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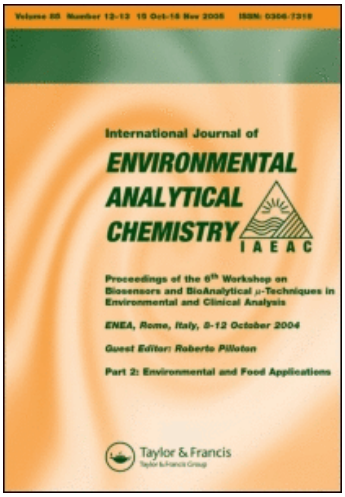

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Determination of Polycyclic Aromatic Hydrocarbons in Marine Sediments, using Solvent Extraction, Thin-Layer Chromatography and Spectrofluorimetry

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Polycyclic aromatic hydrocarbons (PAH) are present in bottom sediments of rivers, estuaries and coastal waters. Few analyses of sediments for PAH have been reported previously, and they have generally determined benzo[a]pyrene (B[a]P) only. The method described here was developed and used to determine B[a]P and perylene quantitatively, and to identify several other PAH in a range of sediments from south-east Australia. A sequence of liquid-liquid extraction using cyclohexane and dimethylsulphoxide, and two stages of thin-layer chromatography, was used for purification and separation of individual PAH which were identified and determined by spectrofluorimetry. The minimum concentrations detectable using this method were 2.5 μg/kg for B[a]P and 1.5 μg/kg for perylene starting with a 30 g sample of dried sediment. At the 500 μg/kg level both PAHs were determined with a coefficient of variation of 5–7%.

KEY WORDS: Polycyclic aromatic hydrocarbons, marine sediments, solvent extraction, thin-layer chromatography, spectrofluorimetry.

INTRODUCTION

Since the discovery that many polycyclic aromatic hydrocarbons (PAH) are carcinogenic, investigations have been undertaken to identify PAHs and to measure their concentration in a variety of environmental samples. Much of the work has been directed towards the most carcinogenic of the

PAHs tested, benzo[a]pyrene (B[a]P). This compound is widely distributed in the marine environment, having been detected in marine fauna (1,2), algae (3), plankton (4), and bottom sediments (2, 4). Although sediments by acting as potential sinks for PAHs may play a major role in controlling levels of PAHs in waters and aquatic organisms, reported studies of B[a]P in sediments are few (5,6,7,8), and they contain only small numbers of results for a very limited range of locations. In environmental samples B[a]P is only one of several PAHs expected to be present, and the distribution and role of other PAHs needs examination. In a previous study of marine sediments from Buzzards Bay, Massachusetts, U.S.A. (3), PAHs have been separated by a sequence of gel filtration, adsorption chromatography and charge-transfer complexation. Identification and quantitative determination using U.V. spectroscopy and mass spectrometry showed at least 14 PAHs to be present.

Analytical techniques used for identification of PAHs from a variety of matrices have been dominated by mass spectrometry, after separation by thin-layer chromatography (9), gas-liquid chromatography (10), or high-pressure liquid chromatography (11). These procedures have value in the positive identification of PAHs, and in speed and precision of analysis, particularly when large numbers of samples must be analysed. In some circumstances a simpler technique using inexpensive equipment may be more appropriate. The method described in the present work was developed as part of a study of PAHs in the marine environment. It has been used in the analysis of sediments from rivers, estuaries and the continental shelf of south-east Australia, and is being extended to tissues of marine organisms.

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EXPERIMENTAL

Materials

All chemicals were analytical grade, with additional purification of some to reduce the level of fluorescent impurities. Purification methods were as follows

Cyclohexane 3L of cyclohexane was shaken with 100 g of activated charcoal, filtered through Whatman No. 1 paper then distilled, rejecting the final 300 ml. This procedure was repeated until a suitably low level of fluorescence was reached.

Dimethylsulphoxide (DMSO) 1.5 L of DMSO was mixed with 6 mL of phosphoric acid and 50 g activated charcoal, stirred for 15 minutes, filtered, and distilled under vacuum.

Water Triple-distilled in an all glass still.

Pentane, methylene chloride, diethyl ether, ethanol Twice distilled.

Benzene, methanol Distilled once

Substrates for thin-layer chromatography
Silica Gel G. (E. Merck, Germany).
Cellulose acetate—30% methylation (Magery & Magel, Germany).
Aluminium oxide, Type E basic (E. Merck, Germany).

For each substrate, plates were coated then developed with purified diethyl ether before use.

Glass-fibre filters Heated at 400°C in a muffle furnace for 30 minutes.

Molecular sieves Washed with distilled water and heated 400°C for 60 minutes.

Cellulose thimbles Pre-extracted with purified cyclohexane for 2 hours.

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Equipment

Two spectrofluorimeters were used to study the separated PAHs in cyclohexane solution.

Hitachi Model 204 for quantitative analyses.

Perkin-Elmer MPF 3L to produce corrected spectra for identification of individual PAHs.

Sediment squeezer (12) operated by pressure from a nitrogen cylinder was used to remove interstitial water from sediment samples.

Sample collection and storage

Sediments were collected with a stainless-steel Smith-McIntyre grab, and stored in glass jars fitted with screw caps lined with pre-washed aluminium foil. Samples were stored frozen and in darkness to minimise possible changes by bacterial action or photo-oxidation, and were allowed to thaw overnight before preparation.

Preparation

The PAHs were separated from the dried, ground sediment by solvent extraction. Attempts to extract wet sediment with organic solvents by

shaking, stirring or Soxhlet were not successful as the organic solvents did not wet the sediment. All sediments were, therefore, dried before extraction. Excess water was removed using the sediment squeezer, then the damp sediment allowed to dry in air at ambient temperature for 48 hr, before grinding to less than 50 μm with a mortar and pestle. The extracts were treated by liquid-liquid partitioning, followed by thin-layer chromatography to isolate the individual PAHs.

Primary extraction

30 g of dried sediment was extracted with 200 mL of cyclohexane for 48 hours in a Soxhlet apparatus. The extract was reduced to 30 mL by evaporation under vacuum at 40°C in a rotary evaporator.

Liquid-liquid extraction

The cyclohexane concentrate (30 mL) was extracted with DMSO (3 $\times$ 30 mL) and the combined DMSO extract acidified with 90 mL of 1.7 M HCl. The acidified DMSO was extracted with cyclohexane (2 $\times$ 180 mL), and the cyclohexane extract washed successively with water (400 mL), 0.9 M KOH (400 mL), and water (2 $\times$ 400 mL), then dried over molecular sieve for 12 hours. The dry cyclohexane extract (360 mL) was reduced to 5 mL by evaporation in a rotary evaporator under vacuum then to 1 mL under a stream of nitrogen at 25°C.

Chromatographic isolation of the PAH fraction

The 1 mL of cyclohexane extract was streaked in a 15 cm line across the top of a silica-gel coated plate (1 mm gel thickness, activated for 1 hour at 100°C). The plate was developed for 2 hours using cyclohexane/benzene (4/1 v/v), and the PAH band located by its fluorescence under a U.V. lamp. The gel containing the PAH band was scraped from the plate and transferred to a glass column 25 mm long and 3 mm diameter. The PAHs were eluted from the column in 60 mL of diethylether, and the eluate evaporated to dryness under a stream of nitrogen at 25°C. The residue containing the PAHs was redissolved in 5 mL of cyclohexane.

Chromatographic isolation of the individual PAHs

100 μl of the solution of PAHs in cyclohexane was spotted on a TLC plate prepared by spreading a 0.5 mm thick layer of a 2/1 w/w mixture of aluminium oxide/cellulose acetate (30% methylated), and activating at

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100 C for 30 minutes. The plate was developed in two dimensions. The first development was 30 minutes with pentane/diethylether (19/1 v/v). The second development was 2 hours with ethanol/methylene chloride/water (20/20/1 v/v). The individual PAH spots were located by their fluorescence under a U.V. lamp, and scraped into separate columns prepared from Pasteur pipettes. Each column was eluted with 6 mL of diethylether, and the solution evaporated to dryness under a stream of nitrogen at 25°C. Each residue was redissolved in cyclohexane and made up to 5.0 mL.

Spectrofluorimetry

Identification of the PAHs present in the cyclohexane solutions was carried out by comparing the corrected excitation and emission spectra of these solutions with spectra reported in the literature. Although seven PAHs were isolated and identified, quantitative measurements were confined to B[a]P and perylene. Concentrations were determined by measuring the height of main emission peaks, using the baseline technique of Cooper (13) and comparing them with peak heights of calibration standard solutions. Two main emission peaks were chosen and used for each PAH, the concentration calculated from both and the average values reported. A linear relation between fluorescence emission intensity and concentration, over the range 0–300 ng/mL, was found for both B[a]P and perylene solutions in cyclohexane. Solutions were diluted where necessary to bring them within this linear range. The excitation and emission wavelengths used were:

B[a]P; excitation 298 nm, emission 403 and 428 nm;
perylene; excitation 440 nm, emission 440 and 468 nm.

RESULTS AND DISCUSSIONS

Concentrations of PAHs in sediments are small by comparison with the massive excess of other organic material. During extraction, a fraction of this additional organic material, some of which is fluorescent or coloured, is also removed leading to interference with the separation of the PAHs at a later stage. Three solvents were tested for their ability to extract PAHs from dried sediment whilst removing the minimum quantity of additional organic material. A dried sediment sample was divided into three 30 g portions, and each portion in a Soxhlet apparatus was extracted for 50 hours with 200 ml of one of the solvents. Extraction was repeated for a further 24 hours with 200 ml of fresh solvent. Comparison of the first and

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second extracts showed that none of the three solvents removed any significant quantities of fluorescent material in the second extraction. The total amount of solids extracted, measured as the residue after evaporation, was also negligible after the second extraction. A comparison of results using the three solvents is made in Table I.

TABLE I

Comparison of solvents for initial extraction of dried sediment

Solvent	Colour of extract	Total solids extracted (mg/g)	B(a)P extracted (μg/g)
Benzene	Dark-brown	6.0	3.9
Toluene	Dark-yellow	6.5	3.4
Cyclohexane	Yellow	5.6	3.7

Although it was possible with some difficulty to determine the B[a]P content of the benzene and toluene extracts, the presence of other material prevented the resolution and identification of other PAHs. Cyclohexane was as effective for extracting B[a]P, but removed much less interfering material and allowed good resolution of all the PAHs. Cyclohexane was chosen as the most suitable solvent for further work.

Cyclohexane still extracted unwanted material, and liquid–liquid partitioning was necessary to remove this before chromatographic separation. The work of Matsushita *et al.*, (14) showed that the partition coefficients for PAHs between DMSO and cyclohexane are such that three extractions should extract PAHs quantitatively from the cyclohexane phase into DMSO. The PAHs were back extracted into cyclohexane to leave unwanted inorganic material in the DMSO before proceeding to the next step. This back extraction would require a very high ratio of cyclohexane to DMSO if pure solvents were used, but acidification of the DMSO with HCl causes a useful reversal of solvent affinities (14) illustrated by the partition coefficients listed in Table II.

TABLE II

Partition coefficients for PAH between (a) DMSO and cyclohexane and (b) cyclohexane and DMSO diluted 1:1 v/v with 1.7 M HCl (Ref. 14).

PAH	Partition coefficient	
	(a) DMSO/cyclohexane	(b) Cyclohexane/acidified DMSO
B(a)P	13.0	100
Fluoranthene	5.3	100
Chrysene	9.8	100

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Two extractions of acidified DMSO with an equal volume of cyclohexane remove more than 99% of PAHs from the DMSO phase. Although this partitioning reduced the amount of unwanted organic material, a preliminary chromatographic separation was necessary before resolution of all the individual PAHs was possible. Two stationary phases, silica gel and alumina, with various mobile phases were examined. The combination of silica gel developed with cyclohexane/benzene was found to separate the PAH group from other materials. The PAH band was located by comparison with the position of pure standards of B[a]P and perylene when the developed plates were examined under a U.V. lamp.

The most effective solvent for eluting the PAHs from silica gel was found to be diethylether. After elution it was necessary to evaporate the diethylether to dryness and dissolve the residue in cyclohexane before proceeding to the final stage. In preliminary tests diethylether was evaporated by the method of McGuirk (15) whereby the eluate was contained in a test-tube with a side-arm, and the mouth closed with a fine porous-glass frit. The side-arm was connected to a vacuum line and air drawn through the test-tube with the solution at 35–40°C. This method gave PAH recoveries of about 70%. An evaporation technique was developed which increased the yields to better than 95%. The diethylether eluate was collected in a small flask, and a stream of nitrogen blown through a Pasteur pipette directed on to the surface of the solution. The nitrogen flow rate was adjusted to produce uniform evaporation and the flask was maintained at 25°C to prevent excessive cooling.

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The final separation, which resolved individual PAHs, was two-dimensional TLC on a mixed alumina/cellulose acetate (30% methylated) substrate. Three solvents were examined for the first development, pentane, pentane/ether, and hexane; and three ternary mixtures for the second development, ethanol/toluene/ether, propanol/water/acetone and ethanol/methylene dichloride/water. The best resolution was obtained with pentane/ether (19 : 1 v/v) for the first development, and ethanol/methylene dichloride/water (20 : 10 : 1 v/v) for the second development. The location of spots on a typical chromatogram as revealed by fluorescence under a U.V. lamp is shown in Figure 1. The most efficient solvent for the recovery of PAHs from individual spots on the chromatogram was again found to be diethylether. The diethylether solutions were evaporated and the PAHs redissolved in cyclohexane before spectrofluorimetry.

In a number of cases the PAHs extracted by this procedure were sufficiently pure to be identified from their fluorescence spectra. The degree of correspondence between the spectra of compounds taken from the spots in the positions expected for B[a]P and perylene and the spectra of the pure compounds is illustrated in Figure 2. The compounds

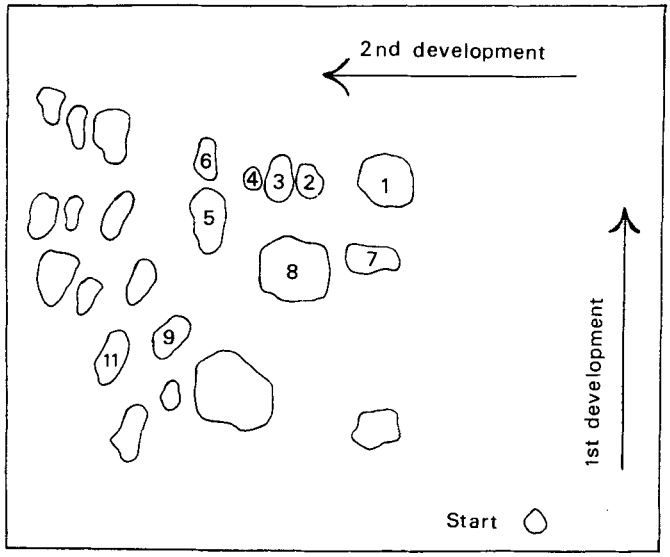

FIGURE 1 Location of PAH spots as revealed by U.V.-fluorescence of the two-dimensional chromatogram of a sediment extract.

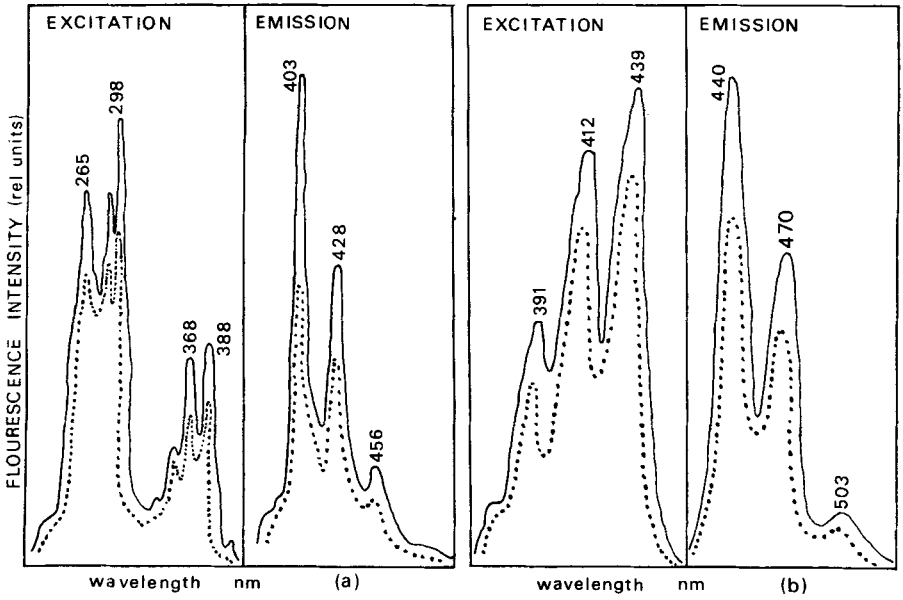

FIGURE 2 Excitation and emission spectra of PAHs in cyclohexane. Comparison of pure standards with extracts of spots shown in Figure 1.

2(a)....Extracts of Spot No. 1. 2(b)....Extract of Spot No. 5.

——B[a]P standard. ——Perylene standard.

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identified in this manner are listed in Table III with references to their location on the chromatogram in Figure 1. The lower limits of concentration of B[a]P and perylene that could be detected were governed by visual detection of fluorescent spots on the chromatogram under the U.V. lamp. Addition of known quantities of PAHs to the alumina/cellulose acetate substrate showed that 1.0 ng of B[a]P and 0.5 ng of perylene gave spots that were just visible. These minimum quantities produced concentrations of 0.2 ng/mL B[a]P and 0.1 ng/mL perylene in the cyclohexane solution used in the fluorimeter cell. The Hitachi Model 204 fluorimeter had an instrumental detection limit of 0.05 ng/mL for both PAHs and so this was not the limit on overall sensitivity.

TABLE III

PAHs identified in marine sediment extracts

Spot No. (Refers to chromatogram in Figure 1)	Colour under U.V. illumination	Compound	Note
1	Blue-violet	Benzo(a)pyrene	a
2	Blue-white	Benzo(b)fluoranthene	b
3	Orange-red	Benzo(j)fluoranthene	c
4	Blue	Benzo(k)fluoranthene	b
5	Pale blue	Perylene	a
8	Yellow-green	Indeno(1-2, 3-cd)pyrene	c
11	Light-blue	Benzo(ghi)perylene	a

[a]Identified by corrected fluorescent emission spectra.
[b]Identified by main excitation and emission peaks in corrected spectra (no corrected emission spectra were found for these compounds).
[c]Identified by fluorescent excitation spectra.

To convert these detection limits for PAHs into the equivalent concentration in the sediment it was necessary to allow for the overall efficiency of the extraction and separation procedures. To determine the recovery, replicate 100 g samples of a typical sediment which had been cleaned by extraction with cyclohexane for 7 days were taken and standard additions of B[a]P and perylene made in amounts falling within the range occurring naturally. The treated sediments were allowed to stand in darkness for 48 hours to allow sorption of the added PAHs. The samples taken through the complete extraction and analytical procedures gave overall recoveries of $72 \pm 5\%$ for B[a]P and $61 \pm 5\%$ for perylene. Assuming that the same recovery applies to naturally-occurring PAHs in

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the sediments, then with visible detection being limiting, the minimum detectable concentrations in an initial 30 g of dried sediment are 2.5 μg/kg for B[a]P and 1.5 μg/kg for perylene. Reproducibility of the method was determined from replicate analyses of four sub-samples of a carefully mixed dry sediment. The results summarised in Table IV show that B[a]P and perylene at the 500 μg/kg level were determined with a coefficient of variation of 5–7%. The method described here was used in a study of PAHs in sediments taken from different localities on the coast of South-East Australia. Some results are given in Table V and will be fully reported elsewhere. Lowest concentrations, 20 μg/kg B[a]P and 60 μg/kg

TABLE IV

Results of replicate analyses of a dried sediment

	Concentration of PAH (μg/kg)	
Sub-sample	B[a]P	Perylene
a	515	513
b	562	470
c	506	438
d	510	490
Mean	523	478
Standard deviation	26	32
Coefficient of variation	5.0%	6.7%

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TABLE V

Concentration of B[a]P and perylene (μg/kg dried sediment) in some estuarine sediments of South-East Australia.

		PAH μg/kg	
Location	Date of sampling	B[a]P	Perylene
Mallacoota Inlet	27.3.76	20	60
Yarra River (fresh water)	14.9.76	520	480
Yarra River (estuary)	15.9.76	6800	4000

Concentrations are corrected for recovery using 70% for B[a]P and 60% for perylene.

perylene were found in the sediments of the Mallacoota Inlet, a region free from urban and industrial discharges. The Yarra River which drains a large urban catchment, gave higher values ranging up to 6,800 μg/kg for B[a]P and 4,000 μg/kg for perylene.

CONCLUSIONS

The procedure described here allows the separation of a range of PAHs found in sediments and their identification from fluorescence spectra. Two PAHs, benz[a]pyrene and perylene can be determined quantitatively by spectrofluorimetry. The combination of solvent extraction and thin-layer chromatography gives good separation of individual PAHs from each other and from the great excess of potentially interfering organic material also found in sediments.

Recovery through the entire procedure was 70% for B[a]P and 60% for perylene. Sensitivity is high enough to allow quantitative determination of both compounds at the lowest levels observed in sediments from clean estuaries. The limit of detection is 2.5 μg/kg for B[a]P and 1.5 μg/kg for perylene, starting with 30 g of dry sediment.

The method uses inexpensive equipment and simple calibration procedures, making it suitable for use in a wide range of laboratories.

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