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Analysis of polycyclic aromatic hydrocarbons in HPLC fractions by laser-excited time-resolved Shpol'skii spectrometry with cryogenic fiber-optic probes

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Abstract

Laser-excited time-resolved Shpol'skii spectrometry at liquid helium temperature (4.2 K) is presented for the analysis of polycyclic aromatic hydrocarbons in high-performance liquid chromatography fractions. Fluorescence measurements are rapidly done with the aid of a fiber-optic probe, pulsed tunable dye laser, spectrograph, and intensified charge-coupled device. Analyte identification and peak-purity checking are made through wavelength–time matrix formats, which give simultaneous access to spectral and lifetime information. Sample preparation is rapid and simple. It involves liquid–liquid extraction or solid–liquid extraction of chromatographic fractions at the tip of the fiber-optic probe. The potential of both approaches is demonstrated with the semi-quantitative analysis of priority pollutants in heavily contaminated water samples.

Keywords: Laser-excited time-resolved Shpol'skii spectroscopy; Polycylic aromatic hydrocarbons; High-performance liquid chromatography; Solid–liquid extraction; Cryogenic fiber-optic probes; Wavelength–time matrices

1. Introduction

The ability of polycylic aromatic hydrocarbons (PAHs) to induce cancer has been documented by epidemiological studies of workers in coal tar, creosote, coal gas, coke, and cutting oil industries. The mutagenic and/or carcinogenic potential of individual PAH, although not fully documented, varies over a wide range and synergistic effects are suspected [1].

Among the numerous PAHs present in the environment, the US Environmental Protection Agency (EPA) has selected 16 as "Consent Decree" priority pollutants. These PAHs are monitored routinely for regulatory purposes. PAH methodology follows classical chromatographic analysis based on high-performance liquid chromatography (HPLC) coupled to ultraviolet absorption and room temperature fluorescence detection. The major drawback of this technique for the analysis of heavily contaminated samples is

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the use of a detection scheme non-specific for compound identification. Since PAH identification is solely based on retention times, unambiguous compound identification requires complete chromatographic resolution of sample components [2].

Because PAH separation might not be achieved in the HPLC column, use of a support analytical technique is recommended. Gas chromatography–mass spectrometry (GC–MS) often provides compound identification and peak-purity check of HPLC fractions. Unfortunately, elution times of 30–60 min are typical, and standards must be run periodically to verify retention times. If the concentrations of target species are found to lie outside the detector's response range, the sample must be diluted and the process repeated. On the other end of the concentration range, many samples are considered uncontaminated because PAH concentrations are below the detection limits of the analytical method.

One technique that can provide direct PAH determination in HPLC fractions is Shpol'skii spectroscopy [3]. Typically, the HPLC fraction is spiked into an appropriate solvent and frozen to 77 K or below, wherein three conditions are met: (a) the solvent freezes to an ordered polycrystalline matrix, not a random glass; (b) the guest–host interactions are weak; and (c) the guest and host molecular dimensions match-up well enough that the guest molecules occupy a small number of crystallographic sites (ideally just one) in the host lattice. The reduced thermal and inhomogeneous broadening leads to quasi-linear spectra for PAH identification. In comparison to GC–MS, no further chromatographic separation is needed [4–8].

In this paper, we present an improvement for the analysis of HPLC fractions by Shpol'skii spectroscopy. Fluorescence measurements are easily made with the aid of a cryogenic fiber-optic probe at helium temperature (4.2 K). Upon sample excitation with a pulsed tunable dye laser, wavelength–time matrices (WTM) are collected with a multi-channel detection system consisting of a spectrograph and an intensified charge-coupled device (ICCD) [9]. Prior to Shpol'skii spectrometry, the chromatographic fractions are submitted to a pre-concentration step either by liquid–liquid extraction (LLE) or solid–liquid extraction (SLE). LLE is carried out in the sample vessel of the fiber-optic probe mixing microliters of HPLC fraction and Shpol'skii solvent. PAH partitioning into the Shpol'skii solvent provides strong fluorescence signal and the fraction of mobile phase partitioning into the organic layer does not deteriorate the resolution of Shpol'skii spectra. The fiber-optic probe allows direct access to the organic layer of the biphasic solution, which results in high-quality Shpol'skii spectra [10]. SLE is performed on extraction disks that fit into the sample vessel of the fiber-optic probe. Prior to sample freezing, microliters of Shpol'skii solvent is spiked on the surface of the membrane and because PAH partition into the Shpol'skii solvent highly resolved spectra are obtained [11].

2. Experimental

2.1. Chemicals

All chemicals were analytical reagent grade and were used without further purification. Nanopure water was employed throughout. SPEC® octadecyl membranes were obtained from Ansys Diagnostics, Inc. Their composition consisted of bonded silica particles enmeshed in a glass fiber support. The average size and mean pore size of the silica particles were 30 μm and 7 nm, respectively. The mean thickness and diameter of the membranes were 1000 μm and 47 mm, respectively. Acenaphthylene, acenaphthene, anthracene, benzo[*g*,*h*,*i*]perylene, dibenz[*a*,*h*]anthracene, indeno[1,2,3-*c*,*d*]-pyrene, naphthalene, phenanthrene, fluorene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, pyrene, fluoranthene, and benzo[*a*]pyrene were purchased from Aldrich at their highest purity available. HPLC-grade acetonitrile, methanol, *n*-pentane, *n*-hexane, *n*-heptane, and *n*-octane were acquired from EM Science.

2.2. Water sample collection

The sample source selected for these experiments was the output water stream of the Amer-

ican Petroleum Institute (API) separator at a North Dakota refinery. The samples were collected from a sampling valve on the output pipe of the API separator. Water at this point had been largely stripped of visible petroleum but had received no further treatment to remove or destroy dissolved components. This ensured the collection of samples with highly complex PAH composition. Prior to sample collection, the valve was allowed to thoroughly flush to obtain a sample consistent with the throughput water. Samples were collected in 1-l glass bottles with PTFE-lined caps and stored at 4 °C. PAH extraction and HPLC analysis were completed within 72 h of sample collection.

2.3. Sample preparation for HPLC analysis

Samples from the API separator contain a significant quantity of suspended solids. For the sample under consideration, the total residue analysis per EPA method 160.3 revealed a total residue of 2520 mg l^{-1}. Because separate pre-filtration and solid removal treatment indicated PAH loss by adsorption on suspended particles, subsequent extraction for HPLC analysis was performed in the presence of suspended solids. SLE and concentration of the extract solvent were both performed per EPA method 550.1. SLE was carried out processing 0.5 l of water sample through 47-mm C-18 disks (Ansys Diagnostics, Inc.) with a Supelco "ENVI-Disk" filtration apparatus. The compounds were eluted from the disk with a small quantity of methylene chloride, dried, and concentrated further to 1 ml. A 3.0-ml portion of acetonitrile was added to the extract and concentrated to a final volume of 0.5 ml.

2.4. HPLC analysis

It was performed using a Beckman HPLC system with model 110B gradient pumps, a model 166 UV detector, and a model 406 control interface. HPLC operation was controlled with Beckman System Gold software. A Supelco Supelcosil LC-PAH column (25 cm in length, 4.6 mm in diameter, and 5 μm particle size; catalog number 58229) was selected for PAH separation. Flow conditions were as stipulated by Supelco, i.e. 1.5 ml min^{-1} flow rate, isocratic elution with water/acetonitrile (60:40, v v^{-1}) for 5 min, then linear gradient to 100% acetonitrile over 20 min. Detector response was monitored for 20 min following the latest eluting component to ensure the absence of any additional absorbing components. The column was allowed to re-equilibrate for a minimum of 15 min at initial conditions between all runs. All sample and standard injections were at a volume of 20 μl using a fixed volume injection loop. An AccuStandard PAH mixture (Product M-610A) was used as a reference standard for the 16 EPA-PAH. For compound identification, a working standard was prepared by diluting 1.00 ml of the AccuStandard mix to a final volume of 10.00 ml in acetonitrile. 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.

2.5. 4.2 K laser-excited time-resolved Shpol'skii spectrometry (LETRSS) analysis

The fiber-optic probe consisted of one delivery and six collection fibers (silica-clad silica; 500 μm core diameter, 2 μm cladding diameter, and 2 m long). At the sample end, the fibers were arranged in a conventional six-around-one configuration with the delivery fiber in the center. The collection fibers at the measurement end were arranged in a slit configuration. Vacuum epoxy (Torr-Seal®, Varian Vacuum Products) was used to bundle the fibers in place at the sample and analysis ends, which were polished after fabrication with a diamond-coated rotating disc. All fibers were fed into a 1.25-m long section of copper tubing that provided mechanical support for lowering the probe into the liquid helium. At the analysis end, the copper tubing was flared stopping a swage nut tapped to allow for the threading of a 0.75 ml polypropylene sample vial.

The excitation fiber was aligned with the beam of a Northern Lights tunable dye laser (Dakota Technologies, Inc.). The dye laser was operated through a KDP frequency-doubling crystal on rhodamine 6G (excitation wavelength range = $\Delta\lambda = 272–292$ nm), DCM ($\Delta\lambda = 310–330$ nm), or

LDS 698 ($\Delta\lambda = 325–380$ nm). When pumped with approximately 30 mJ of the second harmonic generator of an Nd:YAG Q-switched laser (Quanta Ray), the dye laser produced more than 5 mJ at peak of rhodamine 6G in a spectral bandwidth less than 0.03 nm and a pulse width of 5 ns. Fluorescence spectra were recorded with an Instrument S.A. spectrometer (270M) operating in the spectrograph mode. The spectrometer was equipped with a flat, 1200 grooves mm^{-1} grating and had a reciprocal linear dispersion of 3.1 nm mm^{-1}. The spectrograph was controlled with custom LabVIEW software (National Instruments) via an RS-232 interface. Fluorescence was detected with a front-illuminated ICCD (model No. DH520, Andor Technology) positioned at the lateral port of the spectrograph. Data with ICCD were acquired with LabVIEW software (National Instruments) operating in the external trigger mode. A delay generator (Stanford Research System DG535) was used to communicate between the excitation pulse and the intensifier of ICCD. The laser's pre-pulse trigger sent a trigger signal to the delay generator ~ 130 μs before the laser output to account for inherent delays in the electrical cables and components. Once triggered by the laser, the pulse generator used this information to determine when the image intensifier in CCD head was gated on (gate delay), for how long it was gated on (gate width) and the duration of the steps by which the gate delay was progressively increased in the course of the sequence of acquisitions (gate step). The acquired data were transferred from the detector head to the controller card (32-bit Intelligent Bus-Mastering PCI card) in the computer. The parameters for data acquisition (gate delay, gate width, and the gate step) were sent to the pulse generator via a GPIB interface.

2.6. LETRSS analysis of HPLC fractions

Prior to PAH determination, the HPLC fractions were submitted to either LLE or SLE. The main steps for each procedure are presented in Fig. 1. LLE-LETRSS analysis was performed as follows: for each fraction tested, 300 μl of the mobile phase was pipetted into the sample vial followed by 200 μl of Shpol'skii solvent. In both cases, the aliquot volumes were arbitrarily chosen. The sample vial was vigorously shaken for ~ 2 min and then allowed to stand for ~ 1 min to separate the two liquid layers. The sample vial was attached to the fiber-optic probe and the fiber vertically adjusted so that the tip was ~ 1 cm below the surface of the Shpol'skii layer. Sample freezing was accomplished by lowering the copper tubing into the liquid helium held in Dewar with a 60-l storage capacity. Complete sample freezing took less than 90 s. The approximately 1-min probe cleanup 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 cleanup cycle took no longer than 5 min.

SLE of HPLC fractions were performed with a syringe kit following a procedure for aqueous solutions previously described [11]. A cork borer was used to dissect 47 mm SLE disks into 5.5 mm diameter disks. The 5.5-mm membrane was placed into a stainless steel filter syringe kit (Alltech, IL) and attached to a 10-ml syringe (Hamilton, NV). Positive pressure was used to force the liquid solutions through the membrane. Prior to sample application, the disk was conditioned with 1 ml of methanol and 1 ml of nanopure water. A volume of HPLC fraction was then pushed through the membrane at ~ 30 ml min^{-1} flow rates. Following extraction, void water was mechanically removed with a 100-ml syringe forcing three 100 ml volumes of air through the disk. Placing the extraction disk in the sample vial of the low-temperature cell made measurements from octadecyl membranes easy. The membrane had been previously spiked with 100 μl of Shpol'skii solvent using a microliter syringe (Hamilton, NE). The distance between the fiber-optic probe and the substrate was adjusted to irradiate the entire surface of the membrane. In all cases, the position of the probe was held constant with the screw cap of the sample cell. Sample freezing was accomplished by lowering the copper tubing and the cell into the liquid cryogen. Because there was no physical contact between the probe and the

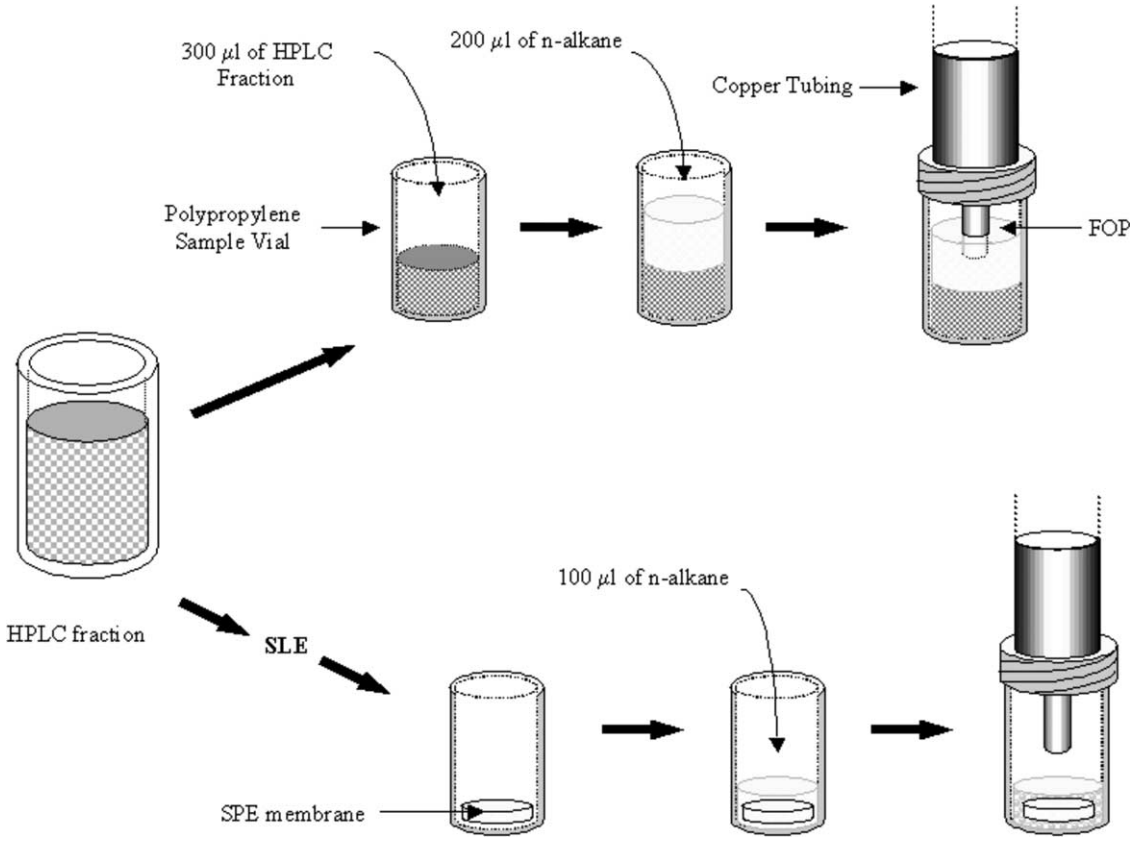

Fig. 1. Diagram showing LLE and SLE procedures. FOP, fiber-optic probe.

membrane, probe cleanup between membrane measurements was not necessary.

2.7. Lifetime analysis

Fluorescence lifetimes were determined via a three-step procedure: (1) full sample and background WTM were collected; (2) the background decay curve was subtracted from the fluorescence decay curve at the wavelength of maximum fluorescence for each PAH; and (3) the background corrected data were fit to single exponential. In cases where the exact sample composition was unknown and the formulation of a correct blank for lifetime background subtraction was impossible, the fluorescence decay at the base of the target peak was used for background subtraction at the target wavelength. The accuracy of this procedure has been demonstrated previously [12]. Origin software (version 5, Microcal Software, Inc.) was used for curve-fitting of fluorescence lifetimes. The decay curve data were collected with a minimum of 10 ns interval between opening of the ICCD gate and the rising edge of the laser pulse, which is sufficient to avoid the need to consider convolution of the laser pulse with the analyte signal. Fitted decay curves ($y = y_0 + A_1 \exp(-(x - x_0)\tau_1)$) were obtained by fixing y_0 and x_0 at a value of zero. In all cases, the residuals between the calculated and observed points were

less than 1% within the first two lifetimes of the decays and showed no systematic errors.

3. Results and discussion

3.1. HPLC analysis of water samples

The second column in Table 1 shows the retention times for the HPLC analysis of EPA-PAH. If present in the sample, these compounds would be collected in the following six fractions: naphthalene and acenaphthylene between 15 and 20 min of chromatographic time (ct = 15–20 min); acenaphthene, fluorene, phenanthrene, and anthracene between ct values of 20 and 25 min; fluoranthene and pyrene between ct values of 25 and 28 min; benzo[*a*]anthracene and chrysene between ct values of 28 and 34 min; benzo[*b*]fluoranthene and benzo[*k*]fluoranthene between ct values of 34 and 37 min; and benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene, and indeno[1,2,3-*c*,*d*]pyrene between ct values of 37 and 46 min.

The HPLC analysis of water samples yielded very complex chromatograms with many overlapping and unresolved peaks in the elution range of the 16 EPA-PAH. At least 40 definitive detector responses were identified in the chromatograms within the working time range. Based on their retention times and standard additions, HPLC was able to identify only three EPA-PAH, namely naphthalene, fluorene, and phenanthrene. Making additional assignments and positive identifications on the later portion of the chromatogram was not possible because of the overwhelming number of components.

3.2. LETRSS parameters for PAH analysis of HPLC fractions

Table 1 relates the LETRSS parameters for EPA-PAH determination. Seeking for convenience and simplicity, we used only one Shpol'skii solvent per HPLC fraction. As a result, the employed solvent did not fit the linear dimensions of all the priority pollutants in the fraction, a criterion often employed in Shpol'skii spectrometry for best spectral resolution. Similarly, site-selective excitation was not attempted. All emission spectra were collected using excitation wavelengths within the dye laser tunable range at which maximum fluorescence intensity was observed. In some cases, the narrow-band laser excitation did not have much impact with regard to site selection because the excitation was into the broad, higher excited states

Table 1
Qualitative parameters for monitoring EPA-PAH in HPLC fractions by LETRSS at 4.2 K

PAH	Retention time (min)	Solvent	$\lambda_{exc}/\lambda_{em}$[a] (nm)	$\tau_{f,STD}$[b] (ns)
Naphthalene	16.38±0.19	*n*-Pentane	291.5/319.8	200.4±0.7
Acenaphthene	20.30±0.09	*n*-Hexane	289.0/*320.8*, 321.3	49.9±0.2
Fluorene	20.98±0.18	*n*-Hexane	289.0/*302.2*, 303.9, 305.7	7.3±0.3
Phenanthrene	22.62±0.10	*n*-Hexane	287.1/*347.2*, 365.2	34.8±1.2
Anthracene	24.32±0.03	*n*-Hexane	362.2/*377.4*, 379.7, 383.0	12.4±0.9
Fluoranthene	25.87±0.12	*n*-Hexane	360.4/*408.9*, 417.6, 436.5	55.0±1.9
Pyrene	27.10±0.17	*n*-Hexane	328.0/*370.2*, 371.1, 376.0, 376.8	506.9±1.2
Benzo[*a*]anthracene	31.00±0.29	*n*-Octane	362.2/*385.2*, 412.4	52.5±1.6
Chrysene	32.18±0.47	*n*-Octane	326.2/*359.1*, 378.5	57.8±2.3
Benz[*b*]fluoranthene	34.84±0.36	*n*-Octane	370.4/*396.8*, 403.8, 410.2, 427.5	43.8±1.1
Benzo[*k*]fluoranthene	36.61±0.44	*n*-Octane	380.4/399.6, *402.3*, 411.4, 426.9	8.9±0.3
Benzo[*a*]pyrene	38.29±0.59	*n*-Octane	290.0/*402.9*, 403.0	38.6±0.3
Dibenz[*a*,*h*]anthracene	41.10±0.85	*n*-Octane	302.5/*393.0*, 394.1, 394.7	41.9±0.5
Benzo[*g*,*h*,*i*]perylene	43.06±0.86	*n*-Octane	290.0/406.3, 415.5, *419.5*	127.2±1.5
Indeno[1,2,3-*c*,*d*]pyrene	45.26±1.00	*n*-Octane	302.5/*461.2*, 464.9, 469.1, 470.0	10.4±0.1

[a] Wavelengths of excitation (λ_{exc}) and emission (λ_{em}).
[b] Fluorescence lifetime based upon three replicate measurements of standard solutions extracted into appropriate Shpol'skii solvent.

where the lines are broadened by the short lifetimes. All spectra were collected with an ~0.324 nm spectral bandpass, which is the limiting resolution of our instrumental setup. Considering the reciprocal linear dispersion (3.1 nm mm^{-1}) of the spectrograph, the number of active pixels (690 × 256) in the CCD array, and the dimensions (18 mm × 6.7 mm) of the active area of the CCD chip, the spectral range of one pixel should give a 0.081 nm limiting resolution. However, because of the detector cross-talk and the intensifier, the factual limiting resolution corresponds to 4–5 pixels [13], i.e. 0.324–0.405 nm. Although much narrower spectra can be obtained with a higher resolution spectrograph, our studies will prove the feasibility of our approach to target EPA-PAH in the HPLC fractions.

The target wavelengths for the remaining EPA-PAH were selected from synthetic mixtures containing the compounds expected to appear in the HPLC fractions. Fig. 2 shows the 4.2 K fluorescence WTM recorded from the synthetic mixture containing benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene, and indeno[1,2,3-*c*,*d*]pyrene. If present in the sample, these PAH should appear between ct values of 37 and 46 min. The comparison to individual standards showed no spectral overlapping among the components of the mixture. The fluorescence decays—stripped from WTM at the target wavelengths—were single exponential decays confirming the spectral purity of the target wavelengths. The fluorescence lifetimes in the mixture matched those from individual standard solutions showing no synergistic effects. The same type of results was obtained for the other five synthetic mixtures. The only EPA-PAH with no fluorescence emission was acenaphthylene. Purging its standard solutions with nitrogen gas for 15 min showed no effect on fluorescence enhancement. At present we have no

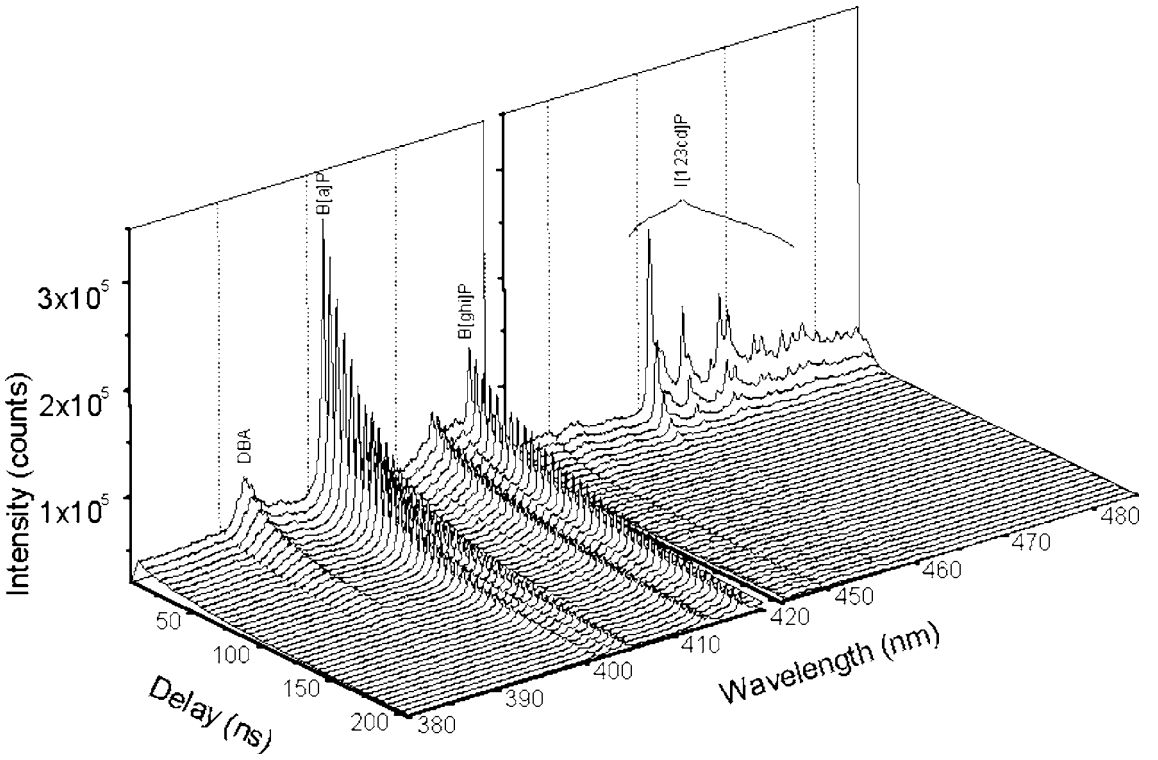

Fig. 2. Wavelength–time matrix of PAH standards eluting in HPLC fraction collected from 37 to 46 min. Initial delay was 10 ns, gate width was 200 ns, and delay step was 5 ns. DBA, dibenz[*a*,*h*]anthracene; B[*a*]P, benzo[*a*]pyrene; B[*g*,*h*,*i*]P, benzo[*g*,*h*,*i*]perylene; and I[1,2,3-*c*,*d*]P, indeno[1,2,3-*c*,*d*]pyrene.

explanation for the observed phenomenon, which persisted in several solvents.

3.3. LETRSS analysis of HPLC fractions

Fig. 3 shows the LETRSS spectra of the HPLC fraction collected between 28 and 34 min. The fraction was liquid–liquid extracted with octane. Upon sample excitation at 326.2 nm, several emission peaks appeared in the spectrum (see Fig. 3A). A relatively small peak at 359.1 nm matched one of the target wavelengths of chrysene. Because a single exponential decay was obtained at 359.1 nm and its fluorescence lifetime matched the one in Table 1, the peak assignment was confirmed. Upon standard addition, the intensity of the peak was enhanced (see Fig. 3B), the fluorescence time profile still matched a single exponential decay, and no change in fluorescence lifetime was observed. These facts guaranteed the spectral purity of the target wavelength. In contrast, the fluorescence at the other target peak (378.5 nm) of chrysene fitted a multi-exponential decay before and after standard addition. This fact clearly shows the advantage of having lifetime information to check for spectral purity and guide quantitative analysis to the target peak with no spectral overlapping. Because our system rapidly generates WTM there is no cost in analysis time.

Fig. 4 shows the LETRSS spectra recorded from the HPLC fraction collected between 25 and 28 min. The spectrum in Fig. 4A was recorded from 200 μl of hexane previously spiked with 300 μl of fraction. Upon sample excitation at 328 nm, spectral and lifetime data confirmed the presence of pyrene. Fig. 4B shows the spectrum of the fraction recorded from an extraction membrane spiked with 100 μl of hexane. Two-milliliter aliquot of HPLC fraction was processed through the membrane. The spectral comparison clearly shows the advantage of using SLE. The improvement of the signal-to-noise ratio (S/N) facilitates the spectral identification of target peaks and improves the statistical fitting for lifetime analysis (data not shown). The S/N improvement (2 ×) is mostly due to the larger pre-concentration obtained with SLE. Because the extraction efficiency for pyrene was rather low (77%), optimization of this parameter prior to SLE could have led to a better S/N. Similar results could have been obtained by processing a larger volume of mobile phase through the extraction membrane. However, careful examination of Fig. 4 shows the appearance of new peaks in the SLE spectrum within 400

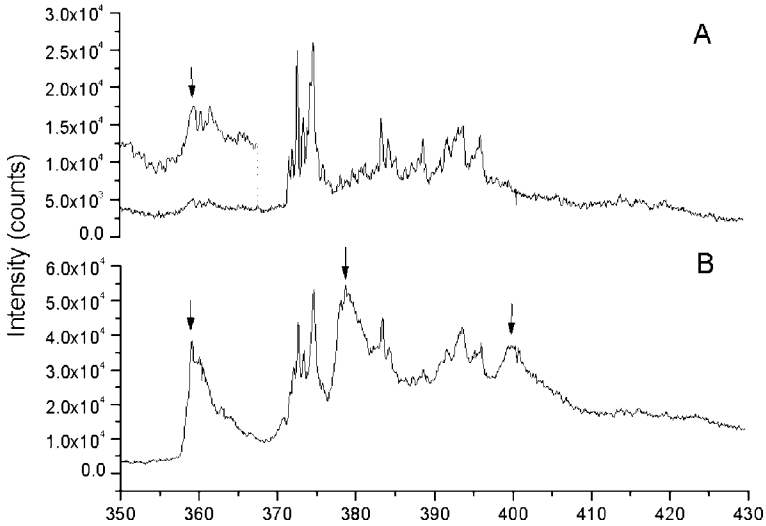

Fig. 3. (A) Fluorescence spectra of HPLC fraction collected from 28 to 34 min following LLE. Spectra were taken with delay of 10 ns and gate of 200 ns. Inset shows expanded region of spectra from 350 to 367 nm. (B) Fluorescence spectra of same HPLC fraction in (A) spiked with 5 μl of chrysene standard following LLE. Timing parameters were same as in (A).

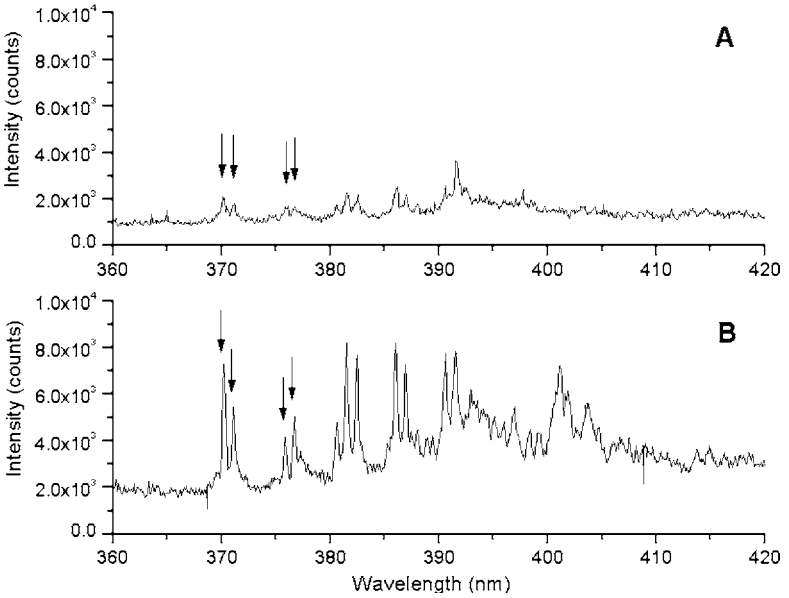

Fig. 4. (A) Fluorescence spectra of HPLC fraction collected from 25 to 28 min following LLE. Delay and gate times were 10 and 2000 ns, respectively. (B) Fluorescence spectra of same fraction in (A) following SLE.

and 405 nm. These peaks do not match any of the target wavelengths in Table 1 but demonstrate the capability to measure lower PAH concentrations than the LLE procedure.

Table 2 shows the nine EPA-PAH identified in the water sample. Quantitative analysis was performed with the multiple standard addition method. Six standard additions (5 μl of standard in acetonitrile) were made to the chromatographic aliquot (300 μl) prior to LLE. The limits of quantitation were calculated as 10 × the standard deviation of 16 blank measurements divided by the slope of the standard addition curve. The measurements were taken at the peak base of the HPLC fraction. PAH concentrations were calculated based on three independent samples. At the limit of quantitation, the relative standard deviations of analyte signals were lower than 8%, which is excellent for fluorescence measurements at 4.2 K. The enrichment factors were calculated taking into

Table 2
Quantitative analysis of EPA-PAH in HPLC fractions using 4.2 K LETRSS[a]

Compound	Limit of quantitation[b] (μg l^{-1})	Enrichment factor[c]	Concentration in water sample (μg l^{-1})
Naphthalene	4.2	4	2.8±0.2
Acenaphthene	3.0	4	3.8±0.4
Fluorene	5.4	4	40±4
Pyrene	0.3	6.7	0.30±0.03
Chrysene	0.3	2.8	0.88±0.07
Benzo[*k*]fluoranthene	1.8	10	0.40±0.03
Benzo[*a*]pyrene	0.06	2.2	7.3±0.7
Dibenz[*a*,*h*]anthracene	5.4	2.2	8.5±0.8
Benzo[*g*,*h*,*i*]perylene	0.6	2.2	60±5

[a] LETRSS analysis was done using excitation and emission wavelengths reported in Table 1.

[b] Limits of quantitation were calculated based on the equation $10S_B/m$, where S_B is the standard deviation of 16 baseline measurements and m the slope of the standard addition curve.

[c] Enrichment factors were calculated based on the equation $C_{SS} = 3/2 \times 20/V_{HPLC} \times C_{H_2O}$, where V_{HPLC} is the volume of HPLC fraction collected, and C_{SS} and C_{H_2O} are the analyte concentrations in the Shpol'skii layer and the water sample, respectively.

consideration all the steps of the experimental procedure, namely the pre-concentration of the water sample (SLE), HPLC analysis, and LLE of chromatographic aliquots. The concentrations of pyrene and benzo[*k*]fluoranthene demonstrate the capability for precise analysis at the parts per trillion level.

4. Conclusions

We have presented a valuable tool for PAH analysis in HPLC fractions of heavily contaminated water samples. In comparison to Shpol'skii spectrometry at 77 K, lowering the temperature to 4.2 K promotes significant enhancement of spectral narrowing. Because the fiber-optic probe retains the simplicity of dunking the sample into the liquid cryogen, the analysis can be made at liquid helium temperature with no cost in analysis time. The entire procedure—from HPLC collection to LETRSS measurement—takes less than 10 min per sample. Because the 60 l of liquid helium would typically last 3 weeks of daily use averaging 15–20 samples per day, the cost per sample is well suited for routine analysis.

The combination of the fiber-optic probe, pulsed tunable dye laser, spectrograph, and ICCD allows for the rapid collection of 4.2 K WTM. This data format gives simultaneous access to both spectral and lifetime information. The highly resolved fluorescence spectra provide several target wavelengths for PAH identification. Fluorescence lifetimes give an additional parameter for PAH identification and provide a unique way for confirming wavelength assignments and checking spectral purity in HPLC fractions. The high selectivity of our approach is completed with the scanning capability of the tunable dye laser, which allows one to selectively excite small wavelength shifts in PAH excitation spectra, collect excitation-emission matrices (EEM), and time-resolved EEM.

SLE-LETRSS accomplishes pre-concentration of HPLC fractions via a one-step procedure with minimum cost in analysis time and solvent consumption. Because spiking microliters of Shpol'skii solvent on the extracted membrane provide highly resolved spectra and matching lifetimes to those from standards [10], SLE-LETRSS retains the selectivity of LLE-LETRSS with the capability to reach concentration levels below the LOD of LETRSS.

Acknowledgements

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