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Development of a Flow-Injection Fluorescence Method for Estimation of Total Polycyclic Aromatic Compounds in Asphalt Fumes

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Traditionally, measurements of specific polycyclic aromatic compounds (PACs) have been attempted as an estimate of asphalt fume exposure. However, asphalt fumes contain numerous alkyl substituted PACs, including PACs containing heteroatoms of nitrogen, oxygen, and sulfur. Many of these compounds coelute precluding the resolution of the individual compounds resulting in ambiguous data. Moreover, many researchers believe that some observed health hazards are associated with PACs overall and not just a few select PACs. Therefore, NIOSH method 5800 was developed to evaluate total PACs as a chemical class in asphalt fumes. Asphalt fume samples were collected on a poly(tetrafluoroethylene) filter backed by an XAD-2 sorbent tube. The samples were extracted with hexane; then, a cyano-solid-phase-extraction column was used to remove the polar compounds while the aliphatic and aromatic compounds were eluted with hexane. An equal volume of dimethyl sulfoxide (DMSO) was added to the hexane extract, causing the aromatic compounds to partition into the DMSO, thus isolating the PACs. The PACs were then analyzed for fluorescence using a flow-injection method with two fluorescence detectors. Wavelength settings for the first detector (254-nm excitation, 370-nm emission) emphasized the 2- to 4-ring PACs that may cause eye and respiratory tract irritation. Wavelength settings of the second detector (254-nm excitation, 400-nm emission) emphasized the 4- and higher-ring PACs that are often mutagenic and possibly carcinogenic.

Keywords: analytical method development, asphalt fumes, construction, flow injection analysis, polycyclic aromatic compounds, PACs

Asphalt is a mixture of aliphatic and polycyclic aromatic compounds (PACs) including heterocyclic compounds containing nitrogen, oxygen, or sulfur.[1] Because asphalt is the residuum resulting from the distillation and processing of crude petroleum, its chemical composition is extremely complex and variable, containing a multitude of compounds in low concentrations, having no dominant compounds.[2] When asphalt products are applied at elevated temperatures, complex and variable organic emissions are released, thus exposing the workers to a fume. Although the fume is primarily aliphatic, PACs are present in small amounts.[3]

PACs belong to a chemical class that contain two or more fused benzenoid rings. If the PACs contain only carbon and hydrogen, they are often called polycyclic aromatic hydrocarbons (PAHs). PACs also include compounds in which one or more of the carbon atoms in the benzenoid rings are replaced by heteroatoms of nitrogen (N-PACs), oxygen (O-PACs), or sulfur (S-PACs). Besides these PACs, numerous substituted compounds exist that contain various substituent groups, such as alkyl, amino, chloro, cyano, hydroxy, oxy, or thio groups.[4]

Many 2- and 3-ring PACs (naphthalene, anthracene, and phenanthrene) are irritants.[5] Also, certain 4- and 5-ring PACs (benz[*a*]anthracene, chrysene, and benzo[*a*]pyrene) are mutagenic and possibly carcinogenic.[5,6] Consequently,

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some adverse health effects associated with asphalt fume exposure have been attributed to PACs.[3,7] Chemical analyses of asphalt fume samples indicate that the PACs include PAHs with two to six fused rings and N-, O-, and S-PACs with two to four fused rings. Many of the PACs have alkyl side chains of one to two carbons in length and a maximum of four saturated carbons. Besides alkyl substituent groups, hydroxy, oxy, and thio substituent groups have been identified.[3,7–10]

Concerns about PACs and their relationship to cancer have resulted in the investigation of select PACs in asphalt fumes.[11–14] These investigations have centered on the analysis of 16 select PACs. The *NIOSH Manual of Analytical Methods* (NMAM) contains two analytical methods that can be used for individual PACs.[15,16] Both methods have been successfully used to analyze PACs in coke-oven emissions, coal-tar-pitch volatiles, and various combustion matrices. In either method, PACs are collected on a poly(tetrafluoroethylene) (PTFE) filter, backed by an XAD-2 sorbent tube. NMAM method 5506[15] uses high-performance liquid chromatography (HPLC). Separation is achieved on a polymeric C_{18} reversed-phase column with an acetonitrile/water gradient. The eluting compounds are monitored by an ultraviolet (UV) detector (254 nm) and a fluorescence detector (340-nm excitation, 425-nm emission). The limits of detection (LODs) for select PACs range from 0.001 to 5 μg per sample. NMAM method 5515[16] uses gas chromatography (GC) with a flame ionization detector (FID) for sample analysis. Separation is achieved on a 5% diphenyl 95% dimethyl polysiloxane column with a temperature gradient. The LODs for select PACs range from 0.3 to 0.5 μg per sample.

For asphalt fumes, the identification and quantitation of individual PACs based on retention data are complicated because of numerous coeluting compounds. Figure 1 shows the fluorescence response of a typical filter sample, collected at an asphalt paving site and analyzed using an HPLC procedure. Despite the specificity of fluorescence monitoring, this characteristic chromatographic profile shows little baseline separation, making identification or quantitation of select components questionable. Compared with HPLC fluorescence methods, NMAM 5515[16] yields even more complex results because the FID is a nonspecific detector and responds to all the components in asphalt fumes. Consequently, application of these methods provided almost no information concerning exposure because of the lack of resolution and complexity of the mixture comprising asphalt fumes.

Although the sampling train used in NMAM 5506[15] and 5515[16] is useful for collecting PACs, separation of the PACs into discrete compounds was not possible. Consequently, and because some potential adverse health effects may be due to exposure to these coeluting compounds, a flow-injection fluorescence method was developed to determine the total PAC content in asphalt fumes. This article reports the development of the method (NMAM 5800[17]). The objectives of the method development were to (1) isolate the PACs from the aliphatic and polar components, (2) maximize the recovery of PACs, (3) analyze the PACs as a chemical class, and (4) automate as much of the procedure as possible.

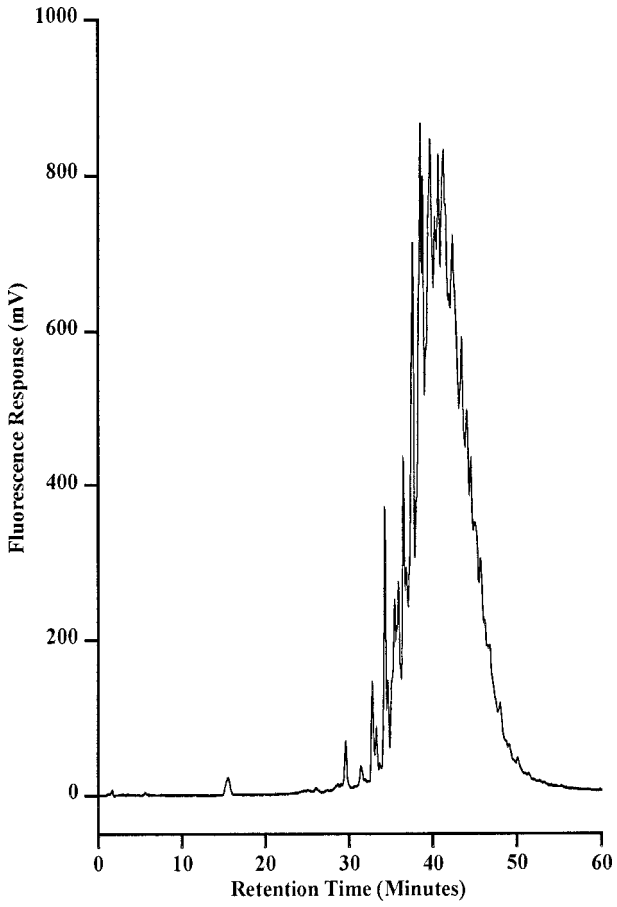

FIGURE 1. Fluorescence chromatogram (254 nm excitation, 370 nm emission) of a typical paving asphalt fume sample collected on a PTFE-membrane filter. Obtained using the reversed-phase HPLC procedure.

METHODS AND MATERIALS

Reagents

The PAH test mixture (containing varying concentrations between 4 and 2000 μg/mL of naphthalene; acenaphthylene; acenaphthene; fluorene; phenanthrene; anthracene; fluoranthene; pyrene; benz[*a*]anthracene; chrysene; benzo[*b*]fluoranthene; benzo[*k*]fluoranthene; benzo[*a*]pyrene; dibenz[*a,h*]anthracene; benzo[*ghi*]perylene; and indeno[1,2,3-*cd*]pyrene) and the QTM PAH test mixture (2000 μg/mL of the same PACs as the PAH test mixture except benzo[*k*]fluoranthene was replaced with 2-bromonaphthalene) were obtained from Supelco (Bellefonte, Pa.). Hexane, methanol, methylene chloride, acetonitrile, and dimethyl sulfoxide (DMSO) were HPLC grade and distilled in glass (Burdick and Jackson; Muskegon, Mich.). Water used for the HPLC mobile phase was obtained using an EASYpure UV Compact Ultrapure Water System (Barnstead/Themolyne; Dubuque, Iowa).

HPLC Equipment and Conditions

The HPLC system consisted of a Waters 600-MS System Controller (Milford, Mass.), membrane degasser (ThermoSeparation Products; San Jose, Calif.), a Waters 717+ Autosampler, a Supelcosil LC-PAH column (25-cm × 2.1-mm i.d.; polymeric C_{18} bonded to 5-μm silica; Supleco); a CH-30 column heater (30°C; Eppendorf; Madison, Wisc.); a Shimadzu RF-551 Spectrofluorometric Detector (254-nm excitation, 370-nm emission; Tokyo, Japan) in series with a Waters 990+ photodiode array detector (254 nm); and a laboratory data management system (Dionex AI-450; Sunnyvale, Calif.). The chromatographic conditions were: (1) solvent gradient program starting at 40:60 acetonitrile/water for 5 min, followed by a linear gradient to 100% acetonitrile over the

next 25 min and then held for 40 min, (2) the flow rate was 1.0 mL/min, (3) and the injection volume was 25 μL.

Wavelength Selection

Analysis of standards prepared from the PAH test mixture was used to evaluate various excitation and emission wavelengths.

Flow-Injection Equipment and Conditions

The flow-injection system was the same as the HPLC system except that the chromatographic column was removed and the photodiode array detector was replaced with a Shimadzu RF-535 Fluorescence HPLC Monitor (254-nm excitation, 400-nm emission). The mobile phase was 100% acetonitrile, the flow rate was 1.5 mL/min, and the injection volume was either 5 or 25 μL.

Air Collection

The air sampler consisted of a PTFE-membrane filter (37-mm diameter, 2-μm pore size, Zeflour filter, Supelco) in a two-piece filter cassette with a cellulose o-ring (37-mm o.d. × 32-mm i.d.; Supelco), placed above and below the membrane filter. The filter cassette was followed by a sorbent tube (ORBO 42 LG; Supelco) containing 100/50 mg of washed XAD-2 resin in the front and back sections, respectively. (For early field work and for media standards, a sorbent tube [ORBO 43; Supelco] containing XAD-2 resin was used. However, because of pressure drop problems, the ORBO 42 LG sorbent tube was used during field work.) Full-work-shift air samples were collected at a flow rate of 2 L/min. To avoid exposure to light, the filter cassettes were wrapped with aluminum foil. When possible, the samplers were kept cold in the field but were shipped at ambient temperature. After arrival at the laboratory, all samplers were refrigerated at 5°C.

Sample Preparation

The samplers were removed from the refrigerator and allowed to equilibrate at room temperature for at least 120 min. The membrane filters and each section of the sorbent tubes were placed separately in screw-capped test tubes (16 × 100 mm), and 4 mL of hexane was added to each test tube. The test tubes were capped (PTFE-faced caps), and the PACs were extracted using a 360° rotating shaker for at least 12 hours. The sample extracts were transferred with Pasteur pipettes to smooth-walled test tubes (16 × 100 mm); these test tubes were loaded into the sample position on a Zymark Benchmate II Workstation (ZBW; Hopkinton, Mass.). A solid-phase-extraction (SPE) column holder (Zymark) and a cyano-solid-phase-extraction (CN-SPE) column (Supelco) were placed on top of each test tube. Screw-capped test tubes (16 × 100 mm) were loaded into the collection position of the ZBW. Plastic sleeves were placed over the threaded portion of these tubes to insure proper orientation with the robotic arm.

The ZBW was programmed to perform the following steps: (1) the CN-SPE column was conditioned sequentially with 3 mL each of methanol, methylene chloride, and hexane (0.25 mL/sec); (2) 2 mL of the sample extract was eluted through the CN-SPE column (0.15 mL/sec) and collected in a screw-capped test tube; (3) 2 mL of hexane was passed through the CN-SPE column (0.15 mL/sec) and combined with the eluate from Step 2; (4) 4 mL of DMSO was added to the eluate (0.25 mL/sec). The test tubes were capped (PTFE-faced caps), transferred to a 360° rotating shaker and mixed for at least 12 hours. Using Pasteur pipettes, the DMSO layer (bottom layer) was transferred to amber autosampler vials for analysis using either the HPLC procedure or the flow-injection method.

Calibration Curves, LODs, and LOQs Studies

The QTM PAH test mixture was diluted with DMSO, and two calibration curves were prepared to cover the range of 0.006 to 0.94 and 0.65 to 6.54 μg total PACs/sample for both sets of fluorescence wavelengths. Initially, samples were analyzed based on the higher calibration range using a 5-μL injection volume and both detectors set on low sensitivity. For samples with concentrations higher than 6.54 μg total PACs/sample, the samples were diluted and reanalyzed until they fell within the high calibration range. The samples with a concentration less than 0.75 μg total PACs/sample were reanalyzed using the lower calibration range with a 25 μL injection volume and both detectors set on high sensitivity.

To determine the LODs and limits of quantitation (LOQs), separate filters and tubes were fortified with the QTM PAH test mixture. Six standards on each sample medium were prepared to cover the range of 0.0032 to 0.320 μg of total PACs/media. After allowing solvent to evaporate overnight, the standards were prepared as described previously and analyzed by the flow-injection method.

Recovery for Individual PACs

Analyte recoveries were determined individually for the select 16 PACs in the PAH test mixture. Six each of filters and sorbent tubes were fortified with 10 μL of a 1:10 dilution of the PAH test mixture in hexane. After the solvent was allowed to evaporate overnight, the sampling media were prepared as described previously and analyzed by the HPLC procedure.

Recovery and 30-Day Stability Studies for Total PACs

Recovery and 30-day stability studies for total PACs as a chemical class were performed.[18] For each study the sampling media were fortified with dilutions of the QTM PAH test mixture in hexane. Using a 25-μL syringe, four sets of six samplers (filters and sorbent tubes) for each study were fortified with 0.16, 0.32, 0.64, and 1.28 μg of PACs. Samplers were kept at ambient conditions for 2 hours to allow for solvent evaporation. On the following day the recovery study samples were prepared as described previously and analyzed by the flow-injection method. The stability study samples were refrigerated (5°C) for 30 days before analysis by the flow-injection method.

RESULTS AND DISCUSSION

Wavelength Selection

Each of the unsubstituted PACs exhibit a distinctive excitation and emission maxima that are similar to those of their alkylated derivatives.[19] Furthermore, the spectra of these compounds are broad, causing varying fluorescence contributions at different wavelength settings. Therefore, experiments were conducted to select two sets of fluorescence wavelengths: one to emphasize the 2- and 4-ring PACs and the other to emphasize the 4- and higher-ring PACs. Various excitation (225 to 302 nm) and emission (310 to 500 nm) wavelengths were investigated using the HPLC equipment and the PAH test mixture. Due to a strong response for all the monitored PACs, 254 nm was chosen as the excitation wavelength.

Figures 2 and 3 show the resultant chromatograms when monitored at an excitation wavelength of 254 nm and emission wavelengths of 370 nm and 400 nm, respectively. The compounds

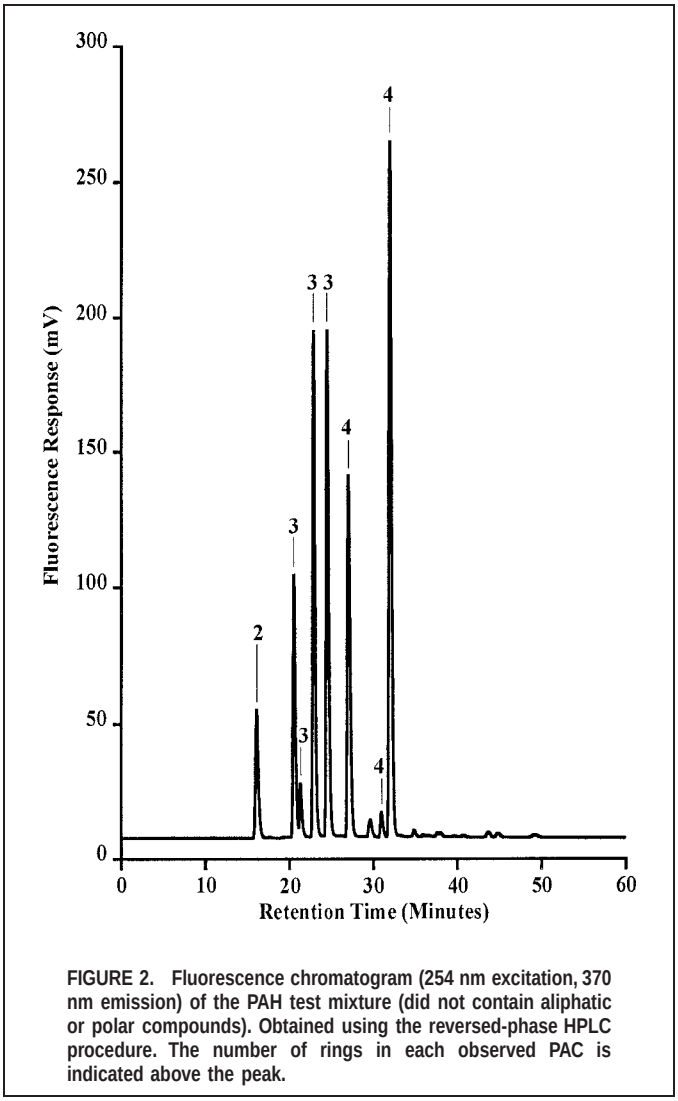

FIGURE 2. Fluorescence chromatogram (254 nm excitation, 370 nm emission) of the PAH test mixture (did not contain aliphatic or polar compounds). Obtained using the reversed-phase HPLC procedure. The number of rings in each observed PAC is indicated above the peak.

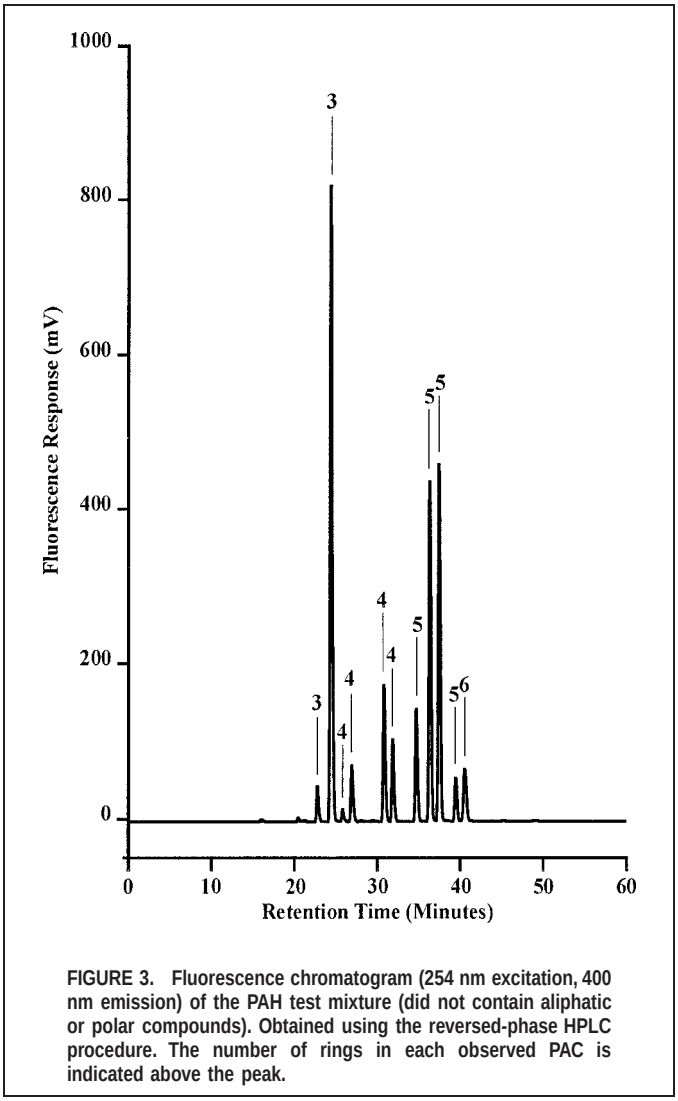

FIGURE 3. Fluorescence chromatogram (254 nm excitation, 400 nm emission) of the PAH test mixture (did not contain aliphatic or polar compounds). Obtained using the reversed-phase HPLC procedure. The number of rings in each observed PAC is indicated above the peak.

emphasized at 370 nm were, in their order of elution, naphthalene; acenaphthene; fluorene; phenanthrene; anthracene; pyrene; benz[*a*]anthracene; and chrysene. Similarly, the compounds emphasized at 400 nm were, in the order of elution, phenanthrene; anthracene; fluoranthene; pyrene; benz[*a*]anthracene; chrysene; benzo[*b*]fluoranthene; benzo[*k*]fluoranthene; benzo[*a*]pyrene; dibenz[*a,h*]anthracene; and benzo[*ghi*]perylene. Because acenaphthylene does not fluoresce and indeno[1,2,3-*cd*]pyrene is a poor fluorophore at these fluorescence wavelengths, these two compounds were not detected.

These emission wavelengths were chosen to emphasize either 2- to 4-ring PACs (many of which may cause irritation) or 4- and higher-ring PACs (many of which are mutagens and possible carcinogens). It should be noted that five of the PACs (phenanthrene, anthracene, pyrene, benz[*a*]anthracene, and chrysene) contribute to the fluorescence response for both emission wavelengths. Except anthracene and benz[*a*]anthracene, these five compounds exhibited a greater response at 370 nm. In retrospect, an emission wavelength shorter than 370 nm should have been chosen to better emphasize the 2- and 3-ring PACs. Also, longer excitation and emission wavelengths should have been chosen to better emphasize the 4- and higher-ring PACs.

Sample Preparation and Analysis

The petroleum industry has long employed a separation procedure that yields four chemical classes: saturate, aromatic and polar compounds, and hexane insolubles (asphaltenes).[20] The interest here was on the PACs as a chemical class, so it was decided that the polar and conjugated aliphatic compounds should be removed from the asphalt fume samples because these compounds may affect the fluorescence response. The sample preparation procedure is diagrammed in Figure 4. Initially, the samples were extracted from the sampling media with hexane. The hexane extract was passed through a CN-SPE column, and the polar compounds were retained on the column. The aromatic and aliphatic compounds passed through the column with the hexane and were collected in a second test tube. Next, DMSO was added to the test tube resulting in the partitioning of the PACs into the DMSO while the aliphatic compounds remained in the hexane. An aliquot of the DMSO was analyzed for total PACs as a chemical class.

In addition, aliquots of the DMSO and the hexane extract before and after passing through the CN-SPE column were analyzed using the HPLC procedure to determine if removing the polar and conjugated aliphatic compounds was necessary. Because the fluorescence chromatogram was different for each aliquot, this was

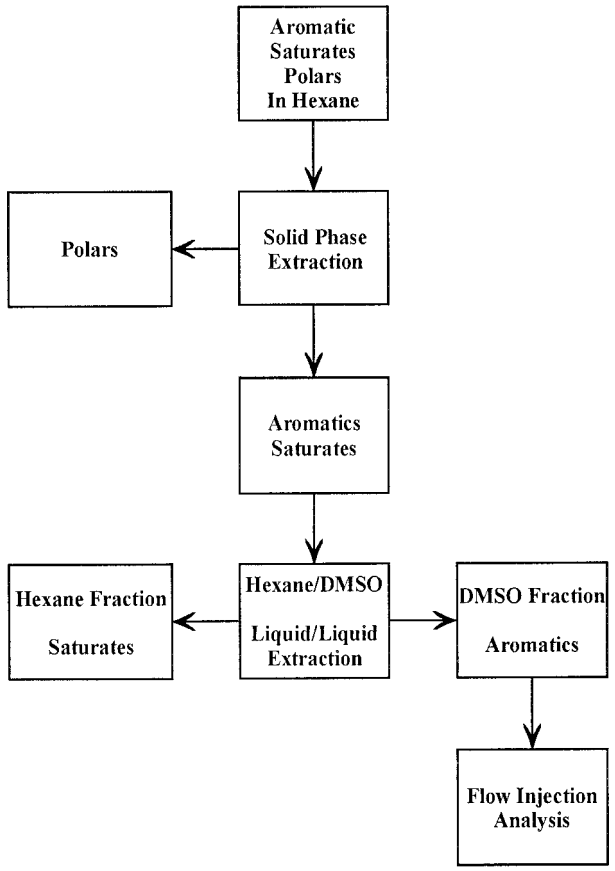

FIGURE 4. Diagram of the sample preparation procedure

considered sufficient to conclude that both the polar and conjugated aliphatic compounds needed to be removed; however, the effectiveness of these extraction steps was not determined.

Hexane was chosen for its ability to dissolve the saturate, aromatic, and polar compounds. Although cyano, amino, or silica could be used to remove the polar compounds, a CN-SPE column was chosen because it does not irreversibly retain some polar compounds, such as aldehydes and ketones.[20] (If needed, the polar compounds retained on the CN-SPE column may be eluted using methanol.) After removal of the polar compounds, several liquid/liquid extraction procedures could be used to isolate the aromatics.[21] These procedures are based on solvents having a high aromatic partition ratio with respect to hydrocarbon solvents. Of the partition solvents investigated (nonpolar: hexane and benzene; polar: DMSO, acetonitrile, methanol, dimethylformamide, and tetrahydrofuran), equal volumes of hexane and DMSO provided the highest aromatic partition ratio.

The SPE portion of the sample preparation procedure was directly amenable to automation using a ZBW. The automated steps included sample transfer, solvent addition, and conditioning of the SPE column, the SPE, and system rinses to prevent cross contamination.

The flow-injection method allows the partial automation of the fluorometric measurements of total PACs as a chemical class. The solvent system and injector deliver the entire sample injected to the detector flow cell for measurement and sweeps the flow cell with solvent. Elimination of the chromatographic separation enhances the sensitivities of the total PACs because the sample arrives at the flow cell as a sharp, single band with minimum broadening. Use of HPLC equipment for sample analysis rather than a traditional spectrofluorometric procedure eliminated the need to transfer individual samples into cuvettes. As a partially automated procedure, not only was the analysis time decreased but also unattended operation was possible. The data were collected by the laboratory data management system, thereby eliminating transcription errors and simplifying data reduction.

Calibration Curves, LODs, and LOQs

Several options were considered for reporting the total PAC concentration. Calibration curves based on naphthalene, pyrene, or benzo[*a*]pyrene were considered. Because the fluorescence signal due to two- and three-ring PACs was emphasized at 370-nm emission, the concentration could be expressed as naphthalene. Similarly, the fluorescence signal due to 4- and higher-ring PACs was emphasized at 400-nm emission, the concentration could be expressed as pyrene or benzo[*a*]pyrene. However, because some 3- and 4-ring PACs contribute to the fluorescence at both emission wavelengths, a mixture of PACs was considered a better choice for standardization, because it would better mimic the chemical complexity of field samples. Moreover, it must be emphasized that it is not possible to combine the results from the two emission wavelengths. At best, the results are estimates of the 2- to 4-ring PACs versus the four- and higher-ring PACs.

To prevent a bias toward an individual PAC, a mixture of PACs of constant concentration was desired. The QTM PAH test mixture was chosen because it contains equal concentrations of each PAC (2000 μg/mL of each PAC or 32,000 μg/mL of total PACs). Serial dilutions of the QTM PAH test mixture with DMSO were used to establish the calibration curves using the sum of the individual PAC concentrations as the concentration of total PACs.

The LODs and the LOQs were determined as 3 and 10 times the standard error of the low-level calibration curves divided by their slope, respectively, according to NIOSH guidelines.[18] The LOD and LOQ for the PACs monitored at 254-nm excitation and 370-nm emission were 0.01 and 0.03 μg/sample, respectively. The LOD and LOQ for the PACs monitored at 254-nm excitation and 400-nm emission were 0.02 and 0.07 μg/sample, respectively.

Recovery and Stability Studies

For the recovery study of select PACs, the sampling media were fortified with the PAH test mixture. These fortified samples were prepared as described previously, and an aliquot of the DMSO was analyzed by the HPLC procedure. Figure 5 shows the individual PAC recoveries. The recoveries for the two- and three-ring PACs were between 74 and 91%, except acenaphthene, which was 60%, whereas the 4-, 5-, and 6-ring PACs showed recoveries between 83 and 108%.

Similarly, for the recovery study of total PACs the sampling media were fortified with the QTM PAH test mixture. These fortified samples were prepared as described previously, and an aliquot of the DMSO was analyzed by the flow-injection method. The average recoveries were 90% (8% relative standard deviation, RSD) and 95% (5% RSD) for the total PACs monitored at the two emission wavelengths of 370 and 400 nm, respectively.

The 30-day stability study was conducted like the total PAC recovery study. The results were within 10% of the original recovery values, indicating that the PACs were stable on the sampling media when refrigerated.

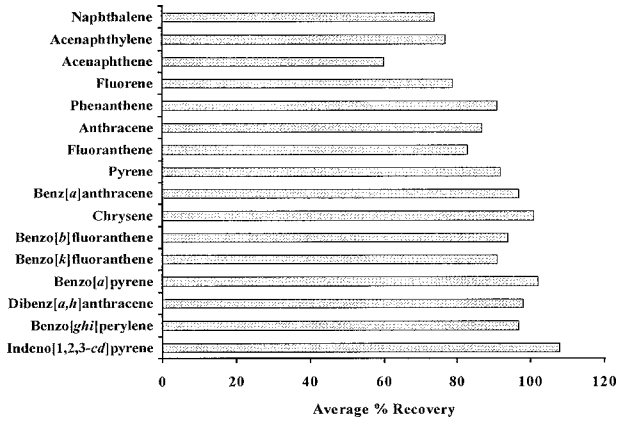

FIGURE 5. Average percentage recoveries of the PACs in the PAH test mixture from fortified sampling media. Determinations were by reversed-phase HPLC with a UV detector set at 254 nm.

CONCLUSIONS

A flow-injection method was developed to monitor PACs as a chemical class in asphalt fumes, NMAM 5800.[17] This approach was necessary because the complexity of asphalt fumes precluded the separation of the PACs. Fluorescence measurement at 254-nm excitation and 370-nm emission wavelengths emphasized the two- to four-ring PACs, exposure to which may cause irritation. Similarly, fluorescence measurement at 254-nm excitation and 400-nm emission wavelengths emphasized the 4- and higher-ring PACs, exposure to which may cause mutations or cancers. Moreover, it must be emphasized that it is not possible to combine the results from the two emission wavelengths. At best, the results are estimates of the 2- to 4-ring PACs versus the four- and higher-ring PACs.

This method is selective, specific, and sensitive for simultaneous monitoring of total PACs as a chemical class. The selectivity and specificity are due to the isolation of the PACs from the asphalt fumes and the use of fluorescence measurements. For this method, the LOD is 0.01 μg/sample for the 370 nm measurement and 0.02 μg/sample for the 400 nm measurement. These sensitivities are possible for this method because, relative to chromatographic methods, the entire PAC sample arrives at the flow cell as a sharp, single band with minimal band broadening.

This method was used to make relative comparisons of asphalt fume exposures during several NIOSH investigations. The comparisons were used to determine whether changes in asphalt formulations resulted in more hazardous asphalt fume exposures, that is, total PACs. Also, these comparisons are being used to determine whether newly developed engineering controls are effective at reducing worker exposure. Once these comparison studies are complete, future work will investigate other wavelengths to reduce or eliminate the fluorescence contributions observed for several three- and four-ring PACs.

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