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Routine Liquid Chromatographic Method for Assessing Polynuclear Aromatic Hydrocarbon Pollution in Fresh Water Environments

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A portion of the Western New York Great Lakes aquatic environment is being examined for the presence of carcinogenic and/or mutagenic hazards using an ecological approach. During preliminary investigations of tumor pathology encountered in several bottom feeding species of fish (BLACK et al. 1978), we found it useful to develop a routine method for assessing the relative degree of polynuclear aromatic hydrocarbon (PAH) pollution in fresh water environments.

DUNN (1976) published a specific method for the analysis of the PAH benzo(a)pyrene in marine organisms and sediments. This method utilizes alkaline digestion, partition into iso-octane, Florisil chromatography, and a dimethylsulfoxide sample enrichment step prior to analysis of PAH by TLC and spectroscopy.

We have used a technique incorporating alkaline digestion and partitioning steps similar to those described by Dunn, to analyze bottom sediments collected from polluted fresh water environments, for the presence of PAH contamination.

METHODS AND MATERIALS

High Pressure Liquid Chromatography: Using a fixed volume injection, 20_{uL} samples are injected onto a Waters C_{18} u-Bondapak, reversed phase column and eluted isocratically with a 60/40 solution of acetonitrile/water at a flow rate of 0.7 mL/min. Compounds are detected by their absorbance at 254 nm.

Sample Extraction: Samples of wet sediment (10-25 g) are transferred into a 500-mL r.b. flask with successive washings of methanol, up to a total volume of 250 mL. Each successive addition is stirred thoroughly and then decanted so as to prevent heavy particulates from being transferred to the flask. KOH (12 g) is added along with several boiling chips.

After refluxing the mixture for 4 h, the contents are allowed to settle. While still hot, i.e. about 60°C, the hydrolysate is decanted into a 2000-mL separatory funnel containing 250 mL of cyclohexane over 100-mL of 4:1 methanol:water. The remaining residue in the flask is washed with three 50-mL portions of methanol: water and the washings decanted carefully so as to prevent coarse particulates from being transferred into the funnel. The funnel

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and its contents are shaken for 2 min and then allowed to settle briefly. (Since, at this point, the heaviest sediment particles have a tendency to clog the stop-cock bore, a separatory funnel having a large drain opening is best suited for this operation. If necessary, a small amount of material can be removed after brief settling, to prevent clogging.)

The aqueous phase is transferred to a second 2000-mL separatory funnel containing a fresh 250-mL volume of cyclohexane and extracted again. (Additional extractions may be useful in heavily polluted samples.) The non-polar phases are combined and washed repeatedly with warm (45-50°C) water until the cyclohexane phase is free of all suspended particulates. At this point the cyclohexane phase is pale to deep yellow. This fraction is concentrated to about 30-mL in a flash evaporator prior to further cleanup on a column of 2% deactivated Florisil. The Florisil, heated overnight at 300°C, is deactivated with 2.0 mL of water per 100 g of adsorbant. A 22 x 400 mm column with a porous glass frit, is dry packed with 30 g of the Florisil and topped with a 1½ inch layer of sodium sulfate. The column is prewashed with 100 mL of hexane.

The cyclohexane concentrate is added to the column and allowed to percolate onto the Florisil. A first fraction is eluted with 150-200 mL of hexane. A second fraction is collected by eluting with 250 mL of 30% dichloromethane in hexane. During the elution progress, the column is monitored occasionally with a U.V. light source (365 n) to locate the fluorescent PAH fraction.

In our samples, a fluorescent violet band generally precedes a bright blue band as visualized under the U.V. light. These materials migrate very slowly under the influence of hexane, but come off the column readily with dichloromethane.

The hexane fraction (1st fraction) generally is heavily contaminated with aliphatics and may be discarded. However, samples may be evaluated for the presence of U.V. absorbing materials by concentrating this fraction 10-40 times and injecting a sample onto the HPLC. A polar column, such as Partisil, is suitable for this purpose and will be less readily fouled by the aliphatics in this fraction.

The 2nd fraction is consistent with a PAH containing fraction and may be routinely assessed for the presence of PAH molecules by injection onto a reversed phase column, e.g. Waters C_{18} u-Bondapak, ODS Zorbax, etc. Since the dichloromethane solvent is not "seen" by the 245 n detector, this fraction is simply concentrated and directly evaluated for its PAH content.

The reversed phase column can be eluted with combinations of water and acetonitrile, water and methanol, or water and U.V. grade tetrohydrofuran, etc. We have used an isocratic elution of 60/40 acetonitrile/water for our samples, but elution gradient techniques could also be used and should improve peak resolution for later eluting PAH which tail badly with isocratic techniques.

As an aid to the interpretation of data, it was useful to adjust sample concentrations and/or instrument sensitivity so as to produce a legible chromatogram in which the majority of peaks were approximately 1/4 of the maximum amplitude. After suitable instrument/concentration conditions have been established, a second chromatogram may be run with the inclusion of one or more internal standards. The inclusion of internal PAH standards permits more accurate comparison of chromatographic data from samples of diverse origins.

RESULTS AND DISCUSSION

A method is described for processing bottom samples for PAH analysis that leads to a cleaned up fraction analyzed for its PAH composition by means of HPLC. A combined extraction-hydrolysis-partitioning method utilized for this fractionation has a number of advantages. This technique appears to yield reliable quantitative extractions of many aromatic hydrocarbon species (DUNN 1976, NEGISHI 1978). In addition, a number of pesticides that might interfere with the PAH analysis are destroyed by this treatment (NEGISHI 1978).

GRITZ and SHAW (1977) attempted to evaluate alkaline hydrolysis tissue digestion as an extraction method for both aromatic and aliphatic hydrocarbons, unfortunately their added aromatic fortifications were obscured by other peaks in their samples when analyzed by gas chromatography. This occurrence is unlikely in HPLC analysis since the U.V. detector is relatively specific for aromatic species.

Figure 1a shows typical reversed phase HPLC chromatograms as obtained from alkaline hydrolysis of samples of river sediment, utilizing the described extraction and analysis scheme. The major peaks, identified by co-chromatography, are indicated. Packed column GC traces performed on the same samples (flame ionization detector) are shown for comparison in Figure 1b. The FID traces are considerably more complex than the corresponding HPLC chromatograms since the flame ionization detector does not show the selectivity for aromatics that is given by U.V. absorption at 254 n in the case of the HPLC detector.

An example of co-chromatography of several unknown peaks in a river sediment fraction with those of a mixed PAH standard containing benzene, anthracene, benz(a)anthracene, and benz(a)pyrene, is shown in Figure 2. While co-chromatography alone can <u>not</u> be used as evidence for the existence of a molecule in environmental samples, absorption spectra of peaks isolated from HPLC can be used to substantiate that suspect PAH peaks are at least consistent with such an identification. Peaks can be isolated by fraction collection since the HPLC method is non-destructive.

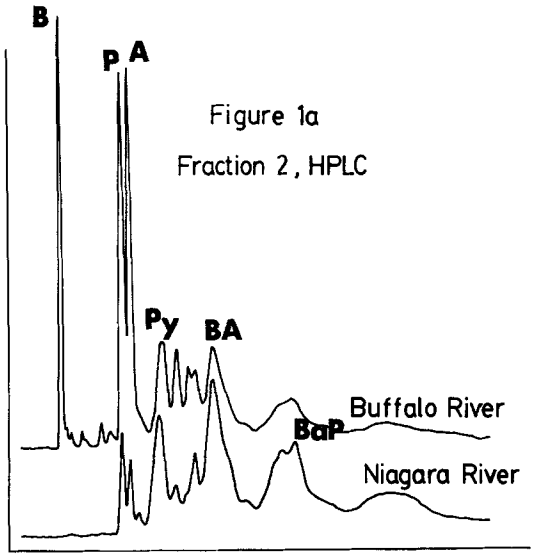

Fig. 1a HPLC chromatograms derived from bottom sediment samples. Sample concentrations: Buffalo River 2 x, Niagara River 20x. B=Benzene, P=phenanthrene, A=Anthracene, Py=pyrene, BA=benzanthracene, BaP=Benz(a)pyrene.

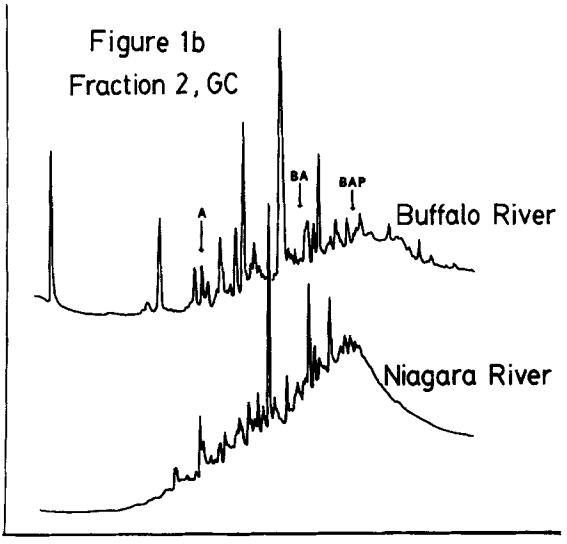

Fig. 1b Packed column GC-FID chromatograms of the same samples in 1a (column SP2250, 6 ft x ¼", T_1=100°C, T_2=300°C, rate= 7°C/min). Approximate PAH locations, A=anthracene, BA= benzanthracene, BaP=benz(a)pyrene.

Figure 2
Co-Chromatography Of Standard PAH'S
With Sediment Unknowns

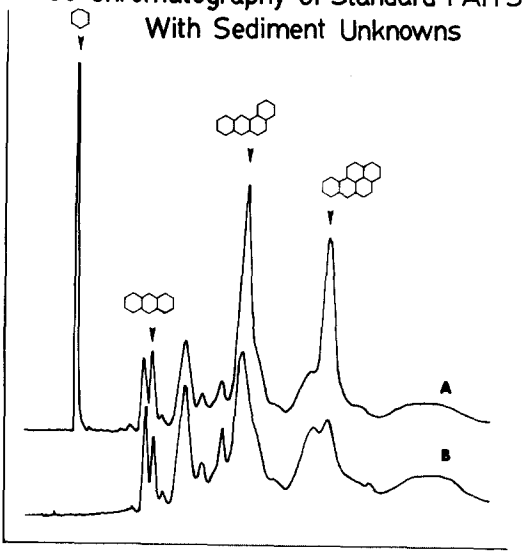

Fig. 2 HPLC Chromatograms: A) PAH containing fraction from sediment fortified with PAH standards as indicated. B) Same PAH containing fraction prior to addition of PAH standards.

Our studies of PAH contamination in several widely separated aquatic environments has indicated a general similarity in the resulting chromatograms obtained from the HPLC analysis of the Florisil derived, PAH fractions, to the extent that homologous peaks can readily be located through comparisons with standards and use of R_f values, or by simple inspection of superimposed chromatograms.

By keeping instrument conditions fixed and simply noting the sample concentration, it is comparatively easy to assess the relative distribution of PAH contamination within a particular environment. For example, a point source of PAH pollution was readily detected by comparing the chromatograms produced from samples collected above, below, and adjacent to the wastewater outfall of a local chemical manufacturing plant. These data are shown in Figure 3.

While in detailed studies of PAH pollution, it is necessary to confirm the molecular nature of peaks detected with HPLC, the technique described here can be used to rapidly screen a series of samples for their degree of PAH pollution. Where homologous peaks can be deciphered through a series of samples, a single GC-MS run on one sample could suffice to substantiate the presence of suspected molecular species as identified in the entire homologous peak/sample series. In our use of this method, a single GC-MS run did, in fact, verify as major PAH components, all of the PAH species identified by co-chromatography.

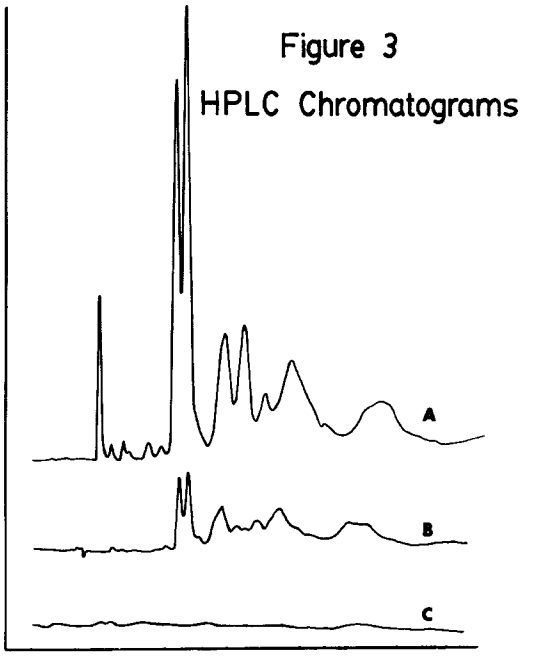

Fig. 3 HPLC chromatograms from sediment collected:
A) immediately adjacent to dye industry (2.5x) sample conc.
B) 1/2 mile down river (5x)
C) 1 mile above industry (5x)

While GC on both packed and capillary columns has been widely used for PAH analysis (GIGER & SCHAFFNER 1978, LAO et al. 1976, BRUNNERMAN & HOFFMAN 1976, GRIMMER & BOHNKE 1975, HITES & BIEMAN 1972) HPLC has been exploited by relatively few researchers for analysis of PAH contamination in complex environmental samples NOVOTNY et al. 1974, DONG & LOCKE 1976). As used in our studies of PAH pollution in fresh water ecosystems, it appears to be a relatively inexpensive and efficient method for determining the relative degree of PAH contamination in environmental samples. Although some PAH isomers are poorly resolved with the simple isocratic HPLC techniques used in this study, others, such as phenanthrene and anthracene, that are only poorly resolved by GC are well resolved in our chromatograms.

HPLC, as a method for PAH monitoring and analysis has much to recommend it. A variety of columns and solvent systems are available, including aminosilane columns that efficiently separate PAH's according to the number of condensed rings (WISE et al. 1977). This separation could be performed in a preparatory mode prior to further analysis of the resulting fractions.

The use of sophisticated instrumentation techniques such as elution gradient programming with multisolvent systems, solvent

scouting, recycling of peaks, dual wave length detection or variable wave length detection should enable the separation of most PAH isomers as encountered in fresh water sediment samples or other complex environmental samples.

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