 0.14% (three samples) of the original sediment sample weight. These weight ratios were essentially invariant with the weight of sample extracted (0.5 g to 5.0 g) using ultrasonication.

Table 2 shows a comparison of the quantitation of PAH extracted from SRM 1649 urban dust using the ultrasonic extraction method and the NIST certified values. Other PAH extracted from the SRM 1649 were comparable with the non-certified values reported by the NIST.

The extractions using the ultrasonic method required much less time and much less organic solvent than extractions using the Soxhlet method. Ultrasonic extraction, using the

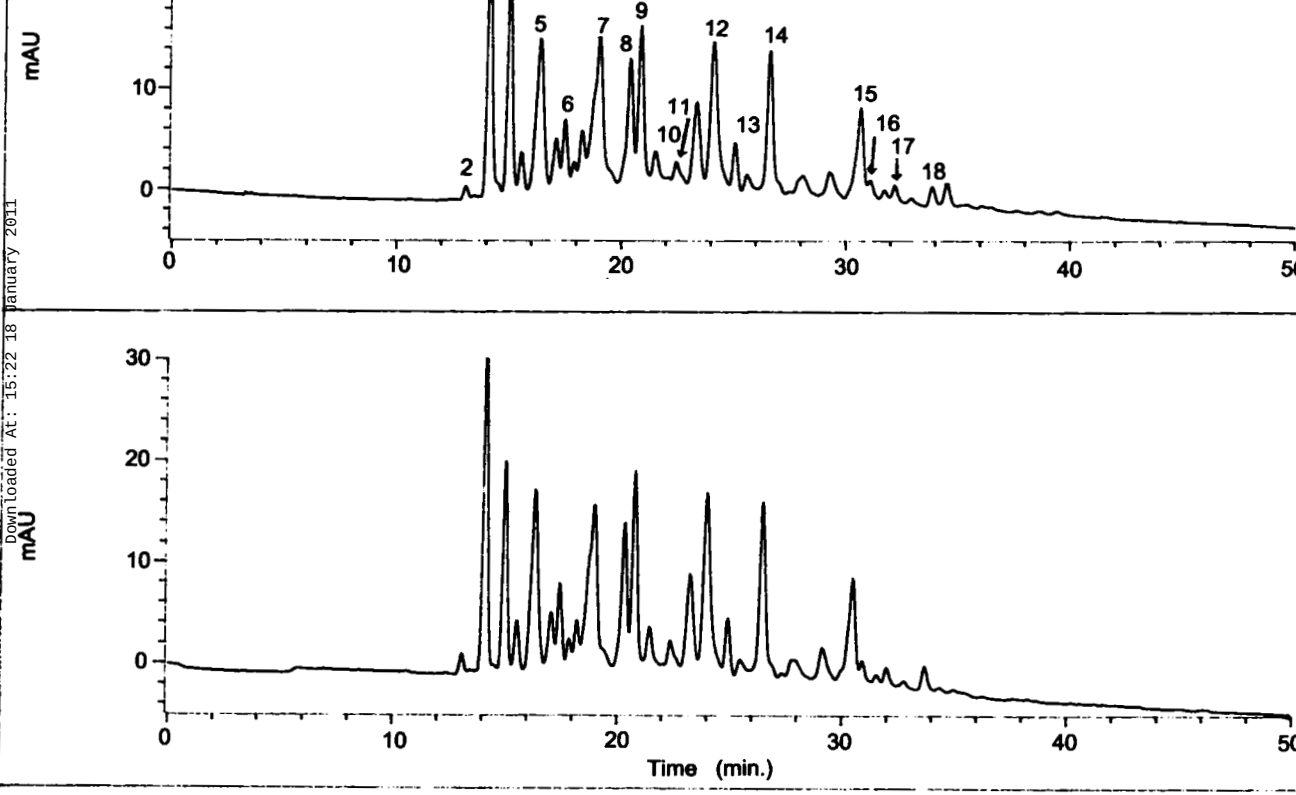

Figure 2 Reverse phase HPLC chromatograms of the PAH extracts (fraction P) prepared from equal weights of sediment using Soxhlet extraction (extraction (2B). These samples have been processed using the described fractionation scheme. The peaks are numbered to correspond with compound The injection was equivalent to 1.70 mg of sample. The analytes were detected at 254 nm.

Table 2 A comparison of PAH levels determined in a Standard Reference Material 1649 urban dust sample prepared by ultrasonication, with the certified values.

Compound	*Conc.(ug/g)*	*Cert. Value (ug/g)*
Fluoranthene	7.2 ± 1.0 (4)	7.1 ± 0.5
Benz[a]anthracene	2.8 ± 0.4 (4)	2.6 ± 0.3
Benzo[a]pyrene	3.4 ± 0.3 (5)	2.9 ± 0.5
Benzo[ghi]perylen	4.1 ± 0.5 (5)	4.5 ± 1.1
Indeno[1,2,3-cd]pyrene	3.4 ± 0.8 (5)	3.3 ± 0.5

Numbers in () indicate the number of measurements.

developed method, was performed in 45 minutes, making it comparable with the time required for SFE[10, 18]. Ultrasonic extraction does not suffer from the problems associated with SFE, including the need for sample matrix or fluid system modifiers, and restrictor clogging. The Soxhlet extraction method using dichloromethane and methanol sequentially, extracts a substantial number of polar compounds. Based on comparison of weights of the methanol/methanol-water extracts from the alumina column and their reverse phase HPLC chromatograms, we have found the extraction of polar materials using ultrasonication with two solvents to be as efficient as Soxhlet extraction. Compared to SFE, we feel that the ultrasonication method is more efficient for the extraction of polar organics since modifiers must be added to supercritical carbon dioxide to achieve efficient extractions of higher molecular weight PAH such as benzo[a]pyrene. More than one SFE fluid system may be needed for extraction of samples containing compounds with varying polarities. The extraction of organic compounds containing polar functional groups, such as carboxyls and hydroxyls, can be very difficult or impossible when using SFE[19, 20].

The optimum conditions for ultrasonic extraction are dependent upon sample size and sample matrix. In this work, we have used 8 pulses of ultrasonic power and 50 ml of solvent per extraction cycle for the extraction of sample sizes varying from several hundred milligrams to 5 g of sample. We have successfully applied the ultrasonication method to air particulate samples, as well as sediment samples.

CONCLUSIONS

The method of ultrasonication for the extraction of organics from sediment samples results in efficient extractions, based upon comparison with extraction using the Soxhlet method. Ultrasonic extraction can be accomplished in short periods of time and is applicable to a wide range of sample sizes. The percentage of the original mass of material extracted was found to be constant over a ten-fold sample weight range. The ultrasonic probe apparatus is very easy to operate and the technique can be applied to the extraction of organics from various sample matrices. The described chromatographic fractionation scheme was effective in separating the components of complex environmental samples into chemical classes.

Acknowledgements

The authors would like to thank Wynn Booth, Mike Fox, and Murray Charlton for technical support, and the Great Lakes University Research Fund, the Ontario Ministry of the Environment, and the Natural Sciences and Engineering Research Council for financial support.

References

1. E. Sawicki, *Health Lab. Sci.*, **12**, 407–414 (1975).
2. G.A. Eiceman, A.C. Viau, and F.W. Karasek, *Anal. Chem.*, **52**, 1492–1496 (1980).
3. T.S. Koh, *Anal. Chem.*, **55**, 1814–1815 (1983).
4. G.A. Junk, and J.J. Richard, *Anal. Chem.*, **58**, 962–965 (1986).
5. H.H. Hill, Jr., K.W. Chan, and F.W. Karasek, *J. Chromatogr.*, **131**, 245–250 (1977).
6. W.L. Fitch, E.T. Everhart, and D.H. Smith, *Anal. Chem.*, **50**, 2122–2126 (1978).
7. R.C. Pierce, and M. Katz, *Anal. Chem.*, **47**, 1743–1747 (1975).
8. A.T. Gimmarise, D.L. Evans, M.A. Butler, C.B. Murphy, D.K. Kiriazides, D. Marsh, and R. Mermelstein, in: *Polycyclic Aromatic Hydrocarbons: Physical and Biological Chemistry* (M. Cooke, A.J. Dennis, and G.L. Fisher, Eds.; Battelle Press, Colombus, OH, 1982) pp. 325–334.
9. S.B. Hawthorne, and D.J. Miller, *J. Chromatogr.*, **403**, 63–76 (1987).
10. S.B. Hawthorne, and D.J. Miller, *Anal. Chem.*, **59**, 1705–1708 (1987).
11. I.L. Davies, M.W. Raynor, J.P. Kithinji, K.D. Bartle, P.T. Williams, and G.E. Andrews, *Anal. Chem.*, **60**, 683(A)-702(A) (1988).
12. S.B. Hawthorne, *Anal. Chem.*, **62**, 633(A)-642(A) (1990).
13. D.H. Swanson, and J.F. Walling, *Chromatogr. Newsl.*, **9**, 25–27 (1981).
14. R.H. Soderburg, in: *Measurement and Monitoring of Non-criteria (Toxic) Contaminants in Air* (E.R. Frederick, Ed., Publishers Choice MFG. Co., Mars, PA) pp. 489–499.
15. C. Golden, and E. Sawicki, *Int. J. Environ. Anal. Chem.*, **4**, 9–23 (1975).
16. J. Grimalt, C. Marfil, J. Albaigés. *Int. J. Environ. Anal. Chem.*, **18**, 183–194 (1984).
17. D.W. Bryant, and B.E. McCarry, in: *Genotoxicity Testing of Polycyclic Aromatic Compounds Associated with Respirable Air Particulate*, McMaster University, 1990 (unpublished data).
18. B.W. Wright, S.R. Frye, D.G. McMinn, and R.D. Smith, *Anal. Chem.*, **59**, 640–644 (1987).
19. E.A. Brignole, *Fluid Phase Equilib.*, **29**, 133–144 (1986).
20. E. Stahl, and K.W. Quirin, *Fluid Phase Equilib.*, **8**, 93–105 (1983).