Improvements on Instrumentation to Explore the Multidimensionality of Luminescence Spectroscopy

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IMPROVEMENTS ON INSTRUMENTATION TO EXPLORE THE MULTIDIMENSIONALITY OF LUMINESCENCE SPECTROSCOPY

by

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B.S. University of Central Florida, 2006

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 Science at the University of Central Florida Orlando, Florida

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Major Professor: Andres D. Campiglia
ABSTRACT

This dissertation presents experimental and instrumentation developments that take full advantage of the multidimensional nature of line narrowing spectroscopy at liquid nitrogen (77 K) and liquid helium (4.2 K) temperatures. The inconvenience of sample freezing procedures is eliminated with the aid of cryogenic fiber optic probes. Rapid collection of multidimensional data formats such as wavelength time matrices, excitation emission matrices, time-resolved excitation emission matrices and time resolved excitation emission cubes is made possible with the combination of a pulsed tunable dye laser, a spectrograph and an intensifier-charged coupled device. These data formats provide unique opportunities for processing vibrational luminescence data with second order multivariate calibration algorithms.\textsuperscript{1} The use of cryogenic fiber optic probes is extended to commercial instrumentation. An attractive feature of spectrofluorimeters with excitation and emission monochromators is the possibility to record synchronous spectra. The advantages of this approach, which include narrowing of spectral bandwidth and simplification of emission spectra, were demonstrated with the direct analysis of highly toxic dibenzopyrene isomers.\textsuperscript{2} The same is true for the collection of steady-state fluorescence excitation-emission matrices.\textsuperscript{3} These approaches provide a general solution to unpredictable spectral interference, a ubiquitous problem for the analysis of organic pollutants in environmental samples of unknown composition. Since commercial spectrofluorimeters are readily available in most academic institutions, industrial settings and research institutes, the developments presented here should facilitate the widespread application of line-narrowing spectroscopic techniques to the direct determination, no chromatographic separation, of highly toxic compounds in complex environmental matrixes of unknown composition.
References:


This dissertation is dedicated to my mother, Loreta Lucero Moore, for her continued love and support, and to my late father, Tom Paul Moore, who exposed me to the classic processes used for developing photographic prints, which initially piqued my interest in chemistry.
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>PMT</td>
<td>Photomultiplier Tube</td>
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<tr>
<td>PSB</td>
<td>Phonon Side-Band</td>
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<tr>
<td>RBL</td>
<td>Residual Bilinearization</td>
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<tr>
<td>RTF</td>
<td>Room Temperature Fluorescence</td>
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<td>RTL</td>
<td>Residual Trilinearization</td>
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<tr>
<td>RTP</td>
<td>Room-temperature Phosphorescence</td>
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<td>RSD</td>
<td>Relative Standard Deviation</td>
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<td>SFS</td>
<td>Synchronous Fluorescence Spectroscopy</td>
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<td>SFSS</td>
<td>Synchronous Fluorescence Shpol’skii Spectroscopy</td>
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<td>SRM</td>
<td>Standard Reference Material</td>
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<td>TLC</td>
<td>Thin-Layer Chromatography</td>
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<tr>
<td>TREEC</td>
<td>Time-Resolved Excitation-Emission Cube</td>
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<td>TREEM</td>
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<td>U-PLS</td>
<td>Unfolded–Partial Least Squares</td>
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<td>UV</td>
<td>Ultraviolet</td>
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<td>VR</td>
<td>Vibrational Relaxation</td>
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<tr>
<td>WTM</td>
<td>Wavelength-Time Matrix</td>
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<tr>
<td>ZPL</td>
<td>Zero–Phonon Line</td>
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CHAPTER 1: INTRODUCTION

1.1 Photoluminescence Spectroscopy

Photoluminescence is a process by which deactivation of an excited molecule results in the emission of a photon. A luminescing analyte has the potential to be selectively determined by its excitation wavelength, emission wavelength, and lifetime of emission. The numerous parameters for analyte identification, which are referred as multidimensionality of photoluminescence, offer significant advantages as an analytical method. Additionally, the number of compounds that emit radiation is limited by a variety of factors, further enhancing the selectivity of the method.

A Jablonski diagram (Figure 1.1) is a partial energy diagram which graphically represents the possible pathways of excitation and deactivation of a photoluminescent molecule exposed to light in the near-UV and visible spectrum. The lower energy limits of each of the electronic states are represented by bolded horizontal lines. The thinner horizontal lines represent vibrational energy levels within each electronic state. $S_0$ represents the energy of the molecule in the ground state. $S_1$ and $S_2$ represent the energies of the molecule in the first excited singlet state and the second excited singlet state, respectively. $T_1$ represents the energy level for the first excited triplet state.
1.1.1 Absorption

Absorption of energy by a molecule results in the promotion of a molecule to an electronic state of higher energy (excited state). Absorption of organic molecules in the near ultraviolet and visible regions of the electromagnetic spectrum are due to $n\rightarrow\pi^*$, and $\pi\rightarrow\pi^*$ transitions. Absorption of samples is monitored by measuring the ratio between of incident light and light transmitted through a sample. Different competing processes allow for the release of energy of the molecule in an excited state, to relax, or deactivate, back to the ground state, which are further discussed in the proceeding sections.

Absorbance, $A$, is related to the transmittance of incident light though the sample (Equation 1.1), where $I_0$ is the intensity of incident radiation, and $I_t$ is the intensity of the fraction of incident radiation transmitted through the sample. Proportionality of absorbance with path-length of the cell and concentration was established by Johann Heinrich Lambert and August
Beer, respectively, giving the combined Beer-Lambert Law shown in Equation 1.2, where \( \varepsilon \) is the absorptivity, \( b \) is the path-length of the sample cell, and \( C \) is the concentration of absorbing species in the sample cell. Deviations from the law may occur at higher analyte concentrations, where absorption is inhomogeneous across the path length of the sample cell.

\[
A = \log_{10} \frac{I_0}{I_t} \quad (1.1)
\]

\[
A = \varepsilon b C \quad (1.2)
\]

1.1.2 Non-Radiative Deactivation Processes

Non-radiative deactivating processes include vibrational relaxation (VR), internal conversion (IC), external conversion (EC), and intersystem crossing (ISC). VR involves processes by which a molecule in an excited state passes to lower vibrational levels within the same excited state, releasing energy as heat through interactions of the molecules in an excited state with solvent molecules. VR is a very efficient process (lifetime of \( 10^{-12} \) s, or less). IC is a process by which a molecule passes to a lower electronic state, e.g., \( S_2 \rightarrow S_1 \). Overlapping vibrational levels, as shown for \( S_2 \) and \( S_1 \) in Figure 1.1, increase the probability of deactivation by IC. Efficiency is expected to decrease as the energy difference between the electronic states increases. While the mechanisms IC are not well understood, IC is likely to be very efficient since relatively few compounds exhibit fluorescence.

EC is a process where excess energy of molecules in an excited state is transferred to another species, e.g., solute and solvent molecules. A primary mechanism of EC is dynamic
quenching where energy transfer takes place via collisions of excited state molecules (donor) with another species present (acceptor). Dynamic quenching follows a Stern-Volmer relationship (Equation 1.3), where $I_0$ and $I_q$ is the fluorescence intensity in the absence and presence of the quencher, respectively, $[Q]$ is the concentration of quencher, and $K_q$ is the Stern-Volmer quenching constant, defined by Equation 1.4, where $k_q$ is the quenching rate constant. Methods that minimize EC, such as lowering the temperature of the sample, or immobilizing the sample, will generally lead to an enhancement of luminescence.¹

$$\frac{I_0}{I_q} = 1 + K_q [Q]$$ (1.3)

$$K_q = \frac{k_q}{k_f+k_{ic}+k_{isc}}$$ (1.4)

ISC is a transition between a singlet excited state and a triplet excited state. ISC will be discussed in more detail with regards to phosphorescence (Section 1.1.3.2).

1.1.3 Radiative Deactivation Processes

1.1.3.1 Fluorescence

Fluorescence is a radiative deactivation process, which typically involves a transition from the lowest vibrational level of the lowest excited singlet state ($S_1$), to the vibrational levels of the ground state ($S_0$). This is due to the relative lifetimes of each of the transitions, where more rapid transitions have a higher probability of occurring. The lifetime of the fluorescence transition is generally on the order of $10^{-9}–10^{-6}$ s. When compared to VR ($\leq 10^{-12}$ s), lifetime of
fluorescence is three orders of magnitude (or more) longer and consequently has a much lower probability of occurring. Similarly, IC between excited singlet states is typically faster ($10^{-12}$ s) than fluorescence, so fluorescence emission generally occurs from the $S_1 \rightarrow S_0$ transition. The greater efficiency of VR and IC can also be observed when comparing the absorption and fluorescence spectra, where the fluorescence transition is generally lower in energy, resulting in emission of photons at a higher wavelength, relative to absorption. The difference in the wavelength maxima is referred to as the Stokes shift. Absorption and fluorescence bands will overlap at the 0–0 transition, i.e., the transition between the lowest limits of $S_1$ and $S_0$ in Figure 1.1.²

The efficiency of fluorescence, or fluorescence quantum yield, denoted $\phi_f$, is defined as the ratio of the number of fluorescing photons to the total number of absorbing photons upon exposure to incident light. The fluorescence quantum yield can also be expressed in terms of the rate constants (Equation 1.5) of fluorescence ($k_f$) and the sum of the rate constants of competing non-radiative transitions ($k_{nr}$), which include internal conversion ($k_{ic}$), external conversion ($k_{ec}$), and intersystem crossing ($k_{isc}$).

$$\phi_f = \frac{k_f}{k_f + k_{nr}}$$

(1.5)

Fluorescence is proportional to the amount of incident radiation absorbed, as shown in Equation 1.6, where intensity of light absorbed is the difference between $I_0$ and $I_t$. Substitution of $I_t$ in Equation 1.6, using Equation 1.1 and Equation 1.2, gives Equation 1.7. Unlike absorption, fluorescence does not vary linearly with concentration. Expansion of exponential in Equation 1.7
via a Maclaurin series gives Equation 1.8. At very dilute concentrations, typically when absorption is less than 0.01, the magnitude of the polynomial terms are insignificant, and can be neglected, giving the expression shown in Equation 1.9. If working with dilute concentrations of a fluorophore, the relationship between $I_f$ and concentration can be considered linear, allowing for linear calibration methodologies to be used for determining the amount of fluorophore present. Deviations from linearity may also be attributed to the inner-filter effect, where absorption is not homogeneous across the path of the cell, absorption of light due to the presence of concomitants in the sample, or self-absorption of emitted fluorescence.

$\begin{align*}
I_f &= \phi_f(I_0 - I_t) \\
I_f &= \phi_f I_0(1 - 10^{-ebC}) \\
I_f &= 2.3\phi_f I_0 ebC \left[ 1 - \frac{2.3ebC}{2!} + \frac{(2.3ebC)^2}{3!} - \cdots - \frac{(2.3ebC)^n}{n!} \right] \\
I_f &= 2.3\phi_f I_0 ebC
\end{align*}$

1.1.3.2 Phosphorescence

Phosphorescence is a radiative transition from the lowest vibrational level of the first excited triplet state, to the vibrational levels of the ground state. For phosphorescence to occur, deactivation of the excited state molecule must proceed via ISC to the triplet state. While the
spins of electrons in an excited singlet state remain paired, i.e., opposed, the spins of one pair of electrons in an excited triplet state become unpaired, i.e., electron-spins parallel to each other.

As shown previously for fluorescence, the quantum yield of phosphorescence can be expressed in terms of the rate constants of the competing processes (Equation 1.10). Overlapping of vibrational energy levels may be due to both intramolecular and intermolecular interactions increasing the probability of singlet-triplet transitions. The presence of an atom with high atomic number, e.g., bromine and iodine, enhance $k_{isc}$, and thus enhance $k_p$. Enhancement is attributed to spin-orbit coupling between excited state species and the heavy atom, an experimental observation referred to as the “heavy-atom effect.” Molecular oxygen (O$_2$) will also enhance $k_{isc}$, however, O$_2$ will also quench phosphorescence. Since the ground electronic configuration of O$_2$ is a triplet state, excited triplet state energy may also be transferred to O$_2$ via dynamic quenching, promoting O$_2$ to an excited singlet state (singlet oxygen). Oxygen is ubiquitous in both the atmosphere and in solution, so its absence is critical in phosphorescence experiments.

$$\phi_p = \frac{k_{isc}}{k_f + k_{nr}} \left( \frac{k_p}{k_p + k_{nr}'} \right)$$  \hspace{1cm} (1.10)

Because the probability of a singlet–triplet transition occurring is lower than a singlet–singlet transition, the average lifetime of the transition ($10^{-3}$–10 s) is relatively longer, compared to singlet–singlet transitions, such as fluorescence. The longer lifetime of the excited state increases the probability of quenching by non-radiative deactivation processes. As suggested in Section 1.1.2, to marginalize or completely eliminate the competing quenching processes,
phosphorescence measurements are typically performed with analytes in condensed phases at low temperature, or adsorbed onto solid substrates.

1.1.3.3 *Lifetime of Luminescence*

Deactivation is generally assumed to occur through first-order rate processes. From classical kinetics, the rate of deactivation of an excited species can be expressed using Equation 1.11, where $[A]$ is the concentration of an absorbing species, $[A^*]$ is the concentration of the species in an excited state following absorption of a photon. Integration of Equation 1.11 gives Equation 1.12, where $\tau$ is lifetime of radiative deactivation, given by Equation 1.13. Lifetime of fluorescence, $\tau_f$, would then be given as $(k_f + k_{nr})^{-1}$, where $k_{nr}$ is the rate constant for non-radiative deactivation from the first singlet excited state. Likewise, lifetime of phosphorescence, $\tau_p$, is given as $(k_p + k'_{nr})^{-1}$, where $k'_{nr}$ is the rate constant for non-radiative deactivation from the first triplet excited state.

$$\frac{-d[A^*]}{dt} = (k_r + k_{nr})[A^*] \quad (1.11)$$

$$[A^*]_t = [A^*]_0 e^{-t/\tau} \quad (1.12)$$

$$\tau = \frac{1}{k_r + k_{nr}} \quad (1.13)$$

Experimentally, lifetime of luminescence intensity can be determined by plotting the luminescence signal collected at different delay times from the excitation pulse, and fitting the
decay to the first order exponential function (Equation 1.14), where $I_t$ is the intensity at time $t$, and the intensity at time $\tau$, is equal to $I_0/e$.

$$I_t = I_0 e^{-t/\tau}$$  \hspace{1cm} (1.14)

1.2 Line-Narrowing Spectroscopy

While photoluminescence techniques, specifically fluorescence, have the potential to be very selective and sensitive method, spectra collected at room temperature are generally very diffuse. The line-shape can be thought of as the population-distribution of the absorbing and emitting species in solution. The broad, diffuse nature is due to Brownian motion of molecules in a solution. The molecules may therefore be exposed to a wide variety of interactions with the solute and solvent molecules, resulting in a wide distribution of transition energies.

The diffuse nature of room temperature spectra is especially problematic when analyzing a complex matrix. Even matrixes containing only a few fluorescent components can be difficult if their spectra significantly overlap. A popular approach in analytical chemistry to eliminate spectral overlapping is to physically separate sample components prior to determination via chromatographic methods. This dissertation deals with an alternative approach, which is based on a high-resolution spectroscopic technique known as Shpol’skii spectroscopy. This technique deals with frozen samples at 77 K or below.
1.2.1 Homogeneous and Inhomogeneous Band-Broadening

Homogeneous band-broadening ($\Gamma_{\text{hom}}$) is the natural linewidth of the transition, which is the same for all chemically identical molecules (hence homogeneous). The natural linewidth of an isolated molecule is due to the uncertainty in the measured energy of the excited state transition, which has a lifetime of $10^{-5} - 10^{-8}$ s.\textsuperscript{1,4} The natural linewidth of a vibrational transition consists of a zero–phonon line (ZPL) and phonon side-band (PSB). Figure 1.2 shows a representation of a fluorescence band. The ZPL generally fits a Lorentz function and the PSB a Poisson distribution function. The absorption band would be similar, but the relative positions mirrored, with the PSB on the higher energy, i.e., shorter wavelength, side of the ZPL band.

![Figure 1.2: The emission spectral profiles and relative positions of the ZPL and PSB.](image)

The PSB arises from electron-phonon coupling, which results from interactions of the electrons of the guest (solute) molecules and the vibrations of the rigid host (solvent) lattice. The Debye-Waller factor, $\alpha$, is a parameter that indicates the relative strength of the electron-phonon coupling, where $I_{ZPL}$ is the intensity of the ZPL, and $I_{PSB}$ is the intensity of the PSB (Equation 1.15).\textsuperscript{5} A Debye-Waller factor value approaching unity indicates a system with a relatively weak
electron-phonon coupling interactions, which is ideal for observing quasi-line spectra. As temperature increases, the Debye-Waller factor decreases exponentially, and as a consequence, the ZPL is only observed at very low temperatures.

\[ \alpha = \frac{I_{ZPL}}{I_{ZPL} + I_{PSB}} \]  \quad (1.15)

Inhomogeneous band broadening (\( \Gamma_{\text{inh}} \)) arises from the interaction of each molecule with its microenvironment. As the sample is frozen, the guest molecules will become trapped within a solvent cage, and become what can be thought of as impurity centers, defects, or sites within the host lattice. The number of possible orientations of the solvent cage will depend on the host–host and guest-host geometries and interactions. Figure 1.3 compares a homogeneously broadened spectrum within one site of a crystalline host, to an inhomogeneously broadened spectrum within an amorphous host. The slightly differing microenvironments of each site result in slightly shifted spectra. Highly resolved quasi-line spectra with relatively few sites will have a multiplet structure revealing the slightly shifted spectra from guest molecules oriented in each of the different sites. The narrowing (or broadening) of spectra is typically given as the “full-width at half-maximum” (FWHM), which is the width of peak measured at half of the peak-height (width between the arrow points, as illustrated in Figures 1.3A and 1.3B).
1.2.2 Methods for Reducing Inhomogeneous Band-Broadening

Several methodologies have been developed in order to reduce the effect of inhomogeneous broadening revealing detailed vibronic spectra of the analytes. Early studies dispersed analytes in glasses, e.g., boric acid, and in polymers. Other studies took advantage of compounds of similar dimensions which were known to form an optically transparent crystalline matrix, e.g., naphthalene in durene\(^6\) and pentacene in \(p\)-terphenyl\(^7\) but applicability was limited to a few systems.\(^8\) The latter half of the 20\(^{th}\) century saw the development of more widely applicable techniques, such as Matrix-Isolation Spectroscopy, Fluorescence Line-Narrowing Spectroscopy, and Shpol’skii Spectroscopy. These techniques reduce the effect of inhomogeneous broadening in one of two ways, either by reducing the number of sites by using crystalline host, or by site-selective excitation of the guest analytes within a single, specific site.

Figure 1.3: Emission spectra of guest molecules in (A) a single site of a crystalline host, and (B) an inhomogeneously broadened spectrum consisting of spectra slightly shifted spectra, each from one of several possible sites of an amorphous host.
1.2.2.1 Matrix-Isolation Spectroscopy

Matrix isolation spectroscopy involves the vaporization of sample into a chamber containing an inert gas and its subsequent deposition onto a solid surface at low temperature. The analyte and gas molecules are layered onto the substrate providing an ordered host matrix for the guest, minimizing inhomogeneous broadening mechanisms. Choice of gas can have an effect on the luminescence transitions observed, with gasses containing heavy atoms favoring ISC and phosphorescence. Samples may also be deposited in the presence of an n-alkane vapor, minimizing inhomogeneous broadening, similar to observations via the Shpol’skii effect.3,9,10

1.2.2.2 Shpol’skii Spectroscopy

Shpol’skii Spectroscopy is a technique based on observations reported by Shpol’skii and co-authors in 1952, where quasi-linear spectra were observed for dilute solutions of planar aromatic hydrocarbons, e.g., polycyclic aromatic hydrocarbons (PAHs), in an n-alkane solvent.11 This has been coined the “Shpol’skii effect.” In general, the three conditions that need to be met for observing the Shpol’skii effect are (1) solvent host freezes to a polycrystalline matrix, (2) solute-solvent interactions are weak, and (3) molecular structures of the guest and host dimensionally match.12 These conditions serve to minimize both electron-phonon coupling, and the number of possible crystallographic sites the analyte guest resides in. Optimum spectral narrowing is typically obtained with an n-alkane, in which the length most closely matches the largest dimension of the PAH molecular structure.13 Examples of guest-host pairs suitable for collecting Shpol’skii spectra of the guest are shown in Figure 1.4. The Shpol’skii effect is generally considered a non-equilibrium effect, where the solute is supersaturated within
substitutional sites in the solid matrix,\textsuperscript{13} and best results are typically achieved when samples are rapidly cooled. Slow-cooling may result in aggregation of solutes due to poor solubility at low temperatures resulting in broad spectra.\textsuperscript{4,13,14} While narrowing is observed at 77 K (FWHM of 10–30 cm\textsuperscript{−1}), narrowing is optimal at temperatures below 20 K (FWHM of 1–10 cm\textsuperscript{−1} at 4.2 K).\textsuperscript{8}

1.2.2.3 Fluorescence Line-Narrowing Spectroscopy (FLNS)

FLNS is a technique which uses selective excitation, in which only a subset of the population of guest molecules within a specific site is excited (iscochromat), resulting in quasi-line spectra. Unlike Shpol’skii spectroscopy, where spectra of the guest are collected in a polycrystalline matrix, FLN spectra are collected from an analyte in a condensed amorphous matrix. Because the absorption spectra are expected to be inhomogeneously broadened (FWHM of 100–500 cm\textsuperscript{−1}),\textsuperscript{15} a line-source, such as a tunable laser, is necessary for selective excitation. The energy of the line-source (laser-line) must overlap with the 0–0 energy transition to avoid exciting guest molecules occupying other sites.\textsuperscript{15}

Figure 1.4: A selection of PAH–n-alkane pairs for collecting Shpol’skii spectra of the corresponding PAH.
In comparison to Shpol’skii Spectroscopy, FLNS is more applicable overall since the choice of solvent is less stringent. It only requires that the electron-phonon coupling is weak and that the solvent is a glass, i.e., optically transparent, when frozen. Due to the amorphous nature of the matrix, the guest may reside in one of several possible sites. Since only a subset of the population is excited in any particular site, the sensitivity of FLNS is usually worse than the one achieved with Shpol’skii Spectroscopy, where the $n$-alkane solvent reduces the number of possible sites in the frozen matrix and provides a larger subset of the population for analyte excitation.\(^{16}\)

### 1.3 Sample Procedures

1.3.1 **Fluorescence**

Typical fluorescence measurements are carried out at room temperature, from samples dissolved in a liquid matrix, and measured using either a fluorimeter or a spectrofluorimeter. Cuvettes composed of an optically transparent material are suitable for measuring luminescence from liquid solutions at room temperature. While glass and some plastics are suitable for spectroscopic measurements in the visible spectrum, measurements in the UV and visible spectrum require cuvettes composed of quartz. The sample holder within the sample compartment of the spectrofluorometer is designed to prevent positional variation.

Room temperature fluorescence may also be carried out using solid substrates. Sensitivity is often enhanced due to immobilization of analytes through adsorption onto the solid surface, reducing collisional interactions with the excited state (Section 1.1.2). Consequently, solid surface fluorescence may be advantageous when small volumes of samples are necessary or
desired. Particular attention has also been made to extraction membranes for typically used for pre-concentrating organic pollutants or biomarkers as solid surface fluorescence-based screening methodologies. Several types of solid substrates have been used for solid surface room temperature fluorescence, including filter paper, \(^{17-19}\) polymer resins, \(^{20}\) silica gel, \(^{20,21}\) and functionalized–silica membranes \(^{22,23}\).

As suggested in Section 1.2, freezing of the sample can be particular advantageous for fluorescence applications, where line-narrowing of spectra is desired. Solutions or samples immobilized on solid substrates may be placed within a cryostat which is designed for lowering and maintaining a sample at low-temperature. Solvents used should form a glass when frozen to cryogenic temperatures to avoid excitation scatter. Cryogenic methods may also be applied to solid-substrates, and has been demonstrated with TLC plates (hyphenated with HPLC).\(^{24,25}\) Cryostats will be discussed specifically in Section 1.3.3.

1.3.2 Phosphorescence

Classical methodologies used for measuring phosphorescence typically involve immobilization of analytes into a rigid matrix in order to minimize quenching processes. If necessary, the environmental conditions are adjusted so that intersystem-crossing is favored. Classical method involves freezing of a sample solution at 77 K and performing measurements using an optical Dewar flask,\(^{3}\) similar to low-temperature fluorescence measurements. The appropriate solvent should have low (ideally no) phosphorescence background and forms a glass (optically transparent matrix) upon freezing. Common host solvents include \(n\)-alkanes, and solvent mixtures that form glasses upon freezing, e.g., ethanol:isopentane:diethyl ether (2:5:5)\(^{3}\).
Solvent additives containing heavy atoms, e.g., sodium iodide, silver acetate, or halogenated \( n \)-alkanes, may be added to help favor ISC and phosphorescence, via the external heavy atom effect. Degassing of solvents may be necessary to avoid quenching by \( O_2 \).

Solid-surface room-temperature phosphorescence (SS-RTP) is a technique of interest due to its simplicity and low sample volumes (as little as 5 \( \mu L \)).\textsuperscript{26} Filter paper is the most common solid substrate cited in the literature,\textsuperscript{18,19,27,28} however, other substrates found in the literature include silica gel,\textsuperscript{21,29-31} functionalized–silica membranes,\textsuperscript{32-34} and polyamide membranes\textsuperscript{35,36}. Similar to low temperature measurements, additives which contain heavy atoms, e.g., \( I^- \) (NaI), or \( Pb^{2+} \) (PbCl\(_2\)), may be added which help favor ISC and phosphorescence, via the external heavy atom effect.\textsuperscript{26,27} While SS-RTP avoids the complications of cryogenic measurements, lower phosphorescence signal, and luminescence background of the solid surface may have a detrimental effect on detection limits.\textsuperscript{37}

Room-temperature phosphorescence (RTP) has also been carried out in solution. Analytes can be protected from quenching by adding an appropriate concentration of surfactant to the solution, which in ideal conditions will aggregate to form micelles which act as a host for the analyte to reside within. Alternatively, RTP in solutions can be carried out using cyclodextrins.\textsuperscript{38,39} Cyclodextrins are a family of molecules composed of an oligosaccharide in which the ends are joined to form a ring-shaped molecule. An analyte may reside within the cavity of the ring, forming what is referred to as an inclusion complex, protecting the analyte from collisional quenching. As with the above examples, samples may be spiked with inorganic salts containing heavy atoms to enhance ISC and phosphorescence via the external heavy atom effect.
effect. Degassing of solutions and the sample compartment is necessary to avoid quenching by dissolved oxygen.

1.3.3 Cryostats

Cryostats for low-temperature spectroscopy are used for cooling of samples to cryogenic temperatures. Measurements of samples at cryogenic temperatures often require far more elaborate setups. Samples are cooled either through direct immersion in cryogen, or through thermal conductance. The freezing of samples via immersion can be carried out using an optical Dewar flask. A liquid sample is contained in a quartz tube and immersed into liquid cryogen. 77 K measurements can be carried out by immersing the sample into liquid nitrogen. Sharp, quasi-line spectra often require sample temperatures below 20 K. Measurements at those temperatures require the use of more elaborate cryostats which are able to maintain the lower temperatures for longer periods of time. Sample cryostats freeze samples either through immersion, e.g., bath cryostats, or by thermal conductance. Apparatuses such as closed-cycle helium refrigerators use thermal conductance freeze samples by contact through an intermediary, or “cold-finger”. The cold-finger is composed of a thermally conductive material, e.g., copper, which is in direct contact with both the sample and the cryogen.

1.4 Instrumentation

1.4.1 Excitation Sources

The choice of excitation source depends on the photoluminescence technique under consideration. For Shpol’skii spectroscopy, the number of available sites with impurity centers is
minimized, resulting in relatively narrow excitation bands, and allowing for the use of broadband excitation lamps, e.g., mercury and xenon arc lamps, for obtaining quasi-line emission spectra. A monochromator allows for the selection of an excitation wavelength (Section 1.4.2). Improving signal-to-noise can be accomplished by opening the slit-width. Commercial spectrofluorometers are a very convenient means of collecting spectra with a broad excitation source. Lifetime measurements require the use of a pulsed source, via a mechanical chopper, or a flash lamp.

Methods utilizing site-selective excitation require the use of a line source, i.e., laser, which provides monochromatic excitation. Frequency mixing allows for the further tuning of the laser source. Further, the laser output may be tuned through the pumping of a dye-laser. A variety of tunable lasers have been utilized as excitation sources for recording luminescence spectra. The output power of laser line sources are generally much higher than broadband excitation lamps, allowing for the potential of achieving better sensitivities, and for the use of smaller sample sizes. Line-sources may either be continuous or pulsed; pulsed line-sources are necessary for time-resolved measurements.

1.4.2 Emission Wavelength Selectors

Wavelength selection is accomplished by spatially dispersing light. Monochromators for UV-visible spectroscopy typically use a diffraction grating, e.g., echelette (or ruled) grating or holographic grating, which is a reflective surface containing several narrow grooves. Echelette gratings are machined, while photolithography is used to produce holographic gratings. In general, holographic gratings are preferred, since the manufacturing process produces a near-perfect uniform surface, minimizing the stray light effects due to surface defects.
Multiple emission monochromators in a series, e.g., double-monochromator, or a triple-monochromator, serve to eliminate excitation scatter in cases where cutoff filters are inadequate.16 Scanning of a wavelength range is accomplished by adjusting the angle of the grating via rotation, changing the wavelength projected onto the exit slit of the monochromator, and allowing for the collection of spectra. Spectrographs are similar to monochromators with the exception of the larger exit aperture, in place of an exit slit, projecting a broad wavelength range onto a corresponding multichannel detector (Section 1.4.3). Optical designs may differ depending on the choice of a single or multichannel detector. Instrumentation for high-resolution spectroscopy generally requires a spectral resolution of 0.2 nm or less in order to resolve vibrational transitions.16,42

1.4.3 Detectors

Detection of luminescence emission is carried out by means of a transducer, which converts radiating energy into an electrical signal. Single-channel detectors are often used in conjunction with a scanning monochromator to record spectra. Single-channel detectors are typically placed at the exit aperture of the emission monochromator. For the work described in this dissertation, photomultiplier tubes (PMT) are typically used for single channel detection. PMTs are an extension of vacuum phototubes, and amplify electrical signal using a series of dynodes via secondary emission of electrons. Alternatively, electrical signal gain can be accomplished with microchannel plate–photomultiplier tubes (MCP-PMT). This type of detector consists of a thin plate made up of an array of narrow capillary-like channels, which walls are coated with a secondary-emissive material. Compared to a PMT, a MCP-PMT has a faster
response time, and is capable of measuring relatively short lifetime decays. Time-resolved measurements require electronic gating to control the sampling window. Detector noise due to dark current, attributed to thermally generated electrons, can be suppressed by cooling the detector, increasing the sensitivity of the detector.

Multichannel detectors contain an array of responsive elements allowing for spatial resolution in one dimension, e.g., linear photodiode array (LPDA), or in two-dimensions, e.g., charge-coupled device (CCD). Designs of LPDAs are based on reverse-biased p-n junction semiconductors, while the designs of CCDs are based on metal-oxide semiconductors. The advantage of multichannel detectors is the ability to record data from a range of wavelengths allowing for a spectrum to be collected all at once. This is particularly advantageous if faced with photosensitive samples or if the excitation source output is not stable. An intensified CCD (ICCD) takes advantage of the signal gain and the spatial resolution of an MCP, increasing sensitivity for applications where low photon counts are expected. The image intensifier also provides a gating function by reversing the voltage bias, allowing for time-resolved measurements. As with PMTs, thermoelectric cooling serves to reduce dark current and improve sensitivity.
CHAPTER 2: LASER-EXCITED TIME-RESOLVED SHPOL’SKII SPECTROSCOPY (LETRSS)

2.1 Cryogenic Fiber-Optic Probes

2.1.1 Classical Sample Cooling Methods

Routine freezing of samples for luminescence measurements typically involves the immersion of a quartz tube with the liquid sample into liquid cryogen. Dewar flasks are often available as an accessory for a spectrofluorometer, for use with liquid nitrogen. While luminescence experiments utilizing a Dewar flask are simple, there are several disadvantages. The cryogen is thermally insulated by a silvered vacuum jacket, which minimizes heat transfer to the liquid cryogen. Consequently, the vacuum jacket, in addition to the sample cell, introduces several interfaces, which scatter excitation light, increasing the background signal collected. Inconsistent bubbling of N₂ is attributed to the presence of ice, which act as nucleation sites, is a source of random error affecting the precision of measurements. Condensation, which may build up on the outer part of the flask can also have a detrimental effect on the reproducibility of measurements. Additionally, typical Dewar flasks for a spectrofluorometer are limited to use with liquid N₂, restricting the temperature reduction to 77 K, the boiling point of N₂. Optical bath cryostats, utilizing liquid helium allow for cooling of samples to 4.2 K, but may contain additional interfaces, such as from a liquid nitrogen jacket, and suffer similar disadvantages as 77 K Dewar flasks.

An alternative to immersion for sample cooling is thermal conductance. Heat is transferred to the cryogen through an intermediary “cold finger” or sample holder, reducing the amount of scattering interfaces encountered by excitation light. Examples of cryostats that cool
samples via thermal conductance are continuous-flow helium cryostats, closed-cycle helium refrigerators, and Joule-Thomson refrigerators. Closed-cycle refrigerators are particularly advantageous because the liquid cryogen is recycled and does not require resupply of liquid cryogen. Joule-Thompson refrigerators do not require liquid cryogen,\textsuperscript{44,45} instead sample cooling is accomplished through heat exchange with a gas cooled through free expansion. While temperatures obtained by these cooling methods are adequate for high-resolution luminescence spectroscopy,\textsuperscript{46} a significant disadvantage is the time necessary to cool samples to the desired temperature. Depending on the cryostat, sample, and desired temperature, freezing times may range from 30–60 minutes.\textsuperscript{44} The relatively slow cooling times significantly affects sample throughput, especially if samples are to be cooled and analyzed one by one. Strategies to increase sample throughput typically involve sample chambers designed to hold several samples which are all cooled at the same time, with a mechanism for switching between samples.\textsuperscript{4,44-46} Additionally, slow-cooling of a sample may also affect the population distribution within the various crystallographic sites and the formation of aggregates of solute molecules, diminishing the quasilinear profile (the Shpol’skii effect) in favor of a broadened spectral profile. These effects usually lead to a decrease in the intensity of the corresponding peak.\textsuperscript{4,13} An extra cooling step, i.e., immersing the sample in liquid nitrogen prior to further cooling by thermal conductance, can prevent the formation of aggregates,\textsuperscript{46} and potentially reduce the cooling time. Overall, the complications of traditional sample cooling hamper the implementation of low–temperature luminescence techniques for routine analysis.
2.1.2 Sampling via Optical Fiber Probes

A novel improvement to sampling procedures was introduced by the Campiglia group, utilizing cryogenic fiber-optic probes (FOP) for collecting luminescence spectra from samples at cryogenic temperatures. The FOP is similar to the sample cell reported by Hieftje and co-workers,\textsuperscript{47} for collecting fluorescence and phosphorescence at 77 K, which was composed of seven fibers, one excitation and six emission fibers, epoxied in a six-around-one-configuration within a quartz tube, with a quartz window at the end of the tube. The quartz tube was secured to a cap allowing for collection of fluorescence and phosphorescence from samples within a microcentrifuge tube. The probes designed by the Campiglia group excites and collects emission from the sample directly with the optical fibers, without the added interface of the quartz window, while still preserving the simple “dunking” procedure for instantaneously freezing the sample, and the use of a commercially available sample holder.

Optical fibers provide a means of transmitting excitation light to, and collecting luminescence emission from, the sample. Two important parameters that should be considered for the fiber bundle include the attenuation and the numerical aperture (NA) of the optical fibers. Multi-mode optical fibers transmit light from one end to the other with minimal loss of energy by total internal reflection, as illustrated in Figure 2.1. The optical fibers described here consist of core, a cladding layer, and an outer buffer layer. By design, the refractive index of the cladding is less than that of the core. The buffer coating provides mechanical strength to the optical fiber. The NA of the optical fiber is a measure of its ability to gather light, and is defined by Equation 2.1, where $n$ is the refractive index of the medium, $n_{\text{core}}$ and $n_{\text{clad}}$ are the refractive indices of the core and cladding of the optical fiber, respectively, and $\theta_{\text{max}}$ is the half-angle of the acceptance.
cone. Total internal reflection will occur as long as \( n \sin \theta_0 \leq (n_{\text{core}}^2 - n_{\text{clad}}^2)^{1/2} \), where \( \theta_0 \) is the angle of the light ray entering the core of the optical fiber. Therefore any light rays within the acceptance cone will propagate within the optical fiber core. Any incident light rays entering at angles greater than \( \theta_{\text{max}} \), e.g., \( \theta_2 \) in Figure 2.1, will be lost in the cladding and propagation thru the fiber will not occur.

\[
NA = n \sin \theta_{\text{max}} = \sqrt{n_{\text{core}}^2 - n_{\text{clad}}^2}
\]  

(2.1)

Figure 2.1: Propagation of light through an optical fiber.

Attenuation of the optical fiber is a measure of the loss of incident light over the length of the fiber. It is often expressed as ten times the logarithm of the ratio of incident to output power \( (P_0) \) to the power of light transmitted \( (P) \), over a given length \( (l) \), with units of dB·km\(^{-1}\) (Equation 2.2). Loss of intensity is often due to effects of absorption of defects and impurities, and the scattering of the light as it propagates through core, and is dependent on the wavelength of light.
\[ \text{Attenuation (dB \cdot km}^{-1}) = \frac{10}{l} \times \log_{10} \frac{P_0}{P} \quad (2.2) \]

When choosing an optical fiber, it is important to know the wavelength range necessary for the application, and to choose an optical fiber of appropriate composition. Fused silica is generally used for optical fibers for spectroscopy due to its applicability spanning the UV–visible–IR spectrum. For applications in the near-infrared (near-IR), a fused silica core containing low amounts of terminal hydroxyl groups (low –OH) is used due to hydroxyl absorption bands at 980 nm, 1250 nm, and 1383 nm, however, transmittance in the ultraviolet (UV) is poor. Water dissolved into silica, produces silica fibers with high –OH content, improving UV transmittance at the expense of transmittance in near-IR spectrum. Transmission loss in the UV is attributed to the breaking of strained Si–O bonds from UV irradiation forming defects, which act as absorption centers.

Figure 2.2 is a diagram of the cryogenic FOP developed in Campiglia’s lab. It consists of a bifurcated optical fiber bundle fabricated in-house, which utilizes a total of seven multi-mode optical fibers (FVP500550590, Polymicro Technologies, Phoenix, AZ, USA). Each fiber is composed of a fused silica core diameter of 500 µm, surrounded by a doped silica cladding and a polyimide buffer coating, with an overall outer diameter of 590 µm. The NA of the each fiber is 0.22, and has a transmission range of 180–1150 nm. The fused silica core has a high hydroxyl concentration which improves the transmission of ultraviolet light wavelengths through the fiber, at the expense of poor transmittance in the near-infrared region, where hydroxyl groups absorb. At the distal end of the FOP (relative to the instrument), the seven fibers are arranged in a six-around-one configuration, with the center-fiber delivering excitation light to the sample,
while the six surrounding fibers collect the emission from the sample. The fibers are fed into a hollow brass tube, and immobilized using a vacuum epoxy (Torr Seal, Agilent Technologies, Lexington, MA, USA).

Figure 2.2: Cryogenic fiber-optic probe.

The excitation fiber and the six collection fibers are bifurcated and fed into tubing to facilitate their interfacing with instrumentation. The excitation fiber is centered within its interfacing tube. The six collection fibers are vertically aligned into a slit-configuration, and centered within its interfacing tube. The excitation and emission fibers are immobilized within their respective interfacing tubes with the aid of vacuum epoxy.
All three legs of the bundle are surrounded by heat shrink tubing. Additionally, the distal leg containing both excitation and emission fibers, is fed into a ~1.2 m long copper tube, which provides mechanical support for immersion of the bundle into a cryogenic Dewar, and facilitates the attachment of a sample vial to the distal end of the FOP. The copper tubing is flared at the distal end, retaining a phenolic auto-sampler vial screw cap, threaded for 8-425 vials. The sample is then simply placed in the vial, and the vial screwed onto the end. The bundle is not immobilized within the copper tubing, so fine adjustments to the relative positions of the sample and probe are simple to perform.

### 2.2 Instrumentation

Figure 2.3 provides a schematic of the instrumental components used for LETRSS. When coupled to the cryogenic FOP, this instrument provides low-temperature luminescence data within the fluorescence (ns–µs) and phosphorescence (ms–s) time domains.
The excitation source is a tunable dye laser (Northern Lights, Dakota Technologies, Inc.) pumped by the second harmonic of a Q-switched Nd:YAG laser (Quanta Ray). The dye-laser output is directed through a frequency doubling crystal composed of potassium dihydrogen phosphate (KDP), and the resulting excitation energy is focused onto the excitation fiber of the FOP. Tuning of the excitation is performed by selecting an appropriate dye-solution. Several parameters govern the tuning range of a dye-solution, including the concentration of the dye, solvent composition, and the source for “pumping” the dye-laser. Table 2.1 gives a selection of dye-solutions and the corresponding tuning range, pumped using the second harmonic of an Nd:YAG laser (532 nm). With an output power of approximately 30 mJ from the pump source, the dye-laser utilizing a Rhodamine 6G dye produces an output greater than 5 mJ at the peak maximum with a bandwidth of less than 0.03 nm. The narrow bandwidth matches well with typical bandwidths of Shpol’skii excitation spectra.

Emission collected by the FOP is focused onto the entrance slit of the spectrograph (Spex 270M). Emission is dispersed by a holographic grating (1200 grooves·mm⁻¹, blazed at 500 nm, and a reciprocal linear dispersion of 0.8 nm·mm⁻¹), and directed to the exit aperture of the

Table 2.1: A selection of laser dyes and corresponding tuning characteristics.a

<table>
<thead>
<tr>
<th>Dye</th>
<th>Concentrationb (x 10⁻⁴ mol·L⁻¹)</th>
<th>λmaxc (nm)</th>
<th>Tuning rangec (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhodamine 590</td>
<td>2.2</td>
<td>560</td>
<td>552–580</td>
</tr>
<tr>
<td>DCM</td>
<td>5.8</td>
<td>650</td>
<td>624–689</td>
</tr>
<tr>
<td>LDS 698</td>
<td>3</td>
<td>692</td>
<td>659–732</td>
</tr>
<tr>
<td>LDS 759</td>
<td>3</td>
<td>757</td>
<td>708–812</td>
</tr>
</tbody>
</table>

a. Data and characteristics obtained from Exciton.⁵¹
b. All dyes listed in this table were dissolved in methanol.

c. Characteristics when pumped with second harmonic of Nd:YAG laser (532 nm).
spectrograph. At the focal plane of the exit aperture is a front-faced ICCD detector (DH520, Andor Technologies). The ICCD has an active area $18 \times 6.7$ mm corresponding to $690 \times 256$ pixels. Based on the reciprocal linear dispersion of the spectrograph and the dimensions of the active area of the CCD chip, theoretical limiting resolution of one pixel corresponds to $0.02$ nm, which agrees with previous evaluation utilizing a monochromator and PMT ($0.03$ nm).\(^{40}\) Evaluation with a mercury calibration lamp ($313$ nm line) determined the experimental limiting resolution of the spectrograph–ICCD system to be $0.09$ nm,\(^ {40,52}\) which corresponds to $4–5$ pixels ($0.08–0.10$ nm), and is a consequence of pixel crosstalk and the spatial resolution of the image intensifier ($46$ µm).\(^ {53}\) The ICCD is cooled to $-10^\circ$C, via thermoelectric cooling (Peltier cooling), to minimize dark current noise.

External triggering is controlled by a digital delay-generator (DDG) (DG535, Stanford Research Systems). A pre-pulse TTL signal is sent by the Nd:YAG laser $140$ ns prior to the laser pulse to account for delays in electrical connections and components.\(^ {53}\) Depending on the application, the DDG controls the timing of the mechanical shutter and the ICCD intensifier. The mechanical shutter is capable of timing as fast as the millisecond time domain, with a rise (open) time of $1.5$ ms, fall (close) time of $3.0$ ms, and maximum pulse width of $6.5$ ms. The ICCD intensifier is capable of ultra-fast gating with a minimum gate time of $2$ ns (FWHM).\(^ {53}\)

Custom software developed in LabVIEW (version 6.0, National Instruments) provides the graphical user interface for controlling the instrumental parameters, i.e., excitation wavelength, emission wavelength range, entrance slit of the spectrograph, the delay and gating parameters, and the viewing and exporting of raw data collected from the ICCD. Raw data is imported to and post-processed using OriginLab (version 8.5, OriginLab Corporation).
2.2.1 Collection of Fluorescence Data

Average lifetime of the fluorescence transition is within the ns–µs time domain. Gating is accomplished by the ICCD image intensifier, with the mechanical shutter open. Figure 2.4A illustrates the method of collecting time-resolved fluorescence data. In this case the mechanical shutter remains in the open position, and DDG triggers the gate delay \((D)\) and gate width \((G)\) times of the ICCD intensifier. \(D\) is set to avoid convolution of data by the laser pulse.

Fluorescence spectra are collected using the accumulation of emission resulting from a certain number of laser pulses, which ultimately depends on the fluorescence quantum yield of the targeted analyte. With a repetition rate of 10 Hz, i.e., 100 ms per laser pulse, a typical spectral acquisition generated with 100 laser pulses takes 10 s. The spectrograph–ICCD is able to collect a maximum spectral range of 40 nm; if a wider spectral range is desired, the spectrograph grating can be tuned to a different central wavelength, the acquisition repeated, and the spectra combined in post-processing mode.

2.2.2 Collection of Phosphorescence Data

The Nd:YAG laser has a repetition rate of 10 Hz, i.e., a laser pulse is fired every 100 ms. Accounting for a the data transfer and storage time \((DTS)\) of 50 ms, gate times longer than 40 ms would result in convolution of collected data with the following laser pulse. To avoid the convolution of luminescence data, a mechanical shutter, in the closed position, is utilized to block excitation light from reaching the sample.

The average lifetime of the phosphorescence transition may range from milliseconds to several seconds. Depending on the gate times necessary to collect the phosphorescence data, one
of two approaches may be used. In the first approach, phosphorescence data can be collected similar to how fluorescence data is collected (Figure 2.4A), provided sufficient amount of phosphorescence photons are produced from one laser pulse, and that the summation of gate times are less than or equal to 40 ms. A gate delay time greater than 20 µs is typically used to avoid the collection of residual fluorescence. The mechanical shutter in this case remains open.

An alternative approach (Figure 2.4B) is used for weakly phosphorescing species with long lifetimes. In this case, the mechanical shutter is open during the excitation cycle, and then closed for the collection of emission. First, the mechanical shutter is triggered to open, and the sample is excited with several laser pulses (typically 20 pulses) in order to build up the triplet-state population to an acceptable signal-to-noise ratio. The mechanical shutter is triggered to close, which also serves as the zero–reference time for programming the gating times ($D$ and $G$) of the ICCD for collecting the phosphorescence data. Because the mechanical shutter blocks the laser pulses from reaching the sample, gate times greater than 40 ms can be utilized.
Figure 2.4: Schematic of collection of time-resolved luminescence spectrum via LETRSS (A) when sufficient luminescence photons can be generated with one laser pulse and the corresponding luminescence decay can be collected with a gate less than or equal to 40 ms; (B) luminescence, i.e., phosphorescence is too weak to generate photons with one laser pulse, and/or a gate greater than 40 ms is required to collect the luminescence decay. L = laser pulse; $t_0$ = time zero; $t_s$ = shutter time; $D$ = delay time; $G$ = gate time; $DTS$ = time necessary for data transfer and storage. O (outlined letter) = mechanical shutter is open; X (outlined letter) = mechanical shutter is closed.
2.3 Multidimensional Luminescence Data Formats

Typically, luminescence data is collected as a two-dimensional plot of emission intensities versus emission wavelengths (emission spectrum). The emission intensities are recoded using a constant excitation wavelength specifically selected for the luminophore of interest. For samples containing a mixture of luminescing components, which is often the case in real-world samples, the selected excitation wavelength may not be capable to excite all the luminophores in the sample. In these cases, two-dimensional spectra (intensity of emission versus emission wavelength) only yield partial information on the total luminescence of the sample. This type of data format does not provide complete information on the total luminescence of the sample.

Monitoring the intensity of luminescence at several excitation wavelengths offers the possibility to add a third variable (excitation wavelengths) to the luminescence data format. The addition of excitation wavelengths (third variable) provides a three-dimensional plot consisting of luminescence intensities as a function of excitation and emission wavelengths. This type of data format is known as excitation-emission matrix (EEM). Other types of data formats that explore the multidimensional nature of luminescence spectroscopy are wavelength-time matrices (WTM) and time-resolved excitation emission matrices (TREEM). Multidimensional data formats carry with them the second-order advantage. This property is extremely useful for the analysis of complex samples of unknown composition.\textsuperscript{54,55}
2.3.1 Wavelength-Time Matrices

WTMs were originally introduced by Knorr and Harris. A WTM contains three dimensions: emission wavelengths, luminescence intensities, and delay times. The original reports on WTM collection followed a tedious and time-consuming procedure that made them unpractical for analytical chemistry purposes. A set of luminescence decays were collected at a series of sequentially stepped emission wavelengths with a single-channel (monochromator–PMT) system. This limitation was later removed by Campiglia’s group with the instrumental system previously described in Figure 2.3.

Figure 2.5 illustrates the procedure for collecting fluorescence WTM using the LETRSS system in Figure 2.3. It involves the acquisition of several time-resolved fluorescence spectra, as described in section 2.2.1, in which the gate delay \((D)\) is incrementally increased with a constant step size \((\Delta D)\). The gate times used for fluorescence WTM are typically within the nanosecond time domain. Phosphorescence WTM can be collected in a similar fashion provided the target species are strongly phosphorescent, i.e., one laser pulse produces a sufficient amount of photons for emission collection with gate times of 40 ms or less. Compared to fluorescence WTM, the gate times used for phosphorescence are relatively longer, i.e., in the millisecond time domain.

For weak phosphors with relatively long phosphorescence lifetimes, WTM acquisition as previously described in Figure 2.5 might take one hour or more. An alternative approach involves the accumulation of several WTM. First, the mechanical shutter is opened, and the sample is excited with several laser pulses (typically 20 pulses), to build up the triplet-state population. The shutter is then closed, and the ICCD gating is set with \(D = 0\) and \(G = 40\) ms. A spectrum is collected every 100 ms, equal to the repetition rate of the Nd:YAG laser, effectively

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acting as the delay increment, $\Delta D$. Analogous to the accumulation of several fluorescence spectra, the contribution of noise is minimized by accumulating a certain number of WTMs over a certain number of laser pulses. The elimination of a programed $D$ for the collection of each individual emission spectrum significantly reduces the overall WTM acquisition time. Assuming 20 laser pulses (2 s excitation cycle at 10 Hz), 30 spectra per WTM (100 ms each), and an accumulation of 100 WTMs, leads to a total acquisition time shorter than 9 minutes.
Figure 2.5: Schematic diagram illustrating the collection of a fluorescence WTM via LETRSS.
2.3.2 Excitation-Emission Matrices and Time-Resolved Excitation-Emission Matrices

Figure 2.6 depicts two possible ways of plotting EEM data. Additional selectivity for the direct analysis of targeted compounds in complex samples of unknown composition is obtained by adding the temporal dimension to the orthogonal spectral dimension of EEMs. It is possible to choose an appropriate time window for EEM collection that enhances the fluorescence or phosphorescence of targeted compounds over the emission interference of sample concomitants. This type of data format is known as time-resolved excitation emission matrix (TREEM). Two different instrumental procedures have been developed in our lab to record TREEMs. One possibility is to record TREEMs as series of WTMss acquired at different excitation wavelengths. The superposition of WTMss produce a third order data format known as excitation-modulated WTM (EMWTM) data. Another possibility is to record a series of EEMs at different delay
times after the short duration of the excitation pulse. All the intensity values, as a function of excitation and emission wavelengths, for a particular decay time are assembled into an EEM format specific for that decay time. The superposition of EEMs produces a data format known as time-resolved excitation-emission cube (TREEC).\textsuperscript{59} Processing EMWTMs and/or TREECs with second order multivariate calibration algorithms, such as parallel factor analysis (PARAFAC) and unfolded–partial least squares/residual trilinearization (U-PLS/RTL), provide a general solution to unpredictable spectral interference, a ubiquitous problem in samples of unknown composition.\textsuperscript{58,59}
CHAPTER 3: LETTERS ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOIL SAMPLES*

3.1 Introduction

Considerable efforts have been made to develop analytical techniques capable to determine trace concentration levels of polycyclic aromatic hydrocarbons (PAHs) in environmental samples.60-69 One of the main reasons for this motivation is the carcinogenic and toxicological nature of some PAHs. Under this prospective, particular attention has been paid to the 16 PAHs included in the U.S. Environmental Protection Agency (EPA) priority pollutant list. Their monitoring in air, water and soil samples is recommended to prevent human exposure to PAH contamination.

Environmental monitoring of EPA-PAHs follows the general pattern of sample clean up, pre-concentration and chromatographic analysis. Sample preparation simplifies matrix composition and pre-concentrates PAHs to achieve detectable concentrations by chromatographic techniques. High-performance liquid chromatography (HPLC) with absorption and/or fluorescence detection and gas chromatography–mass spectrometry (GC-MS) are the basis of current EPA methodology. When HPLC is applied to unfamiliar samples, a supporting technique such as GC-MS is often used for confirmation purposes.70-73

The time consuming procedures of traditional methodology makes the development of screening techniques particularly attractive for the routine monitoring of numerous samples.

Screening techniques capable to provide a “yes or no” answer to PAH contamination prevent unnecessary scrutiny of un-contaminated samples via conventional methods, reduce analysis cost, and expedites turnaround time for decision making and remediation purposes. A recent trend for the direct determination, i.e., no chromatographic separation, of targeted species in matrixes of unknown composition refers to processing multidimensional spectroscopic data with second-order multivariate calibration methods. The determination of phenanthrene and benzo[k]fluoranthene in urban run-off water samples, benzo[a]pyrene and dibenzo[a,h]anthracene in underground, tap and mineral water samples, and the analysis of chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]anthracene, benzo[a]pyrene and dibenzo[a,h]anthracene in river water and sludge samples, has been demonstrated on the basis of room-temperature fluorescence excitation-emission matrices (EEMs) combined to either parallel factor analysis (PARAFAC) or unfolded–partial least squares/residual-bilinearization (U-PLS/RBL).

This chapter deals with the direct determination of 15 EPA-PAHs in soil samples. Soil is one of the most important reservoirs for PAHs, which are deposited in the gaseous state or associated to airborne particles, even at sites far from the petroleum industry. Once present in the soil, PAHs become a long-term source of environmental health risk due to their rather low water solubility, intrinsic chemical stability and high resistance to bio-degradation. The approach we present here is based on LETRSS. It takes advantage of the full dimensionality of fluorescence spectroscopy combining spectral and lifetime information in WTM and TREEMs.
As previously mentioned, a WTM consists of a series of emission spectra recorded under one excitation wavelength and different time delays after the laser excitation pulse. A TREEM is an excitation-emission matrix recorded at a certain time window during the total fluorescence decay of the sample. The term time window refers to the variation of both the delay and the gate times after the short duration of the excitation pulse. Recording WTM during the fluorescence or phosphorescence decay of the sample provides an additional parameter for PAH identification (lifetime). Unambiguous PAH determination is made possible on the basis of spectral and lifetime information. Fluorescence or phosphorescence lifetimes also report on spectral peak purity, i.e., an essential condition for the accurate quantitative determination of PAHs without previous chromatographic separation. The choice of an appropriate time window for TREEM collection enhances the fluorescence of targeted compounds over the fluorescence interference of sample concomitants.

The main reason for reducing the sample temperature is to promote the spectral narrowing that one needs to determine numerous EPA-PAHs without previous chromatographic separation. LETRSS measurements were made at liquid helium temperature (4.2 K) with the aid of the cryogenic FOP described previously. The FOP provides a straightforward procedure for the LETRSS analysis of soil samples. Upon sample sonication with microliters of n-octane in the vessel of the FOP, PAHs partition into the organic solvent for WTM collection. Since sample handling is limited to weighing milligrams of soil into the vial of the FOP, contamination risks and/or PAH loss is kept to a minimum. The small vial of the FOP (750 µL) and the small volume of extracting solvent facilitate the simultaneous extraction of numerous samples. The entire experimental procedure, including the 30 min of sonication time, takes less
than 40 min per sample. The method is environmentally friendly as the complete screening of the 15 EPA-PAHs takes only 250 µL of organic solvent per sample.

Accurate determination of EPA-PAHs via 4.2 K WTM analysis forces the analyst to check for potential interference via lifetime analysis. This is particularly true for the analysis of samples with unknown composition. A single exponential decay with a lifetime equivalent to the lifetime of the pure standard provides a strong argument to claim accurate PAH determination.\textsuperscript{52,91,93}

The approach we present in this chapter is fundamentally different as we base PAH determination on 4.2 K fluorescence TREECs. 4.2 K fluorescence TREECs result from the superposition of fluorescence TREEMs recorded at different time windows from the laser excitation pulse. Potential interference from unknown sample concomitants is handled by processing four-way 4.2 K fluorescence TREEC data arrays with PARAFAC and U-PLS/RTL.

To the extent of our literature search, this was the first report on the analysis of soil samples via 4.2 K fluorescence TREEC coupled to second-order multivariate calibration methods. The same was true for the hyphenation of PARAFAC or U-PLS/RTL to 4.2 K fluorescence TREEC data. The only article that exists on processing high-resolution data with second-order calibration methods deals with the combination of PARAFAC to 4.2 K excitation modulated phosphorescence WTM (EMWTMs). EMWTMs were generated with the superposition of five 4.2 K phosphorescence WTM recorded at five excitation wavelengths. Each WTM was recorded using the same delay (50 µs) and gate (1100 ms) times. The EMWTMs/PARAFAC approach was successfully applied to the analysis of 2,3,7,8-tetrachlorodibenzo-para-dioxin in solid-phase water extracts.\textsuperscript{58}
The four-way 4.2 K fluorescence TREEC data arrays used here for the determination of the 15 EPA-PAHs were recorded during the nanosecond time domain of the fluorescence decay. TREECs were generated from 4.2 K fluorescence TREEMs recorded using an excitation range common to the 15 EPA-PAHs. The sensitivity of TREEC/four-way modeling made possible to determine PAHs at the ng·g\(^{-1}\) to pg·g\(^{-1}\) concentration level with no need for sample pre-concentration. Its selectivity is demonstrated with analytical recoveries statistically equivalent to those obtained with classic methodology.

3.2 Experimental Section

3.2.1 Chemicals

All solvents were HPLC grade and purchased from Fisher. Unless otherwise noted, Nanopure water, from a Barnstead Nanopure Infinity water system, was used throughout. All chemicals, including those used for PAH extraction, were analytical reagent grade and used without further purification. A soil sample of known composition, Natural Matrix Reference Material CRM 104-100–PAH contaminated soil/sediment from Southern Branch of the Elizabeth River, Chesapeake Bay area, was acquired from Resource Technology Corporation, Laramie, WY. A Supelco PAH mixture (EPA 610) in methanol:methylene chloride (1:1 v/v) from Supelco was used as the reference standard for HPLC analysis. PAH standards for LETRSS analysis were purchased from Aldrich at their highest available purity. Rhodamine 6G was acquired from Exciton and used with the tunable dye laser according to specifications.
3.2.2 Analysis of Soil Samples via Classic Methodology

Saponification and sonication procedures for PAH extraction from soil samples followed previously reported methodology. Methanolic saponification was performed as follows: 20 g of dried soil were mixed with 100 mL of 2M KOH/CH3OH and heated at 70°C for 2 h. After cooling, the mixture was filtered with a 9.0 cm GF/C glass micro-fiber filter (Whatman International, UK), and the methanol phase was extracted three times with 50 mL of hexane. The non-polar phase was concentrated to approximately 1–2 mL and submitted to sample clean-up. The procedure for sonication was the following: 20 g of dried soil was mixed in an Erlenmeyer flask with 100 mL of hexane:acetone:toluene (10:5:1 v/v/v). The mixture was sonicated at room-temperature in a sonication bath (Branson, Model 3210) for 30 min with occasional swirling to prevent sticking on the bottom of the flask. The mixture was filtered with a 9.0 cm GF/C glass micro-fiber filter (Whatman International, UK), concentrated to approximately 1–2 mL and submitted to sample clean-up. Clean-up of saponification and sonication extracts was carried out with the following procedure: prior to loading the extracts onto the solid-phase extraction silica gel cartridges (Supelco 6 mL LC-18 SLE cartridges), small amounts of Na2SO4 were added to the cartridges to protect the silica gel surface. Each cartridge was preconditioned twice with 5 mL of hexane and the extracts were loaded by means of a Pasteur pipette. PAH elution was carried out with a total hexane volume of 10 mL.

HPLC analysis was performed with a computer controlled HPLC system from Hitachi equipped with the following basic components: a pump model L-7100, a UV detector (model L-7400 UV) and a fluorescence detector (model L-7485), and an online degasser (model L-761). A Supelco Supelcosil TM LC-PAH column (15 cm length, 4 mm diameter and 5 µm particle size)
provided adequate resolution of the 16 EPA-PAH. The mobile phase was a mixture of methanol:water. Column conditions include a 1.5 mL·min⁻¹ flow rate, isocratic elution with 40:60 water:methanol (v/v) for 5 min and then linear gradient to 99% methanol over 20 min. The total separation time was approximately 40 min. All sample injections were at a volume of 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 the absence of interfering contamination. PAH detection was carried out in the fluorescence mode using time programmed excitation/emission wavelengths: 0 min, 220/330 nm (naphthalene); 7 min, 225/315 nm (acenaphthene and fluorene); 10.1 min, 244/370 nm (phenanthrene and anthracene); 13 min, 237/460 nm (fluoranthene); 14.3 min, 237/385 nm (pyrene); 16 min, 277/376 nm (benzo[a]anthracene and chrysene); 21.2 min, 255/420 nm (benzo[b]fluoranthene, benzo[k]fluoranthene and benzo[a]pyrene); 25.5 min, 300/415 nm (dibenz[a,h]anthracene and benzo[g,h,i]perylene; 27.5 min, 250/495 nm (indeno[1,2,3-cd]pyrene). For the detection of individual PAH, the selected wavelengths corresponded to the maximum excitation and emission wavelengths. In cases where PAH elution was too fast for individual wavelength optimization, the selected wavelengths corresponded to a compromise among the maximum excitation and emission wavelengths of eluted PAHs.⁹³

3.2.3 PAH Screening via 4.2 K LETRSS

Complete description of the instrumentation for LETRSS analysis is provided in Chapter 2. The same is true for the cryogenic FOP. A known amount (0.05 grams) of soil sample was mixed with 250 µL of n-octane in the vessel of the FOP. The mixture was submitted to 30 min of
sonication at room temperature in a Branson sonication bath (Model 3210). After 5 min of settling time, the sample extract was analyzed via LETRSS. 4.2 K measurements were done by coupling the sample vial of the FOP to the copper tubing of the fiber assembly. The tip of the fiber assembly was kept ~0.5 cm above the surface of the liquid layer. Sample freezing was accomplished by lowering the copper tubing into the liquid helium, which was held in a Dewar flask with 60 L storage capacity. The liquid helium would typically last 3 weeks with daily use, averaging 15–20 samples per day. Complete sample freezing took less than 90 s per sample. Replacing the frozen sample involved removing the sample vial from the cryogen container and melting the frozen sample with a heat gun. Because no physical contact between the tip of the fiber-optic bundle and the sample ever occurred during measurements, probe cleanup between measurements was not necessary. The entire freeze, thaw, and sample replacement cycle took no longer than 5 min. PAH concentrations were determined with the multiple standard additions method.

3.2.4 Collection of TREEC Data Arrays

The four-way 4.2 K fluorescence TREEC data arrays used for EPA-PAH determination result from the superposition of four TREECs recorded from soil extracts containing different concentrations of individual PAHs. PAH concentrations were adjusted via the multiple standard additions procedure. The first TREEC reflected the original PAH composition of the soil sample, i.e., no standard addition. The other TREECs, one per standard addition, were obtained after adding 5, 10, and 15 µL of a standard EPA-PAH mixture to the 250 µL volume of soil extract in the sample vial of the FOP. All standard additions were made at room temperature.
The 4.2 K fluorescence TREEMs used to generate the four TREECs were recorded using an excitation range (280–295 nm) common to the 15 EPA-PAHs. The tunable dye laser was stepped at 0.5 nm increments, generating a total of twenty-two (22) excitation wavelengths. Fluorescence was recorded within the 300–500 nm wavelength range using the following delay times: 10, 30, 60, 90, 120 and 150 ns. The gate width for the first TREEM \((D = 10 \text{ ns})\) was 20 ns. The gate widths for the remaining TREEMs were 30 ns. These parameters provided the following time windows \((D–D+G)\) for TREEM collection: TREEM\(_1\) = 10–30 ns, TREEM\(_2\) = 30–60 ns, TREEM\(_3\) = 60–90 ns, TREEM\(_4\) = 90–120 ns, TREEM\(_5\) = 120–150 ns, and TREEM\(_6\) = 150–180 ns.

### 3.2.5 Chemometric Algorithms and Software

All calculations were done using MATLAB 7.6\(^9_5-9^9\) with the aid of the MVC3 graphical toolbox, and a user friendly MATLAB graphical interface available on internet.\(^1^0^0\) These calculations were made by Prof. Hector C. Goicoechea at the Universidad Nacional del Litoral, Santa Fe, Argentina.

### 3.3 Results and Discussion

#### 3.3.1 Analysis of Soil Samples with Classic Methodology

Several methods exist to extract PAHs from soil samples.\(^9^4,1^0^1-1^0^4\) Our choice for methanolic saponification was based on its comparatively high PAH extraction efficiency.\(^9^4\) Saponification under methanolic conditions breaks down polymeric structures of organic matter, which is frequently present in soil samples and chemically associated to PAHs, and increases the
accessibility of the solvent for PAH extraction. The sonication method was selected because of its relatively short extraction time and easy implementation.\textsuperscript{94,101} The solvent we used for sonication, hexane:acetone:toluene (10:5:1 v/v/v), was reported to yield higher extraction efficiencies than other sonication solvents.\textsuperscript{94,101} It should be noted, however, that even after careful optimization of extraction parameters, such as solvent composition and extraction time, PAH recoveries with sonication tend to be lower than with other extraction techniques.

HPLC analysis of soil extracts was done with previously reported methodology.\textsuperscript{93} Figure 3.1 depicts a typical chromatogram of the soil sample. Peak assignments were solely based on the retention times of pure standards. Acenaphthylene showed no fluorescence under the conditions of the separation. Table 3.1 summarizes the retention times and the limits of detection (LOD) of the HPLC method along with the PAH recoveries obtained via saponification–HPLC and sonication–HPLC analysis. The HPLC–LOD were calculated according to the formula, LOD = $3 \times S_B / m$, where $S_B$ is the standard deviation of the average blank signal estimated from one-fifth of the peak-to-peak noise ($N_{p-p}/5$) and $m$ is the slope of the calibration curve.\textsuperscript{105} The $N_{p-p}$ was measured at the base peak of each PAH elution over a sufficiently wide region of the chromatogram. Calibration curves were built with synthetic mixtures of pure standards using a minimum of five linear concentrations per PAH. The slopes of the calibration curves were calculated from the linear dynamic ranges (data not shown) using the least squares method for statistical fitting.\textsuperscript{106}

All PAH concentrations determined in the soil extracts were at the parts-per-million (µg·mL$^{-1}$) level. Average saponification values varied from 0.55 µg·mL$^{-1}$ (fluorene) to 18.5 µg·mL$^{-1}$ (fluoranthene). Sonication concentrations varied from 0.27 µg·mL$^{-1}$ (fluorene) to 5.46
µg·mL\(^{-1}\) (pyrene). All LODs were at the parts-per-billion level (ng·mL\(^{-1}\)) and, therefore, well below the concentrations of EPA-PAHs in soil extracts. As expected, the PAH recoveries with methanolic saponification were considerably higher than those obtained with sonication. The relative standard deviations (RSD) of the average recoveries obtained with saponification varied from 2.4% (fluoranthene) to 8.9% (fluorene). The RSD values obtained with the sonication method varied from 1.5% (chrysene) to 12.5% (naphthalene). These facts can be attributed to several factors, including uncontrolled PAH loss during extraction via saponification and sonication.\(^{94,101}\)

**Figure 3.1:** HPLC chromatogram obtained from soil extract of reference standard material CRM 104-100. Identified PAH are the following: (1) naphthalene, (2) acenaphthene, (3) fluorene, (4) phenanthrene, (5) anthracene, (6) fluoranthene, (7) pyrene, benzo[\(a\)]anthracene (8), chrysene (9), benzo[\(b\)]fluoranthene (10), benzo[\(k\)]fluoranthene (11), benzo[\(a\)]pyrene (12), dibenzo[\(a,h\)]anthracene (13), benzo[\(g,h,i\)]perylene (14) and indeno[1,2,3-cd]pyrene. Partial magnification of chromatogram aims to facilitate visualization of peaks 13, 14 and 15.
Table 3.1: Analysis of EPA-PAHs in soil samples via established methodology.

<table>
<thead>
<tr>
<th>PAH**</th>
<th>HPLC Analytical Figures of Merit</th>
<th>Extraction recoveries©</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Retention time b (min)</td>
<td>λ_{exc}/λ_{em} c (nm)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>7.5 ± 0.08</td>
<td>240/330</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>14.8 ± 0.05</td>
<td>296/325</td>
</tr>
<tr>
<td>Fluorene</td>
<td>15.5 ± 0.06</td>
<td>258/404</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>17.0 ± 0.09</td>
<td>258/404</td>
</tr>
<tr>
<td>Anthracene</td>
<td>19.4 ± 0.08</td>
<td>258/404</td>
</tr>
<tr>
<td>Fluoranthen</td>
<td>20.8 ± 0.11</td>
<td>278/395</td>
</tr>
<tr>
<td>Pyrene</td>
<td>22.5 ± 0.07</td>
<td>278/395</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>26.8 ± 0.03</td>
<td>278/395</td>
</tr>
<tr>
<td>Chrysene</td>
<td>27.9 ± 0.06</td>
<td>278/395</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>32.1 ± 0.07</td>
<td>363/412</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>32.9 ± 0.12</td>
<td>363/412</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>33.8 ± 0.09</td>
<td>363/412</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>36.6 ± 0.03</td>
<td>341/440</td>
</tr>
<tr>
<td>Dibenzo[g,h,i]perylene</td>
<td>36.9 ± 0.02</td>
<td>304/421</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>38.2 ± 0.05</td>
<td>308/467</td>
</tr>
</tbody>
</table>

a. Sample volume is 20 µL
b. Average retention time of three chromatographic runs. Column conditions include a 1.5 mL·min⁻¹ flow rate, isocratic elution with 60:40 water:methanol for 5 min and then linear gradient to 99% methanol over 20 min.
c. Excitation and emission wavelength for fluorescence detection.
d. Limits of detection (LOD) = 3×Sₙ/m; where Sₙ is the standard deviation of the average blank signal estimated from one-fifth of the peak-to-peak noise (Nₚ₋ₚ/5) and m is the slope of the calibration curve.
e. PAH recoveries were calculated according to the equation: Recovery (%) = [mass of PAH in extract / mass of PAH in soil sample] × 100; where mass of PAH in extract (ng) = CPAH in EXTRACT (ng·mL⁻¹) × 0.25 mL and mass of PAH in soil sample (ng) = CPAH in SOIL (ng·g⁻¹) × weight of soil sample (g). The PAH concentration in the soil extract (CPAH in EXTRACT) was determined via multiple standard additions. The PAH concentration in soil (CPAH in SOIL) is the certified PAH concentration of the reference standard. Reported values are an average of three soil extractions.
3.3.2 4.2 K LETRSS Analysis of EPA-PAHs

For the specific case of EPA-PAHs, the solvent matching criterion leads to one of the following five \( n \)-alkanes: \( n \)-pentane (naphthalene, acenaphthene, and acenaphthylene), \( n \)-hexane (phenanthrene and pyrene), \( n \)-heptane (fluorene, fluoranthene, benzo[\( g,h,i \)]perylene, benzo[\( b \)]fluoranthene and anthracene), \( n \)-octane (benzo[\( a \)]pyrene, dibenz[\( a,h \)]anthracene, chrysene and benz[\( a \)]anthracene), and \( n \)-nonane (indeno[\( 1,2,3-cd \)]pyrene and benzo[\( k \)]fluoranthene). Using the best matching solvent for each PAH would provide the best spectral resolution possible (see Figure 3.2). Considering the use of five organic solvents per sample practically unattractive for screening purposes, we carried out the analysis of the 15 EPA-PAHs with \( n \)-octane. Our preference was based on preliminary studies showing better sonication extractions with \( n \)-octane than with the other four solvents.
Figure 3.2: The structures of 16 EPA-PAHs (black) and corresponding best matching $n$-alkane solvents (red).
Acenaphthylene was the only compound with no fluorescence emission in frozen octane. Its lack of fluorescence persisted after sample de-oxygenation or analyte freezing in \( n \)-pentane, \( n \)-hexane, \( n \)-heptane and \( n \)-nonane. Table 3.2 summarizes the 4.2 K fluorescence figures of merit of the remaining PAHs in \( n \)-octane. The excitation and emission ranges include all the peaks analytically meaningful for each PAH, i.e., with a signal-to-noise ratio equal to or higher than 3 (\( S/N \geq 3 \)). All PAHs showed well-behaved single exponential fluorescence decays in \( n \)-octane. The reported lifetimes (\( \tau \)) were measured at the maximum emission (\( \lambda_{em} \)) wavelength of each PAH. Sample excitation was at 283.2 nm, i.e., an excitation wavelength common to the 15 EPA-PAHs that provides their determination at trace concentration levels (ng·mL\(^{-1}\)). The LODs were calculated with the formula LOD = \( 3 \times S_B/m \); where \( S_B \) is the standard deviation of the blank (\( N = 16 \)) and \( m \) is the slope of the calibration curve.\(^{108} \) Calibration curves were built with pure standards using a minimum of five linear concentrations per PAH. The slopes of the calibration curves were calculated from the linear dynamic ranges using the least squares method for statistical fitting.
Table 3.2: Fluorescence figures of merit of EPA-PAHs in n-octane at 4.2 K.

<table>
<thead>
<tr>
<th>PAH a</th>
<th>Excitation/emission range b (nm)</th>
<th>Emission maxima (nm)</th>
<th>Fluorescence lifetime c (ns)</th>
<th>LOD d (ng·mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>224–295/315–352</td>
<td>320.8</td>
<td>200.5 ± 0.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>229–300/315–353</td>
<td>320.9</td>
<td>42.3 ± 0.3</td>
<td>1.0</td>
</tr>
<tr>
<td>Fluorene</td>
<td>265–303/302–317</td>
<td>308.2</td>
<td>6.4 ± 0.1</td>
<td>1.8</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>258–300/346–384</td>
<td>365.2</td>
<td>43.8 ± 1.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Anthracene</td>
<td>255–366/378–407</td>
<td>384.4</td>
<td>5.0 ± 0.1</td>
<td>2.8</td>
</tr>
<tr>
<td>Fluoranthen</td>
<td>278–364/407–467</td>
<td>408.2</td>
<td>54.9 ± 1.3</td>
<td>2.5</td>
</tr>
<tr>
<td>Pyrene</td>
<td>242–345/370–394</td>
<td>371.7</td>
<td>520.2 ± 4.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>271–300/380–406</td>
<td>384.2</td>
<td>51.7 ± 0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Chrysene</td>
<td>261–330/350–402</td>
<td>361.9</td>
<td>55.2 ± 1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>291–386/395–450</td>
<td>397.9</td>
<td>40.7 ± 0.9</td>
<td>0.7</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>253–397/400–430</td>
<td>403.6</td>
<td>8.6 ± 0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>268–372/395–431</td>
<td>401.4</td>
<td>42.8 ± 1.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>253–391/460–503</td>
<td>462.6</td>
<td>11.9 ± 0.1</td>
<td>1.0</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>289–353/390–420</td>
<td>394.4</td>
<td>41.4 ± 0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>292–391/406–446</td>
<td>419.5</td>
<td>123.8 ± 2.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

a. All PAH solutions were prepared in n-octane.
b. Excitation and emission ranges include all the peaks with a S/N ≥ 3.
c. Fluorescence lifetimes correspond to the average of three individual measurements taken from three frozen aliquots at 283.2 nm excitation and maximum emission wavelength of each PAH.
d. LOD = limit of detection; LOD = 3×SB/m; where SB is the standard deviation of 16 blank measurements (n-octane) and m is the slope of the calibration curve at 283.2 nm excitation and maximum emission wavelength. Delay and gate times were adjusted for maximum fluorescence intensity.
Figure 3.3 shows the normalized fittings of the fluorescence decays of the 15 PAHs. According to their time decay profiles, EPA-PAHs belong to one of the following three groups: short-lived ($\tau \leq 11.9$ ns), medium-lived ($40.7$ ns $\leq \tau \leq 55.2$ ns) and long-lived ($\tau \geq 123.8$ ns) PAHs. Within the context of time-resolved measurements with fixed time delays, discrimination of short-lived and medium-lived PAHs should be possible with time delays of 30 and 170 ns, respectively. These delay values are based on the residual and often negligible fluorescence observed after delay times greater than or equal to $3 \times \tau$. Because time-discrimination of long-lived PAHs is not possible, their potential interference to the determination of both short-lived and medium-lived PAHs is a matter of concern. The same is true for the determination of short-lived PAHs and the potential interference from medium-lived PAHs.

Figure 3.3: Exponential fittings of fluorescence decays recorded from EPA-PAHs. (A) short- and medium-lived PAHs. Short-lived PAHs include anthracene, fluorene, benzo[k]fluoranthene and indeno[1,2,3-cd]pyrene. Medium-lived PAHs include benzo[b]fluoranthene, dibenzo[a,h]anthracene, acenaphthene, benzo[a]pyrene, phenanthrene, benzo[a]anthracene, fluoranthene and chrysene. (B) Long-lived PAHs. All exponential decays were normalized for maximum fluorescence intensity. All decays were recorded at 4.2 K from pure PAH standards in n-octane. All measurements were made at the maximum fluorescence wavelength of each PAH (see Table 3.2). Sample excitation was at 283.2 nm.
3.3.3 TREEC Analysis

One possibility to circumvent this limitation is based on the spectral narrowing EPA-PAHs experience under Shpol’skii conditions at 4.2 K. Because WTM provides the analyst with numerous peaks, there is always the possibility to find a set of excitation and fluorescence wavelengths free from matrix interference. In the analysis of samples with unknown composition, the analyst should always check for potential interference via lifetime analysis. A single exponential decay with a lifetime equivalent to the lifetime of the standard constitutes a strong argument for the accurate determination of the targeted PAH.

The approach we present here is fundamentally different as we base PAH determination on the appropriate selection of time windows for TREEM collection. By shortening the gate of the ICCD, one can reduce the spectral contribution of medium-lived and long-lived PAHs and still collect most of the fluorescence emitted by short-lived PAHs. By placing the time window at an intermediate time interval within the total fluorescence decay of the sample, it is possible to enhance the spectral features of medium-lived PAHs, time-discriminate the fluorescence of short-lived PAHs and reduce the contribution of long-lived PAHs.

Figure 3.4 depicts TREEM$_1$ ($D−D+G = 10–30$ ns), TREEM$_3$ ($D−D+G = 60–90$ ns) and TREEM$_6$ ($D−D+G = 150–180$ ns) recorded from the soil extract with no standard addition. Visual comparison of the spectral profiles of EPA-PAHs within the emission range of the TREEM (375–425 nm) leads to the following PAHs as the main contributors of the total fluorescence of the extract: benzo[a]anthracene, anthracene, dibenzo[a,h]anthracene, benzo[b]fluoranthene, benzo[a]pyrene, benzo[k]fluoranthene, and fluoranthene. The relative contribution of each PAH to the total fluorescence of the sample varies with the time window of
the TREEM. The fluorescence of benzo[a]pyrene, which appears in TREEM₁ and TREEM₃ at ~401.2 nm, practically vanishes in TREEM₆. Benzo[a]pyrene is a medium–lived PAH with τ = 42.8 ± 1.6 ns. The same is not true for the prominent fluorescence at 422.5 nm. The main reason for its presence in TREEM₆ is that belongs to benzo[g,h,i]perylene, a long-lived PAH (τ = 123.8 ± 2.5 ns). The superposition of TREEM₁-₆ leads to a TREEC with 540,672 data points. The superposition of TREEM₁-₆ corresponds to the TREEC of the soil extract.

Figure 3.4: 4.2K TREEM recorded from soil extract with the following time windows: 10–30 ns (EEM₁), 60–90 ns (EEM₃) and 150–180 ns (EEM₆). Excitation wavelength was stepped at 0.5 nm increments. Each emission spectrum corresponds to the accumulation of 100 laser pulses.
3.3.4 PARAFAC and U-PLS/RTL

The theory of PARAFAC and U-PLS/RTL is well documented.95-98 Briefly, the decomposition of the four-way TREEC data array with PARAFAC allows the analyst to extract emission, excitation, and time profiles of EPA-PAH along with their relative concentrations. The score of each PAH is then used to predict its concentration in the unknown sample. The principle of operation of U-PLS/RTL is fundamentally different as the original cube data is transformed into uni-dimensional arrays (vectors) by concatenating (unfolding) the original three-dimensional information. Concentration information is first used with no data from the unknown sample. A usual U-PLS model is calibrated with the included data and the vector of calibration concentrations. If there are no unsuspecting interferences in the test sample, the concentration of each PAH can then be estimated using the same parameters as the calibration step. The sample scores will be un-suited for PAH prediction when un-calibrated components occur in the test sample. In this case, the residuals of the U-PLS prediction step are abnormally large in comparison to the typical instrumental noise assessed by replicate measurements. The residual tri-linearization procedure, which is based on a Tucker3 decomposition, makes possible to model the interference effects and accurately predict the concentration of each PAH in the unknown sample.

EPA-PAH concentrations in the soil extracts were obtained via the multiple standard additions procedure. This calibration method accounts for possible matrix interference due to inner filter and synergistic effects. Table 3.3 summarizes the concentrations of PAH standards used in the synthetic mixtures for the first, second and third standard additions. The three standard additions were made by adding 5 µL increments of the standard mixtures, i.e., 5, 10,
and 15 µL, to the 250 µL volume of soil extract in the sample vial of the FOP. All standard additions were made at room temperature. PARAFAC and U-PLS/RTL modeling were carried out generating a total of four TREECs, i.e., one TREEC for the soil sample, i.e., zero standard addition, and one TREEC per standard addition in Table 3.3.

Table 3.3: Concentrations used for multiple standard additions and chemometric modeling.

<table>
<thead>
<tr>
<th>PAH</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>1.0</td>
<td>3.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>2.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Fluorene</td>
<td>3.0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>2.0</td>
<td>6.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Anthracene</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Fluoranthen</td>
<td>3.0</td>
<td>6.0</td>
<td>9.0</td>
</tr>
<tr>
<td>Pyrene</td>
<td>9.0</td>
<td>3.0</td>
<td>6.0</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>6.0</td>
<td>2.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Chrysene</td>
<td>4.0</td>
<td>6.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>4.0</td>
<td>6.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>2.0</td>
<td>3.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>2.0</td>
<td>1.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>3.0</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>3.0</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylen</td>
<td>1.0</td>
<td>2.0</td>
<td>3.0</td>
</tr>
</tbody>
</table>

- a. All concentrations are in μg·mL⁻¹.
- b. PAH standards were prepared in n-octane.
- c. 5, 10, and 15 µL of standard solution were added to 250 µL of soil extract.
The emission and excitation wavelength ranges, the time windows, the number of PARAFAC factors (responsive components), and the number of latent variables for U-PLS/RTL modeling (U-PLS/RTL factors), are listed in Table 3.4. The number of U-PLS/RTL factors was estimated via the cross validation method. Each PLS model was built by subtracting the TREEC of the sample (zero standard addition) to those recorded after the first, second and third standard additions. The number of PARAFAC factors was estimated following the internal parameter procedure, also known as the core consistency procedure, originally introduced by Bro. Figure 3.5 shows the loading matrices for emission (Figure 3.5A), excitation (Figure 3.5B) and time decay (Figure 3.5C) modes for benzo[a]pyrene predicted with the settings in Table 3.4. The labeling of components 1, 2 and 3 follows the order assigned by the model in the four-way array data of the sample. The order reflects their relative contribution to the overall variance. Benzo[a]pyrene (1) is the main fluorescence contributor in the four-way array data. The fluorescence contribution of interferences 2 and 3 is practically negligible. Comparison of the predicted spectral profiles (Figures 3.5A and 3.5B) to the experimental spectra of benzo[a]pyrene, denoted as short dash–dotted green lines, is confirmed by the values of correlation coefficients, i.e., \( r^2 = 0.889 \) (Figure 3.5A) and \( r^2 = 0.846 \) (Figure 3.5B). The same is true for the predicted decay profile of benzo[a]pyrene. The predicted value of the fluorescence lifetime (34.2 ns) is close to the experimental fluorescence lifetime of benzo[a]pyrene (38.6 ± 0.3 ns, \( N = 3 \)). The fact that the decay profile follows a well behaved, single exponential decay, confirms the negligible contributions of 2 and 3 to the total fluorescence of the sample. Similar results were obtained for the remaining EPA-PAHs.
Table 3.4: Parameters for PARAFAC and U-PLS/RTL analysis.

<table>
<thead>
<tr>
<th>PAH</th>
<th>Excitationa (nm)</th>
<th>Emissiona (nm)</th>
<th>EEM #b</th>
<th>Time windowsb (ns)</th>
<th>PARAFAC factors</th>
<th>U-PLS/RTL factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>280.5–285.5</td>
<td>319.9–320.7</td>
<td>4, 5</td>
<td>90/120, 120/150</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>283.0–289.5</td>
<td>319.3–319.1</td>
<td>1–3</td>
<td>10/30, 30/60, 60/90</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Fluorene</td>
<td>281.5–287.5</td>
<td>303.6–305.1</td>
<td>1, 2</td>
<td>10/30, 30/60</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>286.0–290.0</td>
<td>350.4–351.7</td>
<td>1, 2</td>
<td>10/30, 30/60</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Anthracene</td>
<td>282.5–295.0</td>
<td>383.6–384.4</td>
<td>1–5</td>
<td>10/30, 30/60, 60/90, 90/120, 120/150</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>280.0–290.0</td>
<td>402.7–403.8</td>
<td>1–5</td>
<td>10/30, 30/60, 60/90, 90/120, 120/150</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Pyrene</td>
<td>280.5–290.0</td>
<td>370.1–371.2</td>
<td>4, 5</td>
<td>90/120, 120/150</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>281.0–288.5</td>
<td>381.6–382.4</td>
<td>1–3</td>
<td>10/30, 30/60, 60/90</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Chrysene</td>
<td>280.0–286.5</td>
<td>362.1–362.8</td>
<td>1–3</td>
<td>10/30, 30/60, 60/90</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>284.5–290.0</td>
<td>398.6–399.5</td>
<td>1–4</td>
<td>10/30, 30/60, 60/90, 90/120</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>280.0–289.5</td>
<td>401.5–402.4</td>
<td>1, 2</td>
<td>10/30, 30/60</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>283.0–287.5</td>
<td>400.5–401.5</td>
<td>1, 2</td>
<td>10/30, 30/60</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>283.5–290.0</td>
<td>401.5–402.4</td>
<td>1–3</td>
<td>10/30, 30/60, 60/90</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>281.0–295.0</td>
<td>390.9–392.1</td>
<td>1–3</td>
<td>10/30, 30/60, 60/90</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>283.0–288.5</td>
<td>404.6–405.3</td>
<td>1–5</td>
<td>10/30, 30/60, 60/90, 90/120, 120/150</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

a. Wavelength ranges are fractions of recorded EEMs. The complete wavelength ranges of the recorded EEMs were 280–295 nm for excitation and 300–500 nm for emission.

b. EEM # refers to the TREEM recorded during the following delay (D)/delay + gate (D+G) time windows: 1 = 10/30 ns, 2 = 30/60 ns, 3 = 60/90 ns, 4 = 90/120 ns, 5 = 120/150 ns, 6 = 150/180 ns.
Figure 3.5: PARAFAC modeling profiles obtained from soil extract. (A) Emission spectral profile, (B) excitation spectral profile and (C) fluorescence decay profile of benzo[a]pyrene. Numbers 1, 2 and 3 indicate the factor order. The short dash–dotted (— • —) green line in (A) corresponds to the normalized emission spectrum of benzo[a]pyrene.
3.3.5 Comparison of TREEC–Chemometric Modeling to Sonication–HPLC Data

Table 3.5 summarizes the PAH recoveries obtained via TREEC/Chemometric modeling and HPLC. In all the cases, PAH extraction was carried out via the sonication screening method. The statistical comparisons of PAH recoveries at different concentration levels was carried out with the Bonferroni’s adjustment test.\textsuperscript{111} The alpha value, $\alpha' = 1 - (1 - \alpha)^{1/k}$, was calculated for an overall significance level ($\alpha = 0.05$), and 15 PAH comparisons ($k = 15$). Because the calculated value ($\alpha = 3.5 \times 10^{-3}$) was considerably smaller that the critical $t$-value ($t_{(\alpha', 3–1)} = 13.9$), it was concluded that the experimental recoveries of the 15 EPA-PAHs obtained with the three methods, i.e., TREEC/PARAFAC, TREEC/U-PLS/RTL, and HPLC, were statistically equivalent.

Examination of HPLC data in Table 3.5 and Table 3.1 provides a direct comparison between the screening extraction method and classic sonication methodology. The main difference is the inability of the screening method to detect the presence of anthracene and indeno[1,2,3-cd]pyrene. The observed difference can be attributed to the smaller mass of soil used with the screening method. The extraction of 0.05 g of soil, as opposed to 20 g of sample, probably leads to extract concentrations lower than the LODs of HPLC. The same is not true for the two TREEC/Chemometric modeling methods. The LODs of 4.2 K LETRSS analysis (see Table 3.2) make possible to determine the presence of both anthracene and indeno[1,2,3-cd]pyrene in the extracts of 0.05 g of soil.
Table 3.5: Comparison of PAH recoveries obtained via TREEC/PARAFAC, TREEC/U-PLS/RTL and HPLC analyses.  

<table>
<thead>
<tr>
<th>PAH</th>
<th>HPLC (%)</th>
<th>TREEC/PARAFAC (%)</th>
<th>TREEC/U-PLS/RTL (%)</th>
<th>( t )-student(^b)</th>
<th>( t_1 )</th>
<th>( t_2 )</th>
<th>( t_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>44.8 ± 4.0</td>
<td>42.2 ± 3.8</td>
<td>45.6 ± 3.9</td>
<td>0.17</td>
<td>0.13</td>
<td>0.22</td>
<td></td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>40.9 ± 2.3</td>
<td>39.6 ± 1.7</td>
<td>39.2 ± 1.6</td>
<td>0.52</td>
<td>0.70</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td>Fluorene</td>
<td>43.0 ± 0.3</td>
<td>44.3 ± 0.8</td>
<td>44.1 ± 0.8</td>
<td>2.73</td>
<td>2.19</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>40.5 ± 1.4</td>
<td>42.9 ± 1.3</td>
<td>41.4 ± 1.4</td>
<td>1.31</td>
<td>0.52</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td>Anthracene</td>
<td>—</td>
<td>45.7 ± 2.8</td>
<td>45.1 ± 2.8</td>
<td>—</td>
<td>—</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Fluoranthenene</td>
<td>24.3 ± 0.8</td>
<td>27.0 ± 1.0</td>
<td>26.5 ± 0.9</td>
<td>3.29</td>
<td>3.03</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>30.3 ± 2.3</td>
<td>31.1 ± 1.8</td>
<td>31.3 ± 1.9</td>
<td>0.18</td>
<td>0.29</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>31.8 ± 1.3</td>
<td>27.9 ± 1.8</td>
<td>30.9 ± 1.8</td>
<td>1.58</td>
<td>0.36</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>Chrysene</td>
<td>58.5 ± 5.6</td>
<td>55.4 ± 4.3</td>
<td>57.8 ± 4.1</td>
<td>0.12</td>
<td>0.02</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>55.4 ± 5.0</td>
<td>51.9 ± 3.5</td>
<td>53.8 ± 3.4</td>
<td>0.18</td>
<td>0.10</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>39.3 ± 1.1</td>
<td>43.6 ± 1.4</td>
<td>41.3 ± 1.4</td>
<td>2.71</td>
<td>1.26</td>
<td>1.17</td>
<td></td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>49.9 ± 2.1</td>
<td>40.4 ± 2.1</td>
<td>48.9 ± 1.9</td>
<td>2.15</td>
<td>0.25</td>
<td>2.11</td>
<td></td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>—</td>
<td>46.5 ± 3.6</td>
<td>47.1 ± 3.4</td>
<td>—</td>
<td>—</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>37.5 ± 3.2</td>
<td>46.7 ± 3.1</td>
<td>43.1 ± 3.4</td>
<td>0.95</td>
<td>0.88</td>
<td>0.34</td>
<td></td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>38.6 ± 0.6</td>
<td>40.5 ± 1.0</td>
<td>38.4 ± 0.6</td>
<td>2.75</td>
<td>0.55</td>
<td>3.09</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) HPLC, TREEC/PARAFAC and TREEC/U-PLS/RTL analyses were performed in soil extracts obtained via the sonication screening procedure. Each reported value is the average of three independent extractions of soil samples.

\(^b\) \( t \)-student = experimental values calculated for \( N = 3 \) and \( p < 0.0035 \) (Bonferroni’s adjustment test, \( \alpha' = 1-\alpha/k \); \( t_1 \): comparison between HPLC and TREEC/PARAFAC; \( t_2 \): comparison between HPLC and TREEC/U-PLS/RTL; \( t_3 \): comparison between both chemometric models. Reported values should be compared to \( t \)-critical = 13.9.
The presence of constant and proportional biases in the two TREEC methods was tested by comparing their results to HPLC data in Table 3.5. The statistical comparisons were made with the aid of the bivariate least-squares (BLS) regression method and the elliptic joint confidence region (EJCR) test.\textsuperscript{112,113} The plots of the TREEC/PARAFAC and TREEC/U-PLS/RTL data versus the sonication–HPLC data provided the following slope/intercept results: 0.944 ± 0.20/5.4 ± 7.7 (TREEC/PARAFAC) and 0.941 ± 0.08/2.9 ± 3.2 (TREEC/U-PLS/RTL). The better precision of U-PLS/RTL is in good agreement with its superior predictive ability, which probably results from its latent variable properties.\textsuperscript{98} The EJCRs of the slopes and the intercepts are shown in Figure 3.6. The elliptical domains obtained with TREEC/PARAFAC and with TREEC/U-PLS/RTL include the theoretically predicted value of the slope (1) and the intercept (0). This fact rules out the possible presence of constant and proportional biases in the two approaches.

Figure 3.6: Elliptic joint confidence region obtained with the bivariate least-squares regression method from the plot of the TREEC/PARAFAC and TREEC/U-PLS data in Table 3.5.
3.4 Conclusion

We have demonstrated for the first time the possibility to monitor 15 EPA-PAHs in soil samples processing high-resolution fluorescence data with third-order multivariate calibration methods. Its experimental relies on a previously reported procedure with various desirable features for routine screening of numerous soil samples. PAH determination is based on the collection of 4.2 K fluorescence TREEMs recorded at six time windows away from the laser excitation pulse. Potential interference from unknown sample concomitants was successfully handled by processing four-way 4.2 K fluorescence TREEC data arrays with PARAFAC and U-PLS/RTL. The analytical recoveries of the 15 EPA-PAHs were in good agreement with those obtained via classic sonication–HPLC methodology. When handling intrinsically complex samples of unknown composition, processing 4.2 K WTM data with univariate calibration methods forces the analyst to check for potential interference via lifetime analysis. Depending on the complexity of the sample, situations might arise where finding an appropriate set of excitation and fluorescence wavelengths is not possible. The TREEC/PARAFAC and TREEC/U-PLS/RTL approaches presented here provide a general solution to the ubiquitous problem of spectral interference.
CHAPTER 4: COMBINING CRYOGENIC FIBER OPTIC PROBES WITH COMMERCIAL SPECTROFLUORIMETERS FOR THE SYNCHRONOUS FLUORESCENCE SHPOL’SKII SPECTROSCOPY OF HIGH MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS†

4.1 Introduction

Reducing the sample temperature offers several advantages to molecular fluorescence spectroscopy. Analysis at cryogenic temperatures (77 K or below) often improves spectral resolution, increases fluorescence quantum yields and minimizes, or even eliminates, complications with oxygen quenching and energy transfer. Although an improvement of spectral resolution is almost always observed because the Boltzmann population distribution is narrowed, the temperature effects on spectral narrowing are particularly noticeable in Shpol’skii matrixes.

The term Shpol’skii matrix refers to a dilute solution of a guest molecule (non-polar compound) 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 guest and host molecules match up well enough, guest molecules occupy a small number of crystallographic sites (ideally just one) in the host matrix. Matrix isolation of guest molecules reduces inhomogeneous band broadening. The combination of reduced thermal and inhomogeneous broadening produces vibrational spectra with sharp line widths.

One reason for pursuing spectral narrowing in complex matrixes is to determine the presence and the concentrations of chemically related compounds without previous

chromatographic separation. Numerous applications of the Shpol’skii phenomenon have targeted the direct analysis of polycyclic aromatic hydrocarbons (PAHs) in environmental samples.\textsuperscript{91,93,107,114-117} The main motivation for monitoring PAHs in the environment is to avoid human exposure to contaminated samples. Well-documented epidemiological studies have linked PAH exposure to increasing cancer risks\textsuperscript{118-120} and endocrine disrupting activity.\textsuperscript{121} The primary PAH targets have been the sixteen priority pollutants of the Environmental Protection Agency (EPA) list.\textsuperscript{70} These include benz[\textit{a}]anthracene, benzo[\textit{b}]fluoranthene, benzo[\textit{k}]fluoranthene, benzo[\textit{a}]pyrene, dibenzo[\textit{a,h}]anthracene, indeno[1,2,3-\textit{cd}]pyrene, naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, and benzo[\textit{g,h,i}]perylene.

More recent studies have shifted their attention to the analysis of high–molecular weight (HMW) PAHs, i.e. PAHs with MW $\geq$ 300.\textsuperscript{122-136} A significant portion of the biological activity of PAH contaminated samples is now attributed to the presence of HMW-PAHs. A crucially relevant toxicological example is dibenzo[\textit{a,l}]pyrene (DB[\textit{a,l}]P), the most potent carcinogenic PAH known to date.\textsuperscript{137-139} Its toxicity is considerably higher than that of benzo[\textit{a}]pyrene (B[\textit{a}]P), which is the most carcinogenic EPA-PAH. There are several more isomers of dibenzopyrene which are also carcinogenic, but not to the extent DB[\textit{a,l}]P is. Since the carcinogenic properties of HMW-PAHs differ significantly from isomer to isomer, it is important to determine the most toxic isomers even if they are present at much lower concentrations than their less toxic isomers. This is not a trivial task, as many isomers present very similar chromatographic behaviors and virtually identical mass fragmentation patterns.\textsuperscript{128-131}
The first environmental determination of DB[a,l]P was made in river sediment samples with the aid of laser-excited Shpol’skii spectrometry.\textsuperscript{114,115} Later chromatographic efforts reported its presence in several standard reference materials and particulate matter samples.\textsuperscript{131-136} Recent efforts in our lab have determined HMW-PAHs via laser excited time-resolved Shpol’skii spectroscopy (LETRSS), which refers to the collection of multidimensional data formats during the lifetime decay of luminescence (fluorescence and/or phosphorescence) emission. Adding the temporal dimension to Shpol’skii spectra provides a particularly selective tool for the determination of structural isomers without previous chromatographic separation. Wavelength-time matrices take advantage of the full dimensionality of luminescence spectroscopy by combining spectral and lifetime information for the unambiguous determination of targeted PAHs.\textsuperscript{12} Time-resolved excitation-emission matrices give the analyst the opportunity to select the best time window for minimum (or even none) spectral overlapping in highly complex samples.\textsuperscript{40} The complications of traditional methodology for 77 K and 4.2 K measurements were avoided by using a bifurcated fiber-optic probe (FOP) that delivered the excitation light directly into the frozen matrix. This approach retained the simplicity of dunking the sample into the liquid cryogen for fast and reproducible freezing, eliminated all interfaces that could scatter exciting light into the detection system, and also eliminated the need for an optical Dewar and/or helium cryostat. Frozen samples were prepared in a matter of seconds.

Collection of multidimensional data formats was made with the aid of an in-house instrumental set up consisting of a pulsed tunable dye laser, a pulse delay generator, a spectrograph, and an intensifier-charged coupled device.\textsuperscript{52} In this article, we combine the FOP approach to commercial instrumentation for the environmental analysis of HMW-PAHs. An
attractive feature of spectrofluorimeters with excitation and emission monochromators is the
possibility to record synchronous spectra. Constant energy synchronous spectroscopy refers to
varying simultaneously the excitation ($\lambda'$) and the emission ($\lambda$) wavelengths while keeping a
constant wavelength interval ($\Delta\lambda = \lambda - \lambda'$) between them. The advantages of this approach
include narrowing of spectral bandwidth and simplification of emission spectra. Although
synchronous fluorescence spectroscopy (SFS) has been reported for the environmental analysis
of EPA-PAHs at both room temperature\textsuperscript{140-144} and low temperature,\textsuperscript{145,146} the extent of our literature search
revealed no applications toward the analysis of HMW-PAHs. A similar statement is true for the
combination of the FOP with commercial instrumentation. Previous reports on the low-
temperature analysis of EPA-PAHs were carried out with the aid of a liquid nitrogen Dewar (77 K)\textsuperscript{4,10,41,147} or commercially available cryostats (77 K or below).\textsuperscript{4,41,114,145}

4.2 Experimental

4.2.1 Chemicals

Nanopure water from a Barnstead Nanopure Infinity water system was used throughout. All chemicals were analytical-reagent grade and used without further purification. Benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, B[a]P, dibenzo[a,h]anthracene, indeno[1,2,3-cd]pyrene, naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, chrysene, benzo[g,h,i]perylene were purchased from Sigma-Aldrich at their highest available purity ($\geq 98\%$). DB[a,l]P, 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) were purchased from Accustandard at 100$\%$ purity. HPLC grade methanol, $n$-
hexane, n-heptane and n-octane were acquired from Fischer Scientific. Stock solutions of PAHs were kept in the dark at 4°C. Possible PAH degradation was monitored via room-temperature fluorescence spectroscopy. Working solutions were prepared by serial dilution of stock solutions prior to data collection. Interference studies were made with a soil sample (Loamy Sand, CRM171-100) purchased from Resource Technology Corporation, Laramie, WY.

4.2.2 Instrumentation

The FOP was described in Chapter 2 of this dissertation. Fluorescence measurements were carried out with a FluoroMax–P (Horiba Jobin Yvon, Edison, NJ) equipped with a 150 W xenon arc source. The 1200 grooves·mm$^{-1}$ gratings in the single excitation and emission monochromators were blazed at 330 and 500 nm, respectively. Their reciprocal linear dispersion was equal to 4.25 nm·mm$^{-1}$. The uncooled photomultiplier tube (Hamamatsu, Model R928) detector was operated in the photon-counting mode. Commercial software (DataMax, version 2.20, Horiba Jobin Yvon) was used for automated scanning and fluorescence data acquisition.

Figure 4.1: Schematic diagrams of the fiber optic probe and the fiber optic mount used for low-temperature measurements.
The excitation fiber and the emission fiber bundle of the FOP were coupled to the sample compartment of the spectrofluorimeter with the aid of a commercial fiber optic mount (F-3000, Horiba Jobin Yvon) that optimized collection efficiency via two concave mirrors (see Figure 4.1). Position alignment of each end of the FOP with the respective focusing mirror was facilitated by commercially available adapters (Horiba Jobin Yvon).

4.2.3 Freezing Procedures

The classic procedure for 77 K measurements consisted of immersing the sample solution in a quartz tube into a nitrogen-filled Dewar flask. FOP measurements were made as follows: after microliter volumes (100–750 µL) of undegassed sample solution were pipetted into the sample vial, the tip of the FOP was positioned and held constant with the screw cap below the solution surface. Sample freezing was accomplished by lowering the copper tubing into the liquid cryogen. Liquid nitrogen and liquid helium were held in two separate Dewar containers with 5, and 60 L storage capacity, respectively. The 60 L liquid helium volume would typically last three weeks of daily use, averaging 15–20 samples per day. At both 77 and 4.2 K, complete sample freezing took less than 90 s. The ~1 min probe clean up procedure involved removing the sample vial from the cryogen container, melting the frozen matrix and warming the resulting solution to approximately room temperature with a heat gun, rinsing the probe with n-alkane, and drying it with warm air from the heat gun. The entire freeze, thaw, and clean up cycle took less than 5 min.
4.2.4 **Soil Analysis**

500 µL of \(n\)-octane and 20 µL of methanol were mixed with a known amount (0.05 grams) of soil sample in a 0.75 mL propylene sample vial. The mixture was submitted to 30 min of sonication at room temperature in a Branson sonication bath (Model 3210). After 5 min of shaking time, a 400 µL volume of the sample extract was transferred to the sample vessel of the FOP for Synchronous Fluorescence Shpol’skii Spectroscopy at 4.2 K.

4.3 **Results and Discussion**

4.3.1 **Spectral Accuracy**

The ability to record accurate spectra with the FOP-spectrofluorimeter combination was first investigated with a representative set of three EPA-PAHs. Working solutions of anthracene, pyrene, and benzo[\(\alpha\)]pyrene were prepared in \(n\)-heptane, \(n\)-hexane and \(n\)-octane, respectively. Each one of these solvents is known to provide the best spectral resolution for each EPA-PAH. Figure 4.2 compares the 77 K excitation and fluorescence spectra obtained with the FOP to those recorded with traditional methodology. No attempts were made to optimize spectral resolution, nor were the spectra corrected for instrumental response. Site-selective excitation was not attempted. All spectra showed the quasi-line structure expected from Shpol’skii systems.
Figure 4.2: 77 K excitation (dotted line) and fluorescence (solid line) spectra recorded with a Dewar flask, and the FOP. (A) and (B): benzo[a]pyrene in n-octane; $\lambda_{\text{exc}} = 368$ nm, $\lambda_{\text{em}} = 402$ nm, band-pass = 0.75 nm. (C) and (D): pyrene in n-hexane; $\lambda_{\text{exc}} = 337$ nm, $\lambda_{\text{em}} = 381.5$ nm, band-pass = 1.00 nm. (E) and (F): anthracene in n-heptane; $\lambda_{\text{exc}} = 254$ nm, $\lambda_{\text{em}} = 379.5$ nm, band-pass = 1.00 nm. $\lambda_{\text{exc}}$ and $\lambda_{\text{em}}$ refer to the excitation and emission wavelengths used to record the emission and excitation spectra, respectively. The specified bandpass refers to the excitation and emission band-pass. All PAH concentrations were 10 µg·L$^{-1}$. 
Although the excitation and fluorescence spectra were acquired at identical spectral band-pass, the features in emission appear significantly narrower than those in excitation. The primary reason is that the displayed portions of the excitation spectra correspond to the excitation of singlet states higher than $S_1$ and are therefore subjected to uncertainty broadening from rapid $S_n$–$S_1$ internal conversion. In all cases, the maximum wavelengths and the relative peak intensities of the FOP spectra correlate well to those recorded with conventional methodology.

Table 4.1 compares the FWHM of fluorescence spectra recorded with conventional methodology (Dewar at 77 K) to those collected with the FOP at 77 K and 4.2 K. The agreement between the 77 K values demonstrates that the FOP does not deteriorate the resolution of spectral acquisition. The spectral narrowing that is often gained by lowering the temperature from 77 K to 4.2 K is also noticeable with the FOP. 4.2 K measurements with conventional methodology were not performed due to the unavailability of a helium cryostat.

Table 4.1: Full-width-at-half maxima of EPA-PAHs in $n$-alkanes obtained with conventional methodology (77 K) and with the FOP (77 K and 4.2 K).

<table>
<thead>
<tr>
<th>PAH/$n$-alkane</th>
<th>Excitation/ Emission (nm)</th>
<th>Band-pass (nm)</th>
<th>Dewar (77 K)</th>
<th>FOP (77 K)</th>
<th>FOP (4.2 K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzo[a]pyrene/$n$-octane</td>
<td>368/402</td>
<td>0.50</td>
<td>1.26</td>
<td>1.22</td>
<td>0.99</td>
</tr>
<tr>
<td>Pyrene/$n$-hexane</td>
<td>337/381.5</td>
<td>0.75</td>
<td>2.99</td>
<td>2.96</td>
<td>2.75</td>
</tr>
<tr>
<td>Anthracene/$n$-heptane</td>
<td>254/379.5</td>
<td>0.75</td>
<td>1.97</td>
<td>1.82</td>
<td>1.15</td>
</tr>
</tbody>
</table>

Figure 4.3A shows the emission spectrum of an Hg lamp placed at the analysis end of the FOP. The spectrum was recorded by scanning the emission monochromator at the smallest wavelength step possible, i.e. 0.0625 nm. The slit-widths of the monochromator (~24 µm) were adjusted to reach the narrowest full-width at half maximum (FWHM) possible. Their experimental values 312.56 nm (FWHM = 0.29) and 313.19 nm (0.28 nm) are in good agreement
with the nominal limiting resolution (0.3 nm) of the spectrofluorimeter. This fact demonstrates that the FOP does not deteriorate the limiting resolution of the spectrometer.

Figure 4.3B depicts the fluorescence spectrum of benzo[a]pyrene in \textit{n}-heptane. Upon fast cooling to 4.2 K, this PAH occupies four crystallographic sites in the guest lattice (\textit{n}-heptane).\textsuperscript{41,148} The four crystallographic orientations produce identical fluorescence profiles slightly shifted by small wavelength differences.\textsuperscript{148} The maximum wavelengths of the quartet emissions from the 0–0 transitions match the maximum wavelengths of the four peaks in Figure 4.3B. Although baseline resolution of the four peaks was not possible, the limiting resolution of the FOP/spectrofluorimeter was enough to identify the four crystallographic sites of benzo[a]pyrene in \textit{n}-heptane. Site-selective excitation was not possible due to the wide excitation band-pass (10 nm) we had to use for an acceptable signal-to-noise ratio with the 0.1 nm emission band-pass. This is a disadvantage of the present system when compared to sample excitation with a narrow band-pass laser.\textsuperscript{15}
Figure 4.3: (A) Emission spectra of an Hg-lamp recorded with the FOP, using an emission band-pass of 0.1 nm (left) and 0.05 nm (right). (B) 4.2 K fluorescence spectra recorded with the FOP from a 50 µg·L⁻¹ benzo[a]pyrene sample in n-heptane using a 4 nm excitation and emission band-pass (left), and a 10/0.1 nm excitation/emission band-pass. All spectra were collected using the same monochromator step, i.e. 0.0625 nm.
4.3.2 Analytical Figures of Merit (AFOM)

Table 4.2 summarizes the AFOM of three model PAHs obtained with the FOP.

Fluorescence measurements were made at the maximum excitation and emission wavelengths of each PAH. The excitation and emission band-pass were adjusted to provide a reasonable compromise between spectral resolution and fluorescence intensity. The relative standard deviations (RSD) of the fluorescence intensities were calculated using medium linear concentrations from individual aliquots submitted to the entire freezing procedure. The tabulated values compare favorably to those obtained with the Dewar procedure (RSD ≥ 12%). The correlation coefficients and the slopes of the log-log plots close to unity demonstrate the existence of a linear relationship between PAH concentration and signal intensity. No efforts were made to reach the upper concentration limits of the calibration curves. The limits of detection (LOD) were calculated as $3 \times S_B/m$, where $S_B$ is the standard deviation from 16 blank determinations and $m$ is the slope of the calibration curve (calibration sensitivity). The blank signals were measured at the maximum excitation and emission wavelengths of each PAH. Their values did not change much with lowering the temperature to 4.2 K. Therefore, the better LODs at liquid helium temperature result from the steeper slopes of the calibration curves at 4.2 K. This is in good agreement with literature reports showing better LOD at lower temperatures. The obtained LODs are in good agreement with those previously obtained via 4.2 K LETRSS. The 4.2K fluorescence spectra and calibration plots of benzo[a]pyrene, anthracene and pyrene are provided in Appendix A of this dissertation.
### Table 4.2: Analytical figures of merit of EPA-PAHs in *n*-alkane solvents obtained with the fiber optic probe and the spectrofluorometer.

<table>
<thead>
<tr>
<th>PAH/<em>n</em>-alkane</th>
<th>Analytical Figures of Merit</th>
<th>77 K</th>
<th>4.2 K</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benzo[α]pyrene/<em>n</em>-octane</strong></td>
<td>Excitation/Emission (nm)</td>
<td>367.5/401.5</td>
<td>367.5/401.5</td>
</tr>
<tr>
<td></td>
<td>Excitation/Emission Band-pass (nm)</td>
<td>3/1.25</td>
<td>3/1.25</td>
</tr>
<tr>
<td></td>
<td>S/N&lt;sup&gt;a&lt;/sup&gt;</td>
<td>161.95</td>
<td>390.05</td>
</tr>
<tr>
<td></td>
<td>Reproducibility (RSD, %)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.39</td>
<td>3.54</td>
</tr>
<tr>
<td></td>
<td>LDR (µg·L&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.12–100</td>
<td>0.452–100</td>
</tr>
<tr>
<td></td>
<td>Slope (log-log)</td>
<td>0.9949</td>
<td>0.9787</td>
</tr>
<tr>
<td></td>
<td>Correlation Coefficient (R&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.9899</td>
<td>0.9909</td>
</tr>
<tr>
<td></td>
<td>Calibration Sensitivity (cps·L·µg&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>5691.3</td>
<td>10518</td>
</tr>
<tr>
<td></td>
<td>LOD (µg·L&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>1.12</td>
<td>0.452</td>
</tr>
<tr>
<td><strong>Anthracene/<em>n</em>-heptane</strong></td>
<td>Excitation/Emission (nm)</td>
<td>359.5/379.5</td>
<td>360/359.5</td>
</tr>
<tr>
<td></td>
<td>Excitation/Emission Band-pass (nm)</td>
<td>3/1.25</td>
<td>3/1.25</td>
</tr>
<tr>
<td></td>
<td>S/Na</td>
<td>166.67</td>
<td>342.87</td>
</tr>
<tr>
<td></td>
<td>Reproducibility (RSD, %)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.33</td>
<td>7.08</td>
</tr>
<tr>
<td></td>
<td>LDR (µg·L&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.26–500</td>
<td>2.79–500</td>
</tr>
<tr>
<td></td>
<td>Slope (log/log)</td>
<td>0.8593</td>
<td>0.7961</td>
</tr>
<tr>
<td></td>
<td>Correlation Coefficient (R&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.9983</td>
<td>0.9864</td>
</tr>
<tr>
<td></td>
<td>Calibration Sensitivity (cps·L·µg&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1109.2</td>
<td>2198.2</td>
</tr>
<tr>
<td></td>
<td>LOD (µg·L&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.26</td>
<td>2.79</td>
</tr>
<tr>
<td><strong>Pyrene/<em>n</em>-hexane</strong></td>
<td>Excitation/Emission (nm)</td>
<td>337/380.5</td>
<td>337/380.5</td>
</tr>
<tr>
<td></td>
<td>Excitation/Emission Band-pass (nm)</td>
<td>3/1.25</td>
<td>3/1.25</td>
</tr>
<tr>
<td></td>
<td>S/N&lt;sup&gt;a&lt;/sup&gt;</td>
<td>181.09</td>
<td>409.82</td>
</tr>
<tr>
<td></td>
<td>Reproducibility (RSD, %)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.10</td>
<td>8.01</td>
</tr>
<tr>
<td></td>
<td>LDR (µg·L&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.642–125</td>
<td>0.297–125</td>
</tr>
<tr>
<td></td>
<td>Slope (log-log)</td>
<td>1.0352</td>
<td>0.9926</td>
</tr>
<tr>
<td></td>
<td>Correlation Coefficient (R&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>0.9995</td>
<td>0.9979</td>
</tr>
<tr>
<td></td>
<td>Calibration Sensitivity (cps·L·µg&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7085.7</td>
<td>13131</td>
</tr>
<tr>
<td></td>
<td>LOD (µg·L&lt;sup&gt;−1&lt;/sup&gt;)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.642</td>
<td>0.297</td>
</tr>
</tbody>
</table>

<sup>a</sup> Signal-to-noise ratio at median concentrations, where the noise is the standard deviation of six blank aliquots.

<sup>b</sup> Reproducibility at median concentrations. RSD: relative standard deviation

<sup>c</sup> LDR = linear dynamic range. The upper limit concentration corresponds to the highest concentration experimentally tested.

<sup>d</sup> cps: counts-per-second

<sup>e</sup> LOD = limit of detection, calculated as $3 \times S_B/m$, where $S_B$ is the standard deviation of 16 blank measurements and $m$ is the slope of the calibration curve.
4.3.3 4.2 K Synchronous Fluorescence Shpol’skii Spectroscopy (SFSS) of HMW-PAHs

Previous work in our lab investigated the 4.2 K spectral and lifetime characteristics of DB[a,e]P, DB[a,h]P, DB[a,l]P and N[2,3-a]P in several Shpol’skii matrices. The direct analysis of the five isomers without previous chromatographic separation was best accomplished in n-octane. Table 4.3 summarizes the main characteristics of the synchronous fluorescence spectra of the five HMW-PAHs recorded from n-octane standard solutions at two \( \Delta \lambda \) values, i.e. \( \Delta \lambda_a \) and \( \Delta \lambda_b \). Synchronous spectra recorded with a constant difference between the maximum emission (\( \lambda = \lambda_{em} \)) and excitation (\( \lambda' - \lambda_{exc} \)) wavelengths of each PAH, i.e., \( \Delta \lambda_a = \lambda_{em} - \lambda_{exc} \), yielded one peak per compound. When the \( \lambda_{exc} \) of each PAH was

<table>
<thead>
<tr>
<th>HMW-PAH</th>
<th>( \lambda_{exc} ) (nm)</th>
<th>( \lambda_{em} ) (nm)</th>
<th>Synchronous Offset (nm)</th>
<th>Synchronous Fluorescence Wavelengths (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB[a,e]P</td>
<td>293.5, 306, 342, 362*</td>
<td>394.5, 399.5, 404.5, 417, 443.5</td>
<td>88.5</td>
<td>306, 362, 381</td>
</tr>
<tr>
<td>DB[a,h]P</td>
<td>299, 312.5, 398, 417, 421.5*</td>
<td>447.5, 452.5, 477, 481.5, 513</td>
<td>135</td>
<td>312.5, 421.5, 447.5</td>
</tr>
<tr>
<td>DB[a,l]P</td>
<td>306.5, 320.5, 375.5, 390.5, 397*</td>
<td>417, 422.5, 431.5, 442.5, 447.5</td>
<td>96.5</td>
<td>320.5, 279, 397</td>
</tr>
<tr>
<td>N[2,3-a]P</td>
<td>296.5, 320, 335.5, 406, 431.5*</td>
<td>459, 491, 526.5</td>
<td>123.5</td>
<td>335.5, 431.5, 458.5</td>
</tr>
<tr>
<td>DB[a,i]P</td>
<td>297, 331.5, 354.5, 374.5*, 396.5</td>
<td>430, 445.5, 458, 489</td>
<td>33.5</td>
<td>396.5, 374.5, 391, 396.5</td>
</tr>
</tbody>
</table>

* Excitation (\( \lambda_{exc} \)) and emission (\( \lambda_{em} \)) wavelengths. Underlined peaks denote maximum excitation and emission wavelengths. * denotes excitation wavelengths used for the secondary synchronous offset wavelength (\( \Delta \lambda_b \)) of each compound.

b. \( \Delta \lambda_a \) = primary synchronous offset wavelength = difference between maximum emission and excitation wavelengths of each compound.
replaced by its second most intense excitation peak ($\lambda_{\text{exc-2}}$), i.e., $\Delta\lambda_b = \lambda_{\text{em}} - \lambda_{\text{exc-2}}$, synchronous spectra with more than one peak per PAH were observed in all cases.

Figure 4.4: Normalized 4.2 K SFSS spectra recorded from a synthetic mixture of the five HMW-PAH isomers in n-octane. $\Delta\lambda_a$ values in Table 4.3 were used to target individual isomers in the mixture. A 2 nm excitation and emission band-pass was used in all cases. The concentrations of DB[a,e]P and DB[a,h]P were set at 10 µg·L$^{-1}$. The concentrations of N[2,3-a]P, DB[a,h]P, and DB[a,i]P were set at 1 µg·L$^{-1}$. 
Figure 4.4 shows 4.2 K synchronous fluorescence spectra of a synthetic mixture prepared with the five isomers in \( n \)-octane. PAH concentrations were adjusted to provide signal intensities with the same order of magnitude for the five compounds in the mixture. Spectra were recorded using the \( \Delta \lambda_a \) values listed in Table 4.3. Spectral comparison to individual standards provided perfect matches for the targeted isomers in the mixture. The synchronous signal intensities recorded from standards and mixtures were statistically equivalent (\( N_1 = N_2 = 3; \alpha = 0.05 \))\(^{108} \) showing that no inner effects and/or synergistic effects deteriorated the accuracy of analysis.

Table 4.4 lists the 4.2 K SFSS AFOM of the five isomers in \( n \)-octane. Calibration curves were built with five synthetic mixtures containing each HMW-PAH at five different concentration levels. Their plots are shown in Appendix B of this dissertation. Synchronous spectra were recorded using the primary and secondary \( \Delta \lambda \) values of each isomer. All spectra were recorded using a 2 nm excitation and emission band-pass. Fluorescence measurements were made at the target peak of each isomer. Blank intensities from \( n \)-octane were recorded at the target wavelengths of each PAH. The correlation coefficients close to unity demonstrate linear correlations between the intensities of the synchronous signals and the PAH concentrations in the synthetic mixtures. No efforts were made to reach the upper concentration levels of the LDRs. The LODs were calculated as those in Table 4.2. The best LODs were obtained using the primary wavelength differences (\( \Delta \lambda_a \)), which gave the strongest signals for all the studied PAHs. Their values were of the same order of magnitude as the LODs obtained with 4.2 K LETRSS.\(^{126} \) Although the wavelength difference between the maximum emission and excitation wavelengths of each PAH provide ample opportunity for opening the excitation slits and reach lower LODs, larger excitation band-passes than 2 nm were not attempted.
Table 4.4: Analytical figures of merit for the HMW-PAHs in a mixture via 4.2 K SFSS.

<table>
<thead>
<tr>
<th>HMW-PAH</th>
<th>Analytical Figures of Merit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δλ (nm)³</td>
</tr>
<tr>
<td><strong>DB[a,e]P</strong></td>
<td>88.5</td>
</tr>
<tr>
<td>N[2,3-a]P</td>
<td>123.5</td>
</tr>
<tr>
<td><strong>DB[a,h]P</strong></td>
<td>135</td>
</tr>
<tr>
<td><strong>DB[a,i]P</strong></td>
<td>33.5</td>
</tr>
<tr>
<td><strong>DB[a,l]P</strong></td>
<td>96.5</td>
</tr>
</tbody>
</table>

³. The Δλ on the left corresponds to the primary wavelength offset (Δλₐ) of each PAH. The Δλ on the right corresponds to the secondary wavelength offset (Δλₐₙ) of each PAH.

ᵇ. LDR = linear dynamic range. The upper limit concentration corresponds to the highest concentration experimentally tested.

ᶜ. LOD = limit of detection, calculated as 3×Sᵇ/m, where Sᵇ is the standard deviation of 16 blank measurements and m is the slope of the calibration curve.
4.3.4 Comparison of 4.2 K SFSS to Room-Temperature (RT) Synchronous Fluorescence Spectroscopy

The advantage of lowering the temperature to 4.2 K is demonstrated by targeting DB\[a,i\]P in synthetic mixtures with its other four isomers. The wavelength offsets used for synchronous excitation at room temperature are listed in Table 4.5. Their selection followed the same selection criteria as those in Table 4.3. Figure 4.5 compares the room-temperature synchronous fluorescence spectra of DB\[a,i\]P to those recorded from a five isomer mixture using the $\Delta \lambda_a$ and $\Delta \lambda_b$ offsets of the pure standard. The spectral overlapping due to the presence of DB\[a,l\]P and DB\[a,e\]P prevents the accurate determination of DB\[a,i\]P via room-temperature SFS. The same is not true at 4.2 K (see Figure 4.4).

Table 4.5: Wavelength offsets for the room temperature SFSS of HMW-PAHs.

<table>
<thead>
<tr>
<th>HMW-PAH</th>
<th>$\lambda_{exc}$ (nm) $^a$</th>
<th>$\lambda_{em}$ (nm) $^a$</th>
<th>Synchronous Offset (nm) $^b$</th>
<th>Synchronous Fluorescence Wavelengths (nm) $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\Delta \lambda_a$</td>
<td>$\Delta \lambda_b$</td>
</tr>
<tr>
<td>DB[a,e]P</td>
<td>290, 302, 355, 374*</td>
<td>394, 405, 417, 443</td>
<td>92</td>
<td>20</td>
</tr>
<tr>
<td>DB[a,h]P</td>
<td>297, 310, 396, 419*</td>
<td>447, 476, 508</td>
<td>137</td>
<td>28</td>
</tr>
<tr>
<td>DB[a,l]P</td>
<td>271, 303, 316, 371, 390*</td>
<td>417, 432, 443</td>
<td>101</td>
<td>27</td>
</tr>
<tr>
<td>N [2,3-a]P</td>
<td>293, 317, 332, 403, 427*</td>
<td>456, 486, 522</td>
<td>124</td>
<td>29</td>
</tr>
<tr>
<td>DB[a,i]P</td>
<td>294, 313, 329, 352, 371*, 392</td>
<td>430, 446, 458, 489</td>
<td>38</td>
<td>59</td>
</tr>
</tbody>
</table>

$^a$. Excitation ($\lambda_{exc}$) and emission ($\lambda_{em}$) wavelengths. Underlined peaks denote maximum excitation and emission wavelengths. * denotes excitation wavelengths used for the secondary synchronous offset wavelength ($\Delta \lambda_b$) of each compound.

$^b$. $\Delta \lambda_a$ = primary synchronous offset wavelength = difference between maximum emission and excitation wavelengths of each compound.
The potential of 4.2 K SFSS for the identification of HMW-PAHs in samples of unknown composition was evaluated with a loamy soil sample. Soil is one of the most important reservoirs for PAHs, which are deposited in the gaseous state or associated to air-born particles, even at sites far from the petroleum industry. Once present in the soil, PAHs become a long-term source of environmental health risk due to their rather low water solubility, intrinsic chemical stability and high resistance to bio-degradation. The soil sample chosen for this study was a certified reference material consisting of a natural matrix waste sample fortified with the 16 EPA-PAHs. The additional presence of numerous aliphatic and aromatic compounds provided similar interference challenges as those faced with samples of unknown composition.

Figure 4.5: Room temperature synchronous fluorescence spectra of a (A) DB[a,i]P and (B) synthetic mixture with the five of the HMW-PAHs. All spectra were recorded from n-octane solutions using DB[a,i]P's $\Delta\lambda_a$ and $\Delta\lambda_b$ values listed in Table 4.5. Concentrations were as follows: 20 $\mu$g·L$^{-1}$ DB[a,e]P, 1 $\mu$g·L$^{-1}$ N[2,3-a]P, 1 $\mu$g·L$^{-1}$ DB[a,h]P, 10 $\mu$g·L$^{-1}$ DB[a,i]P, 20 $\mu$g·L$^{-1}$ DB[a,l]P. Excitation and emission band-pass was set at 2 nm in all cases.
Soil extraction followed a slightly modified procedure previously described in the literature. Considering the relatively high content of EPA-PAHs in the Loamy Sand soil sample, we first attempted the direct determination of the five HMW isomers in synthetic mixtures with the sixteen priority pollutants. Based on the recoveries of the extraction procedure, the maximum possible concentrations of EPA-PAHs in the soil extracts should vary between $0.82 \pm 0.11 \, \mu g \cdot L^{-1}$ (benzo[a]pyrene) and $20.3 \pm 2.8 \, \mu g \cdot L^{-1}$ (phenanthrene). Synchronous spectra were recorded in the absence and the presence of EPA-PAHs using the $\Delta \lambda_a$ and $\Delta \lambda_b$ values reported in Table 4.3. The individual concentrations of EPA-PAHs in the synthetic mixtures were $20 \, \mu g \cdot L^{-1}$.

Figure 4.6 shows typical results for DB[a,h]P, DB[a,i]P and N[2,3-a]P in the presence of the sixteen priority pollutants. Upon sample excitation with $\Delta \lambda_a$ values, the presence of EPA-PAHs caused no spectral interference at the maximum peaks of the targeted PAHs. The same is not true for DB[a,l]P and DB[a,e]P (see Figure 4.7), which accurate determination was only possible with $\Delta \lambda_b$ values at 20 and 32 nm, respectively. Under sample excitation with the appropriate $\Delta \lambda_a$ and $\Delta \lambda_b$ values, the synchronous signal intensities of HMW-PAHs in the absence and the presence of EPA-PAHs were statistically equivalent ($N_1 = N_2 = 3; \alpha = 0.05$).

Figure 4.8 compares the 4.2 K synchronous fluorescence spectra of soil extracts recorded in the absence and the presence of the five HMW isomers. The synthetic mixtures of HMW-PAHs were spiked into the soil extracts prior to fluorescence measurements with the FOP. Synchronous spectra were recorded using the $\Delta \lambda_a$ and $\Delta \lambda_b$ values reported in Table 4.3. The synchronous signal intensities recorded from HMW-PAHs in the soil extracts were statistically equivalent ($N_1 = N_2 = 3; \alpha = 0.05$) to those recorded from their synthetic mixtures in $n$-octane. The lack of spectral overlapping at the main target peak of each HMW-PAH and the statistical
equivalence of fluorescence intensities in the absence and the presence of matrix concomitants demonstrates the accurate identification of the five dibenzopyrene isomers in the soil extract.

Figure 4.6: 4.2 K SFSS spectra of (A) DB[a,h]P, (B) N[2,3-a]P, and (C) DB[a,i]P in the presence of the 16 EPA-PAHs in n-octane (black line); SFSS spectrum of the 16 EPA-PAHs in n-octane (red line); and SFSS spectrum of n-octane (green line). The $\Delta \lambda_a$ values used for the identification of each HMW isomer correspond to those in Table 4.3. Each HMW-PAH was at 50 $\mu$g·L$^{-1}$ concentration. Each EPA-PAH was at 20 $\mu$g·L$^{-1}$ concentration. Excitation and emission band-pass was set at 2 nm in all cases.
Figure 4.7: 4.2 K SFSS spectra of HMW-PAHs in the presence of the 16 EPA-PAHs in n-octane (black line), the 16 EPA-PAHs in n-octane (red line), and n-octane (green line). The $\Delta \lambda_a$ and/or $\Delta \lambda_b$ values used for the identification of each HMW isomer correspond to those in Table 4.3. (A) and (B) correspond to DB[a,e]P recorded using $\Delta \lambda_a$ and $\Delta \lambda_a$, respectively. (C) and (D) correspond to DB[a,l]P recorded using $\Delta \lambda_a$ and $\Delta \lambda_b$, respectively. Each HMW-PAH was at 50 µg·L$^{-1}$ concentration. Each EPA-PAH was at 20 µg·L$^{-1}$ concentration. Excitation and emission band-pass was set at 2 nm in all cases.
Figure 4.8: Normalized 4.2 K SFSS spectra of the loamy sand sonicant in the absence (dashed line), and presence (solid line) of HMW-PAHs. Wavelength offsets for each isomer identification followed those listed in Table 4.3: $\Delta \lambda_\text{b}$ for DB[$a,e$]P (A), $\Delta \lambda_\text{b}$ for DB[$a,l$]P (B), $\Delta \lambda_\text{a}$ for DB[$a,h$]P (C), $\Delta \lambda_\text{a}$ for N[2,3-$a$]P (D), and $\Delta \lambda_\text{a}$ for DB[$a,l$]P (E). The excitation and emission band-pass were set to 2 nm.
4.4 Conclusion

We have presented the first successful application of fiber optic probes for Shpol’skii spectroscopy measurements with standard spectrofluorimeters. By coupling the cryogenic FOP to commercial instrumentation, it is possible to perform 77 K and 4.2 K fluorescence measurements in Shpol’skii matrixes via simple, rapid and straightforward procedures. The accurate and reproducible acquisition of fluorescence spectra was demonstrated for both single and multiple-site PAH/n-alkane system. The possibility to adjust the excitation and emission band-pass of the spectrofluorimeter to reach both site-resolution and analytically valuable signal to-noise ratios was illustrated with benzo[a]pyrene in n-octane.

An attractive feature of fluorescence instrumentation with excitation and emission monochromators is the possibility to record synchronous excitation and emission spectra. The advantages of this approach include narrowing of spectral bandwidth and simplification of emission spectra. This feature was here explored to demonstrate the potential of 4.2 K SFSS for the analysis of HMW-PAHs in soil samples. In addition to improving the resolution of synchronous spectra, lowering the temperature for 4.2 K changed the vibrational transition energies of HMW-PAHs to provide additional selectivity of analysis. This intriguing feature will be further explored in our lab within the context of multidimensional data acquisition for chemometrics analysis with second order advantage algorithms.
CHAPTER 5: 4.2 K EXCITATION–EMISSION MATRICES FOR THE DIRECT DETERMINATION OF DIBENZOPYRENE ISOMERS IN COAL-TAR SAMPLES WITH A CRYOGENIC FIBER–OPTIC PROBE COUPLED TO A COMMERCIAL SPECTROFLUORIMETER ‡

5.1 Introduction

The standard reference material (SRM) 1597 is a natural complex sample from Coal Tar originally issued by the National Institute of Standards and Technology (NIST) in 1987. Its original Certificate of Analysis included 12 certified concentrations of polycyclic aromatic hydrocarbons (PAHs) along with 18 noncertified (reference) concentrations that included PAHs and other polycyclic aromatic compounds. Information on the certified concentrations was obtained with combination of gas chromatography with flame ionization detection (GC/FID) and reversed-phase liquid chromatography with fluorescence detection (LC-FL). Noncertified concentrations assignments were solely based on GC/FID.

Since then, SRM 1597 has found widespread and continued use within the analytical community for the validation of methods in environmental matrices. In addition to the compounds originally included in the Certificate of Analysis, this SRM has been applied to develop methods for the determination of polycyclic aromatic sulfur heterocycles (PASHs), methyl-substituted benzo[a]pyrene isomers, other methyl and dimethyl-substituted PAHs, stable carbon isotope values and radiocarbon content of individual PAH, and to investigate the effects of complex

PAH mixtures on the activation of carcinogenic PAHs to DNA-binding derivatives and carcinogenesis.\textsuperscript{156,157}

To address the need for updating the original certified values and expanding the number of PAHs with assigned values, NIST reanalyzed SRM 1597 and reissued it as SRM 1597a.\textsuperscript{158} Its Certificate of Analysis included certified concentrations for thirty-four PAHs and reference values for an additional thirty-six PAHs and ten PASHs. PAH concentrations were assigned based on results from gas chromatography/mass spectrometry (GC/MS) and reversed-phase LC-FL. PASHs concentrations were determined based on GC/MS and GC with atomic emission detection (GC/AED).\textsuperscript{151}

NIST has identified a total of 23 HMW-PAHs with a MM 302 in SRM 1597a.\textsuperscript{158} The assignment of mass fraction values for 17 of these HMW-PAHs was based on the analytical approach shown in Figure 5.1. In methods I–III, the coal tar sample was fractionated by normal phase-LC (NPLC) using a semi-preparative aminopropylsilane (NH\textsubscript{2}) column that isolated isomeric fractions prior to PAH determination via HPLC-FL or GC/MS. HPLC separation was carried out with a silica modified stationary phase (C\textsubscript{18}). GC separation was accomplished with fused silica capillary columns with one of two stationary phases, namely 5\% phenyl-substituted methylpolysiloxane (DB-5ms) or 50\% phenyl-substituted methylpolysiloxane (DB-17ms). DB-17ms was also used as the stationary phase in GC/MS methods IV and V to attempt isomers determination without previous sample fractionation.
This chapter deals with the direct determination of DB[a,l]P and four of its isomers, namely, dibenzo[a,h]pyrene (DB[a,h]P), dibenzo[a,i]pyrene (DB[a,i]P), dibenzo[a,e]pyrene (DB[a,e]P) and naphtho[2,3-a]pyrene (N[2,3-a]P), in a coal-tar standard reference material (NIST SRM 1597a). The approach presented here is based on 4.2 K EEMs, Shpol’skii spectroscopy, and PARAFAC. We take advantage of the FOP coupled to a commercial spectrofluorimeter. The extent of our literature search revealed no reports on the direct analysis of HMW-PAHs in complex environmental samples via low-temperature, steady-state EEM spectroscopy. The broad-band excitation source and the monochromators of the spectrometer facilitate the collection of EEMs throughout a wide range of excitation and emission.
wavelengths. Lowering the temperature to 4.2 K provides sufficient spectral narrowing for the PARAFAC determination of the five dibenzopyrene isomers in an extremely challenging, complex environmental matrix. The robustness of this approach for screening HMW-PAHs in complex environmental extracts is demonstrated with a straightforward experimental procedure and excellent analytical figures of merit.

5.2 Experimental Section

5.2.1 Instrumentation

The FOP and the instrumentation were described in the previous chapters of this dissertation.

5.2.2 Reagents

All chemicals were analytical-reagent grade and used without further purification. DB[a,l]P, DB[a,e]P, DB[a,h]P, and DB[a,i]P were purchased from Accustandard at 100% purity. N[2,3-a]P was purchased from Sigma-Aldrich. n-Octane (extra pure, 99+% ) was acquired from Fischer Scientific. The standard reference material (NIST SRM 1597a) was obtained from NIST.

5.2.3 Solution Preparation for Calibration, Validation, and Coal-Tar Test Samples

Stock solutions of PAHs were kept in the dark at 4 °C. Possible PAH degradation was monitored via room-temperature fluorescence spectroscopy. For each PAH, calibration samples were prepared by serial dilution of stock solutions prior to data collection. A validation sample is
a synthetic mixture of the five HMW-PAHs. Twelve validation samples were devised on the basis of a factorial experimental design, consisting of three concentration levels for each of the five HMW-PAHs. Each level is represented by a concentration near the lower limit, near the median concentration, and near the upper limit of the linear dynamic range of the PAH. For the validation samples, appropriate volumes of PAH stock solutions were combined and diluted to the appropriate volume with \( n \)-octane. Similarly, for the spiked coal-tar test samples, appropriate volumes of the PAH stock solutions and of the coal tar extract were combined and diluted with \( n \)-octane.

5.2.4 Fluorescence Measurements

Room-temperature fluorescence measurements were carried out by pouring liquid solutions into a standard quartz cuvette (1 cm path length). FOP measurements were made as follows: after microliter volumes (100–750 \( \mu \text{L} \)) of undegassed sample solution were pipetted into the sample vial, the sample vial was secured to the sample end of the copper tubing, and the tip of the FOP was positioned at a constant depth below the solution surface. Sample freezing was accomplished by lowering the sample vial into the liquid cryogen. Liquid nitrogen and liquid helium were held in two separate Dewar containers with 5 and 60 L storage capacity, respectively. The 60 L liquid helium volume would typically last for 3 weeks of daily use, averaging 15–20 samples per day. At both 77 and 4.2 K, complete sample freezing took less than 90 s. The \( \sim \)1 min probe cleanup procedure involved removing the sample vial from the cryogen container, melting the frozen matrix, warming the resulting solution to approximately room
temperature with a heat gun, rinsing the probe with \( n \)-alkane, and drying it with warm air from the heat gun. The entire freeze, thaw, and cleanup cycle took less than 5 min.

5.2.5 Software

Chemometric analysis of data was conducted by our collaborator, Prof. H. C. Goicoechea. All calculations were done using MATLAB (version 7.10, The MathWorks Inc., Natick, Massachusetts, USA). PARAFAC was applied with the MVC2 graphical user interface written in MATLAB by Olivieri et al.\textsuperscript{159} and available on the Internet.\textsuperscript{160}

5.3 Results and Discussion

5.3.1 Spectral Features of Dibenzopyrene Isomers in \( n \)-Octane at 77 K and 4.2 K

Previous research in our lab investigated the 4.2 K spectral features and fluorescence lifetimes of DB\([a,l]\)P, DB\([a,e]P\), DB\([a,h]\)P, DB\([a,i]\)P and N\([2,3-a]\)P in three Shpol’skii matrixes, namely, \( n \)-hexane, \( n \)-heptane, and \( n \)-octane. The best spectral narrowing was obtained with \( n \)-octane,\textsuperscript{124,125,127} which is the solvent selected here for all current studies. The excitation and emission band-pass for the collection of EEMs were set to an acceptable compromise between spectral resolution and signal-to-noise ratio at the parts-per-billion concentration levels. The excitation (275–380 nm) and emission (390–550 nm) wavelength ranges were common to the five studied isomers and gathered most of their spectral signatures. The excitation and emission monochromators were stepped at 5 and 1 nm increments, respectively. These settings provided individual EEMs with 22 emission spectra and 161 data points per emission spectrum. A long-
pass filter with 50% transmittance at approximately 320 nm (cutoff wavelength) was used in all cases to minimize instrumental artifacts such as scattered radiation and second-order emission.

Figure 5.2 compares the spectral features of DB[a,l]P at room temperature, 77 K, and 4.2 K. A 3/3 nm excitation/emission band-pass was needed to obtain an acceptable signal-to-noise ratio at room temperature. The low temperature data was recorded using a 1/1 nm excitation/emission band-pass. The spectral features of DB[a,l]P at both 77 and 4.2 K show the quasi-line structure often observed from Shpol’skii systems. Table 5.1 summarizes the fluorescence intensities and the full-width at half maxima (FWHM) of the studied isomers at low temperature. Lowering the temperature from 77 to 4.2 K enhanced the fluorescence intensities of the five studied isomers. With the exception of DB[a,i]P, all the other isomers presented slightly narrower FWHM at 4.2 K. The FWHM of DB[a,i]P was statistically equivalent (N₁ = N₂ = 3; α = 0.05) at both temperatures. The worst (largest) 4.2 K FWHM was observed from N[2,3-a]P. This is probably due to the poorer guest-host compatibility of N[2,3-a]P in n-octane.

<table>
<thead>
<tr>
<th>PAH</th>
<th>77 K</th>
<th>4.2 K</th>
<th>Intensity enhancement factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intensity (cps)</td>
<td>FWHM (nm)</td>
<td>Intensity (cps)</td>
</tr>
<tr>
<td>DB[a,l]P</td>
<td>38820 ± 825</td>
<td>2.21 ± 0.02</td>
<td>84980 ± 1038</td>
</tr>
<tr>
<td>DB[a,i]P</td>
<td>19237 ± 329</td>
<td>2.55 ± 0.05</td>
<td>28687 ± 698</td>
</tr>
<tr>
<td>DB[a,h]P</td>
<td>15827 ± 420</td>
<td>2.98 ± 0.28</td>
<td>31873 ± 1004</td>
</tr>
<tr>
<td>N[2,3-a]P</td>
<td>12263 ± 150</td>
<td>5.18 ± 0.23</td>
<td>34943 ± 346</td>
</tr>
<tr>
<td>DB[a,e]P</td>
<td>22390 ± 406</td>
<td>2.01 ± 0.03</td>
<td>33233 ± 176</td>
</tr>
</tbody>
</table>
Figure 5.2: Spectral features of DB[a,l]P at (A) room temperature, (B) 77 K, and (C) 4.2 K in n-octane. Spectra were recorded with the aid of a cryogenic fiber-optic probe. Excitation/emission band-passes were as follows: (A) 3/3 nm, (B) 1/1 nm, and (C) 1/1 nm.
Table 5.2 compares the analytical figures of merit (AFOM) of the five dibenzopyrene isomers at room temperature, 77 K, and 4.2 K. Fluorescence intensities were extracted from the EEMs at the maximum excitation and emission wavelengths of each PAH. The linear dynamic ranges (LDR) are based on the average intensities (N = 3) of at least five PAH concentrations. No efforts were made to reach the upper concentration limits of the calibration curves. The correlation coefficients close to unity demonstrate linear correlations in all cases. The limits of detection (LODs) were calculated as $3 \times S_B/m$, where $S_B$ is the standard deviation of the average blank signal extracted from three EEMs and $m$ is the slope of the calibration curve. The blank signals were measured at the maximum excitation and emission wavelengths of each PAH. The standard deviations of the blank signals did not change much with lowering the temperature to 4.2 K. The better LODs resulted from the steeper slopes of the calibration curves at liquid nitrogen and helium temperatures.

Figure 5.3 superposes the 4.2 K EEMs recorded from calibration samples of the five dibenzopyrene isomers. Figure 5.4A depicts the 4.2 K EEMs recorded from a validation sample of the five PAHs. Visual comparison of Figures 5.3 and 5.4A leads to at least one pair of excitation ($\lambda_{\text{exc}}$) and emission ($\lambda_{\text{em}}$) wavelengths that is free from the spectral interference of the other four isomers. This condition is met at the excitation and emission maxima of DB[a,l]P ($\lambda_{\text{exc}}/\lambda_{\text{em}} = 320/417$ nm) and DB[a,e]P ($\lambda_{\text{exc}}/\lambda_{\text{em}} = 305/395$ nm). Their determination in a synthetic mixture of the five isomers should then be possible at the concentration levels reported in Table 5.2. However, for DB[a,i]P, DB[a,h]P, and N[2,3-a]P, wavelengths free of spectral overlapping are found away from the excitation and emission maxima. Table 5.3 reports the
AFOM of these isomers at excitation and emission wavelengths free from spectral overlapping. Although the LODs are worse than those in Table 5.2, the direct determination of DB\([a,i]\)P, DB\([a,h]\)P, and N\([2,3-a]\)P in a synthetic mixture of the five isomers would still be possible at the parts-per-billion concentration level. As shown in Figure 5.4B, the presence of unknown fluorescence concomitants in the coal-tar sample leads to strong spectral overlapping within the entire EEM range of excitation and emission wavelengths. The direct determination of the five targeted isomers would not be possible without the aid of chemometrics.

![Figure 5.3: Superposition of 4.2 K excitation-emission matrices recorded from calibration sample solutions of (A) 200 ng·mL\(^{-1}\) DB\([a,l]\)P; (B) 300 ng·mL\(^{-1}\) DB\([a,i]\)P; (C) 200 ng·mL\(^{-1}\) DB\([a,h]\)P; (D) 400 ng·mL\(^{-1}\) DB\([a,e]\)P; and (E) 250 ng·mL\(^{-1}\) N\([2,3-a]\)P in \(n\)-octane. All EEMs were recorded with the cryogenic fiber-optic probe using a 1 nm/1 nm excitation/emission band-pass.](image)
Table 5.2: Analytical figures of merit at maximum excitation and emission wavelengths.

<table>
<thead>
<tr>
<th>PAH</th>
<th>Room temperature a</th>
<th>77 K b</th>
<th>4.2 K b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \lambda_{\text{exc}}/\lambda_{\text{em}} ) (nm)</td>
<td>LDR d (ng·mL(^{-1}))</td>
<td>( R^2 ) e</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>DB[a,l]P</td>
<td>315/418</td>
<td>331–800</td>
<td>0.9944</td>
</tr>
<tr>
<td>DB[a,i]P</td>
<td>295/431</td>
<td>213–400</td>
<td>0.9963</td>
</tr>
<tr>
<td>DB[a,h]P</td>
<td>310/447</td>
<td>4.13–400</td>
<td>0.9954</td>
</tr>
<tr>
<td>N[2,3-a]P</td>
<td>330/456</td>
<td>7.65–600</td>
<td>0.9960</td>
</tr>
<tr>
<td>DB[a,e]P</td>
<td>300/395</td>
<td>110–800</td>
<td>0.9992</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------</td>
<td>-----------------------------</td>
<td>-----------------------------</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \text{a. Excitation/emission band-pass: 3/3 nm} \)

\( \text{b. Excitation/emission band-pass: 1/1 nm} \)

\( \text{c. Excitation and emission wavelengths.} \)

\( \text{d. LDR = linear dynamic range in ng·mL}^{-1} \text{ extending from the limit of detection (LOD) to an arbitrarily chosen upper linear concentration.} \)

\( \text{e. } R^2 \text{ = coefficient of determination of the calibration curve.} \)

\( \text{f. Limit of detection calculated as } 3 \times S_y/m, \text{ where } S_y \text{ is the standard deviation of three blank measurements and } m \text{ is the slope of the calibration curve.} \)
Figure 5.4: 4.2 K excitation-emission matrix of (A) a synthetic mixture of (A) 180 ng·mL$^{-1}$ DB[a,l]P, 180 ng·mL$^{-1}$ DB[a,i]P, 180 ng·mL$^{-1}$ DB[a,h]P, 450 ng·mL$^{-1}$ DB[a,e]P, and 260 ng·mL$^{-1}$ N[2,3-a]P in n-octane; (B) a 1:375 dilution of SRM 1597a in n-octane. Both EEMs were recorded with the cryogenic fiber-optic probe using a 1 nm/1 nm excitation/emission band-pass.

Table 5.3: 4.2 K analytical figures of merit at excitation and emission wavelengths free from spectral overlapping of other isomers.$^a$

<table>
<thead>
<tr>
<th>PAH</th>
<th>$\lambda_{\text{exc}}$/$\lambda_{\text{em}}$ (nm)</th>
<th>LDR$^c$ (ng·mL$^{-1}$)</th>
<th>$R^2$$^d$</th>
<th>LOD$^e$ (ng·mL$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DB[a,l]P</td>
<td>355/430</td>
<td>1.63–400</td>
<td>0.9934</td>
<td>1.63</td>
</tr>
<tr>
<td>DB[a,h]P</td>
<td>300/483</td>
<td>3.00–300</td>
<td>0.9899</td>
<td>3.00</td>
</tr>
<tr>
<td>N[2,3-a]P</td>
<td>335/491</td>
<td>2.32–500</td>
<td>0.9915</td>
<td>2.32</td>
</tr>
</tbody>
</table>

$^a$. Fluorescence was recorded using an excitation and emission band-pass of 1 nm.

$^b$. Excitation and emission wavelengths.

$^c$. LDR = linear dynamic range in ng·mL$^{-1}$ extending from the limit of detection (LOD) to an arbitrarily chosen upper linear concentration.

$^d$. $R^2 =$ coefficient of determination of the calibration curve.

$^e$. Limit of detection calculated as $3 \times S_B / m$, where $S_B$ is the standard deviation of three blank measurements and $m$ is the slope of the calibration curve.
5.3.3 Validation and Test Set Results Obtained by PARAFAC Modeling

The theory of PARAFAC has been extensively discussed in previous articles. Only a brief description, which is directly related to fluorescence EEM data formats, will be provided here. A cube \( \mathbf{X} \) is built by stacking the matrices data of size \( (J \times K) \) corresponding to \( I \) standards plus the sample data. Then, a trilinear decomposition, according to Equation 5.1, is carried out to retrieve the values of \( a_{in} \), \( b_{jn} \), and \( c_{kn} \) from a fitting procedure of the values of the elements \( x_{ijk} \):

\[
x_{ijk} = \sum_{n=1}^{N} a_{in} b_{jn} c_{kn} + e_{ijk} \quad (5.1)
\]

where \( a_{in} \) denotes the values of the profile in the sample mode for constituent \( n \) in sample \( i \) (quantitative information); \( b_{jn} \) and \( c_{kn} \) are the corresponding profile values in both instrumental data modes for constituent \( n \) (qualitative information); and \( e_{ijk} \) collects the model errors, which are often unavoidable in experimental signals that carry noise. If \( a_{in} \), \( b_{jn} \), and \( c_{kn} \) can be reliably obtained, it is customary to arrange them into three matrices: the matrix of scores \( \mathbf{A} \) of size \([ (I+1) \times N ] \), containing all \( a_{in} \) values, and the two matrices of loadings \( \mathbf{B} \) (emission profiles of size \( J \times N \), containing all \( b_{jn} \) values) and \( \mathbf{C} \) (excitation profiles of size \( K \times N \), containing all \( c_{kn} \) values).

In order to exploit the second-order advantage of three-way data, PARAFAC was separately applied to cubes of data formed by the EEMs recorded from (a) the 25 calibration samples (i.e., five standards per dibenzopyrene isomer) and (b) each one of the validation samples (V1–V12) in Table 5.4 or (c) the test samples in Table 5.5. The size of each of the cubes submitted to PARAFAC analysis was then \( 26 \times 161 \times 22 \); i.e., \((I+1) \times J \times K\). The number of spectral components in each of the cubes \( N \) was obtained via core consistency analysis. When
analyzing the validation samples, \( N \) was equal to 6, one spectrum per each of the studied isomers and one for the background signal. When analyzing coal tar samples with and without the addition of the five isomers, \( N \) was equal to 7. This higher value indicates that an additional profile from an unexpected sample concomitant was retrieved by the PARAFAC modeling and informs us that the second-order advantage should be exploited.

Figure 5.5 compares the excitation and emission spectra recorded from calibration samples to the loading matrices \( \mathbf{B} \) and \( \mathbf{C} \) obtained from PARAFAC when processing the spiked coal tar sample #1 and the set of calibration samples. Peak assignments 1–5 in the loading matrices were made based on the similarities of spectral profiles and maximum wavelengths of excitation and emission. Peak 6 was attributed to the background signal and peak 7 to an unexpected sample component that could interfere in the absence of the second order advantage.

Tables 5.4 and 5.5 summarize the prediction results for both sets samples. Due to the high complexity of the coal-tar sample, the REP\% values and the recoveries of the five targeted isomers can be considered satisfactory. A statistical comparison of the prediction results was made via the bivariate least-squares (BLS) regression method and the elliptic joint confidence region (EJCR) test.\(^{163}\) The EJCR plots of the slopes and the intercepts are shown in Figure 5.6. The elliptical domains obtained for the five isomers in both sets of samples include the theoretically predicted value of the slope (1) and the intercept (0). This fact excludes the possible presence of biases in PARAFAC and demonstrates comparable precision and accuracy when analyzing samples with and without unexpected sample components.
Figure 5.5: Excitation (A) and emission (C) profiles extracted with PARAFAC when analyzing a coal-tar sample (sample #1, Table 4.5) spiked with the five targeted isomers; lines: 1 = DB[a,e]P, 2 = DB[a,h]P, 3 = DB[a,i]P, 4 = DB[a,l]P, 5 = N[2,3-a]P and 6 = background; red line 7 corresponds to an unexpected component present in the coal-tar sample. (B) Excitation and (D) emission spectra recorded with the spectrofluorimeter from pure standard solutions of the five targeted isomers at 4.2 K. Spectra were recorded with the cryogenic fiber optic probe using a 2 nm/2 nm excitation/emission band-pass. 1 = 100 ng·mL⁻¹ DB[a,e]P, 2 = 100 ng·mL⁻¹ DB[a,h]P, 3 = 100 ng·mL⁻¹ DB[a,i]P, 4 = 200 ng·mL⁻¹ DB[a,l]P, and 5 = 100 ng·mL⁻¹ N[2,3-a]P.
Table 5.4: Composition of validation samples and predictions by applying PARAFAC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dibenzopyrene isomer (ng·mL(^{-1}))(^a)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DB</td>
<td>a,e</td>
<td>P</td>
<td>DB</td>
<td>a,h</td>
<td>P</td>
<td>DB</td>
</tr>
<tr>
<td>nom.</td>
<td>pred.</td>
<td>error (%)</td>
<td>nom.</td>
<td>pred.</td>
<td>error (%)</td>
<td>nom.</td>
<td>pred.</td>
</tr>
<tr>
<td>V1</td>
<td>750</td>
<td>747</td>
<td>-0.4</td>
<td>60</td>
<td>63</td>
<td>5.0</td>
<td>60</td>
</tr>
<tr>
<td>V2</td>
<td>450</td>
<td>442</td>
<td>-1.8</td>
<td>180</td>
<td>169</td>
<td>-6.1</td>
<td>180</td>
</tr>
<tr>
<td>V3</td>
<td>750</td>
<td>725</td>
<td>-3.3</td>
<td>60</td>
<td>50</td>
<td>-16.7</td>
<td>320</td>
</tr>
<tr>
<td>V4</td>
<td>750</td>
<td>763</td>
<td>1.7</td>
<td>280</td>
<td>265</td>
<td>-5.4</td>
<td>320</td>
</tr>
<tr>
<td>V5</td>
<td>150</td>
<td>147</td>
<td>-2.0</td>
<td>60</td>
<td>52</td>
<td>-13.3</td>
<td>320</td>
</tr>
<tr>
<td>V6</td>
<td>150</td>
<td>153</td>
<td>2.0</td>
<td>60</td>
<td>63</td>
<td>5.0</td>
<td>320</td>
</tr>
<tr>
<td>V7</td>
<td>750</td>
<td>769</td>
<td>2.5</td>
<td>280</td>
<td>302</td>
<td>7.9</td>
<td>320</td>
</tr>
<tr>
<td>V8</td>
<td>150</td>
<td>160</td>
<td>6.7</td>
<td>280</td>
<td>270</td>
<td>-3.6</td>
<td>60</td>
</tr>
<tr>
<td>V9</td>
<td>450</td>
<td>474</td>
<td>5.3</td>
<td>180</td>
<td>193</td>
<td>7.2</td>
<td>180</td>
</tr>
<tr>
<td>V10</td>
<td>450</td>
<td>416</td>
<td>-7.6</td>
<td>100</td>
<td>95</td>
<td>-5.0</td>
<td>180</td>
</tr>
<tr>
<td>V11</td>
<td>450</td>
<td>485</td>
<td>7.8</td>
<td>180</td>
<td>205</td>
<td>13.9</td>
<td>180</td>
</tr>
<tr>
<td>V12</td>
<td>150</td>
<td>141</td>
<td>-6.0</td>
<td>280</td>
<td>277</td>
<td>-1.1</td>
<td>320</td>
</tr>
<tr>
<td>REP (%)(^b)</td>
<td>4.2</td>
<td>-</td>
<td>-</td>
<td>7.6</td>
<td>-</td>
<td>8.1</td>
<td>3.3</td>
</tr>
<tr>
<td>Recovery (%)</td>
<td>100.4</td>
<td>-</td>
<td>99.0</td>
<td>-</td>
<td>101.3</td>
<td>-</td>
<td>102.</td>
</tr>
</tbody>
</table>

\(\text{a. nom.} = \text{nominal concentration; pred.} = \text{predicted concentration.}\)

\(\text{b. REP (\%)} = 100 \times ((\sum_{i=1}^{I} (y_{\text{pred}} - y_{\text{nom}})^2) / I) / (y_{\text{calc}})^2,\) where \(y_{\text{pred}}\) is the predicted concentration for the \(i\) th validation sample, \(y_{\text{nom}}\) is the nominal concentration, \(I\) the number of validation samples and \(y_{\text{calc}}\) is the average of the nominal concentration values of the calibration set samples (450, 167, 187, 187 and 267 ppb, respectively).
Table 5.5: Composition of coal tar samples spiked with the five targeted isomers and PARAFAC predictions.

<table>
<thead>
<tr>
<th>Coal-tar sample</th>
<th>Dibenzopyrene isomer (ng·mL⁻¹)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DB[a,e]P</td>
</tr>
<tr>
<td></td>
<td>nom.</td>
</tr>
<tr>
<td>Original coal-tar sampleᵇ</td>
<td>21</td>
</tr>
<tr>
<td>Spiked test sample #1</td>
<td>150</td>
</tr>
<tr>
<td>Spiked test Sample #2</td>
<td>150</td>
</tr>
<tr>
<td>Spiked test sample #3</td>
<td>450</td>
</tr>
<tr>
<td>Spiked test sample #4</td>
<td>150</td>
</tr>
<tr>
<td>REP (%)ᶜ</td>
<td>–</td>
</tr>
<tr>
<td>recovery (%)</td>
<td>–</td>
</tr>
</tbody>
</table>

ᵃ nom. = nominal concentration; pred. = predicted concentration. All concentrations in ng·mL⁻¹.
ᵇ coal tar extract was diluted with n-octane, 1:375 (v/v).
ᶜ REP (%) = 100 × ((∑ᵢ₌₁(I(yₚᵢᵉᵈ − yₙᵢᵐ)²) / I)^(1/2) / yₙᵢₘ), where yₚᵢᵉᵈ is the predicted concentration for the i th validation sample, yₙᵢₘ is the nominal concentration, I is the number of validation samples, and yₙᵢₘ is the average of the nominal concentration values of the calibration set samples.
Figure 5.6: Elliptic joint confidence regions of PARAFAC predictions on (A) validation samples and (B) coal-tar spiked samples. Solid lines: 1 = DB$[a,e]P$, 2 = DB$[a,h]P$, 3 = DB$[a,i]P$, 4 = DB$[a,l]P$ and 5 = N$[2,3-a]P$. 

\[ \text{Intercept} \]
\[ \text{Slope} \]
5.3.4 Limits of Detection and Limits of Quantitation Based on EEM/PARAFAC Analysis

The LODs and LOQs obtained via multi-way calibration were calculated with the aid of the general sensitivity (SEN) expression reported by Olivieri:164

\[
SEN = s_n\{\delta_n^T[Z_{cal}^T(I - Z_{unx}^+Z_{unx}^+)Z_{cal}]^{-1}\delta_n\}^{-1/2}
\]

(5.2)

where \(s_n\) is the slope of the PARAFAC pseudounivariate plot, \(\delta_n\) is a column vector of size \(N_{cal} \times 1\) with zeros except for a “1” in the position of the analyte of interest, \(Z_{cal}\) is the Khatri-Rao product of the matrices containing the profiles in both modes for the calibrated components \((C_{cal}\) and \(B_{cal}\)), and \(Z_{unx}\) contains profiles in both modes for the unexpected components.

Considering a 95% confidence level for Type I (\(\alpha = 0.05\)) and II errors (\(\beta = 0.05\)) and assuming that the Gaussian curves in the absence and the presence of analyte have identical widths, the LOD and the LOQ can be calculated as \(3.3 \times s_0\) and \(10 \times s_0\), respectively. The term \(s_0\), which corresponds to the uncertainty prediction of the background sample, is computed with Equation 5.3:

\[
s_o = \left[SEN^{-2}\sigma_x^2 + h_oSEN^{-2}\sigma_x^2 + h_o\sigma_{ycal}^2\right]^{1/2}
\]

(5.3)

where \(h_o\) is the leverage for a blank sample, and \(\sigma_x\) and \(\sigma_{ycal}\) are the uncertainties in signal and calibration concentration, respectively. For the five studied isomers, the LOD/LOQ values were the following (ng·mL\(^{-1}\)): \(D[a,l]P = 0.40/1.21\), \(D[a,i]P = 0.11/0.33\), \(D[a,h]P = 0.22/0.67\), \(N[2,3-a]P = 0.31/0.94\), and \(D[a,e]P = 1.51/4.58\). The comparison of these values to those reported in
Table 5.2 show LOD improvements in all cases. This is in good agreement with previous reports showing LOD improvements when going from a one-way to a three-way calibration method.\textsuperscript{161}

5.4 Conclusions

A novel method for the direct determination, i.e., without chromatographic separation, of DB[\textit{a,l}]P, DB[\textit{a,h}]P, DB[\textit{a,i}]P, DB[\textit{a,e}]P, and N[2,3-\textit{a}]P in coal-tar samples has been developed on the basis of the collection of 4.2 K EEM and data processing with PARAFAC. Easy collection of steady-state EEM was accomplished with the aid of a cryogenic FOP and commercial instrumentation. Lowering the temperature to 4.2 K provided sufficient spectral narrowing for the accurate determination of the five dibenzopyrene isomers via PARAFAC at parts-per-billion concentration levels. The simplicity and the environmentally friendly nature of the experimental procedure, associated with the excellent analytical figures of merit, provide a valuable alternative for screening these highly toxic HMW-PAHs in coal-tar samples.
CHAPTER 6: CONCLUDING REMARKS

While research efforts employing established methodology continue to advance our knowledge on organic pollutants, the increasing awareness and public concern with environmental and occupational exposure to contaminants calls for analytical approaches capable to handle numerous samples in short analysis time. Data collection from statistically meaningful population sizes should benefit the development of efficient environmental remediation processes, which is extremely relevant for a globally sustainable environment. Although chromatographic techniques have dominated the environmental and toxicological scenarios, alternative solutions to this challenge require a new generation of analysts with knowledge in a plethora of analytical techniques and instrumentation. A recent trend for the direct determination, i.e., no chromatographic separation, of targeted species in matrices of unknown composition refers to processing multidimensional spectroscopic data with second order multivariate calibration methods. Under this prospective, particular attention has been paid to photoluminescence techniques. The combination of excitation and emission spectra to lifetime information within the time domains of fluorescence or phosphorescence provides a minimum of three qualitative parameters for compound determination, namely, one excitation and emission wavelength and one lifetime.

This dissertation has focused on the advancement of line-narrowing photoluminescence techniques. Line-narrowing techniques deal with the measurement of fluorescence and phosphorescence at cryogenic temperatures. Reducing the sample temperature to 20 K or below often improves spectral resolution. The main reason for seeking spectral narrowing is to differentiate the spectral features of chemically related compounds with significantly different
carcinogenicity and toxicity properties. We have developed LETRSS instrumentation to efficiently collect multidimensional data formats in frozen matrixes during the fluorescence and phosphorescence lifetime decays. Fluorescence and phosphorescence decays report on compound identification and spectral peak purity, an essential condition for the accurate quantitation of targeted contaminants without previous chromatographic separation. The inconvenience of sample freezing procedures was 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. LETRSS instrumentation takes advantage of the full dimensionality of luminescence spectroscopy combining spectral and lifetime information in multidimensional data formats known as WTM\textsuperscript{s} and TREEM\textsuperscript{s}. Processing WTM\textsuperscript{s} and TREEM\textsuperscript{s} with second order multivariate calibration algorithms, such as PARAFAC and U-PLS/RTL, provide a general solution to unpredictable spectral interference, a ubiquitous problem in samples of unknown composition.

The main limitation for the widespread use of LETRSS methodology could be the lack of commercial instrumentation. This dissertation has removed this limitation by coupling cryogenic fiber optic probes to commercial spectrofluorimeters. An attractive feature of commercial instrumentation with excitation and emission monochromators is the possibility to record synchronous spectra. The advantages of this approach, which include narrowing of spectral bandwidth and simplification of emission spectra, were demonstrated with the direct analysis of highly toxic dibenzopyrene isomers. The same is true for the collection of EEM\textsuperscript{s}. Commercial spectrofluorimeters are readily available in most academic institutions, industrial settings and research institutes. The developments presented in this dissertation should facilitate the
widespread use of Shpol’skii spectroscopy for the direct determination of PAHs in complex environmental matrixes.
APPENDIX A: 4.2 K EXCITATION-EMISSION SPECTRA AND CALIBRATION CURVES
Figure A.1: 4.2 K excitation (red) and emission (black) spectra of 100 μg·L⁻¹ solution of benzo[a]pyrene in n-octane. Spectra were collected with a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Excitation/emission wavelengths: 367.5/401.5 nm; excitation/emission band-passes: 3/1.25 nm; step-size 0.5 nm.
Figure A.2: 4.2 K calibration curve for benzo[a]pyrene in n-octane. Spectra were collected with a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Excitation/emission wavelengths: 367.5/401.5 nm; excitation/emission band-passes: 3/1.25 nm; step-size 0.5 nm.

\[ y = 10518x + 27242 \]
\[ R^2 = 0.9909 \]
Figure A.3: 4.2 K excitation (red) and emission (black) spectra of 100 µg·L⁻¹ solution of pyrene in n-hexane. Spectra were collected with a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer.

Excitation/emission wavelengths: 337/382 nm; excitation/emission band-passes: 3/1.25 nm; step-size: 0.5 nm.
Figure A.4: 4.2 K calibration curve for pyrene in \textit{n}-hexane. Spectra were collected with a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Excitation/emission wavelengths: 337/382 nm; excitation/emission band-passes: 3/1.25 nm; step-size: 0.5 nm.
Figure A.5: 4.2 K excitation (red) and emission (black) spectra of 100 μg·L⁻¹ solution of anthracene in n-heptane. Spectra were collected with a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Excitation/emission wavelengths: 254/380 nm; excitation/emission band-passes: 3/1.25 nm; step-size: 0.5 nm.
Figure A.6: 4.2 K calibration curve for anthracene in $n$-heptane. Spectra were collected with a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Excitation/emission wavelengths: 254/380 nm; excitation/emission band-passes: 3/1.25 nm; step-size: 0.5 nm.

$y = 2198.2x + 80984$

$R^2 = 0.9864$
APPENDIX B: 4.2 K SFSS SPECTRA AND CALIBRATION CURVES OF DIBENZOPYRENE ISOMERS
Figure B.1: 4.2 K SFSS spectrum of a synthetic mixture of DB\[a,e\]P, DB\[a,h\]P, DB\[a,i\]P, DB\[a,l\]P, and N[2,3-\text{a}]P in \textit{n}-octane. The concentration of each compound was 19.6 µg·L\(^{-1}\). The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (\(\Delta\lambda\): 88.5 nm) optimized for collection of emission from DB\[a,e\]P recorded at a synchronous excitation wavelength of 306 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.2: 4.2 K calibration curve for DB[a,e]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δλ: 88.5 nm) was optimized for collection of emission from DB[a,e]P recorded at a synchronous excitation wavelength of 306 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.3: 4.2 K SFSS spectrum of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane. The concentration of each compound was 19.6 µg·L\(^{-1}\). The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (\(\Delta \lambda\): 32.5 nm) optimized for collection of emission from DB[a,e]P recorded at a synchronous excitation wavelength of 362 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.4: 4.2 K calibration curve for DB[a,e]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δ\(\lambda\): 32.5 nm) was optimized for collection of emission from DB[a,e]P recorded at a synchronous excitation wavelength of 362 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.

\[
y = 4846.1x + 35931
\]
\[
R^2 = 0.9838
\]
Figure B.5: 4.2 K SFSS spectrum of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane. The concentration of each compound was 19.6 µg·L$^{-1}$. The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset ($\Delta \lambda$: 135 nm) optimized for collection of emission from DB[a,h]P recorded at a synchronous excitation wavelength of 312.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.6: 4.2 K calibration curve for DB[a,h]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset ($\Delta \lambda$: 135 nm) was optimized for collection of emission from DB[a,h]P recorded at a synchronous excitation wavelength of 312.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.7: 4.2 K SFSS spectrum of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane. The concentration of each compound was 19.6 µg·L⁻¹. The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δλ: 26 nm) optimized for collection of emission from DB[a,h]P recorded at a synchronous excitation wavelength of 421.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.8: 4.2 K calibration curve for DB[a,h]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset ($\Delta \lambda$: 26 nm) was optimized for collection of emission from DB[a,h]P recorded at a synchronous excitation wavelength of 421.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.9: 4.2 K SFSS spectrum of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane. The concentration of each compound was 19.6 µg·L$^{-1}$. The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δλ: 33.5 nm) optimized for collection of emission from DB[a,i]P recorded at a synchronous excitation wavelength of 396.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.10: 4.2 K calibration curve for DB[a,i]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset ($\Delta\lambda$: 33.5 nm) was optimized for collection of emission from DB[a,i]P recorded at a synchronous excitation wavelength of 396.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.

$y = 50949x + 3825$

$R^2 = 0.9937$
Figure B.11: 4.2 K SFSS spectrum of a synthetic mixture of DB\(a,e\)P, DB\(a,h\)P, DB\(a,i\)P, DB\(a,l\)P, and N[2,3-\(a\)]P in \(n\)-octane. The concentration of each compound was 19.6 µg·L\(^{-1}\). The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (\(\Delta \lambda\): 55.5 nm) optimized for collection of emission from DB\(a,i\)P recorded at a synchronous excitation wavelength of 374.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.12: 4.2 K calibration curve for DB[a,i]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δλ: 55.5 nm) was optimized for collection of emission from DB[a,i]P recorded at a synchronous excitation wavelength of 374.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.13: 4.2 K SFSS spectrum of a synthetic mixture of DB[e,P, DB[h,P, DB[i,P, DB[l,P, and N[2,3-a]P in n-octane. The concentration of each compound was 19.6 µg·L⁻¹. The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δλ: 96.5 nm) optimized for collection of emission from DB[l,P recorded at a synchronous excitation wavelength of 320.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.14: 4.2 K calibration curve for DB[a,l]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset ($\Delta\lambda$: 96.5 nm) was optimized for collection of emission from DB[a,l]P recorded at a synchronous excitation wavelength of 320.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.

\[ y = 16267x + 17896 \]
\[ R^2 = 0.9958 \]
Figure B.15: 4.2 K SFSS spectrum of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane. The concentration of each compound was 19.6 µg·L⁻¹. The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δλ: 20 nm) optimized for collection of emission from DB[a,l]P recorded at a synchronous excitation wavelength of 397 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.16: 4.2 K calibration curve for DB[a,l]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δλ: 20 nm) was optimized for collection of emission from DB[a,l]P recorded at a synchronous excitation wavelength of 397 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.17: 4.2 K SFSS spectrum of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane. The concentration of each compound was 19.6 µg·L⁻¹. The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δλ: 123.5 nm) optimized for collection of emission from N[2,3-a]P recorded at a synchronous excitation wavelength of 335.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.18: 4.2 K calibration curve for N[2,3-a]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (Δλ: 123.5 nm) was optimized for collection of emission from N[2,3-a]P recorded at a synchronous excitation wavelength of 335.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.19: 4.2 K SFSS spectrum of a synthetic mixture of DB[\(a,e\)]P, DB[\(a,h\)]P, DB[\(a,i\)]P, DB[\(a,l\)]P, and N[\(2,3-a\)]P in \(n\)-octane. The concentration of each compound was 19.6 \(\mu g \cdot L^{-1}\). The spectrum was collected using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset (\(\Delta \lambda: 27.5 \text{ nm}\)) optimized for collection of emission from N[\(2,3-a\)]P recorded at a synchronous excitation wavelength of 431.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.
Figure B.20: 4.2 K calibration curve for N[2,3-a]P in n-octane. A 4.2 K SFSS spectrum was collected of a synthetic mixture of DB[a,e]P, DB[a,h]P, DB[a,i]P, DB[a,l]P, and N[2,3-a]P in n-octane using a cryogenic FOP coupled to a FluoroMax-P spectrofluorometer. Wavelength offset ($\Delta \lambda$: 27.5 nm) was optimized for collection of emission from N[2,3-a]P recorded at a synchronous excitation wavelength of 431.5 nm. Excitation and emission band-pass: 2 nm; step-size: 0.5 nm.

N[2,3-a]P
$\Delta \lambda = 27.5$ nm

$y = 20304x + 20860$
$R^2 = 0.9900$
APPENDIX C: ROOM TEMPERATURE EXCITATION AND FLUORESCENCE SPECTRA OF DIBENZOPYRENE STANDARDS IN N-OCTANE
Figure C.1: Room temperature fluorescence excitation and emission spectra of dibenzo[a,e]pyrene, 100 ng·mL$^{-1}$, in $n$-octane. Spectra were collected with a quartz cuvette within the sample compartment of the spectrofluorometer, an $\lambda_{\text{exc}}/\lambda_{\text{em}}$ of 302/394 nm, and an excitation and emission band-pass of 2 nm.
Figure C.2: Room temperature fluorescence excitation and emission spectra of dibenzo[\(a,h\)]pyrene, 100 ng·mL\(^{-1}\), in \(n\)-octane. Spectra were collected with a quartz cuvette within the sample compartment of the spectrofluorometer, an \(\lambda_{exc}/\lambda_{em}\) of 310/447 nm, and an excitation and emission band-pass of 2 nm.
Figure C.3: Room temperature fluorescence excitation and emission spectra of dibenzo[\textit{a,i}]pyrene, 100 ng·mL\(^{-1}\), in \textit{n}-octane. Spectra were collected with a quartz cuvette within the sample compartment of the spectrofluorometer, an \(\lambda_{\text{exc}}/\lambda_{\text{em}}\) of 392/430 nm, and an excitation and emission band-pass of 2 nm.
Figure C.4: Room temperature fluorescence excitation and emission spectra of dibenzo[a,l]pyrene, 100 ng·mL⁻¹, in n-octane. Spectra were collected with a quartz cuvette within the sample compartment of the spectrofluorometer, an \( \lambda_{\text{exc}}/\lambda_{\text{em}} \) of 316/417 nm, and an excitation and emission band-pass of 2 nm.
Figure C.5: Room temperature fluorescence excitation and emission spectra of naphtho[2,3-a]pyrene, 100 ng·mL$^{-1}$, in $n$-octane. Spectra were collected with a quartz cuvette within the sample compartment of the spectrofluorometer, an $\lambda_{\text{exc}}/\lambda_{\text{em}}$ of 332/456 nm, and an excitation and emission band-pass of 2 nm.
APPENDIX D: 4.2 K EXCITATION AND FLUORESCENCE SPECTRA OF DIBENZOPYRENE STANDARDS IN N-OCTANE
Figure D.1: 4.2 K fluorescence excitation and emission spectra of dibenzo[a,e]pyrene, 100 ng·mL$^{-1}$, in n-octane. Spectra were collected with a FOP coupled to a spectrofluorometer, an $\lambda_{exc}/\lambda_{em}$ of 306/396.5 nm, and an excitation and emission band-pass of 2 nm.
Figure D.2: 4.2 K fluorescence excitation and emission spectra of dibenzo[\(a,h\)]pyrene, 100 ng mL\(^{-1}\), in \(n\)-octane. Spectra were collected with a FOP coupled to a spectrofluorometer, an \(\lambda_{\text{exc}}/\lambda_{\text{em}}\) of 312.5/447.5 nm, and an excitation and emission band-pass of 2 nm.
Figure D.3: 4.2 K fluorescence excitation and emission spectra of dibenzo[a,i]pyrene, 100 ng·mL⁻¹, in n-octane. Spectra were collected with a FOP coupled to a spectrofluorometer, an $\lambda_{exc}/\lambda_{em}$ of 396.5/430 nm, and an excitation and emission band-pass of 2 nm.
Figure D.4: 4.2 K fluorescence excitation and emission spectra of dibenzo[a,l]pyrene, 100 ng·mL⁻¹, in n-octane. Spectra were collected with a FOP coupled to a spectrofluorometer, an $\lambda_{exc}/\lambda_{em}$ of 320.5/417 nm, and an excitation and emission band-pass of 2 nm.
Figure D.5: 4.2 K fluorescence excitation and emission spectra of naphtho[2,3-$a$]pyrene, 100 ng·mL$^{-1}$, in $n$-octane. Spectra were collected with a FOP coupled to a spectrofluorometer, an $\lambda_{\text{exc}}/\lambda_{\text{em}}$ of 335.5/459.5 nm, and an excitation and emission band-pass of 2 nm.
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(95) Öhman, J.; Geladi, P.; Wold, S. *J. Chemom.* **1990**, *4*, 79–90.


(99) *MATLAB* 7.6; The Math Works Inc.: Natick, MA, USA, 2008.


