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# RAPID SIMPLE SAMPLE PREPARATION TECHNIQUE FOR ANALYZING POLYNUCLEAR AROMATIC HYDROCARBONS IN SEDIMENTS BY GAS CHROMATOGRAPHY–MASS SPECTROMETRY

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## SUMMARY

A rapid, simple and reproducible sample preparation technique has been developed for analyzing polynuclear aromatic hydrocarbons (PAHs) in sediments. The PAHs are removed by ultrasonic extraction and isolated by solvent partition and silica gel column chromatography. The sulfur removal step, which is often necessary for gas chromatographic–mass spectrometric (GC–MS) analysis of sediments, is combined into the ultrasonic extraction procedure. Identification of PAHs is carried out by GC alone and in conjunction with MS. Quantitation is achieved by addition of known amounts of standard compounds using flame ionization and multiple ion detectors. Major PAHs in Charles River sediment have been analyzed.

## INTRODUCTION

It is well recognized that some polynuclear aromatic hydrocarbons (PAHs) are highly carcinogenic[1]. Due to this health hazard much attention has been paid to the study of their biological activities as well as to the analysis of PAHs in environmental samples[2].

PAHs are formed primarily by pyrolysis of carbonaceous materials at high temperatures (500–800°). Since pyrolysis can occur naturally as well as in man's activities, PAHs are widely spread throughout the environment. They first exist in air particulates, then they are deposited in water, soil and sediments. Trace quantities have even been detected in foods, pharmaceuticals and tissues[3–6].

Due to their high levels and potential direct health effects, studies on PAHs in the environment have been mostly in air particulates[7–17]. However, increasing attention is being paid to their presence in water and sediments. Several studies were made on the origins of PAHs as indicated by their composition and regional distribution in sediments[18–24], and the levels of PAHs in cored sediments have been used to reveal their chronological depositions and fates[25,26]. Our laboratory is particularly interested in the correlations between PAH levels in the aquatic environment and aquatic organisms so that the uptake and metabolic patterns of PAHs by the aquatic orga-

nisms can be better understood. This is particularly important as the aquatic food chain is one of the major channels through which PAHs find their way to man. To facilitate these studies, optimum analytical methods are required.

For separation, identification and quantitation of PAHs, gas chromatography–mass spectrometry (GC–MS) is one of the most widely used methods[2]. However, various methods for extraction and isolation have been applied[18–21,27]. These procedures are generally very time consuming. The precisions and the recoveries are rarely discussed. As an alternative, a simple, rapid sample preparation technique has been developed in this laboratory. The procedure involves ultrasonic extraction, which combines the sulfur removal step, followed by solvent partition and silica gel column chromatography. The efficiency, reproducibility and recoveries of the method are also discussed.

## EXPERIMENTAL

### *Reagents*

Benzo[*k*]fluoranthene was donated by Dr. R. C. Lao of the Department of the Environment of Canada, benzo[*b*]fluoranthene by Dr. W. Karcher of the Joint Research Center of the Netherlands. The other PAH standard compounds were purchased from Pfaltz & Bauer (Flushing, N.Y., U.S.A.), K & K Labs. (Plainview, N.Y., U.S.A.), Eastman (Rochester, N.Y., U.S.A.), Aldrich (Milwaukee, Wisc., U.S.A.), Analabs (North Haven, Conn., U.S.A.), Polysciences (Warrington, Pa., U.S.A.) and RFR Corp (Hope, R.I., U.S.A.). Spectrograde nitromethane was obtained from Eastman. All other solvents used were distilled-in-glass grade from Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). These PAHs and solvents were used without further purifications.

Silica gel, MCB SX 0144-06 (100–200 mesh), was Soxhlet-extracted with methylene chloride and activated at 170° for 18 h. Copper "C" metal powder from U.S. Metal Products (Niagara Falls, N.Y., U.S.A.) was freshly activated before use by soaking in 6 *N* HCl for 10 min, then rinsed thoroughly with Milli Q water followed by methanol. Glass beads ($\frac{1}{2}$ mm), from Heat Systems-Ultrasonics (Plainview, N.Y., U.S.A.) were washed in methylene chloride in an ultrasonic cleaner, then dried.

Charles River sediment was provided by Dr. R. A. Hites of the Massachusetts Institute of Technology.

### *Equipment*

A Hewlett-Packard 5710A gas chromatograph with flame ionization detector (FID) and a HP 5980A gas chromatograph–mass spectrometer with HP 5974A multiple ion detector were used for separation, identification and quantitation. An HP 5985A gas chromatograph–mass spectrometer with an HP 21MX13E series computer data system was used for mass spectrum confirmations. A Model W-225R Sonicator with standard $\frac{1}{2}$ in. titanium cell disrupter, and Model 50 Rosett cooling cells, both from Heat Systems-Ultrasonics, were used for extraction. The glass column (20 cm × 1.05 cm I.D.) for absorption chromatography, with a water condenser jacket (2.8 cm O.D.), 100 ml reservoir and PTFE stopcock, was custom-made by Kontes (Vineland, N.J., U.S.A.).

*Procedure*

*Sediment handling.* Centrifuge the watery sediment and decant off the excess water. Dry the wet sediment in a freeze dryer. Pulverize the dried sediment then sieve with a No. 40 (0.425 mm) sieve. The fines are collected for analysis.

*Extraction.* Place 20 g of fine sediment in a Rosett cooling cell, then add 2 g of freshly activated Cu powder on top. After the residual methanol in the Cu powder has evaporated, add 60 ml of cyclohexane and stir to wet the whole sediment. Place the Rosett cell in an ice–water bath and fasten the cell in such a way that the disrupter horn sits in the center of the cell and 1 in. deep into the cyclohexane–sediment mixture. Sonicate the mixture at maximum frequency with 80% pulser for 30 min. Add ice, as necessary, to keep the water bath ice-cold. Filter the sonicated mixture with a Kontes filter apparatus. Transfer the sediment back into the Rosett cell to be extracted once again with another 60 ml of fresh cyclohexane.

*Solvent partition.* Combine the two cyclohexane extracts, then evaporate them to 20 ml with a rotary evaporator at 35° under reduced pressure. Extract the condensate six times with six equal volumes of cyclohexane-saturated nitromethane in a 60-ml separatory funnel. Collect the six nitromethane layers together in a 300 ml round-bottomed flask. Evaporate the extract to dryness with a rotary evaporator at 35° under reduced pressure.

*Column chromatography.* Place 15 ml of clean, activated silica gel into a 50 ml erlenmeyer flask. Add 25 ml of refrigerated methylene chloride and swirl to make a slurry. Pour the slurry into a prepared chromatographic column through a funnel. Cool the column by running cold water through the water jacket during the whole procedure. Rinse the silica gel into the column with cold methylene chloride. Drain the methylene chloride through the stopcock until its level just reaches the top of the gel. Stop the flow and top the column with 1 cm of clean glass beads. Replace the methylene chloride in the column with 5 ml, 10 ml, then 20 ml portions of refrigerated pentane. Here, as in subsequent steps, after each addition of portions of solvents or portions of residue solutions, drain the liquid into the column until the level reaches the top of the column before adding subsequent liquid. Transfer the nitromethane-extracted residue from the evaporation flask onto the top of the silica gel column with six 0.5 ml portions of pentane. Wash the residue in the column first with 2 ml, followed by 15 ml of cold pentane. Rinse the evaporation flask further with four 0.5-ml portions of methylene chloride and deposit them onto the column. Elute the column first with 2 ml, followed by another 21 ml, of cold 40% methylene chloride in pentane. Collect the eluate and evaporate it to dryness at room temperature under reduced pressure. Pick up the PAH residue with 1 ml of methylene chloride for GC and GC–MS analysis.

*GC and GC–MS identification and quantitation.* The GC and GC–MS operating conditions are listed in Table I.

The identifications are performed by GC retention times. Duplicate samples, one unspiked and one spiked with standard PAHs, are analyzed simultaneously. The peaks superimposed by the spiked PAHs are identified accordingly. These identifications are further confirmed by their mass spectra. The FID signals of the spikes are also used to quantitate the PAHs based on standard additions. Identifications and quantitations are also cross-checked by GC–MS with multiple ion detector at the masses of their molecular ions.

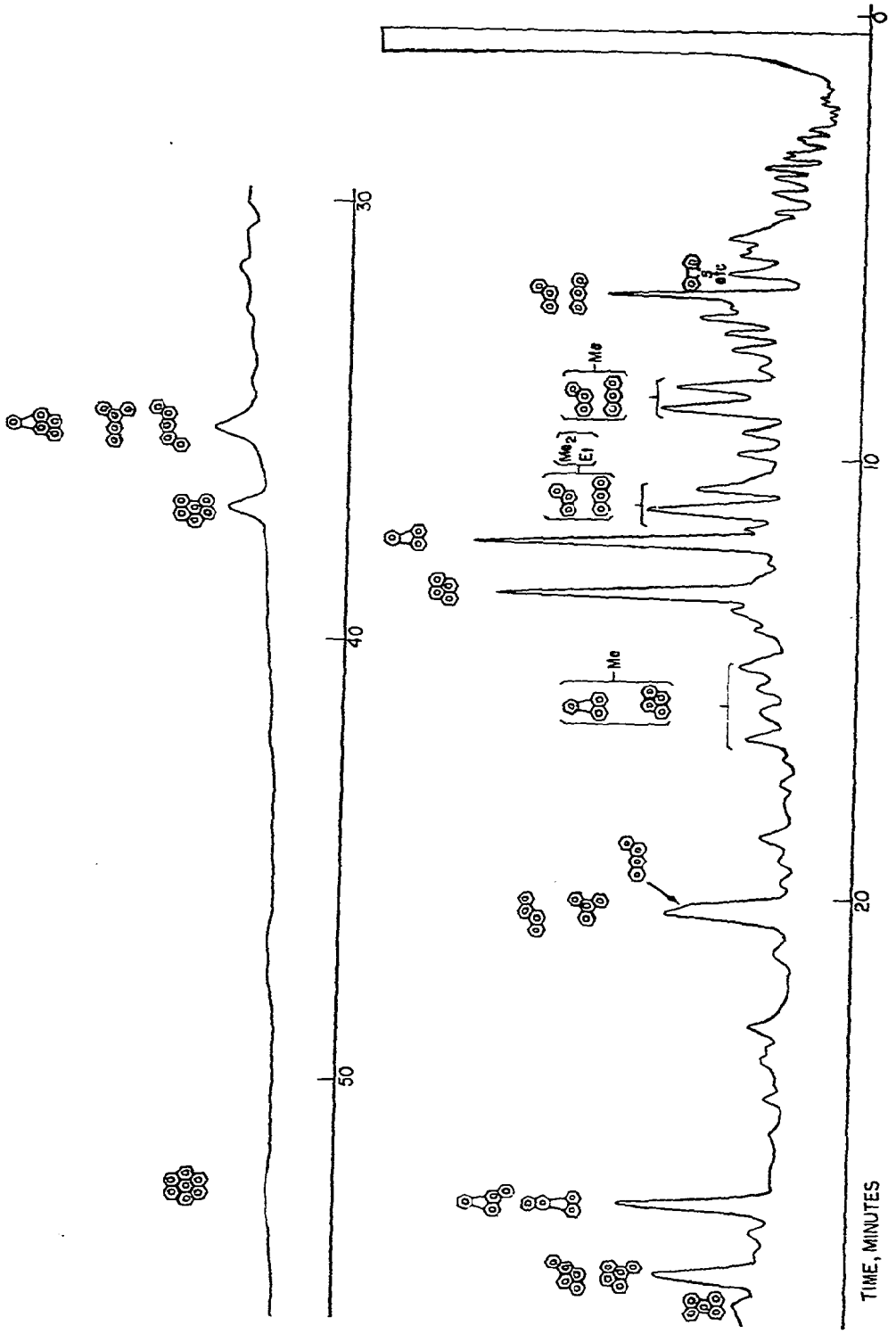


Fig. 1. Gas chromatogram of PAHs in Charles River sediment.

TABLE I

INSTRUMENTAL CONDITIONS

| *Parameters* | *Descriptions* |
|---|---|
| GC column | Packed glass, 8 ft. × 2 mm I.D. 3% Dexil 300 on Supelcoport (100–200 mesh) |
| Oven temperature | 175–300° at 4°/min |
| Injection temperature | 300° |
| Detector temperature | 300° |
| Auxiliary temperature | 300° |
| Carrier gas | Helium, 33 ml/min |
| Sample size | 1 $\mu$l |
| GC detector | FID |
| Ionization mode | Electron impact |
| Electron energy | 70 eV |
| Emission | 200 $\mu$A |
| Ion source pressure | $3 \times 10^{-6}$ Torr |
| Ion source manifold | 270° |
| Analyzer manifold | 130° |

## RESULTS AND DISCUSSIONS

The gas chromatogram of the PAHs in Charles River sediment is shown in Fig. 1. The major PAHs are determined and tabulated in Table II. Obviously, a pyrolytic origin is indicated by the predominant parent PAHs. Some of the components are not determined because of the lack of standard compounds. However, their structures are proposed based on retention times and mass spectra. They are also indicated in Fig. 1.

Sulfur-containing and alkylated PAHs are searched for by spiking with dibenzothiophene and 1-methylpyrene. The spiked dibenzothiophene eluted right before phenanthrene, but further confirmation with mass spectra showed that the GC peak of Charles River sediment in the region of the dibenzothiophene peak contains a mixture of compounds. Dibenzothiophene was then detected using the data system with selected ion monitoring at the mass of its molecular ion; the response indicates a level of less than 500 ng/g. The spiked 1-methylpyrene showed up in the methylated fluoranthene and pyrene region without a precise superimposition on any of the peaks in that region. The level of these methylated PAHs is 0.9–1.1 $\mu$g/g based on their FID signals and abundances of their molecular ions. Dibenz[*a*,*c*]anthracene, dibenz[*a*,*h*]anthracene and *o*-phenylenepyrene all eluted together. Their determination is based only on molecular ion abundances.

The recoveries in Table II are calculated based on the differences in FID signals between the spiked and unspiked samples against the FID signals of the standards. The recoveries are lower for dibenzothiophene and for larger PAHs. In order to trace out the steps which cause these lower recoveries, the following experiments were performed. (a) The sediment was ultrasonically extracted with two additional portions of 60 ml of cyclohexane after the usual two extractions. (b) Standard compounds were partitioned between 20 ml volumes of cyclohexane and cyclohexane-saturated nitromethane and their partition coefficients determined by GC. (c) Standard compounds

TABLE II

MAJOR PAHs IN CHARLES RIVER SEDIMENT

| *PAH* | *Concentration ± S.D. (μg/g)* | *Recovery (%)* |
|---|---|---|
| Phenanthrene/anthracene | 2.9 ± 0.4 | 96.1 |
| Fluoranthene | 7.8 ± 0.3 | 99.1 |
| Pyrene | 7.1 ± 0.2 | 99.2 |
| Benz[*a*]anthracene | 3.4 ± 0.5 | 89.4 |
| Chrysene/triphenylene | 3.8 ± 0.5 | 89.7 |
| Benzo[*b*/*k*]fluoranthene | 6.0 ± 0.3 | 88.1 |
| Benzo[*a*/*e*]pyrene | 5.7 ± 0.7 | 71.5 |
| Perylene | 0.9 ± 0.2 | 85.2 |
| Dibenz[*a*,*c*/*a*,*h*]anthracene | 0.7 ± 0.2 | 80.0 |
| *o*-Phenylenepyrene | 2.3 ± 0.5 | 82.2 |
| Benzo[*g*,*h*,*i*]perylene | 2.8 ± 0.3 | 85.3 |
| Coronene | 0.5 ± 0.1 | 78.1 |
| Dibenzothiophene | <0.5 | 85.1 |
| 1-Methylpyrene | | 92.1 |
| Methyl-(pyrene/fluoranthene) | 0.9–1.1 | |

were deposited onto the silica gel column. Then they were washed, eluted and analyzed exactly as the real samples.

No improvement in recovery is obtained with two additional extractions. Also, Soxhlet extraction for 18 h yielded the same results. Both observations indicated that losses due to incomplete extraction of the spikes are very unlikely.

The partition coefficients of the PAHs of interest between nitromethane and cyclohexane are listed in Table III. They range from 1.2 to 2.8. In general, the coefficient is lower for alkylated PAHs and higher for larger PAHs. Comparison of the coefficients between fluoranthene and pyrene, benzo[*a*]pyrene and perylene, dibenz-[*a*,*c*]anthracene and benzo[*g*,*h*,*i*]perylene indicates that PAHs of the same molecular weight but with more compact molecular structures have lower coefficients. However, even with the lowest partition coefficient (1.2 for 1-methylpyrene) less than 1% is left in the discarded cyclohexane layer after six equal-volume partitions. This loss is within the uncertainty of the final results and will not cause the variation in recoveries. This conclusion agrees with the observation that the final recovery of 1-methylpyrene is not lower than all the others.

The recoveries of standard compounds from a silica gel column are listed in Table IV. They vary from 78.6 to 100.1%. This variation in recovery has a similar trend as that of the whole analysis but much less in magnitude. It appears that irreversible adsorption on the silica gel column partially accounts for the low recoveries.

Since the steps in the sample preparation tested with standard compounds do not show as wide a variation in recovery as the whole analysis, there are two factors left to be considered: stability and matrix effect. It has been suggested that some PAHs are less stable than the others, for example, BaP is less stable than pyrene and the BaA skeleton structure can undergo ring isomerization[8]. These instabilities will show up more in the whole analysis which takes a longer period of time than the single steps. BaP has consistently shown lower recovery than its isomer perylene which elutes quite close to it on the GC.

TABLE III

PARTITION COEFFICIENTS BETWEEN NITROMETHANE AND CYCLOHEXANE

| *PAH* | *Partition coefficient* |
|---|---|
| Dibenzothiophene | 1.8 |
| Phenanthrene | 1.9 |
| Fluoranthene | 1.9 |
| Pyrene | 1.5 |
| 1-Methylpyrene | 1.2 |
| Benz[*a*]anthracene | 2.0 |
| Chrysene | 2.0 |
| Benzo[*a*]pyrene | 2.0 |
| Perylene | 2.5 |
| Dibenz[*a,c*]anthracene | 2.8 |
| Benzo[*g,h,i*]perylene | 2.2 |
| Coronene | 2.2 |

Another difference between single-step analysis and the whole analysis is the matrix effect. In the total analysis, PAHs are much more complex in composition and are presented together with various foreign compounds. The matrix effect is most obviously shown with dibenzothiophene and phenanthrene. Their recoveries are lower from a silica gel column when alone, than from the whole analysis. It seems that the foreign compounds, mostly aliphatics, being selectively washed out by pentane, have helped dibenzothiophene and phenanthrene to remain on the column. The matrix might also affect the sample transfer from one step to the next. When the nitromethane-extracted residue was transferred onto the silica gel column, only a very small amount of solvent was used and the larger PAHs with lower solubilities might not be completely transferred. This loss is usually enhanced by the solubility suppression by co-solubles in a complex matrix.

Both freeze-dried and desiccator-dried sediments were analyzed. The desiccator-dried material has a 20–25% lower content of PAHs. The discrepancy might be attributed to the difference in moisture content which resulted from the two different drying processes. Also, since desiccation only removes water while freeze drying removes all volatiles indiscriminately, some volatiles which remained in

TABLE IV

RECOVERIES FROM SILICA GEL COLUMN CHROMATOGRAPHY

| *PAH* | *Recovery (%)* |
|---|---|
| Dibenzothiophene | 78.6 |
| Phenanthrene | 91.4 |
| Fluoranthene | 99.9 |
| Pyrene | 100.1 |
| 1-Methylpyrene | 100.0 |
| Benz[*a*]anthracene | 98.5 |
| Chrysene | 98.5 |
| Benzo[*a*]pyrene | 93.0 |
| Perylene | 90.4 |
| Dibenz[*a,c*]anthracene | 94.9 |
| Benzo[*g,h,i*]perylene | 90.7 |
| Coronene | 87.0 |

sediment in the desiccator might be pulled out in the freeze dryer. Another important factor could be the degradation of PAHs during drying in the desiccator at room temperature for prolonged periods of time. It took 2 months to dry totally 1 in. thick of wet sediment in a desiccator. Although the desiccator was covered with yellow plastic to cut down the photooxidation under UV light, oxidation and other chemical degradation might not have been completely prevented.

To assure that the procedure is free from contamination, blanks of cyclohexane with activated copper were analyzed. Clean gas chromatograms were obtained at highest GC sensitivity.

When pentane and methylene chloride come into contact during column chromatography, heat of mixing is generated which causes the low boiling solvents to evaporate and form bubbles inside the column. This problem has been solved by circulating cooling water in the water jacket outside the column.

Although other solvents, such as benzene–methanol[2] and methylene chloride[3], have been used as extraction solvents for PAHs in sediments, it was found in this experiment, that cyclohexane is just as efficient but more selective.

Soxhlet extraction with cyclohexane for 18 h has been compared to the described ultrasonic extraction. No significant difference is observed. Ultrasonic extraction is a much faster procedure and it avoids the prolonged exposure which can lead to contamination. Also, by adding Cu powder into the sediment to be extracted, the coextracted sulfur simultaneously forms black CuS precipitate wich is filtered off together with the extracted sediment. Therefore, another distinct advantage of this procedure is that it combines the sulfur-removal step which is usually required for the analysis of PAHs in sediments by GC–MS.

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