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NOVEL DEVELOPMENTS ON THE EXTRACTION AND ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN ENVIRONMENTAL SAMPLES

by

WALTER B. WILSON
B.S. Greensboro College, 2005
M.S. University of North Carolina at Greensboro, 2007

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Chemistry in the College of Sciences at the University of Central Florida Orlando, Florida

Summer Term 2014

Major Professor: Andres D. Campiglia
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ABSTRACT

This dissertation focuses on the development of analytical methodology for the analysis of polycyclic aromatic hydrocarbons (PAHs) in water samples. Chemical analysis of PAHs is of great environmental and toxicological importance. Many of them are highly suspect as etiological agents in human cancer. Among the hundreds of PAHs present in the environment, the U.S. Environmental Protection Agency (EPA) lists sixteen as "Consent Decree" priority pollutants. Their routine monitoring in environmental samples is recommended to prevent human contamination risks.

A primary route of human exposure to PAHs is the ingestion of contaminated water. The rather low PAH concentrations in water samples make the analysis of the sixteen priority pollutants particularly challenging. Current EPA methodology follows the classical pattern of sample extraction and chromatographic analysis. The method of choice for PAHs extraction and pre-concentration is solid-phase extraction (SPE). PAHs determination is carried out via high-performance liquid chromatography (HPLC) or gas chromatography/mass spectrometry (GC/MS). When HPLC is applied to highly complex samples, EPA recommends the use of GC/MS to verify compound identification and to check peak-purity of HPLC fractions.

Although EPA methodology provides reliable data, the routine monitoring of numerous samples via fast, cost effective and environmentally friendly methods remains an analytical challenge. Typically, 1 L of water is processed through the SPE device in approximately 1 h. The rather large water volume and long sample processing time are recommended to reach detectable concentrations and quantitative removal of PAHs from water samples. Chromatographic elution times of 30 – 60 min are typical and standards must be run periodically to verify retention times.
If concentrations of targeted PAHs are found to lie outside the detector’s response range, the sample must be diluted (or concentrated), and the process repeated.

In order to prevent environmental risks and human contamination, the routine monitoring of the sixteen EPA-PAHs is not sufficient anymore. Recent toxicological studies attribute a significant portion of the biological activity of PAH contaminated samples to the presence of high molecular weight (HMW) PAHs, i.e. PAHs with MW ≥ 300. Because the carcinogenic properties of HMW-PAHs differ significantly from isomer to isomer, it is of paramount importance to determine the most toxic isomers even if they are present at much lower concentrations than their less toxic isomers. Unfortunately, established methodology cannot always meet the challenge of specifically analyzing HMW-PAHs at the low concentration levels of environmental samples. The main problems that confront classic methodology arise from the relatively low concentration levels and the large number of structural isomers with very similar elution times and similar, possibly even virtually identical, fragmentation patterns.

This dissertation summarizes significant improvements on various fronts. Its first original component deals with the unambiguous determination of four HMW-PAHs via laser-excited time-resolved Shpol’skii spectroscopy (LETRSS) without previous chromatographic separation. The second original component is the improvement of a relatively new PAH extraction method - solid-phase nanoextraction (SPNE) - which uses gold nanoparticles as extracting material for PAHs. The advantages of the improved SPNE procedure are demonstrated for the analysis of EPA-PAHs and HMW-PAHs in water samples via GC/MS and LETRSS, respectively.

Additional work related to the development of methodology for the analysis of PAHs has been reported in the following publications:


ACKNOWLEDGMENTS

Foremost, I would like to express my sincere gratitude to my advisor Dr. Andres D. Campiglia for his continuous and enormous support during my Ph.D. research with his patience, enthusiasm, and immense knowledge. His guidance helped me through the duration of my Ph.D. experience and preparation of this thesis.

Besides my advisor, I would like to thank the rest of my dissertation committee: Dr. Kevin D. Belfield, Dr. James Harper, Dr. Matthew Rex and Dr. Jay Hoffman. It’s truly a privilege to have them on my dissertation committee.

I would also like to thank my fellow lab members and collaborators for their valuable help throughout my research, Dr. Huiyong Wang, Dr. Andréia Acosta, Dr. José A. Dias, Dr. Korina Calimag-Williams, Dr. Krishnaveni Appalaneni, Dr. Gaston Knobel, Dr. Emily Heider, Anthony F. T. Moore, Bassam F. Alfarhani, Cristina Bisson, Udienza Hewitt, Mattheu Miller, Nirvani Mujumdar, Khang Trieu, Maddie Johnson, Josh Knorr, Maha Al-Tameemi and Jongseok Jung.

Last but not least, I would like to thank my parents and family, for their unconditional love, support, and encouragement to pursue my interests.
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CHAPTER 1 POLYCYCLIC AROMATIC HYDROCARBONS

1.1 Environmental Protection Agency Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are important environmental pollutants originating from a wide variety of natural and anthropogenic sources. PAHs are generally formed during incomplete combustion of organic matter containing carbon and hydrogen.\(^4\) Due to the carcinogenic nature of some PAHs, their chemical analysis is of great environmental and toxicological importance.\(^7\) Among the hundreds of PAHs present in the environment, the U.S. Environmental Protection Agency (EPA) lists sixteen as "Consent Decree" priority pollutants. Their molecular structures are shown in Figure 1.1. According to the EPA, these PAHs should be routinely monitored to avoid human exposure to contaminated sites.

In addition to air inhalation and dermal absorption, direct routes of human exposure to PAHs include food consumption\(^10\) and the ingestion of contaminated water.\(^21\) This dissertation focuses on the analysis of PAHs in water samples. On drinking waters, the EPA recommends the routine monitoring of benzo\([a]\)pyrene. This is the most toxic PAH in the EPA list, and its concentration alone is often used as a measure of risk. According to the EPA, its maximum contaminant level (MCL) should not exceed 200 ng L\(^{-1}\).\(^26\) The European Union and the World Health Organization (WHO) have regulated benzo\([a]\)pyrene, fluoranthene, benzo\([b]\)fluoranthene, benzo\([k]\)fluoranthene, benzo\([ghi]\)perylene and indeno\([1,2,3-cd]\)pyrene. MCL values were set at 10 ng L\(^{-1}\) for the highly toxic benzo\([a]\)pyrene and 200 ng L\(^{-1}\) for the remaining PAHs.\(^27\)
Figure 1.1 Molecular structure of 16 EPA-PAHs.
Established methodology for the monitoring EPA-PAHs in public water supplies follows the classical pattern of sample preparation and chromatographic analysis. Sample preparation pre-concentrates PAHs, simplifies matrix composition, and facilitates analytical resolution in the chromatographic column. Among the numerous approaches that exist to pre-concentrate PAHs from water samples (see Table 1.1), the EPA recommends the use of solid-phase extraction (SPE). The use of liquid-liquid extraction has dropped dramatically following obligatory reduction of chlorinated usage in analytical laboratories. When compared to liquid-liquid extraction, SPE reduces solvent consumption, has fewer laborious steps, provides better extracting efficiency, enables easy sample collection, and is more amenable to automation.

**Table 1.1 Extraction Techniques for PAHs in Water Samples.**

<table>
<thead>
<tr>
<th>Sample Preparation Technique</th>
<th>Sample Volume (mL)</th>
<th>Volume of Organic Solvent per Sample (mL)</th>
<th>Extraction Time per Sample (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solid-Phase Extraction</td>
<td>36-49</td>
<td>5 – 1000</td>
<td>10 – 150</td>
</tr>
<tr>
<td>Liquid-Liquid Extraction</td>
<td>48-53</td>
<td>20 – 1000</td>
<td>20 – 250</td>
</tr>
<tr>
<td>Solid-Phase Microextraction</td>
<td>48, 54-67</td>
<td>5 – 30</td>
<td>0</td>
</tr>
<tr>
<td>Stir Bar Sorptive Extraction</td>
<td>50, 68-74</td>
<td>10 – 500</td>
<td>0 – 1</td>
</tr>
</tbody>
</table>

\(^a\) Extraction time was not reported.\(^{49-53}\)

High-performance liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS) are the basis of current standard methodology.\(^{26, 34, 35}\) Ultraviolet-visible (UV-VIS) absorption and room temperature fluorescence (RTF) detection are both widely used in HPLC, but the specificity of these detection modes is modest. Since PAHs identification is solely based on retention times, unambiguous PAHs determination requires the complete
chromatographic resolution of sample components. When HPLC is applied to “unfamiliar” samples, EPA recommends the use of a supporting analytical technique – such as GC/MS – to verify compound identification and to check peak-purity of HPLC fractions.  

1.2 Solid-Phase Extraction

SPE of is typically carried out with the aid of non-polar sorbents such as octadecyl-silica particles. The effectiveness of octadecyl-silica sorbents is based on the same sorption-desorption phenomena as in reversed-phase liquid chromatography. The main mechanism for isolating PAHs is a non-polar interaction, i.e. van der Waals forces. SPE materials are packed in two formats, discs and cartridges. A SPE cartridge consists of a small column (generally an open syringe barrel) containing a sorbent with an average particle size of about 40 µm packed between plastic frits. The sorbent bed occupies about one-third of the syringe barrel volume. The remaining volume is used as sample reservoir. SPE discs are particle-loaded membranes in the form of flexible discs of various diameters and 0.5 - 1.0 mm of thickness. They are packed in a way that the sorbent is enmeshed into a web of some other inert polymer, e.g., Teflon, or is trapped in a glass fiber or paper filter. SPE discs are preferred over cartridges because their larger cross-sectional area and shorter bed depth allow higher flow rates and shorter analysis time.

The experimental procedure for SPE consists of four steps: conditioning, retention, rinsing and drying. The solid sorbent must undergo proper conditioning to wet the packing material before the sample passes through the SPE device. In its dry form, the octadecyl (C_{18}) bonded-phase material is randomly oriented on the surface. If the sorbent is placed in contact
with an aqueous sample, the environment surrounding the bonded organic moiety would be highly polar and entirely incompatible with a C$_{18}$ bonded-phase. The bonded groups try to minimize the exposure to the high polarity medium by forming clusters among them that are close to each other. In such a configuration, the organic surface that is exposed to the solute is very small. This arrangement of the bonded organic groups diminishes the extraction efficiency of SPE. The conditioning step “activates” the sorbent by treatment with an organic solvent, typically methanol. Under these conditions, the bonded-organic moiety is more open and available for interaction with the solute. This process can be determined as one of the most important steps for a successful SPE.

After conditioning, the sample is loaded into the SPE device by gravity feed, pumping, or vacuum. Depending on the type of sample, micro-liters to liters of liquid sample can be applied through the sorbent phase. During this step, PAHs are extracted and pre-concentrated in the solid sorbent. Some of the matrix concomitants may also be retained along with PAHs, making matrix composition more challenging for further chromatographic analysis. A rinsing step is then used to remove potential interferences. For aqueous samples, a water-organic-solvent mixture is usually used as the rinsing solvent.

The final step is the elution of PAHs from the sorbent with an appropriate solvent specifically chosen to disrupt PAHs-sorbent interactions. For efficient PAHs elution, one would desire the capacity factor to approach zero in the eluting organic solvent. Unfortunately, non-reversed-phase interactions take place in C$_{18}$ sorbent materials that make the elution of PAHs difficult. Non-reversed phase interactions result from the existence of residual silanol groups on the surface of silica particles. These groups, which interact with PAHs through both hydrogen
bonding and dipole-dipole interactions, make difficult PAHs desorption for subsequent chromatographic analysis.\textsuperscript{75}

1.3 High-Performance Liquid Chromatography

According to current EPA methodology (EPA method 550.1), the HPLC analysis of the 16 EPA-PAHs should use a combination of UV-VIS and RTF detection.\textsuperscript{34} UV-VIS should be used for the detection of naphthalene, acenaphthylene, acenaphthene and fluorene. The determination of the remaining PAHs should be based on RTF spectroscopy. In both cases, PAHs identification is solely based on the comparison of retention times recorded from samples and standards. Separation is carried out on a Supelco LC-PAH column with the following characteristics: 25 cm length, 4 mm diameter and 5 µm average particle diameters. The settings and conditions of analysis include 2.0 mL min\textsuperscript{-1} flow rate, isocratic elution with 65/35 acetonitrile/water for 2 min and then linear gradient to 100\% acetonitrile over 22 min. The total separation time is approximately 30 min while consuming 60 mL of mobile phase (acetonitrile-water) per sample. Considering the gradient elution of chromatographic separation, this mobile phase volume is equivalent to 42 mL of acetonitrile. Numerous reported methods use methanol as the organic solvent in the mobile phase.\textsuperscript{36-38, 76-90} Separation of the 16 EPA-PAHs using a medium (15 cm) column length takes 53 min and consumes approximately 66 mL of methanol per sample (see Figure 1.2). The main reason methanol is preferred over acetonitrile is its lower cost.
Figure 1.2 Typical HPLC-RTF chromatogram of a 25 ng mL⁻¹ standard of 16 EPA-PAHs in n-octane. The peaks are labeled as follows: (1) naphthalene; (2) acenaphthene; (3) fluorene; (4) phenanthrene; (5) anthracene; (6) fluoranthene; (7) pyrene; (8) benz[a]anthracene; (9) chrysene; (10) benzo[b]fluoranthene; (11) benzo[k]fluoranthene; (12) benzo[a]pyrene; (13) dibenzo[a,h]anthracene; (14) benzo[ghi]perylene and (15) indeno[1,2,3-cd]pyrene. Acenaphthylene is not represented in the chromatogram because it shows no fluorescence.

1.4 Gas Chromatography/Mass Spectrometry

When HPLC is applied to highly complex samples and numerous unfamiliar peaks appear in the chromatogram, the use of a supporting analytical technique is recommended to verify compound identification and to check peak-purity of HPLC fractions. The EPA method of choice (method 525.1) is based on GC/MS. Separation of the 16 EPA-PAHs is carried out with a 5% phenyl methyl siloxane stationary phase (0.25 µm film thickness) packed in a 30 m length column with a 0.25 mm internal diameter. Ultra-pure helium carrier gas is used as the mobile phase – flow rate of 33 cm s⁻¹ – with either a single (160 – 320 °C at 6 °C min⁻¹) or a multi-ramp (130 – 180 °C at 12 °C min⁻¹, 180 °C – 240 °C at 7 °C min⁻¹ and 240 – 320 °C at 12
°C min\(^{-1}\)) temperature oven program. The total separation time is approximately 32 min and 25 min for the single and multi-ramp temperature program, respectively. PAH identification is based on retention times and mass spectra. For positive identification, the retention time of the unknown peak should be within 10 s of the reference standard retention time. All ions that are present above 10% relative abundance in the mass spectrum of the standard should be also present in the mass spectrum of the unknown within an agreement of 20% (see Figure 1.3A-B).

Although considerable efforts have been made to develop methods capable to determine trace concentration levels of EPA-PAHs in water samples, the routine monitoring of numerous samples via simple, cost effective and environmentally friendly methods remains an analytical challenge. Typically, 1 L of water is processed through the SPE device in approximately 1 h. The rather large water volume and long sample processing time are recommended to reach detectable PAH concentrations and quantitative PAH removal from water samples. Chromatographic elution times of 30 – 60 min are typical and standards must be run periodically to verify retention times. If concentrations of targeted PAHs are found to lie outside the detector’s response range, the sample must be diluted (or concentrated), and the process repeated. The numerous preparation steps open ample opportunity for PAH loss and collection of inaccurate data. Table 1.2 summarizes the overall recoveries of EPA-PAHs obtained from water samples via HPLC\(^{36-41}\) and GC/MS\(^{42-46}\) analyses. The reported data show relatively high standard deviations that affect the precision and the accuracy of PAHs determination. The poor reproducibility of average recoveries can be attributed to the difficulty of eluting PAHs from SPE sorbents and/or chromatographic columns as well as PAHs lost during the evaporation of the eluting solvent.\(^{40,42}\)
Figure 1.3 (A) GC/MS chromatogram of a standard mixture of the 16 EPA-PAHs at the 10 ng mL\(^{-1}\) concentration level in n-octane. The peaks are labeled as follows: (1) naphthalene; (2) acenaphthylene; (3) acenaphthene; (4) fluorene; (5) phenanthrene; (6) anthracene; (7) fluoranthene; (8) pyrene; (9) benz[a]anthracene; (10) chrysene; (11) benzo[b]fluoranthene; (12) benzo[k]fluoranthene; (13) benzo[a]pyrene; (14) indeno[1,2,3-cd]pyrene; (15) dibenz[a,h]anthracene and (16) benzo[ghi]perylene. (B) Full scan mass spectra of benzo[a]pyrene obtained from a reference sample and unknown sample.
<table>
<thead>
<tr>
<th>PAH</th>
<th>Recovery (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HPLC</td>
<td>GC/MS</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>(65.5 ± 1.1)(^{39}), 63(^{39}), 10(^{37}), 62(^{38}), 64(^{41}), (86.0 ± 4.3)(^{39})</td>
<td>61(^{43}), 89.5(^{43}), 108(^{45}), 71(^{45}), 89.4(^{46})</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>81(^{59}), 39(^{147}), 40(^{39}), 67(^{41}), (91.4 ± 4.4)(^{49})</td>
<td>76(^{43}), 91.2(^{44}), 97(^{45}), 67(^{45}), 60.1(^{46}), (39 ± 3.3)(^{48}), (50 ± 4.8)(^{48})</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>(65.5 ± 1.8)(^{36}), 79(^{39}), 25(^{37}), 75(^{38}), 77(^{41}), (90.7 ± 4.5)(^{49})</td>
<td>66(^{43}), 94.0(^{44}), 112(^{45}), 71(^{45}), 81.1(^{46})</td>
</tr>
<tr>
<td>Fluorene</td>
<td>(58.5 ± 0.3)(^{39}), 82(^{39}), 52(^{37}), 68(^{38}), 73(^{41})</td>
<td>81(^{43}), 94.8(^{44}), 109(^{45}), 68(^{45}), 98.6(^{46}), (48 ± 6.1)(^{48}), (57 ± 5.2)(^{48})</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>(57.0 ± 0.5)(^{36}), 95(^{39}), 69(^{37}), 93(^{38}), 56(^{41}), (90.7 ± 4.5)(^{49})</td>
<td>70(^{43}), 95.6(^{44}), (51 ± 7)(^{42}), 23(^{45}), 57(^{45}), 64.0(^{46}), (70 ± 6.4)(^{48}), (71 ± 7.3)(^{48})</td>
</tr>
<tr>
<td>Anthracene</td>
<td>(67.6 ± 1.6)(^{36}), 97(^{39}), 77(^{37}), 102(^{38}), 71(^{41}), (66.0 ± 3.2)(^{39})</td>
<td>79(^{43}), 95.7(^{44}), 85(^{45}), 53(^{45}), 81.5(^{46}), (63 ± 4.9)(^{48}), (70 ± 4.3)(^{48})</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>(65.6 ± 1.2)(^{36}), 101(^{39}), 93(^{37}), (85.0 ± 12)(^{40}), 64(^{38}), 75(^{41}), (91.1 ± 4.4)(^{49})</td>
<td>103(^{43}), 85.8(^{44}), 73(^{45}), 46(^{45}), 83.1(^{46})</td>
</tr>
<tr>
<td>Pyrene</td>
<td>(65.7 ± 1.0)(^{36}), 94(^{39}), 88(^{37}), 101(^{38}), 90(^{41}), (90.0 ± 4.4)(^{39})</td>
<td>95(^{43}), 83.8(^{44}), (73 ± 11)(^{42}), 72(^{45}), 45(^{45}), 79.1(^{46}), (73 ± 8.2)(^{48}), (75 ± 6.7)(^{48})</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>(67.7 ± 1.7)(^{36}), 91(^{39}), 98(^{57}), 107(^{58}), 90(^{41})</td>
<td>83(^{43}), 74.9(^{44}), 51(^{45}), 46(^{45}), (64 ± 4.0)(^{48}), (77 ± 4.3)(^{48})</td>
</tr>
<tr>
<td>Chrysene</td>
<td>96(^{39}), 97(^{37}), 105(^{38}), 102(^{41}), (80.5 ± 4.5)(^{39})</td>
<td>109(^{43}), 78.4(^{44}), (70 ± 9)(^{42}), 63(^{45}), 54(^{45}), (60 ± 6.1)(^{48}), (75 ± 3.5)(^{48})</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>(67.7 ± 1.7)(^{36}), 92(^{39}), 98(^{37}), (92 ± 15)(^{40}), 100(^{38}), 69(^{41})</td>
<td>115(^{43}), 62.4(^{44}), (72 ± 11)(^{42}), 53(^{45}), 50(^{45}), 87.2(^{46}), (58 ± 6.4)(^{48}), (66 ± 3.6)(^{48})</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>(68.3 ± 0.3)(^{36}), 89(^{39}), 96(^{37}), (86 ± 13)(^{40}), 99(^{38}), 69(^{41})</td>
<td>85(^{43}), 72.5(^{44}), 27(^{45}), 55(^{45})</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>95(^{39}), 97(^{37}), (86 ± 13)(^{40}), 85(^{38}), 40(^{41})</td>
<td>80(^{43}), 66.8(^{44}), (74 ± 11)(^{42}), 57(^{45}), 47(^{45}), 62.3(^{46}), (59 ± 3.7)(^{48}), (53 ± 2.2)(^{48})</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>91(^{39}), 95(^{37}), 75(^{38}), 36(^{41}), (75.6 ± 4.2)(^{49})</td>
<td>85(^{43}), 67.9(^{44}), 61(^{45}), 61(^{45}), (50 ± 9.1)(^{48}), (48 ± 2.0)(^{48})</td>
</tr>
<tr>
<td>Benzo[ghi]perylen</td>
<td>88(^{39}), 97(^{37}), (83 ± 11)(^{40}), 71(^{38}), 38(^{41})</td>
<td>82(^{43}), 78.1(^{44}), 53(^{45}), 53(^{45}), 76.2(^{46}), (54 ± 8.2)(^{48}), (49 ± 2.2)(^{48})</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>87(^{59}), 95(^{47}), (75 ± 15)(^{40}), 91(^{48}), 44(^{41})</td>
<td>116(^{45}), 71.1(^{44}), (61 ± 9)(^{42}), 57(^{45}), 56(^{45}), (63 ± 2.3)(^{48}), (46 ± 1.3)(^{48})</td>
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</table>
It is within this context that the development of new extraction approaches and instrumental methods become relevant for the routine monitoring of EPA-PAHs in water samples. Equally relevant is the development of analytical methodology for a sustainable environment. From the prospective of green analytical chemistry, HPLC presents a distinct disadvantage over GC/MS, which is the consumption of organic solvents for PAHs separation. Under HPLC methodology, the analysis of the sixteen priority pollutants consumes 75 mL of mobile phase (methanol-water) per sample. Considering the gradient elution of the chromatographic separation, this mobile phase volume is equivalent to 66 mL of methanol. Adding the volumes of eluting solvents from the SPE procedure – i.e., 10 mL of methylene chloride - the total volume of organic solvents is approximately 76 mL per sample.

1.5 High Molecular Weight – PAHs

In order to prevent environmental risks and human contamination, the routine monitoring of the sixteen EPA-PAHs is not sufficient anymore. Recent toxicological studies attribute a significant portion of the biological activity of PAH contaminated samples to the presence of high molecular weight (HMW)-PAHs, i.e. PAHs with MW ≥ 300. The first group of HMW-PAHs, i.e. the MW 300 and MW 302 isomers, has been identified in combustion related samples with high PAH content such as coal tar, carbon black, and fuel combustion exhaust. Their presence has also been confirmed in environmental samples such as air particulate matter, mussels, sediments and soils. Because the carcinogenic properties of HMW-PAHs differ significantly from isomer to isomer, it is of paramount importance to determine the most toxic isomers even if they are present at much lower
concentrations than their less toxic isomers. A crucial example is dibenzo[a,l]pyrene (DB[a,l]P). This compound is the most potent carcinogenic PAH known to date.\textsuperscript{110-112} Its carcinogenic potency is approximately 100 times higher than that of Benzo[a]pyrene. There are several more isomers of dibenzo[pyrene – including dibenzo[a,e]pyrene (DB[a,e]P), dibenzo[a,h]pyrene (DB[a,h]P), dibenzo[a,i]pyrene (DB[a,i]P) and naphtho[2,3-a]pyrene (N[2,3-a]P) – which are also carcinogenic, but not to the extent DB[a,l]P is.\textsuperscript{113} Their molecular structures are shown in Figure 1.4. Thus, unambiguous identification of DB[a,l]P in environmental samples is highly relevant even if the other isomers are present at 10 to 100 times higher concentrations. Although DB[a,l]P is the most potent carcinogenic PAH known to man, there are only limited data on its formation and presence in the environment. As a matter of fact, only recently the U.S. Department of Health and Human Services listed DB[a,l]P, DB[a,e]P, DB[a,h]P and DB[a,i]P as potential carcinogens to humans.\textsuperscript{114} Unfortunately, established methodology cannot always meet the challenge of specifically analyzing HMW-PAH at the low concentration levels of environmental samples.\textsuperscript{115-117} The main problems that confront classic methodology arise from the relatively low concentration levels and the large number of structural isomers with very similar elution times and similar, possibly even virtually identical, fragmentation patterns.

This dissertation addresses methodology with the ability to efficiently and reliably detect the presence and determine the amounts of the most toxic HMW-PAHs, even if their less toxic isomers are present in far higher concentrations. We present fast, selective and sensitive methodology easily interfaced with classic standard methodology to assist compound identification and peak purity analysis in HPLC mobile phase fractions. We demonstrate the ability to
perform the direct analysis (no chromatographic separation) of PAHs in water samples without
the need of laborious and time-consuming clean-up and separation procedures.

Figure 1.4 Molecular structure of 5 HMW-PAHs.

1.6 Line Narrowing Spectroscopy

The main limitation of room-temperature luminescence (fluorescence and/or phosphorescence) spectroscopy towards the selectivity of analysis is the broad nature of excitation and emission spectra. The diffuse character of such spectra limits the information content and, therefore, drastically reduces the possibility to directly determine targeted compounds in complex matrixes without previous chromatographic separation. Several strategies exist to improve the specificity of luminescence techniques, including reducing the sample temperature to enhance vibronic spectral resolution,\textsuperscript{118, 119} collection of multidimensional data formats,\textsuperscript{120, 121} and time-resolved spectroscopy (lifetime measurements)\textsuperscript{122-124}. 
Reducing the sample temperature offers several advantages. Luminescence quantum yields often increase, and the complications of oxygen quenching and energy transfer are eliminated. But the temperature effects on excitation and luminescence spectra are specially pronounced in the so-called high-resolution techniques, namely, Shpol’skii spectroscopy and fluorescence line narrowing spectroscopy. In these techniques, the sharp spectra with vibrational information result from homogeneous and inhomogeneous band-broadening reduction. Homogeneous broadening affects all solute molecules to the same extent. It arises from vibronic coupling to the rapidly fluctuating surrounding matrix and from the limited lifetimes of the states involved in the electronic transition. In an ideal situation, i.e. when all solute molecules experience exactly the same environment, the only remaining source of band broadening (apart from instrumental contributions) is homogeneous. Inhomogeneous broadening results from a distribution of solute molecules with different vibronic transition energies. For amorphous matrixes in the condensed phase, characterized by their lack of long-range order, the local conditions (microenvironments) that influence the transition energies of the solute molecules (predominantly through electron-electron interactions) differ from one solute molecule to another. The resulting differences in electronic transition energies lead to a Gaussian broadening of excitation and emission spectra.

Shpol'skii spectroscopy has long been recognized for its capability in providing direct determination of individual PAHs in environmental samples without the need of previous chromatographic separation. Its capability results from the resolution observed from excitation and fluorescence spectra in Shpol’skii matrices. The term Shpol'skii matrix refers to a dilute solution of a guest molecule (PAH) in a solvent host (usually an n-alkane) where the solvent
freezes to 77 K or below into an ordered polycrystalline matrix. If the dimensions of the PAH and solvent match up well enough, PAH molecules occupy a small number of crystallographic sites in the host matrix. Matrix isolation of guest molecules reduces inhomogeneous band broadening. The combination of reduced thermal and inhomogeneous broadening produces vibrationally resolved spectra with sharp line widths with tremendous potential for qualitative and quantitative determination. Higher spectral resolution can be obtained by site-selective excitation, which refers to the excitation of guest molecules occupying one type of crystallographic site. PAHs occupying the same crystallographic site produce identical excitation and emission spectra. PAHs occupying different sites produce identical spectral profiles, which are slightly shifted by small wavelength increases (typically less than 1000 cm\(^{-1}\)). Multi-site excitation produces spectra with contributions from PAH molecules in all crystallographic sites. The simplest spectra, and therefore the narrowest full-width at half maxima (FWHM), are obtained by site-selective excitation, which is best accomplished with narrow-band laser sources.

1.7 Laser Excited Time-Resolved Shpol’skii Spectroscopy

Prior to our involvement with Shpol’skii spectroscopy, most of the applications towards the analysis of PAHs were based on the interpretation of fluorescence spectra.\(^{127, 128}\) Although time-resolved spectroscopy had shown to reduce spectral overlapping,\(^{126}\) information on fluorescence lifetimes as qualitative parameters for PAHs identification was practically in existence. The same was true for phosphorescence spectra and lifetimes. One contribution we have made to this area is the introduction of methodology to efficiently collect multidimensional data formats during the lifetime decays of fluorescence (nanoseconds to microseconds) and
phosphorescence (milliseconds to seconds) emission. \cite{129-131} Our approach – which we have coined laser excited time-resolved Shpol’skii spectroscopy (LETRSS) - takes advantage of the full dimensionality of luminescence spectroscopy combining spectral and lifetime information in multidimensional formats known as wavelength time matrices (WTM) and time-resolved excitation-emission matrices (TREEM). Adding the temporal dimension to the highly resolved Shpol’skii spectra provides an extremely selective tool for the determination of structural isomers in complex mixtures with numerous PAHs. A single instrument was developed to collect multidimensional data formats in both the fluorescence (microseconds to nanoseconds) and phosphorescence (milliseconds to seconds) time domains. Unambiguous isomer identification is made possible on the bases of spectral and lifetime analysis. In addition to providing a qualitative parameter for PAHs identification, fluorescence decays report on spectral peak purity, an essential condition for the accurate quantitative determination of PAHs without previous chromatographic separation. TREEM give the analyst the opportunity to select the best time window for minimum (or even none) spectral overlapping in highly complex matrixes. Another significant improvement we made was the introduction of cryogenic fiber optic probes for sample freezing at 4.2 K. It is now possible to easily perform reproducible measurements at liquid nitrogen and liquid helium temperatures in a matter of seconds.

1.8 Cryogenic Fiber Optic Probes

The classic sample preparation procedure for 77 K measurements consists of immersing a solution-filled small-diameter tube into an optical Dewar filled with liquid nitrogen. The fragility of the Dewar flask, bubbling in the cryogen at irregular intervals from small ice particles that act
as nucleation sites, and condensation on the outside of the Dewar are nuisances. But scattering at each of the interfaces encountered by the excitation light on its way to the sample is a much more severe problem, as stray light in the emission monochromator degrades the reproducibility of measurements and the limits of detection. The classic approach typically involves three air/glass interfaces, two liquid nitrogen/glass interfaces, and one matrix/glass interface. The number of scattering interfaces can be reduced with closed-cycle refrigerators or Joule-Thomson miniature refrigerators that employ contact cooling or cold vapor directed onto the sample cell. Trade-offs includes higher cost, reduced sample throughput, and possibly less efficient or slower cooling of the sample. Depending on the cooling device and final temperature, and sample size and solvent, freezing times can take between 40 and 100 min per sample. The constancy of the freezing rate is a concern, because it can affect the population distribution in the various sites and the corresponding signal intensity for a given set of excitation and emission wavelengths. Several attempts have been made to improve closed-cycle helium refrigerators. Ariese and co-workers developed a user friendly closed-cycle refrigerator that enables the simultaneous cooling of four 10 μL samples placed in a gold-plated copper holder surrounded with sapphire windows. The high thermal conductivity of the sample holder material provides fast sample cooling for good quality Shpol’skii spectra.

We use fiber optic probes for cryogenic measurements at both 77 K and 4.2 K. Our probes are similar to the one reported by Hieftje and co-workers for phosphorescence measurements at 77 K. Hieftje’s probe consisted of one excitation and six emission fibers bundled in a quartz tube with the opposite end connected to a plastic sample vial. Sample excitation and emission collection was made thru a quartz window placed between the quartz
tube and the sample vial. Our probes retain the simplicity of “dunking” the sample into the liquid cryogen for fast and reproducible freezing but eliminate all interfaces that could scatter excitation light into the detection system. Figure 1.5 shows an example of a probe for cryogenic measurements at 4.2 K. At the sample end, the fibers are epoxied in a six-around-one configuration with the delivery fiber in the center. At the collection end, the excitation and collection fibers are separated and vertically aligned with the spectrograph entrance slit. After the sample is introduced into the sample tube, the tip of the probe is positioned above the solution surface as the sample tube is lowered into a container filled with liquid cryogen. The cell is allowed to cool for 90 s prior to luminescence measurements to ensure complete sample freezing. Because there is no physical contact between the fiber and the sample, fiber clean up between samples is not necessary. Preparing frozen samples for luminescence measurements at 77 K and 4.2 K is now a routine technique. Samples are frozen in a matter of seconds.

Figure 1.5 Fiber optic probe (FOP) for 4.2 K laser-induced fluorescence spectrometry.
1.9 Instrumentation for Multidimensional Luminescence Spectroscopy

Pulsed excitation sources offer prospects for time-resolving the emission to improve signal-to-background ratios and reduce spectral interference. Early reports on laser excited Shpol'skii spectrometry implemented time resolution at fixed delay time intervals by using laboratory-constructed gated integrators or commercial boxcar averagers. More recently, advantage has been taken of commercially available delay generators and intensified linear photodiode arrays. Multi-channel detectors can acquire emission spectra at good signal-to-noise much faster than is possible with a scanning monochromator. Collecting the entire emission spectrum at once avoids problems associated with pulse-to-pulse fluctuation, laser intensity drift, and photodecomposition that could degrade analytical figures of merit.

Our group has shown that the combination of a pulsed tunable dye laser, a pulsed delay generator, a spectrograph, and an intensifier-charged coupled device (ICCD) is well suited for the rapid collection of WTM$s$, excitation-emission matrices (EEM$s$) and TREEM$s$ in both the fluorescence and the phosphorescence time domains. Figure 1.6 shows a schematic diagram of the instrumental set-up mounted in our lab. As an excitation source, we have used a compact frequency doubled pulsed tunable dye laser whose bandwidth (< 0.03 nm) is well matched for selective excitation of narrow Shpol'skii excitation spectra. The ICCD is coupled to a spectrograph to rapidly collect a series of emission spectra at different delay times between the laser firing and the opening of the gate on the ICCD. The integration of the mechanical shutter in the path of the dye laser excitation beam facilitates the collection of multidimensional data in the phosphorescence time domain. The shutter has a rise time (open time) of 1.5 ms and a fall time (close time) of 3.0 ms with a maximum pulse width of 6.5 ms. The shutter is controlled either
manually (always open or always closed) via a switch on the controller front panel or programmatically through a transistor-transient logic (TTL) pulse. This TTL pulse is programmed through the A + B outputs of the digital delay generator via a general purpose interface bus (GPIB) interface.

Fluorescence measurements are made with the mechanical shutter in the open position. Time resolution is achieved with the intensifier in front of the ICCD, which acts as a superfast shutter with a minimum gate of 2 ns (full width at half-maximum). Once triggered by the laser, the pulse delay generator uses this information to determine when the image intensifier in the detector head is gated on (gate delay, $D$) and for how long it is gated on (gate width, $G$). These parameters are entered on the control computer with commercial software. The ICCD acquires

Figure 1.6 Instrumental system for 4.2 K laser-induced fluorescence spectrometry with single channel and multi-channel fluorescence detection.
data while the intensifier is gated on. While the intensifier is gated off, the acquired data are transferred from the detector head to the controller card (32-bit Intelligent Bus-Mastering PCI card) in the computer. Typically, fluorescence spectra from PAHs result from the accumulation of emission of 100 laser pulses. This process takes approximately 10 ms/40 nm spectrum. If a wavelength range larger than 40 nm is of interest, the spectrograph is tuned to the new wavelength range and the process is repeated.

Depending on the strength and the duration of the phosphorescence emission, spectra are collected by one of two procedures. For PAHs that generate enough phosphorescence photons per laser pulse and have relatively short lifetimes (less than 40 ms), the use of the mechanical shutter is not necessary. The sequence of events is the same as the one used for collecting fluorescence spectra, but the delay and gate times are set at a much longer time scale. Typical delay and gate times are of the order of 20 ms and 40 ms, respectively. This delay is long enough to eliminate sample and background fluorescence, and the gate time is the maximum gate allowed for each firing of the laser. Our YAG laser fires every 100 ms (10 Hz), and because approximately 50 ms are needed between shots for data transfer and storage, gate times longer than 40 ms would result in convolution of the collected data with the next laser shot. The use of a faster computer could certainly decrease the data transfer and storage time. The mechanical shutter is useful for PAHs that exhibit weak phosphorescence intensity and relatively long phosphorescence lifetimes. The pulse delay generator controls the timing for both the shutter and the intensifier on the ICCD. During the excitation cycle, the pulse generator is triggered by the laser to set the shutter to the open position for a certain number of pulses (typically 20 pulses). During the emission cycle, the shutter is closed and the phosphorescence decay is recorded. The
closing of the shutter sets the “zero reference time” for the delay time and the gate time on the ICCD. This method allows one to build up the triplet-state population to an acceptable signal-to-noise ratio. It also allows for gate times in excess of 40 ms to be used without concern of overlapping laser pulses.

Previous reports on WTM collection utilized a rather tedious and time-consuming procedure, in which the wavelength of either the laser source or the emission monochromator was incremented while the luminescence intensity-luminescence decay waveform was collected at each wavelength. By setting the ICCD gate step parameter, our system automatically increments the time interval between successive fluorescence scans so that WTM is easily built up. The duration of the steps by which the gate delay is progressively increased in the course of the sequence of acquisitions is entered on the control computer with commercial software. For collecting phosphorescence WTMs from PAHs with strong phosphorescence and relatively short phosphorescence lifetimes (less than 40 ms), the procedure is the same with the delay and the gate set at much longer time scales (milliseconds). For collecting WTMs from PAHs with weak phosphorescence and relatively long phosphorescence lifetimes (longer than 40 ms), the series of phosphorescence spectra is taken at incremental delay times from the closing of the shutter. For relatively long phosphorescence lifetimes, this method results in very long acquisition times, typically 1 h or more per WTM. The alternative method involves exciting the PAH for several seconds to build up the triplet-state population, closing the shutter, and then collecting the time-resolved emission over several seconds. The resulting WTM consists of many spectra with a delay step equal to the firing rate of the laser (100 ms). Since each spectrum is an accumulation of only one laser shot, many WTMs (typically 100) need to be accumulated to average out the
effects of noise. This averaging is, in effect, similar to accumulating 100 laser shots at each delay increment, as in fluorescence WTM collection. Because the delay is eliminated from each acquisition cycle, the time it takes to collect a full WTM is considerably reduced. Collecting 100 WTMs over a total period of 5 s each takes approximately 9 min.

Fluorescence and phosphorescence lifetimes are recorded via a three-step procedure: (1) full sample and background WTM collection; (2) Background decay curve subtraction from the fluorescence or phosphorescence decay curve at a wavelength of maximum emission for each PAH; and (3) fitting of the background corrected data to single-exponential decays. Commercial software is used for curve fitting of fluorescence and phosphorescence lifetimes. Fitted decay curves are obtained from equation 1.1 by fixing $x_0$ and $y_0$ at a value of zero.

$$y = y_0 + A_1 e^{-(x-x_0)/\tau_1}$$

(1.1)

### 1.10 Multidimensional Data Formats from Single-Site and Multi-site PAH/n-alkane Systems

The experimental value (0.09 nm) of the full width at half maximum for a mercury (Hg) line (313 nm) emitted from a Hg lamp placed at the analysis end of the fiber optic probe is within the theoretical value calculated from the spectral range of an individual pixel in the CCD array. Based on the reciprocal linear dispersion ($R_L = 0.8$ nm/mm) of the spectrograph, the number of active pixels (690 x 256) in the array, and the dimensions (18 x 6.7 mm) of the active area of the CCD chip, the spectral range of one pixel should give a 0.02 nm limiting resolution. Because of the detector crosstalk and the intensifier, the factual limiting resolution corresponds to 4-5 pixels
(5), i.e. 0.08 - 0.10 nm. This resolution is sufficient to collect accurate spectra from both single-site and multiple-site PAH/n-alkane systems. Figure 1.7 shows an example of a single-site (naphthalene in n-pentane) and a multiple-site system (benzo[a]pyrene in n-heptane). Upon fast cooling in n-heptane, benzo[a]pyrene occupies four crystallographic sites that yield four distinct fluorescence spectra slightly shifted by small wavelength differences. The maximum wavelengths of the quartet emissions from the 0-0 transitions appear at 402.3, 402.7, 403.1 and 403.8 nm. The maximum wavelengths and the relative intensities of the fluorescence peaks correlate well to those previously reported with conventional methodology.\textsuperscript{126-128}

Figure 1.7 4.2 K fluorescence spectrum of 10 µg mL\textsuperscript{-1} naphthalene in n-pentane (A) and 0.2 µg mL\textsuperscript{-1} benzo[a]pyrene in n-heptane (B) recorded with the fiber-optic probe, spectrograph and ICCD camera. The naphthalene spectrum was recorded with an excitation wavelength of 306.0 nm, spectrograph entrance slit of 42 µm, delay of 10 ns and a gate of 1000 ns. The benzo[a]pyrene spectrum was recorded with an excitation wavelength of 366.0 nm, spectrograph entrance slit of 25 µm, delay of 20 ns and a gate of 200 ns. Both spectra correspond to the accumulation of 100 laser shots.
Figure 1.8 shows two WTM plots recorded with the fiber optic probe at 77 K along with their respective $I_f$ vs. $t_d$ plots. The gate delay ($t_d$) was advanced in 20 ns increments for pyrene and 5 ns increments for dibenz[a,h]anthracene. The fluorescence spectrum at each gate delay $t_d$ was averaged over 100 laser pulses. Table 1.3 summarizes the lifetimes of the 6 PAHs obtained by this procedure. In all cases, single exponential decays were observed. The agreement between the calculated and observed points over the first two lifetimes of the decay is within about 1% and the residuals show no systematic trends. Background emissions from n-alkanes have relatively short lifetimes (~ 5 ns). With long-lived compounds, such as benzo[g,h,i]perylene (183 ns) and pyrene (527 ns), the collection of strong PAH fluorescence can start at relatively long delay times ($t_d > 30$ ns), in which case the main contribution to the background is instrumental noise.

For PAHs with shorter fluorescence lifetimes, such as dibenz[a,h]anthracene (41 ns), complete time-resolution of fluorescence background is not possible, but the $t_d$ and gate time ($t_g$) values can be selected to collect most of the fluorescence emitted by the PAH.

Table 1.3 Fluorescence lifetimes ($\tau$) of PAHs at 77 K in n-hexane.

<table>
<thead>
<tr>
<th>PAH</th>
<th>$\lambda_{\text{exc}}/\lambda_{\text{em}}$ (nm)</th>
<th>$\tau$ (ns)</th>
<th>RSD ($%$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>282/337</td>
<td>193 ± 1.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>287/365</td>
<td>57 ± 2.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Pyrene</td>
<td>280/373</td>
<td>527 ± 4.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Chrysene</td>
<td>289/362</td>
<td>63 ± 2.0</td>
<td>3.2</td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>290/394</td>
<td>41 ± 1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>290/421</td>
<td>183 ± 6.0</td>
<td>3.3</td>
</tr>
</tbody>
</table>

$^a$ The following PAH concentrations were used: 0.8 µg mL$^{-1}$ (pyrene), 0.4 µg mL$^{-1}$ (phenanthrene), 1 µg mL$^{-1}$ (dibenz[a,h]anthracene), 0.2 µg mL$^{-1}$ (chrysene), 0.1 µg mL$^{-1}$ (benzo[g,h,i]perylene) and 0.2 µg mL$^{-1}$ (naphthalene). $^b$ Excitation ($\lambda_{\text{exc}}$) and emission ($\lambda_{\text{em}}$) wavelengths. $^c$ No sample degassing was attempted. The following gate times were used 2000 ns (pyrene, benzo[g,h,i]perylene and naphthalene) and 200 ns (phenanthrene, dibenz[a,h]anthracene and chrysene). $^d$ RSD = relative standard deviations based on six replicate measurements. Replicate measurements were taken from six different frozen aliquots of PAH solution.
Figure 1.8 77 K WTMs recorded from 1 µg mL\(^{-1}\) standards of (A) pyrene and (B) dibenz\([a,h]\)anthracene in n-hexane. The following acquisition parameters were used for spectra collection: (A) \(\lambda_{\text{exc}} = 280\) nm, \(t_d = 10\) ns, \(t_g = 200\) ns and gate step = 20 ns; (B) \(\lambda_{\text{exc}} = 290\) nm, \(t_d = 10\) ns, \(t_g = 200\) ns and gate step = 5 ns. Each fluorescence spectrum in the WTMs corresponds to the accumulation of 100 laser pulses. Entrance slit of spectrograph was 100 µm. Fluorescence decays of pyrene (C) and dibenz\([a,h]\)anthracene (D) built with fluorescence intensities stripped from the WTMs at the maximum wavelengths of emission.
Interesting to note are the significant lifetimes differences we have observed from PAH molecules occupying different crystallographic sites in the same matrix. Some examples are summarized in Table 1.4. No other reports exist on this phenomenon but our observations are consistent with the well-known sensitivity of fluorescence lifetimes to the microenvironment of the fluorophore. It is generally accepted that the multiplet structure of fluorescence spectra arise from the different orientations of the PAH molecule in the solid matrix. The different orientations lead to nonequivalent crystal field effects and hence wavelength shifts. Most likely, the field effects are also sensed by the PAH fluorescence lifetime. The lifetime differences from site to site are usually larger than their wavelength shifts. This fact helped us to identify the different orientations of the PAH molecule in the solid matrix. The different orientations lead to nonequivalent crystal field effects and hence wavelength shifts. Most likely, the field effects are also sensed by the PAH fluorescence lifetime. The lifetime differences from site to site are usually larger than their wavelength shifts. This fact helped us to identify the two crystallographic sites of benzo[a]pyrene in n-octane, which had been previously reported as a single site system. Although we have not unfolded the analytical potential of site selective lifetime analysis yet, the rather large lifetime differences anticipate a promising contribution to enhancing the specificity of site-selective excitation. As we will later show in this review, the specificity of lifetime measurements plays an important role in the analysis of closely related structural isomers.
Table 1.4 4.2 K Fluorescence lifetime of PAH molecules occupying different crystallographic sites in the frozen matrix.

<table>
<thead>
<tr>
<th>PAH(^a)</th>
<th>(\lambda_{\text{em}}(^b)) (nm)</th>
<th>(\tau) (ns)</th>
<th>(\lambda_{\text{em}}(^b)) (nm)</th>
<th>(\tau) (ns)</th>
<th>(\lambda_{\text{em}}(^b)) (nm)</th>
<th>(\tau) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[(a)]pyrene</td>
<td>403.56</td>
<td>27.02 ± 0.50</td>
<td>402.31</td>
<td>52.09 ± 0.93</td>
<td>403.10</td>
<td>36.04 ± 0.61</td>
</tr>
<tr>
<td></td>
<td>403.94</td>
<td>43.44 ± 1.00</td>
<td>402.72</td>
<td>46.93 ± 0.49</td>
<td>403.20</td>
<td>45.68 ± 0.84</td>
</tr>
<tr>
<td></td>
<td>404.53</td>
<td>31.95 ± 0.89</td>
<td>403.15</td>
<td>42.77 ± 0.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>403.89</td>
<td>41.29 ± 1.18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>376.63</td>
<td>503.98 ± 6.01</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>372.45</td>
<td>577.43 ± 3.47</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Benz[(a)]anthracene</td>
<td>383.34</td>
<td>53.63 ± 0.50</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>383.94</td>
<td>56.43 ± 0.76</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

\(^a\) PAH solutions were prepared at 0.5 µg mL\(^{-1}\). \(^b\) Wavelength of maximum fluorescence emission. All data obtained using \(\lambda_{\text{exc}} = 288.50\). \(^c\) Spectra and decay data were not measured for this PAH/solvent system.
Recording two-dimensional (2-D) fluorescence spectra – i.e. plots correlating fluorescence intensities to emission wavelengths – from a mixture with numerous fluorescence components only provides partial information on the total fluorescence of the sample. The emission profile of a mixture with numerous fluorescence components varies with the relative position of the excitation wavelength to the excitation maxima of the fluorophores in the mixture. Individual fluorophore contributions to the total fluorescence spectrum of the sample also depend on the fluorescence quantum yields of the fluorophores and possible quenching due to synergistic effects. EEM gather all this information in a single data format to provide a true signature of the total fluorescence of a multi-fluorophore mixture. Most of the work on low-temperature EEM has been performed with high-resolution spectrometers that built up fluorescence EEM with numerous repetitive scans.\textsuperscript{126} The main drawbacks of this approach are the long EEM acquisition times, the need for relatively large excitation and emission bandwidths and the inability to record phosphorescence EEM. The large bandwidths, which are imposed by the low throughput of the high-resolution spectrometers, limit the spectral resolution of the EEM and the selectivity of analysis.

Our system is well suited for rapid collection of low-temperature fluorescence and phosphorescence EEM. Because of the spectrograph and the ICCD, both formats are collected in short analysis time. The tunable dye laser provides intense excitation energy in a narrow excitation bandwidth. Its scanning capability is essential to take full advantage of the specificity obtainable from the highly resolved PAH excitation spectra in Shpol’skii matrixes. Its intense excitation energy promotes strong PAH fluorescence to recording spectra with optimum spectrograph slits for maximum spectral resolution. Figure 1.9 displays the 4.2 K fluorescence
and phosphorescence EEM of chrysene in n-octane. The dye laser was automatically tuned in 0.1 nm steps between 310 and 335 nm. Collecting the entire fluorescence and phosphorescence data sets took no longer than 5 and 8 min, respectively. Both EEM show similar features shifted from each other with regards to emission wavelengths. This pattern is often an indication that multiple sites are present in the frozen matrix. Our lifetime measurements (see Table 2) confirm this multiple structure of fluorescence and phosphorescence emission.

Figure 1.9 4.2 K fluorescence (A) and phosphorescence (B) EEM recorded from a 0.2 µg mL⁻¹ chrysene solution in n-octane. The fluorescence EEM was recorded using a 20 ns delay and a 200 ns gate time. The phosphorescence EEM was collected using 10 µs delay and 40 ms gate times. The excitation wavelength step was 0.1 nm.
In comparison to EEM, chemical analysis based on TREEM is potentially more selective because it adds the temporal dimension to the orthogonal spectral dimension of EEM. TREEM are basically a series of fluorescence WTM acquired at different excitation wavelengths. The complete data set consists of fluorescence intensity as a function of excitation wavelength, emission wavelength, and delay time after the short duration of pulse excitation. All the intensity values (as a function of excitation and emission wavelengths) for a particular decay time can be assembled into an EEM format specific for that decay time. The potential of TREEM is illustrated with the direct determination of DB[a,l]P in a heavily contaminated water sample from the American Petroleum Institute (API). API water is used to desalt and scrub crude oil and oil fractions in the refinery process and shows a visible surface petroleum layer at the separator trough. Previous HPLC analysis of this water has shown heavy contamination with fluorescent pollutants, including several EPA PAHs. The complexity of this sample is illustrated in Figure 1.10 with the UV-VIS chromatogram of the sample extract and the 4.2 K EEMs collected within the wavelength ranges of DB[a,l]P. At the top EEM, the delay (10 ns) and the gate (1000 ns) were selected to collect a substantial portion of the total fluorescence of the sample. The low-intensity features throughout most of the EEM indicate extensive fluorescence background from several concomitants in the sample. The bottom EEM was collected after spiking the API water with DB[a,l]P at the 0.5 ng mL\(^{-1}\) level. Because the delay (10 ns) and the gate (250 ns) were optimized to enhance the spectral features of DB[a,l]P, the fluorescence background disappears and the presence of DB[a,l]P is clearly noted.
Figure 1.10 (A) Chromatogram of API water extract using Supelcosil LC-PAH column. (B) 4.2 K EEM of API water recorded from an extraction membrane spiked with 100 µL of n-octane. Delay and gate times were 10 and 1000 ns, respectively. (C) 4.2 K EEM of API water spiked with 5 ng mL⁻¹ DB[a,l]P prior to SPE. EEM was recorded from an extraction membrane spiked with 100 µL of n-octane. Delay and gate times were 10 and 150 ns, respectively. In both cases, spectra were accumulated over 100 laser shots at each excitation wavelength using increments of 0.2 nm. Spectrograph slits were set at 42 µm.

1.11 LLE-LETRSS and SPE-LETRSS

The use of cryogenic fiber optic probes facilitates the hyphenation of LETRSS to sample pre-concentration techniques for the analysis of aqueous samples. LLE-LETRSS and SPE-LETRSS methods have been developed in our lab for the determination of polycyclic aromatic compounds in HPLC fractions and sample extracts. Figure 1.11A shows the main steps of the experimental procedure for LLE-LETRSS. Milliliter volumes of aqueous sample are
mixed with micro-liters of Shpol’skii solvent (n-alkane) in the vessel of the fiber optic probe. After shaking the vessel for 1 – 2 min, the mixture is allowed to stand for ~ 1 min to separate the two liquid layers. The sample vial is then attached to the optical probe and the fiber assembly is lowered into the liquid cryogen for LETRSS measurements. Quantitative analysis is based on the linear relationship that exists between analyte concentration in the Shpol’skii layer (C_{SS}) and its concentration in the aqueous sample (C_{AS}):

\[
C_{AS} = \left( \frac{1}{K_d} \right) \times \left( \frac{V_{SS}}{V_{AS}} \right) \times C_{SS}
\]

where \( K_d \) is the partition coefficient of the analyte between the Shpol’skii solvent and the aqueous sample (\( K_d = C_{SS}/C_{AS} \)), and \( V_{SS} \) and \( V_{AS} \) are the volumes of Shpol’skii solvent and aqueous sample, respectively.

Two different procedures have been developed for SPE-LETSS analysis. Figure 1.11B depicts the main steps of the eluting method. A 13 mm \( C_{18} \) membrane is placed into a 100 mL extraction syringe. Positive pressure is used to force all liquid solutions through the disk. Prior to sample extraction, the \( C_{18} \) membrane is conditioned with 1mL of methanol. Following water extraction, void water is mechanically removed by forcing three 100 mL volumes of air through the disk. PAHs are eluted from the extraction membrane using an appropriate volume of Shpol’skii solvent. The quantitative correlation between the PAH concentration in the aqueous sample and the layer of Shpol’skii solvent is given by the following equation:

\[
C_{AS} = \left( \frac{f_2}{f_1} \right) \times \left( \frac{V_{SS}}{V_{AS}} \right) \times C_{SS}
\]

(1.3)
where \( f_1 \) and \( f_2 \) are the fractions of extracted and eluted PAH, respectively. For a 10 mL sample volume \( (V_{AS} = 10 \text{ mL}) \) and 5 mL of eluting solvent \( (V_{SS} = 5 \text{ mL}) \), the best sample pre-concentration factor \( ([f_2/f_1] \times [V_{SS}/V_{AS}]) \) that could be obtained would be 2, i.e. when \( f_1 \) and \( f_2 \) are both equal to 1. Table 1.5 summarizes \( f_1 \) and \( f_2 \) values of several EPA-PAHs. The amounts of extracted PAHs are statistically equivalent to 100% \( (P = 0.05; N = 6) \), i.e. \( f_1 = 1 \). The same is not true for the \( f_2 \) values of some PAHs. Two factors are known to deteriorate PAHs recovery from extraction membranes: (a) non-reversed-phase interactions (hydrogen bonding and dipole-dipole interactions) between the PAH and the unreacted (residual) silanol and siloxane groups on the surface of octadecyl silica phases; and (b) the presence of trapped and sorbed water in the silica particles. The strength of hydrogen bonding and dipole-dipole interactions varies with the physical chemical properties of the PAH, which might explain some of the variations in recoveries. The air-drying procedure prior to PAH elution removes the majority of the water in the void volume. The water in the smallest pores (trapped water) and water sorbed to the silica surface remains on the membrane. This water possibly blocks the interaction between n-hexane and PAH, deteriorating the extraction efficiency of the non-polar solvent.

Better pre-concentration factors are obtained with the spiking method. The main steps of the spiking procedure are shown in Figure 1.11C. The 5.5 mm diameter allows for the membrane to fit into the vessel of the fiber optic probe (~6mm inner diameter). Quantitative analysis obeys the following equation:

\[
C_{AS} = \left( \frac{V_{SS}}{f_1V_{AS}} + \frac{KV_M}{f_1V_{AS}} \right) \times C_{SS}
\] (1.4)
where $K$ is the distribution ratio between the analyte concentration on the membrane ($C_M$) membrane and in the Shpol’skii solvent – i.e., $K = C_M/C_{SS}$ - and $V_M$ is the volume of the extraction membrane, i.e. 23.8 µL. $V_M$ is calculated using the standard formula for the volume of a cylinder $V = \pi r^2 h$, where $r$ is the radius of the membrane (2.75 mm) and $h$ is its thickness (~1 mm). For phenanthrene ($K = 3.3 \pm 0.41$), chrysene ($10.80 \pm 2.50$) and fluorene ($K = 8.0 \pm 1.4$), 10 mL of water sample ($V_{AS} = 10$ mL; $f_1 = 1$) and 100 µL of n-hexane ($V_{SS} = 100$ mL), equation 1.4 predicts pre-concentration factors ranging from ~ 34 (fluorene) to ~ 79 (chrysene).

### Table 1.5 Solid-Phase extraction of EPA-PAHs.

<table>
<thead>
<tr>
<th>PAH</th>
<th>$f_1^a$</th>
<th>$f_2^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>0.994 ± 0.003</td>
<td>0.578 ± 0.035</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>0.999 ± 0.016</td>
<td>0.551 ± 0.016</td>
</tr>
<tr>
<td>Fluorene</td>
<td>0.988 ± 0.023</td>
<td>0.534 ± 0.039</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>0.997 ± 0.030</td>
<td>0.571 ± 0.064</td>
</tr>
<tr>
<td>Anthracene</td>
<td>0.999 ± 0.005</td>
<td>0.103 ± 0.033</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>0.999 ± 0.020</td>
<td>0.937 ± 0.061</td>
</tr>
<tr>
<td>Pyrene</td>
<td>0.999 ± 0.019</td>
<td>0.992 ± 0.022</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>0.998 ± 0.021</td>
<td>0.969 ± 0.014</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.997 ± 0.018</td>
<td>0.105 ± 0.065</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>0.994 ± 0.034</td>
<td>0.883 ± 0.056</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>0.954 ± 0.063</td>
<td>0.977 ± 0.035</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>0.949 ± 0.083</td>
<td>0.574 ± 0.076</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>0.723 ± 0.040</td>
<td>0.104 ± 0.048</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>0.959 ± 0.082</td>
<td>0.588 ± 0.026</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>0.929 ± 0.038</td>
<td>0.889 ± 0.018</td>
</tr>
</tbody>
</table>

\(^a\) Based upon fluorescence of PAH solution before and after extraction. \(^b\) Determined by standard additions on eluent.
Figure 1.11 Schematic diagram of the experimental procedures for (A) LLE-LETRSS, (B) eluting SPE-LETRSS and (C) spiked SPE-LETRSS for the analysis of aqueous samples.
The spiking method is particularly useful for the analysis of polychlorinated biphenyls (PCBs) and polychlorinated dibenzofurans (PCDBFs). PCBs and PCDBFs show strong phosphorescence emission in Shpol’skii matrixes at both 77 K and 4.2 K. Many cases exist where the amount of eluted PCB or PCDBF behaves randomly and makes SPE unsuited for analytical use. In other cases, the low amount of eluted PCB or PCDBF provides poor analyte recoveries. In our lab, the worst-case scenario was observed with 4-chlorobiphenyl, which had a zero recovery from the extraction membrane. Table 1.6 summaries the K values for biphenyl, dibenzofuran, three PCBs and three PCDBFs obtained with 100 μL of n-heptane. The tabulated values were obtained from the slopes of the \( C_{SS} \) vs. \( C_M \) plots. The standard deviations of the slopes were calculated via the bivariate least-squares regression method, which takes into account the uncertainties in both axes of the \( C_{SS} \) vs. \( C_M \) plots. The variances close to unity show the linear relationships between \( C_{SS} \) and \( C_M \) and the constant slopes demonstrate that K remains the same within the studied concentration range. The K values demonstrate the highest affinity of 4-chlorobiphenyl for the extraction membrane, which correlates well to the zero percent recovery of the eluting procedure. It is also interesting to note that the distribution ratio of biphenyl (9.78) is lower than the K values of the three PCBs. The same is true for dibenzofuran (K = 4.08) and the three PCDBFs. The higher affinity of the chlorinated pollutants for the membrane can be attributed to non-reversed-phase interactions (hydrogen bonding and dipole–dipole interactions) with the unreacted (residual) silanol and siloxane groups on the surface of octadecyl silica phases. Although hydrogen bonding and dipole–dipole interactions might also occur with biphenyl and dibenzofuran, the presence of chlorine atoms possibly enhances their effect.
Table 1.6 Distribution ratios of organic pollutants between SPE membrane and Shpol’skii solvent.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>$K^a$</th>
<th>$S^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibenzofuran</td>
<td>4.08</td>
<td>0.45</td>
</tr>
<tr>
<td>Biphenyl</td>
<td>9.78</td>
<td>0.88</td>
</tr>
<tr>
<td>2-Chlorodibenzofuran</td>
<td>7.34</td>
<td>0.55</td>
</tr>
<tr>
<td>2,8-Dichlorodibenzofuran</td>
<td>24.75</td>
<td>3.16</td>
</tr>
<tr>
<td>2,3,7,8-Tetrachlorodibenzofuran</td>
<td>9.33</td>
<td>0.10</td>
</tr>
<tr>
<td>4-chlorobiphenyl</td>
<td>133.84</td>
<td>16.32</td>
</tr>
<tr>
<td>3,3’,4,4’-Tetrachlorobiphenyl</td>
<td>13.42</td>
<td>2.21</td>
</tr>
<tr>
<td>2,3’,4,4’,5-Pentaclorobiphenyl</td>
<td>24.58</td>
<td>3.11</td>
</tr>
</tbody>
</table>

$^a$ $K$, distribution ratio defined as $K = C_M/C_{SS}$. Where $C_M$ is the analyte concentration in the SPE membrane and $C_{SS}$ is the analyte concentration in the organic solvent. $^b$ $S = \text{standard deviation of the slope (K)}$. $K$ and $S$ were obtained from linear regression plots.

Despite the rather large $K$ values, the analysis of PCBs and PCDBFs with the spiking procedure is still possible. Table 1.7 summaries the analytical figures of merit (AFOM) obtained with 10 mL of water and 100 μL of n-heptane. The correlation coefficients (R) of the calibration curves are close to unity, the linear dynamic ranges (LDR) extend over two orders of magnitude and the limits of detection (LODs) are at the parts-per-billion level. The relative standard deviations (RSD) measured at medium concentrations within the LDR are lower than 10%, which is excellent for measurements at helium liquid temperature (4.2 K). It is important to note that the RSD represent the precision of the method and include the random errors propagated from water extraction to signal measurement. It is also important to note that the spiking method makes the analysis of 4-chlorobiphenyl feasible.
<table>
<thead>
<tr>
<th>Analyte</th>
<th>(\lambda_{\text{exc}}/\lambda_{\text{em}}) (nm)</th>
<th>(R^2)</th>
<th>LDR (ng mL(^{-1}))</th>
<th>LOD (ng mL(^{-1}))</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Chlorodibenzofuran</td>
<td>284/440</td>
<td>0.9984</td>
<td>0.27-150</td>
<td>0.27</td>
<td>9.1</td>
</tr>
<tr>
<td>2,8-Dichlorodibenzofuran</td>
<td>284/434</td>
<td>0.9998</td>
<td>0.85-200</td>
<td>0.85</td>
<td>8.4</td>
</tr>
<tr>
<td>2,3,7,8-Tetrachlorodibenzofuran</td>
<td>284/440</td>
<td>0.9987</td>
<td>0.76-200</td>
<td>0.76</td>
<td>7.7</td>
</tr>
<tr>
<td>4-chlorobiphenyl</td>
<td>284/482</td>
<td>0.9996</td>
<td>12.8-2000</td>
<td>7.7</td>
<td>7.2</td>
</tr>
<tr>
<td>3,3’,4,4'-Tetrachlorobiphenyl</td>
<td>284/494</td>
<td>0.9996</td>
<td>5.3-1000</td>
<td>5.3</td>
<td>8.8</td>
</tr>
<tr>
<td>2,3’,4,4’,5-Pentaclorobiphenyl</td>
<td>284/491</td>
<td>0.9998</td>
<td>5.5-2000</td>
<td>5.5</td>
<td>6.4</td>
</tr>
</tbody>
</table>

\(^{a}\) Analytical figures of merit were obtained with 10 mL of aqueous standard solution. 100 \(\mu\)L of n-heptane as Shpol’skii solvent were used to spike the SPE membrane.

\(^{b}\) Excitation (\(\lambda_{\text{exc}}\)) and phosphorescence (\(\lambda_{\text{em}}\)) wavelengths.

\(^{c}\) \(R\), correlation coefficient of the calibration curve.

\(^{d}\) LDR, linear dynamic range estimated from the limit of detection to the upper linear concentration.

\(^{e}\) LOD, limit of detection calculated from equation \(\text{LOD} = 3S_B/m\), where \(S_B\) is the standard deviation of the blank based on 16 measurements and \(m\) is the slope of the calibration curve based on five concentrations within the LDR.

\(^{f}\) RSD, relative standard deviation from three sample measurements at medium concentration within LDR.
1.12 Analysis of Water Samples

The full-width at half-maximum (FWHM) of a PAH in a Shpol'skii matrix depends on the similarity between the molecular dimensions of the PAH and the organic solvent. A criteria often employed for solvent selection matches the length of the solvent molecule to the effective length of the PAH. One of five organic solvents best fits the linear dimensions of each pollutant: n-pentane (naphthalene, acenaphthene, and acenaphthylene), n-hexane (phenanthrene and pyrene), n-heptane (fluorene, fluoranthene, benzo[ghi]perylene, benzo[b]fluoranthene and anthracene), n-octane (benzo[a]pyrene, dibenz[a,h]anthracene, chrysene, benz[a]anthracene), and n-nonane (indeno[1,2,3-cd]pyrene and benzo[k]fluoranthene). Determining each PAH under optimum solvent and site selective excitation wavelengths would give the best spectral resolution. The resulting procedure, however, would be time consuming and inconvenient. LLE or PAH elution from the SPE device would have to be performed with five solvents, which would lead to five extractions per water sample. With the aid of time resolution, it is possible to resolve fifteen EPA-PAHs at 77 K under a single set of Shpol'skii conditions, i.e. using one n-alkane and one excitation wavelength. This possibility is illustrated in Figure 1.12, which shows the spectral features of the 15 EPA-PAHs recorded from a river water sample previously submitted to SPE. PAH elution and subsequent LETRSS analysis were carried out using the same solvent, i.e. n-hexane. According to their 77 K standard fluorescence lifetimes (see Table 1.8), EPA-PAHs can be grouped in three distinct groups: PAH with relatively short ($\tau \leq 11.1$ ns), medium ($39.9$ ns $\leq \tau \leq 61.7$ ns) and long ($182.8$ ns $\leq \tau \leq 523.1$ ns) lifetimes. By selecting the appropriate time window - i.e. delay and gate times – during the total fluorescence decay of the sample, it is possible to minimize spectral overlapping and determine each PAH without previous chromatographic separation. Spectral purities at target wavelengths were monitored via
fluorescence decays, which provided single exponential profiles with statistically equivalent lifetimes to those in Table 1.8.

Figure 1.12 77K fluorescence spectra of a river water sample of unknown spiked with the fifteen EPA-PAHs at the ppb level. Shpol’skii spectra were recorded using optimum delay and gate times for each PAH group.
Table 1.8 77 K Fluorescence lifetimes of EPA-PAHs in n-hexane.

<table>
<thead>
<tr>
<th>PAH(^a)</th>
<th>(\lambda_{\text{exc}}/\lambda_{\text{em}})(^b) (nm)</th>
<th>Lifetime(^c) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>282/323</td>
<td>194.4 ± 1.4</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>290/323</td>
<td>45.5 ± 0.9</td>
</tr>
<tr>
<td>Fluorene</td>
<td>282/304</td>
<td>6.2 ± 0.3</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>287/365</td>
<td>57.0 ± 1.0</td>
</tr>
<tr>
<td>Anthracene</td>
<td>362/379</td>
<td>9.6 ± 0.3</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>288/438</td>
<td>52.4 ± 0.3</td>
</tr>
<tr>
<td>Pyrene</td>
<td>280/384</td>
<td>523.1 ± 5.8</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>290/386</td>
<td>52.7 ± 0.7</td>
</tr>
<tr>
<td>Chrysene</td>
<td>286/381</td>
<td>61.7 ± 0.3</td>
</tr>
<tr>
<td>Benzo[h]fluoranthene</td>
<td>290/423</td>
<td>45.8 ± 0.7</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>290/404</td>
<td>8.4 ± 0.2</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>290/404</td>
<td>39.9 ± 0.7</td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>290/394</td>
<td>41.2 ± 0.9</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>290/421</td>
<td>182.8 ± 5.8</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>289/467</td>
<td>11.1 ± 0.3</td>
</tr>
</tbody>
</table>

\(a\) All PAH concentration were in the ng/mL range. \(b\) Excitation (\(\lambda_{\text{exc}}\)) and emission (\(\lambda_{\text{em}}\)) wavelengths. \(c\) Lifetimes and standard deviations based upon six replicate measurements from different frozen aliquots of analyte solution.

Although the Shpol’skii effect is significant at liquid nitrogen temperature, spectra of PAHs only become extremely sharp at temperatures below 20 K. Optimization of spectral narrowing is particularly important for the isomeric analysis of HMW-PAH without previous separation. This fact is shown in Figure 1.13 with the 4.2 K Shpol’skii spectra of DB[a,l]P, DB[a,i]P, DB[a,e]P, DB[a,h]P and DB[e,l]P in n-octane. The spectral and lifetime differences (see Table 1.9) provide ample opportunity for their unambiguous determination without chromatographic separation.\(^{146}\)
Figure 1.13 4.2 K fluorescence spectra from synthetic mixtures with the five dibenzopyrene isomers. Each mixture contained the target isomer at 0.1 µg mL$^{-1}$ concentration and the other four isomers at 10 µg mL$^{-1}$ concentration. In each mixture, the target isomer was the following: (A) DB[a,l]P; (B) DB[a,e]P; (C) DB[a,h]P; (D) DB[a,i]P; (E) DB[e,l]P. Experimental parameters were the following: (A) $\lambda_{exc} = 323$, $t_d/t_g = 10/250$ ns; (B) $\lambda_{exc} = 310$ nm, $t_d/t_g = 10/150$ ns; (C) $\lambda_{exc} = 316.5$ nm, $t_d/t_g = 0/15$ ns; (D) $\lambda_{exc} = 304$, $t_d/t_g = 10/650$ ns; (E) $\lambda_{exc} = 288$ nm, $t_d/t_g = 10/150$ ns. In all cases, spectrograph slits were 40 µm. All spectra correspond to the accumulation of 100 laser pulses.
Table 1.9 Spectral and lifetime analysis of synthetic mixtures dibenzopyrene isomers.

<table>
<thead>
<tr>
<th>HMW-PAH(\text{a})</th>
<th>(\lambda_{\text{exc}})(\text{b}) (nm)</th>
<th>(\lambda_{\text{em}})(\text{c}) (nm)</th>
<th>(t_d/t_g)(\text{d}) (ns)</th>
<th>Standard Lifetimes(\text{e}) (ns)</th>
<th>Mixture Lifetimes(\text{e}) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB(\text{a,l})P</td>
<td>323</td>
<td>418.1, 443.2</td>
<td>10/250</td>
<td>78.0 ± 2.9</td>
<td>77.1 ± 1.6</td>
</tr>
<tr>
<td>DB(\text{a,e})P</td>
<td>310</td>
<td>396.0, 401.8</td>
<td>10/150</td>
<td>55.6 ± 1.4</td>
<td>53.8 ± 1.2</td>
</tr>
<tr>
<td>DB(\text{a,i})P</td>
<td>304</td>
<td>432.5, 459.6</td>
<td>10/650</td>
<td>214.2 ± 6.9</td>
<td>220.1 ± 5.0</td>
</tr>
<tr>
<td>DB(\text{a,h})P</td>
<td>316.5</td>
<td>449.2, 454.6</td>
<td>0/15</td>
<td>5.9 ± 0.2</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>DB(\text{e,l})P</td>
<td>288</td>
<td>383.7, 388.6</td>
<td>10/150</td>
<td>53.2 ± 1.2</td>
<td>50.5 ± 2.7</td>
</tr>
</tbody>
</table>

\(\text{a}\) Standard solutions were prepared in n-octane at 0.1 µg mL\(^{-1}\). \(\text{b}\) Excitation wavelength (\(\lambda_{\text{exc}}\)) corresponding to the maximum excitation wavelength of the PAH. \(\text{c}\) Emission wavelength (\(\lambda_{\text{em}}\)); maximum emission wavelength is underlined. \(\text{d}\) \(t_d\) = delay time; \(t_g\) = gate time. \(\text{e}\) Lifetimes and standard deviations are based on six replicate measurements from different frozen aliquots.
CHAPTER 2  ANALYSIS OF CO-ELUTED ISOMERS OF HIGH-MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS IN HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FRACTIONS VIA SOLID-PHASE NANOEXTRACTION AND TIME-RESOLVED SHPOL’SKII SPECTROSCOPY


2.1 Introduction

In this chapter, we present an accurate method for the determination of isomers of HMW-PAHs co-eluted in HPLC fractions. The feasibility of this approach is demonstrated with two isomers of molecular weight 302 with identical mass fragmentation patterns, namely DB[a,i]P and N[2,3-a]P. Qualitative and quantitative analysis is carried out via LETRSS at liquid helium temperature. Unambiguous identification of co-eluted isomers is based on their characteristic 4.2 K line-narrowed spectra in n-octane as well as their fluorescence lifetimes. Pre-concentration of HPLC fractions prior to spectroscopic analysis is performed with the aid of gold nanoparticles (Au NPs) via an environmentally friendly procedure. In addition to the two co-eluted isomers, the AFOM of the entire procedure were evaluated with DB[a,l]P, DB[a,h]P and DB[a,e]P. The analytical recoveries from drinking water samples varied between 98.2 ± 5.5 (DB[a,l]P) and 102.7 ± 3.2 % (DB[a,i]P). The LODs ranged from 51.1 ng L⁻¹ (N[2,3-a]P) to 154 ng L⁻¹ (DB[a,e]P). The excellent AFOM associated to its HPLC compatibility makes this approach an attractive alternative for the analysis of co-eluted isomers with identical mass spectra.
2.2 Experimental Section

2.2.1 Chemicals

Nanopure water from a Barnstead Nanopure Infinity water system was used throughout. 20nm average diameter Au NPs aqueous solutions (7x10^{11} \text{ particles mL}^{-1}) were purchased from Ted Pella, Inc. (Redding, CA). HPLC grade methanol and acetonitrile was purchased from Fisher Scientific (Pittsburg, PA). Analytical grade 1-pentanethiol and n-octane were from Across Organics (Atlanta, GA). DB[a,l]P, DB[a,e]P, DB[a,h]P and DB[a,i]P were purchased from Accustandard at their highest available purity (100%). N[2,3-a]P was acquired from Sigma-Aldrich (Milwaukee, WI, USA) at 98% purity.

*Note: use extreme caution when handling PAHs that are known to be extremely toxic.*

2.2.2 Solution Preparation

Stock solutions of HMW-PAHs were prepared in methanol, acetonitrile, and n-octane and kept in the dark at 4 °C. Possible HMW-PAH degradation was monitored via room-temperature fluorescence spectroscopy. Working solutions of HMW-PAH were prepared by serial dilution of their stock solutions with the appropriate solvent. Commercial solutions of Au NPs were kept in the dark at 4 °C. The physical integrity of the Au colloids was monitored via ultraviolet-visible absorption spectroscopy. Working solutions of Au NPs were prepared by diluting commercial solutions with Nanopure water.
2.2.3 Sample Mixing and Centrifugation for HMW-PAHs Extraction from HPLC Fractions with AuNPs

Sample mixing and centrifugation were carried out in 2-mL Microplain glass tubes. Sample mixing was done with a Maxi Mix III Rotary Shaker (Type M65800, Barnstead-Thermolyne) equipped with a PT500X6A Vortex Mixer accessory. Centrifugation was performed with a MiniSpin centrifuge (Eppendorf) at the maximum rotational speed (13,400 rotations per minute) of the centrifuge.

2.2.4 GC/MS Analysis

GC/MS was carried out with the aid of a gas chromatograph (6850 GC, Agilent, Avondale PA) coupled to a quadruple mass spectrometer with electron impact (EI) ionization at 70 eV (5975 VL, Agilent). The GC was equipped with an auto sampler using a 10 µL syringes. Splitless injections were performed at 300 °C and purge for 100 mL min\(^{-1}\) at 0.25 min. Separations were performed on a 5% phenyl methyl siloxane column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The carrier gas was ultrapure helium at constant flow rate of 1.8 mL min\(^{-1}\). Oven temperature program conditions were 55 °C for 0.4 min followed by temperature increases to 200 °C at 25 °C min\(^{-1}\), 280 °C at 8 °C min\(^{-1}\), 320 °C at 10 °C min\(^{-1}\) (hold for 2 min at 320 °C) and 335 °C at 25 °C min\(^{-1}\) (hold for 5 min at 335 °C). The temperatures of transfer line, manifold and source of ionization were set at 340 °C, 150 °C and 340 °C, respectively. The solvent delay time was 5 min and the total run time was 30 min. GC/MS analysis was performed in selective ion mode (SIM) ranging using the molecular ion peak at 302 m/z and fragmented ion peaks 77, 128, 202 and 252 m/z in the SIM chromatogram and spectra.
2.2.5 **UV-VIS Absorption Spectroscopy**

Absorbance measurements were acquired with a single-beam spectrophotometer (model Cary 50, Varian) equipped with a 75-W pulsed xenon lamp, 2-nm fixed bandpass, ± 0.1 nm wavelength precision, and 24 000 nm min$^{-1}$ maximum scan rate. Instrumental performance was monitored with a commercial standard (Photon Technology International) consisting of a single crystal of dysprosium-activated yttrium aluminum garnet mounted in a cuvette-sized holder with a well-characterized quasi-line absorption spectrum. Wavelength accuracy was evaluated periodically by comparing the recorded position of several spectral lines to the maximum wavelengths provided by the manufacturer. Wavelength precision was obtained in accordance to instrumental specifications (± 0.02 nm) by calculating the standard deviations of the average maximum wavelengths obtained from repetitive scans within 300-800 nm. All solution measurements were made with a 600 µL quartz cuvette with a 1 cm path length.

2.2.6 **RTF Spectroscopy**

Steady-state excitation and fluorescence spectra were acquired with a commercial spectrofluorimeter (Photon Technology International). The excitation source was a continuous wave 75-W xenon lamp with broadband illumination from 200 to 2000 nm. The excitation and emission monochromator had the same reciprocal linear dispersion (4 nm mm$^{-1}$), accuracy (± 1 nm), reproducibility (± 2 nm) and spectral resolution (0.25 nm). Both monochromators have 100 grooves/nm grating were blazed at 300 and 400 nm, respectively. Detection was made with a photomultiplier tube (model 1527) with spectral response from 185 to 650 nm. In the photon...
counting mode, the maximum count rate was 4 MHz, rise time 20 ns and fall time 100 ns with a 220 ns pulse width. The instrument was computer controlled using commercial software (Felix32) specifically designed for the system. Excitation and emission spectra were corrected for wavelength dependence of excitation light source and detector sensitivity, respectively. Correction was made in the post-acquisition mode using the radiometric correction factors included in Felix32 software. Long pass filters were used when necessary to eliminate second-order emission from the excitation source. Instrumental performance was monitored with a commercial standard described previously (section 2.2.5) with a well-characterized quasi-line excitation and emission spectrum. Wavelength accuracy and precision was evaluated periodically by comparing the recorded position of several excitation and emission spectral lines obtained from repetitive scans within 250 – 800 nm to the maximum excitation and emission wavelengths provided by the manufacturer. Room-temperature measurements were made from un-degassed solutions with a standard 600 µL quartz cuvettes with a 1 cm cell path. 77 K measurements were made with the classical procedure of immersing an un-degassed sample solution in a quartz tube into a nitrogen-filled Dewar flask developed in our lab. A 90° excitation/emission configuration was used in all measurements.

2.2.7 HPLC Analysis

HPLC analysis was carried out using a computer controlled HPLC system from Hitachi (San Jose, CA) equipped with the following basic components: a pump (L-7100), a UV detector (L-7400 UV), a fluorescence detector (L-7485), and an online degasser (L-761). Separation was carried out on a Supelco (Bellefonte, PA) Supelcosil TM LC-PAH column with the following
characteristics: 15-cm length, 4-mm diameter, and 5-μm average particle diameters. All sample injections were held constant at 20 μL using a fixed volume injection loop. Laboratory reagent blanks were run in conjunction with each series of samples using identical conditions of glassware, equipment, solvents, and analysis to ensure absence of interfering contamination. HPLC fractions were collected in 2.0 mL sample vials with the aid of a Gilson fraction collector (model FC 20313).

2.2.8 Instrumentation for 4.2 K LETRSS

See section 1.9 for instrumental and experimental details.

2.2.9 Sample Freezing Procedures

See section 1.8 for instrumental and experimental details.

2.3 Results and Discussion

2.3.1 HPLC Separation of DB[a,l]P, DB[a,e]P, DB[a,i]P, DB[a,h]P and N[2,3-a]P

Purcaro et al. have reported the separation of DB[a,l]P, DB[a,e]P, DB[a,i]P and DB[a,h]P from a complex PAH mixture. Separation was achieved at 27 °C on a C_{18} column with a 1.0 mL min\(^{-1}\) mobile phase flow consisting of isocratic elution of 50% acetonitrile/water (v/v) for 10 min, increasing to 60% acetonitrile/water (v/v) at 15 min, increase to 75% acetonitrile/water (v/v) in 5 min, isocratic elution for 15 min, linearly increase to 100%
acetonitrile at 45 min and isocratic elution for 10 min. After 39 min the flow was increased to 1.5 mL min\(^{-1}\) in 1 min. Total separation time was 55 min with these chromatographic conditions. With the exception of the column temperature, which we had no instrumental capability to reproduce, we attempted to separate the five studied PAH under the same chromatographic conditions. At room temperature, the separation of DB\([a,l]\)P, DB\([a,e]\)P, DB\([a,i]\)P and DB\([a,h]\)P was only achieved after 60 min of chromatographic time and N\([2,3-a]\)P co-eluted with DB\([a,i]\)P. Faster separation time for DB\([a,l]\)P, DB\([a,e]\)P, DB\([a,i]\)P and DB\([a,h]\)P (see Figure 2.1A) was obtained with a mobile phase gradient consisting of isocratic elution for 10 min with a 50% acetonitrile/water (v/v), linear increase to 100% acetonitrile at 30 min and isocratic elution for 20 min. The flow rate was 1.0 mL/min for the first 39 min and 1.5 mL min\(^{-1}\) for the remaining of the separation time. Under these conditions, N\([2,3-a]\)P still co-eluted with DB\([a,i]\)P.

The fastest separation time and the best resolution for N\([2,3-a]\)P and DB\([a,i]\)P (see Figure 2.1B) were obtained with a flow rate of 1.5 mL min\(^{-1}\) using 100% acetonitrile as the mobile phase. These conditions were the same as those previously reported by Wise and et al.\(^{106}\), who achieved baseline resolution of the two isomers by operating the column at 29 °C temperature. Figure 2.1C overlays three chromatograms of their binary mixtures recorded upon elution at room temperature with 100% of acetonitrile and flow rate of 1.5 mL min\(^{-1}\). The concentration of N\([2,3-a]\)P (1.0 ng mL\(^{-1}\)) was held constant in the three mixtures. The concentration of DB\([a,i]\)P was varied as 1, 5 and 10 ng mL\(^{-1}\). The peak intensity at 20.95 ± 0.10 min showed the expected linear correlation with the concentration of DB\([a,i]\)P. Unfortunately, the overlapping between the two peaks caused a similar change in the peak intensity of N\([2,3-a]\)P.
a]P, which demonstrates insufficient resolution of the two peaks for the accurate quantitative determination of N[2,3-a]P and DB[a,i]P.

Figure 2.1 Fluorescence chromatograms of a synthetic mixture containing 10 ng mL\(^{-1}\) of (I) DB[a,l]P, (II) DB[a,e]P, (III) DB[a,i]P, (IV) N[2,3-a]P and (V) DB[a,h]P. (A) The mobile phase gradient consisting of isocratic elution for 10 min with a 50\% acetonitrile/water (v/v), linear increase to 100\% acetonitrile at 30 min and isocratic elution for 20 min. The flow rate was 1.0 mL min\(^{-1}\) for the first 39 min and 1.5 mL min\(^{-1}\) for the remaining of the separation time. (B) The mobile phase was 100\% acetonitrile and had a flow rate of 1.5 mL min\(^{-1}\). (C) The mobile phase was 100\% acetonitrile and had a flow rate of 1.5 mL min\(^{-1}\). The binary mixtures consisted of 1.0 ng mL\(^{-1}\) DB[a,i]P and N[2,3-a]P for the initial HPLC measurement. The concentration was held constant for N[2,3-a]P. The concentration of DB[a,i]P was increased to 5.0 (-----) and 10 ng mL\(^{-1}\) (----).
2.3.2 GC/MS of DB[a,i]P and N[2,3-a]P

The possibility to analyze DB[a,i]P and N[2,3-a]P in HPLC fractions via GC/MS was evaluated in our lab. Our experiments were carried out under the same experimental conditions previously reported for the successful separation of 24 PAH, including DB[a,l]P, DB[a,e]P, DB[a,i]P and DB[a,h]P.\textsuperscript{149} Within a confidence interval of 95%, the retention times of N[2,3-a]P (24.13 ± 0.03) and DB[a,i]P (24.24 ± 0.02) were statistically equivalent (N\textsubscript{1} = N\textsubscript{2} = 3).\textsuperscript{147}
According to Wise et al.\textsuperscript{150}, the separation of these two isomers is possible either with a much longer column (60 m) than ours (30 m) or with a different stationary phase (50% phenyl-substituted stationary phase, DB-17 ms).

The importance to achieve their separation in the GC column prior to MS determination is demonstrated in Figure 2.2. The mass fragmentation patterns of DB[a,i]P (A) and N[2,3-a]P (B) are virtually identical. Both spectra present molecular ion peaks (M\textsuperscript{+}) at 302 m/z and fragmented ion peaks at 77, 128, 202 and 252 m/z. The fragment ion peaks correspond to the fragmentation of 5, 4, 2 and 1 benzene rings from the molecular structures of the isomers. The fragmentation peaks of ± 1 were measured to compare the fragmentation of additional hydrogen and to compare the ratios of these peaks with the benzene ring fragmentation peaks. The ratios are nearly identical for both isomers. The only difference results from the abundance of the two isomers. N[2,3-a]P has a slightly higher abundance than DB[a,i]P. As a result, recording mass spectra of HPLC fractions does not appear to be a feasible alternative for the accurate and unambiguous determination of these two isomers.
Figure 2.2 Mass spectra of (A) DB[a,i]P and (B) N[2,3-α]P standards in 100% acetonitrile.
2.3.3 Absorption and Fluorescence Characteristics of DB[a,i]P and N[2,3-a]P at Room Temperature

Figure 2.3A overlays the absorption spectra of DB[a,i]P and N[2,3-a]P in acetonitrile. Close examination of their absorption profiles reveals two wavelength regions with potential use for the quantitative analysis of the two co-eluted compounds. The negligible absorption of DB[a,i]P above 400 nm should make possible to determine the concentration of N[2,3-a]P by setting the UV-VIS absorption detector at either 428 or 456 nm. Under the same prospective, the accurate determination of DB[a,i]P should be possible at either 371 or 393 nm. Figure 2.3B overlays the excitation and fluorescence spectra of the two isomers in acetonitrile. Selective excitation of DB[a,i]P at 371 nm completely removes the contribution of N[2,3-a]P from the fluorescence chromatogram of the mixture. The same is true for the fluorescence contribution of DB[a,i]P when N[2,3-a]P is selectively excited at 430 nm. These possibilities suggest using a multi-wavelength detector for their determination in the chromatographic flow. In this case, however, the analyst should still keep in mind the potential interference of the residual signals that the two isomers present at the target wavelengths of each other, mainly if one of the isomers is at much higher concentration than the other.
2.3.4 HPLC-RTF AFOM of DB[a,l]P, DB[a,e]P, DB[a,i]P, DB[a,h]P and N[2,3-a]P

Table 2.1 summarizes the AFOM of the five studied compounds determined via HPLC-RTF. PAH detection was carried out in the fluorescence detection mode using the following time-programmed excitation/emission wavelengths: 0 min, 316/424 nm; 7 min, 303/398 nm; 12 min, 371/434 nm (DB[a,i]P) or 430/465 nm (N[2,3-a]P); 24 min, 313/447 nm. With the exception of DB[a,i]P and N[2,3-a]P, all the other PAH were detected at their maximum excitation and fluorescence wavelengths in acetonitrile. Synthetic mixtures with known PAH
concentrations were in 1% methanol-water (v/v). All LDRs were based on the average intensities of at least five PAH concentrations. The average intensities plotted in the calibration graphs correspond to a minimum of three intensity measurements recorded from three independent chromatographic runs. No efforts were made to reach the upper concentration limits of the calibration curves. The plots with the calibration curves are shown in Appendix A. The excellent R values demonstrate the existence of linear relationships in all cases. The rather low RSD values confirm the excellent precision of measurements of the HPLC method. The worst LOD was obtained for N[2,3-a]P. This fact could be attributed to the relatively low fluorescence emitted at the excitation and emission wavelengths we used for its selective determination (see spectra in Figure 2.3B). Although the other isomers presented better LOD than N[2,3-a]P, all the LOD values were well above the MCL of regulated PAH in drinking water samples.\(^{26,27,28}\)

<table>
<thead>
<tr>
<th>HMW-PAH (^a)</th>
<th>Retention Time (min)</th>
<th>Ex/Em (^b) (nm)</th>
<th>LDR (^c) (ng L(^{-1}))</th>
<th>R(^d)</th>
<th>LOD (^e) (ng L(^{-1}))</th>
<th>RSD (^f) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB[a,l]P</td>
<td>5.52 ± 0.02</td>
<td>316/424</td>
<td>1,370-100,000</td>
<td>0.9994</td>
<td>410</td>
<td>1.11</td>
</tr>
<tr>
<td>DB[a,e]P</td>
<td>8.67 ± 0.06</td>
<td>303/398</td>
<td>1,390-100,000</td>
<td>0.9995</td>
<td>420</td>
<td>0.80</td>
</tr>
<tr>
<td>DB[a,i]P</td>
<td>21.02 ± 0.23</td>
<td>371/434</td>
<td>1,430-100,000</td>
<td>0.9993</td>
<td>687</td>
<td>1.24</td>
</tr>
<tr>
<td>N[2,3-a]P</td>
<td>21.97 ± 0.25</td>
<td>430/465</td>
<td>4,810-100,000</td>
<td>0.9950</td>
<td>1,436</td>
<td>2.58</td>
</tr>
<tr>
<td>DB[a,h]P</td>
<td>26.22 ± 0.29</td>
<td>313/447</td>
<td>1,810-100,000</td>
<td>0.9947</td>
<td>542</td>
<td>2.99</td>
</tr>
</tbody>
</table>

\(^a\) All working solutions were made in 1% methanol. Sample volume was 20 \(\mu\)L. \(^b\) Selective excitation and emission wavelength for fluorescence detection. \(^c\) LDR, linear dynamic range; lower concentration limit = limit of quantitation = 10S\(_B\)/m, where S\(_B\) is the standard deviation of 16 blank determinations and m is the slope of the calibration curve. \(^d\) R, correlation coefficient. \(^e\) Limits of detection (LOD) were calculated on the basis of the equation LOD = 3S\(_B\)/m. \(^f\) Relative standard deviation (RSD) = S\(_F\)/I\(_F\) x 100, where S\(_F\) is the standard deviation of the average calculated from six fluorescence measurements at medium linear PAH concentrations.
2.3.5 *AFOM of DB[a,l]P, DB[a,e]P, DB[a,i]P, DB[a,h]P and N[2,3-a]P in N-Octane via 4.2 K LETRSS*

Previous work in our group has shown n-octane to be a well-suited solvent for the direct analysis of DB[a,l]P, DB[a,e]P, DB[a,i]P and DB[a,h]P in Shpol’skii matrices.\textsuperscript{87, 146} Figure 2.4A and 2.4B show the 4.2K WTM recorded from DB[a,i]P and N[2,3-a]P in n-octane, respectively. In both cases, the gate delay was advanced in 1 ns increments. This gate step allowed us to collect a total of 40 fluorescence spectra per WTM. Each fluorescence spectrum at each gate delay was averaged over 100 laser pulses. Figure 2.4C overlays the first two fluorescence spectra of each WTM recorded at 10 ns delays. Both spectra present the vibrational features expected from PAH in Shpol’skii matrices. The fluorescence time decays presented in Figure 2.4D were stripped from the WTM by plotting the fluorescence intensity of each isomer at its maximum emission wavelength as a function of time delay. Well-behaved single exponential decays were observed in both cases. The calculated and observed points over the first two lifetimes of the decay agree to within about 1% and the residuals show no systematic trends.
Figure 2.4 Wavelength-time-matrix (WTM) recorded from 100 ng mL\(^{-1}\) standards of N[2,3-a]P (A) and DB[a,i]P (B). WTM in (A) was recorded upon excitation at 321 nm using a 10ns delay and a 25 ns gate time. WTM in (B) was recorded upon excitation at 315 nm using a 10 ns delay and a 650 ns gate time. Each fluorescence spectrum in the WTM corresponds to the accumulation of 100 laser pulses. Spectrograph slit was 42 µm in all cases. (C) Overlay of 4.2 K fluorescence spectra of N[2,3-a]P (—) and DB[a,i]P (····). (D) Overlay of 4.2 K fluorescence decay of N[2,3-a]P (■) and DB[a,i]P (▲).

Table 2.2 summarizes the AFOM of the five studied compounds obtained via 4.2 K LETRSS in n-octane. Fluorescence excitation at the maximum wavelength of each isomer would require the use of three laser dyes. For practical purposes, we opted to use only one laser dye (DCM) that covered an excitation range (310 – 325 nm) common to the five compounds. As such, the excitation wavelengths we used in Table 2.2 correspond to the best excitation...
wavelength of each isomer between 310 and 325 nm. All fluorescence measurements were made at the maximum emission wavelength of each compound. The 10 ns delay was long enough to avoid the need to consider convolution of the laser pulse with the analytical signal. The measuring gate was optimized to collect most of PAH fluorescence and still avoid instrumental noise. No attempts were made to reach the upper concentration limits of the LDR. The LOD values are of the same order of magnitude as those reported in Table 2.1. The excellent RSD values reflect the reproducibility of measurements of the FOP. The plots with the calibration curves are shown in Appendix B.

Table 2.2 4.2 K LETRSS analytical figures of merit for the HMW-PAHs.

<table>
<thead>
<tr>
<th>HMW-PAH a</th>
<th>$\lambda_{\text{exc}}/\lambda_{\text{em}}$ (nm)</th>
<th>$\tau$ (ns)</th>
<th>$t_d/t_g$ (ns)</th>
<th>LDR (µg L$^{-1}$)</th>
<th>$R^2$ f</th>
<th>LOD (ng L$^{-1}$)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB[a,l]P</td>
<td>322.0/417.0</td>
<td>81.1 ± 1.3</td>
<td>10/250</td>
<td>1.50-200</td>
<td>0.9999</td>
<td>450</td>
<td>1.60</td>
</tr>
<tr>
<td>DB[a,e]P</td>
<td>310.0/393.4</td>
<td>54.8 ± 1.9</td>
<td>10/150</td>
<td>2.97-200</td>
<td>0.9982</td>
<td>890</td>
<td>1.69</td>
</tr>
<tr>
<td>DB[a,i]P</td>
<td>315.0/429.4</td>
<td>207.1 ± 3.4</td>
<td>10/650</td>
<td>1.14-200</td>
<td>0.9978</td>
<td>340</td>
<td>0.74</td>
</tr>
<tr>
<td>N[2,3-a]P</td>
<td>321.0/458.0</td>
<td>7.5 ± 0.2</td>
<td>10/50</td>
<td>0.74-200</td>
<td>0.9990</td>
<td>320</td>
<td>1.07</td>
</tr>
<tr>
<td>DB[a,h]P</td>
<td>316.0/446.6</td>
<td>5.6 ± 0.1</td>
<td>10/15</td>
<td>1.17-200</td>
<td>0.9991</td>
<td>350</td>
<td>1.54</td>
</tr>
</tbody>
</table>

a All HMW-PAH solutions were made in n-octane. b Excitation ($\lambda_{\text{exc}}$) and fluorescence ($\lambda_{\text{em}}$) wavelengths. c The fluorescence lifetime corresponds to the average of three individual measurements taken from three frozen aliquots at the maximum fluorescence wavelength. d Optimum gate delay ($t_d$) and gate width ($t_g$). e LDR, linear dynamic range; lower concentration limit = LOQ = 10S_B/m, where S_B is the standard deviation of 16 blank determinations and m is the slope of the calibration curve. f $R$ = correlation coefficient. g LODs were calculated on the basis of the equation LOD = 3S_B/m. h Relative standard deviation (RSD) = S_F/I_F x 100, where S_F is the standard deviation of the average calculated from six fluorescence measurements at medium linear PAH concentrations.

Figures 2.5A-D compare the 4.2 K fluorescence spectra of one isomer (target isomer) to the spectra of synthetic mixtures with DB[a,i]P and N[2,3-a]P. Spectra in 2.5A and 2.5B were recorded using the excitation wavelength of DB[a,i]P as well as its optimum delay and gate times (see Table 2.2). Spectra in 2.5C and 2.5D were recorded using the instrumental parameters
optimized for N[2,3-a]P. For a confidence interval of 95% (α = 0.05) and six determinations (N_1 = N_2 = 6), the signal intensity of the mixtures at the maximum fluorescence wavelengths of the target compounds were statistically equivalent to the those recorded from their standard solutions. Single exponential decays were observed in all cases demonstrating the emissions of single components in both synthetic mixtures. The fluorescence lifetimes of DB[a,i]P and N[2,3-a]P in the mixtures were statistically equivalent (α = 0.05; N_1 = N_2 = 6) the those recorded from the standards. These results confirm the absence of inner filter effects and/or synergistic effects in the mixtures that could affect the accuracy of analysis for unambiguous isomer determination in the HPLC fractions.
Figure 2.5 4.2 K fluorescence spectra of DB[a,i]P recorded from (A) a 100 ng mL\(^{-1}\) standard solution in n-octane and (B) a binary mixture with 100 ng mL\(^{-1}\) N[2,3-a]P in n-octane. Both spectra were recorded upon excitation at 316 nm using a 10 ns delay and 650 ns gate time. 4.2 K fluorescence spectra of N[2,3-a]P recorded from a (C) 100 ng mL\(^{-1}\) standard solution and (D) a binary mixture with 100 ng mL\(^{-1}\) DB[a,i]P. Both spectra were recorded upon excitation at 321 nm using a 10 ns delay and 25 ns gate time. A-D spectra correspond to the accumulation of 100 laser pulses. The spectrograph slit was 42 µm.
2.3.6 Quantitative Analysis of HPLC Fractions via SPNE-LETRSS at 4.2 K

Previous work in our lab has demonstrated the advantages of performing liquid-liquid extraction\textsuperscript{87} and solid-liquid extraction\textsuperscript{146} prior to the analysis of PAHs in HPLC fractions. Herein, we demonstrate the advantages of pre-concentrating PAHs from HPLC fractions with SPNE, a pre-concentration approach that extracts PAHs with Au NPs. Only a few articles exist on the use of solid-phase nanoextraction (SPNE), which refers to the extraction of PAH from drinking water samples.\textsuperscript{1, 84, 85, 151} This section deals with the extraction of PAH from HPLC mobile phases containing 100\% acetonitrile. PAH extraction results from the physicochemical affinity that exists between them and the surface of Au NPs. PAH desorption is carried out with 1-pentanethiol, which has strong affinity for AuNPs and upon binding to the metallic surface releases PAH molecules to the surrounding medium (n-octane).

The minimum volume of Au NPs solution (min VCS) needed to extract a multi-component mixture from a certain volume of aqueous solution (VAQ) can be estimated with the following equation:\textsuperscript{84}

$$\text{min VCS} = \sum_{i=1}^{n} \frac{[\text{PAH}]_i}{\text{MEMPAHi}} \left(\frac{\text{VAQ}}{C_{CS}}\right)$$  \hspace{1cm} (2.1)

where $[\text{PAH}]_i$ is the concentration (mass/volume) of any given PAH in the aqueous solution, $C_{CS}$ is the concentration of Au NPs in the extracting solution (particles/volume) and MEMPAHi is the maximum extracted mass of any given PAH per Au NP unit. MEMPAHi values can be estimated via an experimental procedure developed in our lab.\textsuperscript{84} For the particular case of the four studied HMW-PAH, the average MEMPAHi values are the following: $4.89 \times 10^{-10}$ (DB[$a,l$]P), $3.24 \times 10^{-10}$ (DB[$a,i$]P), $3.75 \times 10^{-10}$ (DB[$a,h$]P) and $3.16 \times 10^{-10}$ ng particle$^{-1}$
(N[2,3-\textit{a}]P). Keeping in mind that equation 2.1 does not account for the potential co-extraction of sample concomitants and the resulting coverage of particle surface, the analyst should always use a larger volume of extracting solution than its minimum value (VCS > min VCS) and/or adjust the VAQ/CCS ratio accordingly.

Table 2.3 summarizes the overall recoveries of the entire HPLC-SPNE-LETRSS procedure obtained from HMW-PAHs spiked in drinking water samples. Within a confidence interval of 95% and six replicate trials (\(N = 6\)), the obtained recoveries were statistically equivalent to 100%\(^{147}\). All fluorescence decays recorded from the n-octane layers were single exponential decays, indicating the spectral purity of the target wavelengths. The fluorescence lifetimes were statistically equivalent (\(\alpha = 0.05; N_1 = N_2 = 6\)) to those recorded from the pure standards in n-octane\(^{147}\). This fact associated to their identical spectral features provides the basis for unambiguous isomer determination.

Table 2.3 Overall recoveries of 5 HMW-PAH isomers in drinking water via HPLC-SPNE-LETRSS.

<table>
<thead>
<tr>
<th>HMW-PAH (^a)</th>
<th>Recovery (^b) (%)</th>
<th>(\tau) (^c) (ns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB([a,l])P</td>
<td>98.2 ± 5.5</td>
<td>77.5 ± 2.2</td>
</tr>
<tr>
<td>DB([a,e])P</td>
<td>99.5 ± 2.8</td>
<td>52.3 ± 1.6</td>
</tr>
<tr>
<td>DB([a,i])P</td>
<td>102.7 ± 3.2</td>
<td>216.0 ± 4.4</td>
</tr>
<tr>
<td>N([2,3-\textit{a}])P</td>
<td>102.6 ± 4.1</td>
<td>7.4 ± 0.2</td>
</tr>
<tr>
<td>DB([a,h])P</td>
<td>102.2 ± 6.0</td>
<td>5.5 ± 0.3</td>
</tr>
</tbody>
</table>

\(^a\) All working solutions were made in 1% methanol. Sample volume was 20 \(\mu\)L. HMW-PAHs were SPNE from 0.5 mL of mobile phase. \(^b\) HMW-PAH Recovery from drinking water samples. \(^c\) Average fluorescence lifetime of three measurements taken from three frozen aliquots.
Table 2.4 summarizes the AFOM of the five HMW-PAHs via HPLC-SPNE-LETRSS. Each calibration curve was built with at least five standard solutions prepared in methanol. Each signal plotted in the calibration graph was the average of at least three chromatographic runs. No efforts were made to reach the upper linear concentration of the LDR. The plots of the calibration curves are shown in Appendix C. The R values of the linear regression plots close to unity demonstrate the linearity of our approach. All LOD were at the parts-per-trillion concentration levels and lower than the maximum concentration levels of regulated PAH in drinking water samples.

<table>
<thead>
<tr>
<th>HMW-PAH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>LDR&lt;sup&gt;b&lt;/sup&gt; (µg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>R&lt;sup&gt;2&lt;/sup&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
<th>LOD&lt;sup&gt;d&lt;/sup&gt; (ng L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>RSD&lt;sup&gt;e&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB[a,l]P</td>
<td>0.20-10</td>
<td>0.9990</td>
<td>60.0</td>
<td>1.76</td>
</tr>
<tr>
<td>DB[a,e]P</td>
<td>0.51-10</td>
<td>0.9940</td>
<td>154.0</td>
<td>1.96</td>
</tr>
<tr>
<td>DB[a,i]P</td>
<td>0.27-10</td>
<td>0.9994</td>
<td>81.0</td>
<td>2.53</td>
</tr>
<tr>
<td>N[2,3-a]P</td>
<td>0.17-10</td>
<td>0.9955</td>
<td>51.1</td>
<td>1.23</td>
</tr>
<tr>
<td>DB[a,h]P</td>
<td>0.25-10</td>
<td>0.9992</td>
<td>76.1</td>
<td>1.66</td>
</tr>
</tbody>
</table>

<sup>a</sup> All working solutions were made in 1% methanol. Sample volume was 20 µL. PAH were SPNE from 0.5 mL of mobile phase. All measurements were made using the excitation and emission wavelengths and delay and gate widths stipulated in Table 2.  

<sup>b</sup> LDR, linear dynamic range; lower concentration limit = LOQ = 10 S<sub>B</sub>/m, where S<sub>B</sub> is the standard deviation of 16 blank determinations at the peak base and m is the slope of the calibration curve.  

<sup>c</sup> R, correlation coefficient.  

<sup>d</sup> LODs were calculated using equation LOD = 3 S<sub>B</sub>/m.  

<sup>e</sup> Relative standard deviation (RSD) = S<sub>F</sub>/I<sub>F</sub> x 100, where S<sub>F</sub> is the standard deviation of the average (I<sub>F</sub>) calculated from nine fluorescence measurements at medium linear PAH concentrations.
2.4 Conclusion

We have presented an attractive alternative for the analysis of two HPLC co-eluted isomers of molecular weight 302 with identical mass fragmentation patterns, namely D\([a,i]\)P and N\([2,3-a]\)P. Qualitative and quantitative analysis was carried out via LE Travis at liquid helium temperature. Unambiguous identification of co-eluted isomers was based on their characteristic 4.2 K line-narrowed spectra in n-octane as well as their fluorescence lifetimes. Pre-concentration of HPLC fractions prior to spectroscopic analysis was performed with the aid of Au NPs via an environmentally friendly procedure. Complete SPNE-LETRSS analysis was possible with microliters of organic solvent. Excellent overall recoveries (~100%) from drinking water samples were obtained for the five studied compounds. Relative standard deviations lower than 3% demonstrate the excellent reproducibility of measurements at 4.2 K. Limits of detection at the parts-per-trillion level varied between 51.1 ng L\(^{-1}\) (N\([2,3-a]\)P) and 154 ng L\(^{-1}\) (D\([a,e]\)P). The outstanding AFOM associated to its non-destructive nature, which provides ample opportunity for further analysis with other instrumental methods, makes this approach an attractive alternative for the analysis of co-eluted isomers of HMW-PAH in HPLC fractions.
CHAPTER 3  DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS WITH MOLECULAR WEIGHT 302 IN WATER SAMPLES BY SOLID-PHASE NANOEXTRACTION AND LASER-EXCITED TIME-RESOLVED SHPOL’SKII SPECTROSCOPY


3.1 Introduction

In this chapter, we modify the original SPNE procedure to better extract HMW-PAHs and reduce analysis time. The spectral and lifetime characteristics of DB[a,l]P, DB[a,i]P and DB[a,h]P in Shpol’skii solvents were investigated in our lab previously but not those from N[2,3-a]P. To the extent of our literature search, only one article existed on the Shpol’skii characteristics of N[2,3-a]P, which reported its fluorescence spectrum at 63 K using n-hexane as the Shpol’skii solvent. Herein, we present a common Shpol’skii solvent (n-octane) for the direct analysis of N[2,3-a]P, DB[a,l]P, DB[a,i]P and DB[a,h]P at 4.2 K. We demonstrate the potential of SPNE-LETRSS with the analysis of the four isomers in tap water samples with increasing matrix complexity. The excellent AFOM, the short analysis time and relatively small volumes of water and organic solvents facilitate the simultaneous analysis of numerous water samples via an environmentally friendly experimental procedure.

3.2 Experimental Section

3.2.1 Chemicals

Nanopure water from a Barnstead Nanopure Infinity water system was used throughout. 20nm average diameter Au NPs aqueous solutions (7 x 10^{11} particles mL^{-1}) were purchased from
Ted Pella, Inc. (Redding, CA). HPLC grade methanol was purchased from Fischer Scientific (Pittsburg, PA). Analytical grade 1-pentanethiol and n-octane were from Acros Organics (Atlanta, GA). DB[a,l]P, DB[a,h]P and DB[a,i]P were purchased from AccuStandard at their highest available purity (100%). N[2,3-a]P was acquired from Sigma-Aldrich (Milwaukee, WI, USA) at 98% purity. Benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]-pyrene, dibenz[a,h]anthracene, indeno[1,2,3-cd]pyrene, naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene and benzo[ghi]perylene were purchased from Sigma-Aldrich (Milwaukee, WI, USA) at their highest available purity (≥ 98%).

*Note: use extreme caution when handling PAHs that are known to be extremely toxic.*

### 3.2.2 Solution Preparation

Stock solutions of HMW-PAHs were prepared in either methanol or n-octane and kept in the dark at 4 °C. Possible HMW-PAHs degradation was monitored via room-temperature fluorescence spectroscopy. Working solutions of HMW-PAH were prepared by serial dilution of their stock solutions with the appropriate solvent. Commercial solutions of Au NPs were kept in the dark at 4 °C. The physical integrity of the Au colloids was monitored via ultraviolet-visible absorption spectroscopy. Working solutions of Au NPs were prepared by diluting commercial solutions with Nanopure water.
3.2.3 **Sample Mixing and Centrifugation for HMW-PAHs Extraction with Gold Colloids**

See section 2.2.3 for experimental details.

3.2.4 **UV-VIS Absorption Spectroscopy**

See section 2.2.5 for experimental details.

3.2.5 **Room-Temperature and 77 K Fluorescence Spectroscopy**

See section 2.2.6 for instrumental and experimental details.

3.2.6 **Instrumentation for 4.2 K LETRSS**

See section 1.9 for instrumental and experimental details.

3.2.7 **Sample Freezing Procedures**

See section 1.8 for instrumental and experimental details.
3.3 Results and Discussion

3.3.1 Optimization of SPNE for DB[a,l]P, DB[a,h]P, DB[a,i]P and N[2,3-a]P

The extraction of PAHs with Au NPs was originally devised for water analysis of EPA-PAHs. The extraction step consisted of mixing micro-liters of water sample with micro-liters of an aqueous solution of Au NPs. Among the average diameters of the Au NPs tested (20, 40, 60, 80 and 100 nm), colloid solutions with 20 nm average particle diameters showed the best EPA-PAH extraction efficiencies. After mixture centrifugation and supernatant decantation, the precipitate was treated with 2 μL of 1-pentanethiol and 48 μL of octane. Upon binding to the metallic surface, 1-pentanethiol released EPA-PAHs to the surrounding medium. The presence of n-octane favored PAHs desorption by providing a non-polar environment to the surrounding medium. After centrifugation, the supernatant was then analyzed for the presence of the sixteen EPA priority pollutants.

Figure 3.1 shows the schematic diagram of the original and new SPNE procedure for HMW-PAHs. The main difference to the original approach is the addition of 48 μL of methanol to the releasing mixture of 1-pentanethiol and n-octane. The presence of methanol provides a well-defined and visually distinguishable two-phase system (octane and methanol/Au NPs precipitate) that avoids the need of the second centrifugation step prior to instrumental analysis. This modification reduces extraction time from 30 to 20 min per sample. This is a considerable improvement for routine analysis of numerous samples. For the specific case of HMW-PAHs, adding methanol to the precipitate of Au NPs improved the overall recoveries of the analytical method.
The possibility of HMW-PAH adhesion to the walls of the micro-plain vessels and/or PAH precipitation solely due to shaking and/or centrifugation was investigated via room-temperature fluorescence spectroscopy. The main reason for choosing this technique was its ability to directly monitor trace concentrations of HMW-PAH in aqueous solutions. Table 3.1 summarizes their working concentration ranges in 1% methanol- water (v/v). Appendix D shows the calibration plots. This methanol volume was added to facilitate the dissolution of traces of
HMW-PAH in water samples. Extracting solutions of 20 nm Au NPs were also prepared in 1% methanol-water (v/v). Other particle diameters were not investigated in this study. Fluorescence measurements were made at the maximum excitation and emission wavelengths of each PAH. The lower concentration limits of the linear concentration ranges correspond to the LOQ of the calibration method. Upper concentration limits were determined by measuring signal intensities before (I_B) and after (I_A) shaking and/or centrifugation. The tabulated values correspond to working solutions that had statistically equivalent I_B and I_A and were considered free from PAH loss due to shaking and/or centrifugation. Their statistical comparisons were made at the 95% confidence level (α = 0.05) and based on triplicate measurements of three individual aliquots, i.e. N_B = N_A = 9.147. All further experiments were performed within the respective working concentration range of each HMW-PAH.

Table 3.1 Working concentration ranges of HMW-PAHs in 1% methanol/water (v/v).

<table>
<thead>
<tr>
<th>HMW-PAH^a</th>
<th>λ_exc/λ_em(^b) (nm)</th>
<th>LDR(^c) (ng mL(^{-1}))</th>
<th>R(^d)</th>
<th>LOD(^e) (ng mL(^{-1}))</th>
<th>RSD(^f) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB([a,l])P</td>
<td>316/424</td>
<td>4.69-200</td>
<td>0.9985</td>
<td>1.40</td>
<td>3.92</td>
</tr>
<tr>
<td>DB([a,i])P</td>
<td>394/434</td>
<td>1.01-125</td>
<td>0.9976</td>
<td>0.30</td>
<td>4.14</td>
</tr>
<tr>
<td>DB([a,h])P</td>
<td>313/457</td>
<td>2.50-75</td>
<td>0.9982</td>
<td>0.75</td>
<td>1.80</td>
</tr>
<tr>
<td>N([2,3-a])P</td>
<td>332/467</td>
<td>1.86-75</td>
<td>0.9988</td>
<td>0.56</td>
<td>2.48</td>
</tr>
</tbody>
</table>

^a PAH solutions were made in 1% methanol-water (v/v). ^b Room-temperature excitation and fluorescence maximum wavelength for fluorescence detection. ^c LDR, linear dynamic range; lower concentration limit = limit of quantitation = 10S_B/m, where S_B is the standard deviation of 16 blank determinations and m is the slope of the calibration curve. Higher concentration limit = maximum concentration free from precipitation. ^d R, correlation coefficient. ^e Limit of detection (LOD) estimated as 3S_B/m. ^f Relative standard deviation (RSD) calculated from medium concentrations of linear dynamic ranges.
Figure 3.2 compares the overall recoveries (OR) of the original and the new SPNE procedure. PAH extractions were carried out from 950 μL aliquots of Nanopure water (V_W) previously spiked with HMW-PAHS at the 10 parts-per-billion concentration level (C_{PAH} = 10 ng mL^{-1}). All extractions were made with 50 μL of Au NPs extracting solution. The overall recoveries were calculated according to equation 3.1:

\[
OR \,(\%) = 100 \left( \frac{[PAH]_S \cdot V_S}{C_{PAH} \cdot VW} \right) \tag{3.1}
\]

where [PAH]_S correspond to the concentrations of HMW-PAH in the final volumes of supernatants (V_S). [PAH]_S values were calculated from room temperature fluorescence calibration curves built with standard solutions prepared in n-octane (data shown in Appendix E). To account for slight variations in the chemical composition of the n-octane supernatants – and the possible variations of the fluorescence quantum yields of the studied HMW-PAH – the n-octane used for standard solutions was previously exposed to the experimental steps of the entire procedure. The overall recoveries of the new procedure were significantly better for DB[a,l]P, DB[a,i]P and N[2,3-a]P. The overall recoveries of DB[a,h]P were statistically the same (N_1 = N_2 = 3; α = 0.05) for the two procedures. The observed differences among the four isomers probably reflect their relative affinity towards the surface of Au NPs and the supernatant. The better recoveries of the new procedure can be attributed to the presence of methanol, which apparently facilitates the partitioning of DB[a,l]P, DB[a,i]P and N[2,3-a]P into the layer of n-octane. All further studies were then carried out following the experimental procedure in Figure 3.1.
Figure 3.2 Comparison of overall recoveries obtained via the original and the new SPNE procedure. All extractions were carried out 50 μL of 20 nm Au NPs. Extractions were made from 950 μL of Nanopure water spiked with individual HMW-PAH at the 10 ng mL⁻¹ concentrations.

### 3.3.2 Spectral Characteristics of N[2,3-a]P at 77 K

The spectral and lifetime characteristics of DB[α,l]P, DB[α,i]P and DB[α,h]P in Shpol’skii solvents have been investigated in our lab previously.⁷⁷, ¹⁴⁶ Previous to the present work, only one article existed on the Shpol’skii characteristics of N[2,3-a]P,¹⁵² which reports its fluorescence spectrum at 63 K using n-hexane as the Shpol’skii solvent. Figure 3.3 shows the 77 K excitation and fluorescence spectra of N[2,3-a]P in n-octane recorded with a commercial spectrofluorimeter. No attempts were made to adjust slit-widths to optimize spectral resolution,
nor were the spectra corrected for instrumental response. Site-selective excitation was not attempted. Although the excitation and fluorescence spectra were acquired at identical spectral band-pass, the features in emission appear significantly narrower than in excitation. The primary reason is that the displayed portion of the excitation spectrum corresponds to excitation to singlet states higher than $S_1$ and is therefore subject to uncertainty broadening from rapid $S_n - S_1$ internal conversion. When compared to the 77 K spectra of N[2,3-$a$]P in n-hexane and n-heptane (see spectra in Appendix F), n-octane provided the narrowest full-width at half maxima. Because this observation was in good agreement with the previous solvent optimization we made for DB[a,l]P, DB[a,h]P and DB[a,i]P, all further studies were carried out with n-octane.

![Figure 3.3 77 K excitation and fluorescence spectra of N[2,3-$a$]P in n-octane at 10 ng mL$^{-1}$ concentration. The following acquisition parameters were used for spectra collection: 336 nm excitation, 462 nm emission wavelengths, and 1 nm bandpass.](image)

3.3.3 4.2 K LETRSS Analysis of N[2,3-a]P in Synthetic Mixtures with DB[a,l]P, DB[a,i]P and DB[a,h]P

Previous studies in our lab have shown the possibility to perform accurate analysis of DB[a,h]P, DB[a,i]P and DB[a,l]P without previous separation. Similar to N[2,3-a]P, the 77 K excitation spectra of DB[a,l]P, DB[a,i]P and DB[a,h]P (see spectra in Appendix G) provide ample opportunity for fluorescence excitation within the working range of our tunable dye laser, which – depending on the operating laser dye – can extend from 200 to 600 nm. The spectral comparison of pure standards at 77 K revealed a slight overlapping between the fluorescence spectrum of N[2,3-a]P and the spectra of DB[a,i]P and DB[a,h]P. This potential interference was eliminated with selective excitation of N[2,3-a]P at 321 nm, a wavelength that shows no absorption from DB[a,i]P and DB[a,h]P. The fluorescence spectra of DB[a,l]P and N[2,3-a]P showed no overlapping. Fluorescence excitation at the maximum wavelength of each isomer would require the use of three laser dyes. For practical purposes, we opted to use only one laser dye (DCM) that covered an excitation range (310 – 325 nm) common to the four compounds.

Synthetic mixtures were prepared in n-octane and contained the target isomer at the same or at a lower concentration than the other three PAHs. Figure 3.4 compares the 4.2 K fluorescence spectra and decays of a 10 ng mL\(^{-1}\) N[2,3-a]P standard solution to those recorded from a mixture of 10 ng mL\(^{-1}\) of N[2,3-a]P and 100 ng mL\(^{-1}\) of DB[a,l]P, DB[a,h]P and DB[a,i]P. Spectra and decays were recorded using the best excitation (321 nm) and fluorescence (458.3 nm) wavelengths, and gate time (25 ns) for N[2,3-a]P. For a confidence interval of 95\% (\(\alpha = 0.05\)) and six determinations (\(N_1 = N_2 = 6\)), the signal intensity of the mixture was statistically equivalent to the one recorded from the standard solution. Single exponential decays
were observed in both cases demonstrating the emission of a single component in the synthetic mixture. The fluorescence lifetime of N[2,3-a]P in the mixture was statistically equivalent ($\alpha = 0.05$; $N_1 = N_2 = 6$) to the one of the standard. These results confirm the absence of inner filter effects and/or synergistic effects in the mixture that could affect the accuracy of analysis for unambiguous isomer determination without previous separation.

Figure 3.4 4.2 K fluorescence spectra and fluorescence decays of N[2,3-a]P recorded from (A) a 10 ng·mL$^{-1}$ standard solution and (B) a synthetic mixture with the other three HMW-PAH. The mixture contained 10 ng·mL$^{-1}$ of N[2,3-a]P and 100 ng·mL$^{-1}$ of DB[a,l]P, DB[a,h]P and DB[a,i]P. Spectra were recorded upon excitation at 321 nm using a 10ns delay and 50ns gate time. All spectra correspond to the accumulation of 100 laser pulses. The spectrograph slit was 42 µm. Fluorescence decays were recorded at the maximum fluorescence wavelength (458.3 nm) of N[2,3-a]P.
3.3.4 4.2 K LETRSS Analysis of HMW-PAHs in the Presence of EPA-PAHs

The feasibility to monitor DB\([a,l]\)P, DB\([a,h]\)P and DB\([a,i]\)P in heavily contaminated water samples of unknown composition via 4.2 K LETRSS has been previously demonstrated in our lab.\(^{146}\) This section demonstrates the ability to determine the four target isomers in the presence of the 16 EPA-PAHs. Synthetic mixtures were prepared in n-octane containing the sixteen priority pollutants at concentration levels varying between 0.2 and 20 ng mL\(^{-1}\). All synthetic mixtures contained the four HMW-PAHs at the 2 ng mL\(^{-1}\) concentration. Figure 3.5 shows the Shpol’skii spectra of the four mixtures containing the sixteen EPA-PAHs at 20 ng mL\(^{-1}\). Each spectrum was recorded using the best gate and excitation wavelength for the target isomer. Spectral purity for each isomer was confirmed via fluorescence decay analysis at its target fluorescence peak. Single fluorescence decays with fluorescence lifetime values statistically equivalent to those from the standards (\(N_1 = N_2 = 3; \alpha = 0.05\))\(^{147}\) were observed in all cases. The fluorescence intensities of the mixtures and the standards were statistically equivalent as well. These facts demonstrate the feasibility to determine the four isomers in complex mixtures with the 16 EPA-PAHs.
Figure 3.5 4.2 K fluorescence spectra recorded from synthetic mixtures containing the four HMW isomers and the sixteen priority pollutants. $\tau_m$ and $\tau_s$ refer to the fluorescence lifetimes of the target HMW isomer in the mixture and its pure standard, respectively. Each mixture contained HMW-PAH at 2.0 ng mL$^{-1}$ concentration and the sixteen EPA-PAH at 20 ng mL$^{-1}$ concentration. In each mixture, the targeted HMW isomer was the following: (A) N[2,3-a]P; (B) DB[a,l]P; (C) DB[a,h]P; (D) DB[a,i]P. Experimental parameters were the following: (A) $\lambda_{\text{exc}} = 321$ nm, $t_d/t_g = 10/50$ ns; (B) $\lambda_{\text{exc}} = 322$ nm, $t_d/t_g = 10/250$ ns; (C) $\lambda_{\text{exc}} = 316$ nm, $t_d/t_g = 10/15$ ns; (D) $\lambda_{\text{exc}} = 315$ nm, $t_d/t_g = 10/650$ ns. In all cases, the spectrograph slit was 42 µm. All spectra correspond to the accumulation of 100 laser pulse.

### 3.3.5 AFOM

The minimum volume of Au NPs solutions ($\text{minV}_{\text{CS}}$) that one needs to completely extract a multi-component PAH mixture from a certain volume of water sample ($V_{\text{H2O}}$) can be estimated with the following equation 3.2.$^{84}$
where $[\text{PAH}]_i$ is the concentration (mass/volume) of any given component in the water sample, $C_{CS}$ is the concentration of Au NPs in the extracting solution (particles/volume) and $\text{MEM}_{PAHi}$ is the maximum extracted mass of any given PAH per Au NP unit. Table 3.2 lists the $\text{MEM}_{PAHi}$ values of DB[$a,l$]P, DB[$a,i$]P, DB[$a,h$]P and N[2,3-$a$]P estimated via an experimental procedure previously developed in our lab.\textsuperscript{84, 85, 151} Appendix H shows the experimental plots with the obtained data. Keeping in mind that equation 3.2 does not account for the potential co-extraction of sample concomitants and the resulting coverage of particle surface, the analyst should always use a larger volume of extracting solution than its minimum value ($V_{CS} > mV_{CS}$) and/or adjust the $V_{H2O}/C_{CS}$ ratio accordingly.

Table 3.2 Maximum amount of extracted PAH per Au particle.

<table>
<thead>
<tr>
<th>HMW-PAH$^a$</th>
<th>MEM$^b$ ($10^{-10}$ ng·particle$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB[$a,l$]P</td>
<td>4.89 ± 0.20</td>
</tr>
<tr>
<td>DB[$a,i$]P</td>
<td>3.24 ± 0.21</td>
</tr>
<tr>
<td>DB[$a,h$]P</td>
<td>3.75 ± 0.42</td>
</tr>
<tr>
<td>N[2,3-$a$]P</td>
<td>3.16 ± 0.26</td>
</tr>
</tbody>
</table>

$^a$ All HMW-PAH solutions were made in 1% methanol-water (v/v). All extractions were made with 50 µL of 20-nm Au NPs Solution ($7.0 \times 10^{11}$ particles·mL$^{-1}$). Reported values are the averages of 3 independent extractions from the same HMW-PAH solution. $^b$ MEM$^{PAH} = \text{maximum mass of extracted PAH per Au NP unit estimated according to experimental procedures.}$\textsuperscript{84}

Table 3.3 summarizes the AFOM of the four HMW-PAH obtained via LETRSS and SPNE-LETRSS. PAHs extraction was carried out from 500 µL aliquots of Nanopure water previously spiked with synthetic mixtures of HMW-PAH. All extractions were made using 950
μL of Au NPs solution, which would assure the complete extraction of the four PAH at the 1.2 x 10^5 ng L^{-1} concentration level. This concentration is higher than the upper limit concentrations in Table 3.1. Aqueous standard solutions were prepared by spiking milliliters of Nanopure water with micro-litters of HMW-PAH solutions in 1% methanol-water (v/v). Pure standards for LETRSS measurements (no SPNE) were prepared in n-octane. All LDR were based on the average intensities (N ≥ 3) of at least five PAH concentrations. No efforts were made to reach the upper linear concentrations of the calibration curves. All intensities plotted in the calibration graphs (see Appendix B and I) were measured under the appropriate gate time and wavelengths for direct isomer identification in synthetic mixtures. The excellent R values confirm linear correlations between fluorescence signals and HMW-PAH concentrations. The RSDs reflect the outstanding reproducibility of measurements with cryogenic fiber optic probe.¹³¹,¹⁴¹,¹⁵³ A head-to-head comparison of the LOD values clearly shows the advantage of our pre-concentration approach, which provided improvements varying from 6.6x (DB[a,i]P) to 18.8x (DB[a,l]P). One should notice that better LOD could have been obtained by using the maximum excitation and emission wavelength of each isomer.
Table 3.3 Analytical figures of merit via LETRSS and SPNE-LETRSS.

<table>
<thead>
<tr>
<th>HMW-PAH&lt;sup&gt;a&lt;/sup&gt;</th>
<th>(\lambda_{\text{exc}}/\lambda_{\text{em}})&lt;sup&gt;b&lt;/sup&gt; (nm)</th>
<th>t&lt;sub&gt;d&lt;/sub&gt;/t&lt;sub&gt;g&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt; (ns)</th>
<th>LDR&lt;sup&gt;d&lt;/sup&gt; (ng mL&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>R&lt;sup&gt;e&lt;/sup&gt;</th>
<th>LOD&lt;sup&gt;f&lt;/sup&gt; (ng L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>RSD&lt;sup&gt;g&lt;/sup&gt; (%)</th>
<th>LDR&lt;sup&gt;d&lt;/sup&gt; (ng L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>R&lt;sup&gt;e&lt;/sup&gt;</th>
<th>LOD&lt;sup&gt;f&lt;/sup&gt; (ng L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>RSD&lt;sup&gt;g&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB[(a,l)]P</td>
<td>322.0/417.0</td>
<td>10/250</td>
<td>1.50-200</td>
<td>0.9999</td>
<td>450</td>
<td>1.60</td>
<td>80-5000</td>
<td>0.9990</td>
<td>24.0</td>
<td>1.78</td>
</tr>
<tr>
<td>DB[(a,i)]P</td>
<td>315.0/429.4</td>
<td>10/650</td>
<td>1.14-200</td>
<td>0.9978</td>
<td>340</td>
<td>0.74</td>
<td>180-5000</td>
<td>0.9960</td>
<td>55.0</td>
<td>2.63</td>
</tr>
<tr>
<td>DB[(a,h)]P</td>
<td>316.0/446.6</td>
<td>10/15</td>
<td>1.17-200</td>
<td>0.9991</td>
<td>350</td>
<td>1.54</td>
<td>150-5000</td>
<td>0.9990</td>
<td>44.0</td>
<td>1.46</td>
</tr>
<tr>
<td>N[(2,3-a)]P</td>
<td>321.0/458.0</td>
<td>10/25</td>
<td>0.74-200</td>
<td>0.9990</td>
<td>220</td>
<td>1.07</td>
<td>50-5000</td>
<td>0.9980</td>
<td>16.0</td>
<td>2.89</td>
</tr>
</tbody>
</table>

<sup>a</sup> All HMW-PAH solutions were made in 1% methanol-water (v/v).
<sup>b</sup> Excitation (\(\lambda_{\text{exc}}\)) and emission (\(\lambda_{\text{em}}\)) wavelengths.
<sup>c</sup> Optimum gate delay (t<sub>d</sub>) and gate width (t<sub>g</sub>).
<sup>d</sup> Linear dynamic ranges (LDR) are calculated from the limit of quantitation (LOQ), where LOQ = 10S<sub>B</sub>/m.
<sup>e</sup> R = correlation coefficient of linear dynamic range.
<sup>f</sup> Limit of detection (LOD) were calculated on the basis of the equation LOD = 3S<sub>B</sub>/m.
<sup>g</sup> Relative standard deviation (RSD) = S<sub>F</sub>/I<sub>F</sub> x 100, where S<sub>F</sub> is the standard deviation of the average calculated from six fluorescence measurements at medium linear PAH concentrations.
<sup>h</sup> All extractions were made with 950 µL of Au NPs (7 x 10<sup>11</sup> particles mL<sup>-1</sup>).
3.3.6 HMW-PAHs Determination in Water Samples

The recovery of SPNE-LETRSS was first investigated with tap water samples of unknown matrix composition. Each water sample was collected at different locations of the East Orlando area, FL, and spiked with micro-liter of synthetic mixtures prepared in pure methanol. The final concentration of each isomer in the water sample was 2 ng mL\(^{-1}\). SPNE was carried out according to the experimental procedure in Figure 3.1 and quantitative analysis was made via the calibration curve method using the AFOM in Table 3.3.

Table 3.4 summarizes the average values of the overall recoveries and the standard deviations for the three samples. The overall recoveries were calculated with equation 3.1. Within a confidence interval of 95% \((N_1 = N_2 = N_3 = 3)\),\(^{147}\) the overall recoveries of HMW-PAHs from the three samples were statistically equivalent, which indicates no matrix effects from the unknown composition of water samples. Statistical equivalence \((\alpha = 0.05; N_1 = N_2 = N_3 = 3)\)\(^{147}\) was also observed among the overall recoveries in Table 3.5, which were obtained from water sample #1 spiked with the sixteen EPA-PAHs at different concentration levels. These results are in good agreement with the spectral purity observed at the target fluorescence peak of each HMW-PAH. Single exponential decays (see Appendix J) with fluorescence lifetime values equivalent to those in Table 3.3 were obtained in all cases. These facts demonstrate the accurate and unambiguous determination of each isomer in water samples of increasing complexity.
### Table 3.4 HMW-PAH recoveries from drinking water samples.

| HMW-PAHa | Sample 1 | | Sample 2 | | Sample 3 | |
|-----------|----------|----------|----------|----------|----------|
|           | Recoveryb (%) | RSDc (%) | Recoveryb (%) | RSDc (%) | Recoveryb (%) | RSDc (%) |
| DB[a,l]P  | 96.3 ± 3.8  | 3.9      | 106.6 ± 6.2  | 5.9      | 100.9 ± 6.7  | 6.6      |
| DB[a,i]P  | 93.8 ± 6.2  | 6.6      | 100.0 ± 2.9  | 2.9      | 99.2 ± 9.5   | 9.5      |
| DB[a,h]P  | 99.4 ± 8.5  | 8.6      | 93.8 ± 5.6   | 6.0      | 93.0 ± 8.6   | 9.2      |
| N[2,3-a]P | 91.7 ± 3.8  | 4.2      | 98.8 ± 5.0   | 5.0      | 96.8 ± 2.5   | 2.6      |

a All working solutions were prepared in 1% methanol/water (v/v). All HMW-PAH were spiked at the 2.0 ng·mL⁻¹ concentration. b All extractions were made with 950 µL of Au NPs (7 x 10¹¹ particles·mL⁻¹). Tabulated values represent the averages of triplicate measurements taken from three aliquot samples. c Relative Standard Deviation.

### Table 3.5 HMW-PAH recoveries from drinking water samples spiked with 16 EPA-PAH.

<table>
<thead>
<tr>
<th>HMW-PAHa</th>
<th>0.2 ng mL⁻¹ EPA-PAH</th>
<th>2.0 ng mL⁻¹ EPA-PAH</th>
<th>20 ng mL⁻¹ EPA-PAH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recoveryb (%)</td>
<td>RSDc (%)</td>
<td>Recoveryb (%)</td>
</tr>
<tr>
<td>DB[a,l]P</td>
<td>96.8 ± 1.6</td>
<td>1.6</td>
<td>102.8 ± 5.9</td>
</tr>
<tr>
<td>DB[a,i]P</td>
<td>90.6 ± 6.5</td>
<td>7.2</td>
<td>96.7 ± 8.9</td>
</tr>
<tr>
<td>DB[a,h]P</td>
<td>97.9 ± 9.3</td>
<td>9.5</td>
<td>95.0 ± 9.3</td>
</tr>
<tr>
<td>N[2,3-a]P</td>
<td>93.1 ± 3.3</td>
<td>3.6</td>
<td>105.0 ± 7.7</td>
</tr>
</tbody>
</table>

a All working solutions were prepared in 1% methanol/water (v/v). HMW-PAH concentrations were fixed at 2.0 ng mL⁻¹ measurements. b All extractions were made with 950 µL of Au NPs (7 x 10¹¹ particles·mL⁻¹). Tabulated values represent the averages of triplicate measurements taken from three aliquot samples. c Relative standard deviation.
3.4 Conclusions

The chromatographic analysis of structural isomers of HMW-PAH is a challenging problem. HPLC with absorption or fluorescence detection is not specific enough, particularly in the case of co-eluting isomers. In the specific case of water analysis, the rather low concentrations of HMW-PAH force the analyst to pre-concentrate the sample prior to chromatographic analysis. The numerous steps and lengthy experimental procedures make classical methodology laborious and time-consuming. It is under this prospective that the work presented here becomes relevant. The small volume of water sample (500 µL) one needs for complete analysis facilitates the implementation of SPNE for routine analysis of HMW-PAH in numerous samples. In comparison to the SPNE procedure originally developed for EPA-PAHs, the current method reduces extraction time from 30 to 20 min per sample. Quantitative release of DB[a,l]P, DB[a,h]P, DB[a,i]P and N[2,3-a]P into the Shpol’skii matrix (n-octane) is best accomplished with a mixture of 48 µL of methanol and 2 µL of 1-pentanethiol. The migration of these four isomers into the 50 µL layer of n-octane provides highly resolved spectra with distinct fluorescence lifetimes for their unambiguous determination. Under these experimental conditions, the complete analysis of DB[a,l]P, DB[a,h]P, DB[a,i]P and N[2,3-a]P takes less than 30 min per sample and consumes only 100 µL of organic solvents. Although a straightforward comparison to chromatographic methods is not possible because of the lack of AFOM on HMW-PAH, the excellent precision of measurements, limits of detection and overall recoveries turn SPNE-LETRESS into a valuable tool for the water analysis of DB[a,l]P, DB[a,h]P, DB[a,i]P and N[2,3-a]P.
CHAPTER 4  WATER ANALYSIS OF THE SIXTEEN ENVIRONMENTAL PROTECTION AGENCY – POLYCYCLIC AROMATIC HYDROCARBONS VIA SOLID-PHASE NANOEXTRACTION – GAS CHROMATOGRAPHY/MASS SPECTROMETRY


4.1 Introduction

Previous chapters of this dissertation described the development of environmentally friendly (green) analytical methodology based on SPNE-LETRSS. Complete analysis of HMW-PAHs was accomplished in 25 mins per sample with 100 µL of organic solvent. In this chapter, we present an alternative green method based on SPNE-GC/MS for the analysis of 16 EPA-PAHs. Within the concept of green analytical chemistry,91-93 HPLC presents a distinct disadvantage over GC/MS, which is the need of organic solvents for PAHs separation. Under classic EPA methodology,35 the HPLC analysis of the sixteen priority pollutants consumes approximately 75 mL of mobile phase (methanol-water) per sample. Considering the gradient elution of the chromatographic separation, this mobile phase volume is equivalent to 66 mL of methanol. Adding the volumes of eluting solvents from the SPE procedure – i.e., 5 mL of ethyl acetate and 5 mL of methyl chloride - the total volume of organic solvents is approximately 76 mL per sample.

SPNE-GC/MS requires only 100 µL of organic solvent per sample. Separation and determination of the sixteen EPA-PAHs is accomplished in 22 min via an optimized GC/MS procedure. The entire analysis – i.e., SPNE and GC/MS – takes less than 45 min per sample. The LODs obtained with 500 µL of water samples ranged from 4.94 (fluoranthene) to 65.5 ng L⁻¹
(fluorene). The RSDs at medium calibration concentrations varied from 2.4 (acenaphthene) to 7.8% (dibenz[a,h]anthracene). The analytical recoveries from river water samples of unknown composition ranged from 93.4 ± 2.9 (indeno[1,2,3-cd]pyrene) to 100.7 ± 2.1 (benzo[k]fluoranthene). The excellent AFOM – associated to the simplicity, short analysis time and reduced solvent consumption – demonstrated the potential of SPNE-GC/MS for the routine monitoring of EPA-PAHs via environmentally friendly methodology.

4.2 Experimental Section

4.2.1 Chemicals

Nanopure water from a Barnstead Nanopure Infinity water system was used throughout. 20 nm average diameter Au NPs in aqueous solutions (7 x 10^{11} particles mL^{-1}) were purchased from Ted Pella, Inc. (Redding, CA). HPLC grade methanol was purchased from Fischer Scientific (Pittsburg, PA). Analytical grade 1-pentanethiol and n-octane were from Acros Organics (Atlanta, GA). Benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, indeno[1,2,3-cd]pyrene, naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene and benzo[ghi]perylene were purchased from Sigma-Aldrich (Milwaukee, WI) at their highest available purity (≥ 98%).

*Note: use extreme caution when handling PAHs that are known to be extremely toxic.*
4.2.2 Solution Preparation

Stock PAH solutions were prepared in either HPLC grade methanol or n-octane and kept in the dark at 4°C. Possible PAH degradation was monitored via room-temperature fluorescence spectroscopy. Working solutions of PAHs were prepared by serial dilution of stock solutions with the appropriate solvent. Commercial solutions of Au NPs were kept in the dark at 4°C. The physical integrity of Au NPs was monitored periodically via ultraviolet-visible absorption spectroscopy.

4.2.3 Extraction of EPA-PAHs with Au NPs

A 500 μL aliquot of the water samples was mixed with 1 mL of 20 nm Au NPs. The mixture was shaken for 5 min at 1400 rpm and centrifuged for 10 min at 13,400 rpm. The supernatant was separated from the precipitate with a micro-pipette. 2 μL of 1-pentanethiol, 48 μL of methanol, and 50 μL of n-octane were added to the precipitate. The new mixture was shaken for 5 min at 1400 rpm. All mixing and shaking times were previously optimized for best PAH recovery. 85

4.2.4 Sample Mixing and Centrifugation for PAHs Extraction with Gold Colloids

See section 2.2.3 for experimental details.
4.2.5 UV-VIS Absorption Spectroscopy

See section 2.2.5 for instrumental and experimental details.

4.2.6 RTF Spectroscopy

See section 2.2.6 for instrumental and experimental details.

4.2.7 Gas Chromatography/Mass Spectrometry

GC/MS was carried out with the aid of a gas chromatograph (6850 GC, Agilent, Avondale PA) coupled to a quadruple mass spectrometer with electron impact (EI) ionization at 70 eV (5975 VL, Agilent). The GC was equipped with an auto sampler using a 5 µL syringes. Pulsed, 2 µL splitless injections were performed at 275 °C and purged for 100 mL min⁻¹ at 0.25 min. Separations were carried out on a 5% phenyl methyl siloxane column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The temperatures of transfer line, manifold and source of ionization were set at 280 °C, 280 °C and 230 °C, respectively.

The oven temperature program for “slow” separation conditions were 50 °C for 0.8 min followed by temperature increases to 200 °C at 10 °C min⁻¹, 225 °C at 2.0 °C min⁻¹, 266 °C at 2.0 °C min⁻¹ and 285 °C at 2.0 °C min⁻¹. The solvent delay time was 6.0 min and the total run time was 60 min. The oven temperature program for “fast” separation conditions were 80 °C for 1.0 min, temperature increase to 250 °C at 25 °C min⁻¹, held constant for 6.0 min, temperature increase to 300 °C at 10.0 °C min⁻¹ and held constant for 2.0 min. The solvent delay time was 3.5
min and the total run time was 22 min. In all cases, the carrier gas was ultrapure helium at constant flow rates of 0.8 mL min\(^{-1}\) (slow) and 1.8 mL min\(^{-1}\) (fast).

PAH peak identification was based on the retention times and full scan spectra of the standards. A mass range of \(m/z\) 50 – 300 was recorded in the full-scan mode. AFOM in the selective ion monitoring (SIM) mode were obtained at the main molecular ion peaks of individual PAHs, namely \(m/z = 128\) (naphthalene), \(m/z = 152\) (acenaphthylene), \(m/z = 154\) (acenaphthene), \(m/z = 166\) (fluorene), \(m/z = 178\) (phenanthrene and anthracene), \(m/z = 202\) (fluoranthenne and pyrene), \(m/z = 228\) (benz[a]anthracene and chrysene), \(m/z = 252\) (benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene), \(m/z = 276\) (indeno[1,2,3-cd]pyrene and benzo[ghi]perylene) and \(m/z = 278\) (dibenz[a,h]anthracene).

4.3 Results and Discussions

4.3.1 GC Separation of the Sixteen EPA-PAHs

One of the challenges facing GC/MS for the analysis EPA-PAHs is the separation of priority pollutants with the same molecular weight and virtually identical fragmentation patterns.\(^{154, 155}\) The most popular GC column for the separation of EPA-PAHs utilizes a stationary phase composed of 5% phenyl and 95% methylpolysiloxane (DB-5 ms).\(^{54, 154-165}\) Depending on the complexity of the sample matrix, the length of the column may vary between 15 and 60 m. In highly complex environmental matrixes, the individual resolution of the sixteen EPA-PAHs has been accomplish mainly with 60 m DB-5 ms columns.\(^{154-156}\)
The length of the DB-5 ms column chosen for the present studies – 30 m – provides a reasonable compromise between chromatographic resolution and analysis time.\textsuperscript{54, 157-165} Within the sixteen EPA-PAHs, three groups present the main resolution challenge, namely (i) chrysene and benz[a]anthracene; (ii) benzo[b]fluoranthene and benzo[k]fluoranthene; and (iii) dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene. Their complete separation in the chromatographic column is particularly relevant to distinguish between the MW isomers chrysene /benz[a]anthracene and benzo[b]fluoranthene/ benzo[k]fluoranthene, which have very similar mass fragmentation patterns.

Figure 4.1 compares the GC/MS chromatograms of a synthetic mixture with the sixteen EPA-PAHs recorded under slow and fast separation conditions. The fragmentation patterns of the sixteen priority pollutants are shown in Appendix K. Table 4.1 summarizes the averages of separation parameters calculated from three independent chromatographic runs under “slow” and “fast” conditions. Within a confidence interval of 95\%,\textsuperscript{147} the retention times of the sixteen EPA-PAHs were statistical different from each other under the two sets of experimental conditions. For all the studied PAHs, reducing the total analysis time from 60 to 22 mins increased the intensity of their analyte signals (peak height) and reduced the FWHM of their chromatographic peaks.

The extent to which the faster separation conditions affect the resolution of the most challenging EPA-PAHs is illustrated in Figure 4.2. Similar degrees of peak overlapping as those in Figure 4.2 have been previously reported with the same length of DB-5ms column (30 min) but at the expenses of longer analysis times (30 – 40 min).\textsuperscript{161-163} Table 4.2 compares the resolution (R\textsubscript{s}) and number of theoretical plates (N) obtained under the two sets of separation
conditions. Although baseline resolution \( (R_s = 1.5) \) was not achieved in any of the studied cases, quantitative separation was still possible under both slow and fast GC/MS conditions.

Figure 4.1 GC/MS chromatogram of a standard mixture of the sixteen EPA-PAHs at the \( 1.0 \times 10^4 \text{ ng L}^{-1} \) concentration level recorded under slow (A) and fast (B) separation conditions. Peak identities are (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzo[a]anthracene, (10) chrysene, (11) benzo[b]fluoranthene, (12) benzo[k]fluoranthene, (13) benzo[a]pyrene, (14) indeno[1,2,3-cd]pyrene, (15) dibenz[a,h]anthracene and (16) benzo[ghi]perylene.
Table 4.1 Comparison of chromatographic parameters for the GC/MS separation of the sixteen EPA-PAHs under slow and fast separation conditions.

<table>
<thead>
<tr>
<th>PAH</th>
<th>Slow-GC/MS</th>
<th>Fast-GC/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention Time (min)</td>
<td>Signal Intensity (x10^3 r.u.)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>9.67 ± 0.002</td>
<td>4.39 ± 0.09</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>13.31 ± 0.004</td>
<td>2.81 ± 0.05</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>13.74 ± 0.001</td>
<td>3.16 ± 0.05</td>
</tr>
<tr>
<td>Fluorene</td>
<td>14.94 ± 0.001</td>
<td>2.25 ± 0.04</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>17.33 ± 0.002</td>
<td>2.25 ± 0.10</td>
</tr>
<tr>
<td>Anthracene</td>
<td>17.45 ± 0.003</td>
<td>2.96 ± 0.01</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>21.92 ± 0.001</td>
<td>2.17 ± 0.05</td>
</tr>
<tr>
<td>Pyrene</td>
<td>23.03 ± 0.006</td>
<td>1.80 ± 0.02</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>31.24 ± 0.001</td>
<td>1.79 ± 0.01</td>
</tr>
<tr>
<td>Chrysene</td>
<td>31.52 ± 0.006</td>
<td>1.89 ± 0.02</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>40.40 ± 0.010</td>
<td>1.39 ± 0.01</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>40.60 ± 0.008</td>
<td>1.55 ± 0.02</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>42.96 ± 0.009</td>
<td>1.54 ± 0.01</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>52.47 ± 0.012</td>
<td>1.82 ± 0.02</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>52.88 ± 0.005</td>
<td>1.63 ± 0.02</td>
</tr>
<tr>
<td>Benzo[ghi]perylen</td>
<td>54.50 ± 0.007</td>
<td>1.64 ± 0.01</td>
</tr>
</tbody>
</table>

a Individual PAH concentrations = 1.0 x 10^4 ng L^-1. Working solutions were prepared in n-octane. 2 µL injection volume. b FWHM, full-width at half-maximum.
Figure 4.2 Separation of the critical EPA-PAH pairs at the $1.0 \times 10^4$ ng L$^{-1}$ concentration level under slow (A) and fast (B) GC/MS conditions. Peak identities are (9) benzo[a]anthracene, (10) chrysene, (11) benzo[b]fluoranthene, (12) benzo[k]fluoranthene, (14) indeno[1,2,3-cd]pyrene, (15) dibenz[a,h]anthracene.

Table 4.2 Comparison of chromatographic resolution and number of theoretical plates under slow and fast GC/MS conditions.

<table>
<thead>
<tr>
<th>PAH</th>
<th>Slow-GC/MS</th>
<th>Fast-GC/MS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R_s^b$</td>
<td>$N (\times 10^5)^c$</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>1.212</td>
<td>7.31</td>
</tr>
<tr>
<td>Chrysene</td>
<td></td>
<td>6.65</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>0.971</td>
<td>7.61</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td></td>
<td>6.45</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td></td>
<td>8.75</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>1.167</td>
<td>7.07</td>
</tr>
</tbody>
</table>

$^a$ All working solutions were prepared in n-octane. $^b$ Injection volume. $^c$ Resolution ($R_s$) between peaks was calculated with the equation $R_s = 2(t_2 - t_1)/w_2 - w_1$; where $t_1$ and $t_2$ are retention times of peaks 1 and 2, respectively (Table 1), and $w_1$ and $w_2$ are the widths at the base of each peak. $^c$ Number of theoretical plates (N) of each PAH was determined with the equation $N = 5.54(t_m/w_{0.5})^2$; where $t_m$ is the retention time and $w_{0.5}$ is the width at half height of the peak.
4.3.2 **AFOM under Slow and Fast GC/MS**

Table 4.3 summarizes the AFOM of the sixteen priority pollutants obtained via GC/MS under the two set of separation conditions. The plots with the calibration curves are shown in Appendixes L and M. The LDRs of the calibration curves were built with a minimum of six PAH concentrations. The average peak heights plotted in the calibration graphs correspond to a minimum of three measurements recorded from three independent chromatographic runs. No efforts were made to reach the upper concentration limits of the calibration curves. The excellent R values demonstrate the existence of linear relationships in all cases. The lowest linear concentration corresponds to the LOQ. The LODs and the LOQs were calculated for a signal-to-noise (S/Np-p) ratio of 3 and 10, respectively. The Np-p was measured at the base peak of each PAH over a sufficiently wide region of the chromatogram. The increase in peak intensity observed under fast separation conditions led to LOD improvements ranging from 3x (benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene and benzo[ghi]perylene) to 8x (naphthalene, acenaphthylene and acenaphthene). The rather low RSDs reflect the outstanding precision of the measurements under both sets of separation conditions.
Table 4.3 Analytical figures of merit via slow and fast GC/MS methods.

<table>
<thead>
<tr>
<th>PAH a</th>
<th>Slow-GC/MS</th>
<th></th>
<th>Fast-GC/MS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDR b (10³ ng L⁻¹)</td>
<td>R² c</td>
<td>LOD d (ng L⁻¹)</td>
<td>RSD e (%)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>1.70 – 100</td>
<td>0.9992</td>
<td>514</td>
<td>3.1</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>5.32 – 100</td>
<td>0.9996</td>
<td>1610</td>
<td>5.9</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>4.65 – 100</td>
<td>0.9988</td>
<td>1408</td>
<td>5.4</td>
</tr>
<tr>
<td>Fluorene</td>
<td>2.07 – 100</td>
<td>0.9991</td>
<td>628</td>
<td>0.8</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>2.63 – 100</td>
<td>0.9992</td>
<td>796</td>
<td>4.2</td>
</tr>
<tr>
<td>Anthracene</td>
<td>2.06 – 100</td>
<td>0.9980</td>
<td>624</td>
<td>4.2</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>1.35 – 10.0</td>
<td>0.9993</td>
<td>408</td>
<td>2.2</td>
</tr>
<tr>
<td>Pyrene</td>
<td>1.26 – 10.0</td>
<td>0.9991</td>
<td>382</td>
<td>1.1</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>1.91 – 10.0</td>
<td>0.9987</td>
<td>442</td>
<td>4.7</td>
</tr>
<tr>
<td>Chrysene</td>
<td>0.86 – 10.0</td>
<td>0.9988</td>
<td>261</td>
<td>2.6</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>3.22 – 10.0</td>
<td>0.9989</td>
<td>977</td>
<td>1.5</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>3.46 – 10.0</td>
<td>0.9992</td>
<td>1047</td>
<td>2.6</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>3.05 – 10.0</td>
<td>0.9985</td>
<td>925</td>
<td>3.7</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>3.03 – 10.0</td>
<td>0.9927</td>
<td>919</td>
<td>1.3</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>3.34 – 10.0</td>
<td>0.9947</td>
<td>1012</td>
<td>1.0</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>2.43 – 10.0</td>
<td>0.9935</td>
<td>737</td>
<td>2.4</td>
</tr>
</tbody>
</table>

a All working solutions were prepared in n-octane. Injection volume = 2 µL. b Linear dynamic range (LDR); lower concentration limit = limit of quantitation (LOQ) estimated as S/N = 10. c R = correlation coefficient. d Limit of detection (LOD) estimated as S/N = 3. e Relative standard deviation (RSD) calculated from medium concentrations of linear dynamic ranges.
4.3.3 SPNE

SPNE was originally devised for the analysis of EPA-PAHs\(^4\).\(^{166}\).\(^{167}\) The original procedure was later modified for the extraction of HMW-PAHs\(^168\).\(^{169}\) Herein, we compare the two SPNE procedures (see Figure 3.1 of this dissertation) for the extraction of EPA-PAHs. The plot in Figure 4.3 compares the overall recoveries (OR) of the sixteen EPA-PAHs obtained with the two SPNE procedures. All extractions were made from Nanopure water samples previously spiked with the sixteen priority pollutants at the final concentration of 1.0 x 10\(^3\) ng L\(^{-1}\) (1% methanol-water, v/v). The OR values were calculated according to equation 4.1:

\[
OR = 100 \left( \frac{[PAH]_O \cdot V_O}{[PAH]_{H2O} \cdot V_{H2O}} \right)
\]

(4.1)

where \(V_O\) and \(V_{H2O}\) correspond to the volumes of supernatant (n-octane) and water sample, respectively; \([PAH]_{H2O} = 1.0 \times 10^3\) ng L\(^{-1}\) and \([PAH]_O\) is the PAH concentration in the supernatant (n-octane). \([PAH]_O\) values were obtained via the fast GC/MS method. All concentrations in the supernatants were within the linear dynamic ranges of individual PAHs in n-octane (see Table 4.3). Within a confidence interval of 95\% (\(N_1 = N_2 = 3\)),\(^147\) the ORs of the new procedure were better for all the studied PAHs with the exception of acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene and benz[a]anthracene. The observed differences among the ORs of EPA-PAHs reflect differences in relative affinity towards the surface of Au NPs and the supernatants. Similar to HMW-PAHs,\(^168\).\(^{169}\) the presence of methanol appears to facilitate the partitioning of most EPA-PAHs into the layer of n-octane. All further studies were then carried out using a releasing mixture composed of 2 \(\mu\)L of 1-pentanethiol, 48 \(\mu\)L of methanol and 50 \(\mu\)L of n-octane.
Figure 4.3 Comparison of overall recoveries obtained via the original and the new SPNE procedure. All extractions were carried out with 1 mL of 20 nm Au NPs in aqueous solution. Extractions were made from 500 µL of Nanopure water spiked with PAHs at a final concentration of 1.0 x 10^3 ng L\(^{-1}\). All overall recoveries were quantitatively determined via fast GC-MS.

4.3.4 *AFOM of SPNE-GC/MS*

Table 4.4 summarizes the AFOM of the sixteen EPA-PAHs obtained via SPNE-GC/MS. All extractions were made from 500 µL of Nanopure water previously spiked with standard mixtures of the sixteen priority pollutants in 1% methanol-water (v/v). A volume of 1 mL of Au NPs solution was used in all cases, which should be able to extract the sixteen EPA-PAHs at the 6.4 x 10^4 ng L\(^{-1}\) individual concentration. Each LDR was estimated with a minimum of six PAH concentrations. The peak intensities plotted in the calibration graphs (see Appendix N) corresponded to the averages of nine GC/MS measurements made from triplicate aliquots (2 µL.
each) of three complete SPNE trials. No efforts were made to reach the upper concentration limits of the LDRs. The lowest linear concentrations correspond to the LOQ values, which were calculated as those described in Table 4.3. The existence of linear relationships was confirmed with excellent R values in all cases. The RSDs reflect the outstanding reproducibility of SPNE-GC/MS measurements at the parts-per-trillion concentration level. All LODs were at the parts-per-trillion level and well below the MCL of regulated EPA-PAHs. Comparison of SPNE-GC/MS values to those reported in Table 4.3 (fast GC/MS) show an overall LOD improvement of approximately one order of magnitude. ORs were calculated according to equation 4.1.

Table 4.4 SPNE-GC/MS analytical figures of merit for the sixteen EPA-PAHs.

<table>
<thead>
<tr>
<th>PAH a</th>
<th>LDR b (ng L⁻¹)</th>
<th>R² c</th>
<th>LOD d (ng L⁻¹)</th>
<th>RSD e (%)</th>
<th>Recoveries (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>28 – 5,000</td>
<td>0.9965</td>
<td>8.40</td>
<td>3.5</td>
<td>102.4 ± 4.3</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>46 – 5,000</td>
<td>0.9978</td>
<td>14.0</td>
<td>5.0</td>
<td>95.9 ± 5.5</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>60 – 5,000</td>
<td>0.9955</td>
<td>18.3</td>
<td>2.4</td>
<td>95.9 ± 3.3</td>
</tr>
<tr>
<td>Fluorene</td>
<td>216 – 5,000</td>
<td>0.9966</td>
<td>65.5</td>
<td>3.3</td>
<td>42.7 ± 2.2</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>41 – 5,000</td>
<td>0.9998</td>
<td>12.4</td>
<td>3.7</td>
<td>101.6 ± 5.9</td>
</tr>
<tr>
<td>Anthracene</td>
<td>38 – 5,000</td>
<td>0.9997</td>
<td>11.4</td>
<td>4.3</td>
<td>97.3 ± 4.0</td>
</tr>
<tr>
<td>Fluoranthe</td>
<td>16 – 5,000</td>
<td>1.0000</td>
<td>4.94</td>
<td>4.5</td>
<td>101.8 ± 6.8</td>
</tr>
<tr>
<td>Pyrene</td>
<td>21 – 5,000</td>
<td>0.9999</td>
<td>6.40</td>
<td>4.2</td>
<td>100.2 ± 5.3</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>26 – 5,000</td>
<td>0.9950</td>
<td>7.88</td>
<td>6.8</td>
<td>99.8 ± 1.8</td>
</tr>
<tr>
<td>Chrysene</td>
<td>27 – 5,000</td>
<td>0.9984</td>
<td>8.12</td>
<td>5.9</td>
<td>96.6 ± 2.1</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>60 – 5,000</td>
<td>0.9980</td>
<td>18.2</td>
<td>7.1</td>
<td>95.4 ± 4.7</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>61 – 5,000</td>
<td>0.9969</td>
<td>18.5</td>
<td>3.5</td>
<td>103.5 ± 1.2</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>55 – 5,000</td>
<td>0.9945</td>
<td>16.5</td>
<td>5.5</td>
<td>92.8 ± 2.4</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>43 – 5,000</td>
<td>0.9942</td>
<td>13.0</td>
<td>6.1</td>
<td>95.9 ± 3.5</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>45 – 5,000</td>
<td>0.9958</td>
<td>13.5</td>
<td>7.8</td>
<td>98.7 ± 2.8</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>51 – 5,000</td>
<td>0.9969</td>
<td>15.4</td>
<td>6.2</td>
<td>99.9 ± 4.0</td>
</tr>
</tbody>
</table>

a All working solutions were prepared in 1% methanol. b Linear dynamic range (LDR); lower concentration limit = limit of quantitation (LOQ) estimated as S/N = 10. c R = correlation coefficient. d Limit of detection (LOD) estimated as S/N = 3. e Relative standard deviation (RSD) calculated from medium concentrations of linear dynamic ranges.
4.3.5 SPNE-GC/MS Analysis of EPA-PAHs in Water Samples of Unknown Composition

The feasibility to monitor the sixteen EPA-PAHs in samples of unknown composition was investigated with water samples from the St. John’s River collected at different locations of the Hontoon Island State Park (Deland, FL, USA). All samples were collected in 7 mL dark amber vials and kept in the dark at 4 °C until further analysis. Since no EPA-PAHs were detected in the collected samples, potential matrix interference was then investigated by spiking the collected samples with microliter volumes of standard EPA-PAHs mixtures at the parts-per-billion concentration level. SPNE was carried out with 500 µL aliquots according to the new procedure depicted in Figure 3.1. GC/MS analysis was performed under fast separation conditions. The obtained chromatograms are presented in Appendix O. Table 4.5 summarizes the ORs and the RSDs of the analyzed samples. In all cases, the RSDs were lower than 25%. This feature meets the criterion for the analysis of regulated PAHs in water samples. With the exception of naphthalene and benzo[k]fluoranthene, all the other recoveries were statistically equivalent ($\alpha = 0.05, N_1 = N_2 = 3$) to those reported obtained with Nanopure water (see Table 4.4). Based on these results, it is possible to state that the matrix composition of the St. John’s River had minimal effect on the ORs of EPA-PAHs.
Table 4.5 Overall recoveries of sixteen EPA-PAHs in St. John’s river water samples via SPNE-GC/MS.

<table>
<thead>
<tr>
<th>PAH</th>
<th>Recovery (^b) (%)</th>
<th>RSD (^c) (%)</th>
<th>(t_{\text{exp}}) (^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>85.1 ± 2.5</td>
<td>2.89</td>
<td>6.06</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>85.5 ± 5.9</td>
<td>6.89</td>
<td>2.25</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>91.5 ± 1.9</td>
<td>2.11</td>
<td>2.01</td>
</tr>
<tr>
<td>Fluorene</td>
<td>37.1 ± 0.4</td>
<td>1.16</td>
<td>1.75</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>102.3 ± 9.6</td>
<td>9.37</td>
<td>0.11</td>
</tr>
<tr>
<td>Anthracene</td>
<td>93.4 ± 2.2</td>
<td>2.35</td>
<td>1.49</td>
</tr>
<tr>
<td>Fluoranthe</td>
<td>94.5 ± 1.6</td>
<td>1.68</td>
<td>2.07</td>
</tr>
<tr>
<td>Pyrene</td>
<td>96.0 ± 2.4</td>
<td>2.45</td>
<td>1.26</td>
</tr>
<tr>
<td>Benz[a]anthracene</td>
<td>101.5 ± 1.3</td>
<td>1.30</td>
<td>1.33</td>
</tr>
<tr>
<td>Chrysene</td>
<td>93.3 ± 0.8</td>
<td>0.85</td>
<td>2.56</td>
</tr>
<tr>
<td>Benzo[b]fluoranthe</td>
<td>100.7 ± 2.1</td>
<td>2.09</td>
<td>1.79</td>
</tr>
<tr>
<td>Benzo[k]fluoranthe</td>
<td>98.7 ± 2.1</td>
<td>2.15</td>
<td>3.46</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>96.7 ± 2.2</td>
<td>2.25</td>
<td>2.09</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>93.4 ± 2.9</td>
<td>3.13</td>
<td>0.96</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>99.6 ± 2.7</td>
<td>2.71</td>
<td>0.40</td>
</tr>
<tr>
<td>Benzo[ghi]perylene</td>
<td>95.8 ± 2.9</td>
<td>2.98</td>
<td>1.45</td>
</tr>
</tbody>
</table>

\(^a\) All working solutions were prepared in 1% methanol/water (v/v). All EPA-PAHs were spiked at the 1.0 \(\times\) 10\(^3\) ng L\(^{-1}\) concentration levels. Injection volume = 2 \(\mu\)L. \(^b\) All extractions were made with 1 mL of Au NPs in aqueous solution (7.0 \(\times\) 10\(^{11}\) particles mL\(^{-1}\)). Tabulated values represent the averages of triplicate measurements taken from three aliquot samples. \(^c\) Relative Standard Deviation (RSD). \(^d\) \(t\)-test of recovery for EPA-PAHs in nanopure water (Table 4). \(t_{\text{critical}}\) = 2.78

### 4.4 Conclusion

A wide range of analytical recoveries have been reported for the water analysis of EPA-PAHs via SPE-HPLC and SPE-GC/MS (see Table 1.2). The possible low recoveries for several PAHs and the relatively long analysis times of SPE procedures have prompted the development of new adsorbent materials for PAH extraction.\(^{54, 86, 88, 161-165, 171-179}\) Table 4.6 compares the SPNE procedure to those previously combined with GC/MS.\(^{54, 161-165, 171-173}\) SPNE-GC/MS presents several features that meet the concept of green analytical methodology.\(^{91-93}\) The small volumes of organic solvents and extracting solution qualify SPNE-GC/MS as a cost effective
method with low solvent consumption. The total working volume (1.6 mL) facilitates the implementation of simultaneous extraction of numerous samples. By operating our centrifuge at its maximum capacity, we were able to process twelve samples in 20 min of extraction time. These features - associated to the excellent AFOMs - make SPNE-GC/MS a valuable alternative for the routine monitoring of EPA-PAHs in water samples.
Table 4.6 Comparison of different adsorbent material combined with GC/MS for the analysis of 16 EPA-PAH in water samples.

<table>
<thead>
<tr>
<th>Extraction Material</th>
<th>Sample Size (mL)</th>
<th>Organic Solvent (mL)</th>
<th>Extraction Time (min)</th>
<th>Analysis Time (min)</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
<th>LOD (ng L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AuNPs a</td>
<td>0.5</td>
<td>0.1</td>
<td>20</td>
<td>22</td>
<td>37 – 102.3</td>
<td>1 – 8</td>
<td>4 – 66</td>
</tr>
<tr>
<td>PDMS b</td>
<td>30</td>
<td>0</td>
<td>45</td>
<td>70</td>
<td>-</td>
<td>1 – 20</td>
<td>1 – 29</td>
</tr>
<tr>
<td>PDMS b</td>
<td>10</td>
<td>0</td>
<td>90</td>
<td>60</td>
<td>69 - 105</td>
<td>2 – 24</td>
<td>30 – 270</td>
</tr>
<tr>
<td>Fe₃O₄ c</td>
<td>20</td>
<td>4.5</td>
<td>10</td>
<td>20</td>
<td>35 – 99</td>
<td>2 – 10</td>
<td>800 – 36000</td>
</tr>
<tr>
<td>OPA/MMNPs d</td>
<td>10</td>
<td>0.5</td>
<td>2</td>
<td>30</td>
<td>54 – 119</td>
<td>1 – 12</td>
<td>14 – 70</td>
</tr>
<tr>
<td>SCX-2 e</td>
<td>500</td>
<td>15</td>
<td>60</td>
<td>45</td>
<td>67 – 136</td>
<td>1 – 18</td>
<td>18.4 – 238</td>
</tr>
<tr>
<td>Fe₃O₄ c</td>
<td>20</td>
<td>0.1</td>
<td>46</td>
<td>28</td>
<td>-</td>
<td>4 – 10</td>
<td>15 – 335</td>
</tr>
<tr>
<td>MWCNTs f</td>
<td>20</td>
<td>0.1</td>
<td>35</td>
<td>28</td>
<td>72 – 98</td>
<td>4 – 12</td>
<td>4 – 47</td>
</tr>
<tr>
<td>MWCNTs f</td>
<td>500</td>
<td>36</td>
<td>60</td>
<td>40</td>
<td>70 – 127</td>
<td>1 – 12</td>
<td>2 – 8</td>
</tr>
<tr>
<td>MWCNTs f</td>
<td>50</td>
<td>2</td>
<td>50</td>
<td>65</td>
<td>72 – 93</td>
<td>4 – 14</td>
<td>1 – 150</td>
</tr>
</tbody>
</table>

a Gold Nanoparticles b Polydimethylsiloxane fibres c Silica with strong cation-exchange groups d Fe₃O₄ microspheres e n-Octadecylphosphonic acid modified mesoporous magnetic nanoparticle f Multi-walled Carbon Nanotubes g Based on 12 water samples. h Relative Standard Deviation (RSD). i Limit of detection (LOD) estimated as S/N = 3.
CHAPTER 5  CONCLUDING REMARKS

There has been considerable growth in measurement techniques over the past few decades for the analysis of PAHs with environmental and toxicological importance. Because their carcinogenic properties depend strongly on molecular structure and differ significantly from isomer to isomer, it is of paramount importance to determine the most toxic isomers even if they are present at much lower concentrations than their less toxic isomers. The need for unambiguous isomer identification is further intensified by recent findings attributing a significant portion of the biological activity of PAH contaminated samples to the presence of HMW-PAHs, i.e. PAHs with MW ≥ 300. Unfortunately, classic methodology cannot always meet the challenge of specifically analyzing HMW-PAH at the low concentration levels of environmental samples. The main problems arise from the relatively low concentration levels and the large number of structural isomers with very similar elution times and similar, possibly even virtually identical, fragmentation patterns.

Shpol'skii spectrometry has long been recognized for its capability in providing efficient and adequate resolution of structural isomers at the concentration ratios found in environmental samples. Its widespread use has been hampered by several reasons, including inconvenient sample freezing procedures, questions about signal reproducibility for calibration purposes and lack of reference databases. This dissertation presents significant advances in all fronts. The inconvenience of sample freezing procedures has been eliminated with the aid of cryogenic fiber optic probes. It is now possible to easily perform reproducible and accurate measurements at liquid nitrogen (77 K) liquid helium (4.2 K) temperatures in a matter of seconds. We have developed instrumentation to efficiently collect multidimensional data formats in frozen matrixes.
during the lifetime decays of fluorescence (nanoseconds to microseconds) and phosphorescence (milliseconds to seconds) emission. Previous to our involvement with Shpol’skii spectroscopy, the identification of structural isomers was solely based on wavelength assignments. Our approach takes advantage of the full dimensionality of luminescence spectroscopy combining spectral and lifetime information in WTM and TREEM. Adding the temporal dimension to the highly resolved Shpol’skii spectra provides an extremely selective tool for the determination of structural isomers in complex mixtures with numerous PAHs. In addition to providing additional qualitative parameters for isomer identification, fluorescence and/or phosphorescence decays report on spectral peak purity, an essential condition for the accurate quantitative determination of PAHs without previous chromatographic separation. TREEM gives the analyst the opportunity to select the best time window for minimal-spectral overlapping in highly complex matrixes. The use of cryogenic probes facilitates the hyphenation of LETRSS to sample pre-concentration techniques such as LLE, SPE and SPNE.

Although chromatographic techniques have dominated the environmental and toxicological scenarios, the solution to new challenges, such as the one posed by HMW-PAHs, require a new generation of analysts with knowledge in a plethora of analytical techniques and instrumentation. Not much is known on the environmental fate of HMW-PAHs. The carcinogenic nature of some HMW-PAHs poses the need for a detailed insight in their environmental behavior, i.e. their formation and presence in various environmental sites, and their fate, including physical and chemical decomposition as well as biodegradation pathways. To evaluate the eco-toxicological risks related to HMW-PAHs, it is therefore necessary to
develop analytical methods that provide accurate measurements in a variety of environmental samples.

Our work addresses another disadvantage of classic methodology, namely the laborious and time-consuming clean-up and separation procedures. Lengthy sample extraction and clean up steps are performed before the sample is loaded onto the column. Elution times of 30-60 minutes are typical and standards must be run periodically to verify retention times. If the concentrations of target species are found to lie outside the detector’s response range, the sample must be diluted and the process repeated. These are important considerations when routine analysis of numerous samples is contemplated. The research presented here facilitates data collection from statistically meaningful population sizes under environmentally friendly conditions. It is possible now to carry out complete PAHs analysis with micro-litters of organic solvent. This fact represents a solvent consumption reduction of three orders of magnitude.

For decades, conventional low-temperature methodology has been restricted to optically transparent media. This restriction has limited its application to organic solvents that freeze into a glass. We have removed this limitation with the use of cryogenic fiber-optic probes. We have demonstrated the feasibility to perform reproducible luminescence measurements in optically scattering media such as “snowlike” matrixes and solid samples. We have extended our approach to low temperature absorption measurements. Recording absorption spectra via transmittance through frozen matrixes is a challenging task. The main reason is the difficulty to overcome the strong scattering light reaching the detector. This is particularly true when thick samples are necessary for recording absorption spectra of weak oscillators. In the case of strongly fluorescent (and/or phosphorescent) compounds, additional errors in absorbance
measurements arise from the luminescence reaching the detector. Commercial double-beam spectrophotometers – which afford a low stray light level – meet the requirement for accurate transmittance through scattering media but fall short with regard to spectral resolution. Our approach to low temperature absorption measurements is fundamentally different as the sought for information is the intensity of laser excitation returning from the frozen sample to the ICCD. Because the fluorescence is detected with a different group of CCD pixels and the intensity of back-scattering laser radiation is stronger than the fluorescence intensity, the fluorescence does not introduce large errors in the scatter measurements. A direct benefit of this approach is the measurement of fluorescence and/or phosphorescence quantum yields at room-temperature, 77 K and 4.2 K. Considering the capability to perform fluorescence and phosphorescence lifetime measurements, the researcher interested in the photo-physics of aromatic compounds has access now to experimental design and instrumentation able to generate important spectroscopic information. Luminescence quantum yields and lifetimes are related synergistic effects and spectral properties of molecular aggregates. Experimental data in this field is currently lacking for HMW-PAHs and many other environmental pollutants. Understanding their photo-physical behavior at the fundamental level could lead to better remediation approaches. With the increasing availability of user-friendly tunable laser and multichannel detector systems, we hope that more research groups will soon add line narrowing spectroscopy techniques to their analytical tool box and start exploring its exciting possibilities.
APPENDIX A: CALIBRATION CURVES OBTAINED VIA HPLC-RTF FOR HMW-PAHs
y = 3.577x + 6.053
$R^2 = 0.9994$

Figure A-1: Calibration curve obtained via HPLC-RTF for DB[a,l]P.

y = 3.588x + 5.004
$R^2 = 0.9995$

Figure A-2: Calibration curve obtained via HPLC-RTF for DB[a,e]P.
**Figure A-3:** Calibration curve obtained via HPLC-RTF for DB[a,i]P.

\[
y = 0.499x + 0.533 \\
R^2 = 0.9993
\]

**Figure A-4:** Calibration curve obtained via HPLC-RTF for N[2,3-\(\alpha\)]P.

\[
y = 0.617x + 2.079 \\
R^2 = 0.9950
\]
Figure A-5: Calibration curve obtained via HPLC-RTF for DB[a,h]P.
APPENDIX B: CALIBRATION CURVES OBTAINED VIA 4.2 K LEPRSS
FOR HMW-PAHs
$y = 0.0237x + 0.3575$

$R^2 = 0.9999$

Figure B-1: Calibration curve obtained via 4.2 K LETRSS for DB[a,l]P in n-octane.

$y = 0.0036x + 0.3328$

$R^2 = 0.9982$

Figure B-2: Calibration curve obtained via 4.2 K LETRSS for DB[a,e]P in n-octane.
Figure B-3: Calibration curve obtained via 4.2 K LETRSS for DB[a,i]P in n-octane.

Figure B-4: Calibration curve obtained via 4.2 K LETRSS for N[2,3-a]P in n-octane.
Figure B-5: Calibration curve obtained via 4.2 K LETRSS for DB[a,h]P in n-octane.
APPENDIX C: CALIBRATION CURVES OBTAINED VIA SPNE-LETRSS
FOR HMW-PAHs
Figure C-1: Calibration curve obtained via SPNE-LETRSS for DB[a,l]P in HPLC Fraction.

Figure C-2: Calibration curve obtained via SPNE-LETRSS for DB[a,e]P in HPLC Fraction.
Figure C-3: Calibration curve obtained via SPNE-LETRSS for DB\([a,i]\)P in HPLC Fraction.

\[ y = 0.3312x + 0.7582 \]
\[ R^2 = 0.9994 \]

Figure C-4: Calibration curve obtained via SPNE-LETRSS for N\([2,3-a]\)P in HPLC Fraction.

\[ y = 0.2785x + 0.4737 \]
\[ R^2 = 0.9955 \]
Figure C-5: Calibration curve obtained via SPNE-LETRSS for DB[\alpha,h]P in HPLC Fraction.

\[ y = 0.1103x + 0.4425 \]

\[ R^2 = 0.9991 \]
APPENDIX D: CALIBRATION CURVES OBTAINED VIA ROOM-TEMPERATURE FLUORESCENCE FOR HMW-PAHs IN 1% METHANOL
Figure D-1: Calibration curve obtained via room temperature fluorescence for DB[a,l]P in 1% methanol.

Figure D-2: Calibration curve obtained via room temperature fluorescence for DB[a,i]P in 1% methanol.
Figure D-3: Calibration curve obtained via room temperature fluorescence for N[2,3-a]P in 1% methanol. 

\[ y = 0.0396x + 0.0976 \]

\[ R^2 = 0.9988 \]

Figure D-4: Calibration curve obtained via room temperature fluorescence for DB[a,h]P in 1% methanol. 

\[ y = 0.0464x + 0.1191 \]

\[ R^2 = 0.9982 \]
APPENDIX E: CALIBRATION CURVES OBTAINED VIA ROOM-TEMPERATURE FLUORESCENCE FOR HMW-PAHs IN N-OCTANE
Figure E-1: Calibration curve obtained via room temperature fluorescence for DB[a,l]P in n-octane.

Figure E-2: Calibration curve obtained via room temperature fluorescence for DB[a,i]P in n-octane.
Figure E-3: Calibration curve obtained via room temperature fluorescence for N[2,3-α]P in n-octane.

Figure E-4: Calibration curve obtained via room temperature fluorescence for DB[α,β]P in n-octane.
APPENDIX F: ROOM TEMPERATURE FLUORESCENCE SPECTRA OF N[2,3-A]P IN N-HEXANE AND N-HEPTANE
Figure F-1: Fluorescence spectra of N[2,3-α]P (100 ng mL⁻¹) in n-hexane at 77 K.

Figure F-2: Fluorescence spectra of N[2,3-α]P (100 ng mL⁻¹) in n-heptane at 77 K.
APPENDIX G: 77 K FLUORESCENCE SPECTRA OF HMW-PAHs IN N-OCTANE
Figure G-1: Fluorescence spectra of DB[a,i]P (100 ng mL⁻¹) in n-octane at 77 K.

Figure G-2: Fluorescence spectra of DB[a,i]P (100 ng mL⁻¹) in n-octane at 77 K.
Figure G-3: Fluorescence spectra of DB[a,h]P (100 ng mL$^{-1}$) in n-octane at 77 K.
APPENDIX H: “TITRATION” CURVE FOR HMW-PAHs WITH 5% 20 NM AU NPS
Figure H-1 Fluorescence intensity as a function of DB[a,l]P concentration after extracted by 5% 20 nm Au NPs.

Figure H-2 Fluorescence intensity as a function of DB[a,i]P concentration after extracted by 5% 20 nm Au NPs.
Figure H-3 Fluorescence intensity as a function of N[2,3-a]P concentration after extracted by 5% 20 nm Au NPs.

Figure H-4 Fluorescence intensity as a function of DB[a,h]P concentration after extracted by 5% 20 nm Au NPs.
APPENDIX I: CALIBRATION CURVES OBTAINED VIA SNPE-LETRSS FOR HMW-PAHs IN NANOPURE WATER SAMPLES
Figure I-1: Calibration curve obtained via SPNE-LETRSS for DB[a,I]P in water samples.

Figure I-2: Calibration curve obtained via SPNE-LETRSS for DB[a,I]P in water samples.
Figure I-3: Calibration curve obtained via SPNE-LETRSS for N[2,3-\textit{a}]P in water samples.

Figure I-4: Calibration curve obtained via SPNE-LETRSS for DB[a,\textit{h}]P in water samples.
APPENDIX J: FLUORESCENCE LIFETIME DECAY OF HMW-PAHs IN N-OCTANE AT 4.2 K
Figure J-1 Fluorescence decay of DB[a,l]P standard (100 ng mL$^{-1}$) in n-octane at 4.2 K. Ex/Em = 322/417.0 nm; $t_d = 10$ ns; $t_g = 250$ ns; step = 6 ns.

Figure J-2 Fluorescence decay of DB[a,l]P in n-octane from a drinking water sample at 4.2 K. Ex/Em = 322/417.0 nm; $t_d = 10$ ns; $t_g = 250$ ns; step = 6 ns.
Figure J-3 Fluorescence decay of DB[a,i]P standard (100 ng mL\(^{-1}\)) in n-octane at 4.2 K. Ex/Em = 315/429.4 nm; \(t_d = 10\) ns; \(t_g = 650\) ns; step = 21 ns.

Figure J-4 Fluorescence decay of DB[a,i]P in n-octane from a drinking water sample at 4.2 K. Ex/Em = 315/429.4 nm; \(t_d = 10\) ns; \(t_g = 650\) ns; step = 21 ns.
Figure J-5 Fluorescence decay of N[2,3-a]P standard (100 ng mL⁻¹) in n-octane at 4.2 K. Ex/Em = 321/458.3 nm; $t_d = 0$ ns; $t_g = 25$ ns; step = 1 ns.

Figure J-6 Fluorescence decay of N[2,3-a]P in n-octane from a drinking water sample at 4.2 K. Ex/Em = 321/458.3 nm; $t_d = 0$ ns; $t_g = 25$ ns; step = 1 ns.
Figure J-7 Fluorescence decay of DB[\(a,h\)]P standard (100 ng mL\(^{-1}\)) in n-octane at 4.2 K. Ex/Em = 316/446.6 nm; \(t_d = 0\) ns; \(t_g = 15\) ns; step = 1 ns.

Figure J-8 Fluorescence decay of DB[\(a,h\)]P in n-octane from a drinking water sample at 4.2 K. Ex/Em = 316/446.6 nm; \(t_d = 0\) ns; \(t_g = 15\) ns; step = 1 ns.
APPENDIX K: MASS SPECTRA SHOWING THE FRAGMENTATION PATTERN OF 16 EPA-PAHs OBTAINED VIA GC/MS IN N-OCTANE
Figure K-1: Mass spectra for naphthalene in n-octane.
Figure K-2: Mass spectra of acenaphthylene in n-octane.
Figure K-3: Mass spectra of acenaphthene in n-octane.
Figure K-4: Mass spectra of fluorene in n-octane.
Figure K-5: Mass spectra of phenanthrene in n-octane.
Figure K-6: Mass spectra of anthracene in n-octane.
Figure K-7: Mass spectra of fluoranthene in n-octane.
Figure K-8: Mass spectra of pyrene in n-octane.
Figure K-9: Mass spectra of benz[a]anthracene in n-octane.
Figure K-10: Mass spectra of chrysene in n-octane.
Figure K-11: Mass spectra of benzo[b]fluoranthene in n-octane.
Figure K-12: Mass spectra of benzo[k]fluoranthene in n-octane.
Figure K-13: Mass spectra of benzo[a]pyrene in n-octane.
Figure K-14: Mass spectra of indeno[1,2,3-cd]pyrene in n-octane.
Figure K-15: Mass spectra of dibenz[a,h]anthracene in n-octane.
Figure K-16: Mass spectra of benzo[ghi]perylene in n-octane.
APPENDIX L: CALIBRATION CURVES OBTAINED VIA SLOW GC/MS CONDITIONS FOR 16 EPA-PAHs IN N-OCTANE
Figure L-1: Calibration curve obtained via slow GC/MS conditions for naphthalene in n-octane.

\[
y = 291.2x - 8655.1 \\
R^2 = 0.9992
\]

Figure L-2: Calibration curve obtained via slow GC/MS conditions for acenaphthylene in n-octane.

\[
y = 202.82x - 4751.4 \\
R^2 = 0.9996
\]
Figure L-3: Calibration curve obtained via slow GC/MS conditions for acenaphthene in n-octane.

Figure L-4: Calibration curve obtained via slow GC/MS conditions for fluorene in n-octane.
**Figure L-5:** Calibration curve obtained via slow GC/MS conditions for phenanthrene in n-octane.


y = 227.21x - 13563
\[ R^2 = 0.9992 \]

**Figure L-6:** Calibration curve obtained via slow GC/MS conditions for anthracene in n-octane.


y = 186.24x - 6990.1
\[ R^2 = 0.9980 \]
Figure L-7: Calibration curve obtained via slow GC/MS conditions for fluoranthene in n-octane.

\[ y = 161.23x - 5225.8 \]
\[ R^2 = 0.9993 \]

Figure L-8: Calibration curve obtained via slow GC/MS conditions for pyrene in n-octane.

\[ y = 133.42x - 2477.9 \]
\[ R^2 = 0.9991 \]
Figure L-9: Calibration curve obtained via slow GC/MS conditions for benz[a]anthracene in n-octane.

\[ y = 330.7x - 27541 \]
\[ R^2 = 0.9987 \]

Figure L-10: Calibration curve obtained via slow GC/MS conditions for chrysene in n-octane.

\[ y = 166.34x - 8320.7 \]
\[ R^2 = 0.9988 \]
Figure L-11: Calibration curve obtained via slow GC/MS conditions for benzo[b]fluoranthene in n-octane.

\[ y = 237.35x - 16744 \]

\[ R^2 = 0.9989 \]

Figure L-12: Calibration curve obtained via slow GC/MS conditions for benzo[k]fluoranthene in n-octane.

\[ y = 108.73x - 6219.3 \]

\[ R^2 = 0.9992 \]
$y = 186.37x - 12350$

$R^2 = 0.9985$

Figure L-13: Calibration curve obtained via slow GC/MS conditions for benzo[a]pyrene in n-octane.

$y = 369.7x - 32269$

$R^2 = 0.9927$

Figure L-14: Calibration curve obtained via slow GC/MS conditions for indeno[1,2,3-cd]pyrene in n-octane.
Figure L-15: Calibration curve obtained via slow GC/MS conditions for dibenz[a,h]anthracene in n-octane.

\[ y = 203.37x - 13784 \]
\[ R^2 = 0.9992 \]

Figure L-16: Calibration curve obtained via slow GC/MS conditions for benzo[ghi]perylene in n-octane.

\[ y = 185.14x - 12092 \]
\[ R^2 = 0.9994 \]
APPENDIX M: CALIBRATION CURVES OBTAINED VIA FAST GC/MS CONDITIONS FOR 16 EPA-PAHs IN N-OCTANE
Figure M-1: Calibration curve obtained via fast GC/MS conditions for naphthalene in n-octane.

Figure M-2: Calibration curve obtained via fast GC/MS conditions for acenaphthylene in n-octane.
Figure M-3: Calibration curve obtained via fast GC/MS conditions foracenaphthene in n-octane.

Figure M-4: Calibration curve obtained via fast GC/MS conditions for fluorene in n-octane.
Figure M-5: Calibration curve obtained via fast GC/MS conditions for phenanthrene in n-octane.

Figure M-6: Calibration curve obtained via fast GC/MS conditions for anthracene in n-octane.
Figure M-7: Calibration curve obtained via fast GC/MS conditions for fluoranthene in n-octane.

\[ y = 987.70x + 930.56 \]
\[ R^2 = 0.9987 \]

Figure M-8: Calibration curve obtained via fast GC/MS conditions for pyrene in n-octane.

\[ y = 811.38x + 3320.4 \]
\[ R^2 = 0.9985 \]
Figure M-9: Calibration curve obtained via fast GC/MS conditions for benz[a]anthracene in n-octane.

\[ y = 612.03x - 6019.9 \]
\[ R^2 = 0.9978 \]

Figure M-10: Calibration curve obtained via fast GC/MS conditions for chrysene in n-octane.

\[ y = 756.31x - 5795.4 \]
\[ R^2 = 0.9972 \]
Figure M-11: Calibration curve obtained via fast GC/MS conditions for benzo[b]fluoranthene in n-octane.

Figure M-12: Calibration curve obtained via fast GC/MS conditions for benzo[k]fluoranthene in n-octane.
**Figure M-13:** Calibration curve obtained via fast GC/MS conditions for benzo[a]pyrene in n-octane.

\[
y = 682.00x - 13218 \\
R^2 = 0.9917
\]

**Figure M-14:** Calibration curve obtained via fast GC/MS conditions for indeno[1,2,3-cd]pyrene in n-octane.

\[
y = 876.44x - 13445 \\
R^2 = 0.9966
\]
Figure M-15: Calibration curve obtained via fast GC/MS conditions for dibenz[a,h]anthracene in n-octane.

Figure M-16: Calibration curve obtained via fast GC/MS conditions for benzo[ghi]perylene in n-octane.
APPENDIX N: CALIBRATION CURVES OBTAINED VIA SPNE-GC/MS CONDITIONS FOR 16 EPA-PAHs IN WATER SAMPLES
Figure N-1: Calibration curve obtained via SPNE-GC/MS for naphthalene in water samples.

Figure N-2: Calibration curve obtained via SPNE-GC/MS for acenaphthylene in water samples.
Figure N-3: Calibration curve obtained via SPNE-GC/MS for acenaphthene in water samples.

Figure N-4: Calibration curve obtained via SPNE-GC/MS for fluorene in water samples.
y = 81610x + 9430.1
$R^2 = 0.9997$

Figure N-5: Calibration curve obtained via SPNE-GC/MS for phenanthrene in water samples.

y = 89158x - 6980.7
$R^2 = 0.9997$

Figure N-6: Calibration curve obtained via SPNE-GC/MS for anthracene in water samples.
Figure N-7: Calibration curve obtained via SPNE-GC/MS for fluoranthene in water samples.

Figure N-8: Calibration curve obtained via SPNE-GC/MS for pyrene in water samples.
Figure N-9: Calibration curve obtained via SPNE-GC/MS for benz[a]anthracene in water samples.

\[ y = 84431x - 14164 \]
\[ R^2 = 0.9950 \]

Figure N-10: Calibration curve obtained via SPNE-GC/MS for chrysene in water samples.

\[ y = 74378x - 8997.5 \]
\[ R^2 = 0.9984 \]
Figure N-11: Calibration curve obtained via SPNE-GC/MS for benzo[b]fluoranthene in water samples.

Figure N-12: Calibration curve obtained via SPNE-GC/MS for benzo[k]fluoranthene in water samples.
Figure N-13: Calibration curve obtained via SPNE-GC/MS for benzo[a]pyrene in water samples.

Figure N-14: Calibration curve obtained via SPNE-GC/MS for indeno[1,2,3-cd]pyrene in water samples.
Figure N-15: Calibration curve obtained via SPNE-GC/MS for dibenz[a,h]anthracene in water samples.

Figure N-16: Calibration curve obtained via SPNE-GC/MS for benzo[ghi]perylene in water samples.
APPENDIX O: GC/MS CHROMATOGRAMS OBTAINED FROM SPIKED ST JOHN’S RIVER WATER SAMPLE AFTER SPNE
Figure O-1: SIM chromatogram obtained St. John’s river water samples via SPNE-GC/MS. PAH spiking provided the final concentration of $1.0 \times 10^4$ ng mL$^{-1}$. Peak identities are (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benzo[a]anthracene, (10) chrysene, (11) benzo[b]fluoranthene, (12) benzo[k]fluoranthene, (13) benzo[a]pyrene, (14) indeno[1,2,3-cd]pyrene, (15) dibenz[a,h]anthracene and (16) benzo[ghi]perylene.
Figure O-2: SIM chromatogram obtained St. John’s river water samples via SPNE-GC/MS. PAH spiking provided the final concentration of $1.0 \times 10^4$ ng mL$^{-1}$. Peak identities are (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benz[a]anthracene, (10) chrysene, (11) benzo[b]fluoranthene, (12) benzo[k]fluoranthene, (13) benzo[a]pyrene, (14) indeno[1,2,3-cd]pyrene, (15) dibenz[a,h]anthracene and (16) benzo[ghi]perylene.
Figure O-3: SIM chromatogram obtained St. John’s river water samples via SPNE-GC/MS. PAH spiking provided the final concentration of $1.0 \times 10^4$ ng mL$^{-1}$. Peak identities are (1) naphthalene, (2) acenaphthylene, (3) acenaphthene, (4) fluorene, (5) phenanthrene, (6) anthracene, (7) fluoranthene, (8) pyrene, (9) benz[a]anthracene, (10) chrysene, (11) benzo[b]fluoranthene, (12) benzo[k]fluoranthene, (13) benzo[a]pyrene, (14) indeno[1,2,3-cd]pyrene, (15) dibenz[a,h]anthracene and (16) benzo[ghi]perylene.
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