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Photoluminescence spectroscopy of anthrathiophenes and benzonaphthothiophenes in Shpol'skii matrixes

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Abstract

The dispersion of harmful oil components into the ocean waters could pose long-term risks to flora and fauna. Due to the complexity of oil-contaminated sites, the unambiguous identification and quantitation of environmental pollutants often requires the sequence of high-performance liquid chromatography and gas chromatography-mass spectrometry. A classic example is the analysis of polycyclic aromatic hydrocarbons. This article tackles a different aspect of environmental analysis as it focuses on the Shpol'skii spectroscopy of polycyclic aromatic sulfur heterocycles, specifically those belonging to the subgroups often known as anthrathiophenes and benzonaphthothiophenes. Photoluminescence measurements were made with a commercial spectrofluorimeter equipped with a continuous wave excitation source for steady state measurements and a pulsed excitation source for time-resolved measurements in the phosphorescence time domain. To the extent of our literature search, this is the first report on the 4.2 K fluorescence and phosphorescence spectra of anthrathiophenes and benzonaphthothiophenes, and the 77 K and 4.2 K phosphorescence lifetimes of benzonaphthothiophenes. 77 K and 4.2 K analytical figures of merit revealed the possibility to detect the studied compounds at the parts-per-billion (ng mL^{-1}) concentration levels. The spectral and lifetime data gathered in this article provides the required information to choose an appropriate photoluminescence technique for the analysis of four-ring polycyclic aromatic sulfur heterocycles in complex environmental extracts.

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Conflict of interest

There are no conflicts of interest to declare.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2018.10.044.

Keywords

Photoluminescence; Shpol'skii spectroscopy; Polycyclic aromatic sulfur heterocycles; Anthrathiophenes; Benzonaphthothiophenes; Time-resolved phosphorimetry

1. Introduction

Similar to polycyclic aromatic hydrocarbons (PAHs), polycyclic aromatic sulfur heterocycles (PASHs) are formed either from petrogenic (fossil material) or from pyrolytic sources. Their presence in the environment has been confirmed in air particulate matter [1,2], sediments [3,4], coal liquids [5], crude oils [6–9], coal tars [10–12], waste products [13], and other environmental samples [1,14]. Once released in the environment, PASHs can photo-react in aqueous media and affect microbial metabolism [15,16], bio-accumulate [17] and, through combustion reactions, form SO_2 that contributes to the precipitation of acid rain [18].

Since several PASHs have shown potential mutagenic and carcinogenic properties [19,20] the European Union and the National Oceanic and Atmospheric Administration (NOAA) have included a few specific PASHs in their priority pollutants lists [21,22]. These include dibenzothiophene, 4-methyldibenzothiophene, 4,6-dimethyldibenzothiophene, 2,4,6-trimethyldibenzothiophene, benzo[*b*]naphtho[1,2-*b*]thiophene (BbN12T), benzo[*b*]naphtho[2,1-*b*]thiophene (BbN21T) and benzo[*b*]naphtho[2,3-*b*]thiophene (BbN23T).

Current analytical methodology for the analysis of PASHs in environmental samples is based on high-performance liquid chromatography (HPLC) coupled to ultraviolet – visible (UV–VIS) absorption detection or room-temperature fluorescence (RFT) detection and gas chromatography with mass spectrometry detection (GC-MS) [23–27]. Since many PASHs present similar chromatographic behaviors and the detection schemes are not specific enough to resolve co-eluting PASHs, the unambiguous determination of PASHs requires a combination of normal-phase (NP) and/or reversed-phase (RP) liquid chromatographic (LC) steps prior to GC-MS analysis. Recently, Wilson and co-workers [28,29] developed methodology for the analysis of PASHs and alkyl-PASHs in standard reference materials (SRMs). Due to the complexity of the samples and the co-existence of numerous polycyclic aromatic hydrocarbons and their alkylated homologues, the determination of PASHs was best achieved with the combination of NPLC retention times, GC retention times and mass spectra. According to our literature search, those studies represent the most comprehensive characterization of three combustion-related SRMs for PASHs and some alkyl-substituted (alkyl-PASHs) derivatives to date.

The research presented here provides a thorough investigation of the photoluminescence properties of PASHs containing four benzene rings ($\text{MM } 234 \text{ g mol}^{-1}$) in Shpol'skii matrixes, specifically those belonging to the subgroups often known as anthra-thiophenes and benzonaphtho-thiophenes. According to our literature search, only a few articles exist on the spectral characteristics of PASHs in Shpol'skii matrixes. Colmsjö and co-workers [30] reported on the 63 K fluorescence and phosphorescence spectral characteristics of three

benzonaphthothiophenes, namely BbN12T, BbN21T and BbN23T. All spectra were recorded from n-hexane solutions and a commercial spectrofluorimeter. BbN12T and BbN21T presented well resolved fluorescence spectra with vibrational resolution. BbN23T showed weak fluorescence intensity with poor spectral resolution. None of the studied benzonaphtho-thiophenes showed phosphorescence in n-hexane at 63 K [30].

The direct analysis of $C_{16}H_{10}S$ isomers, i.e. no chromatographic separation, via Shpol'skii Spectroscopy in complex environmental extracts was reported by Elsaïd and co-workers [31]. Selective excitation was achieved with a narrow band tunable dye laser. Fluorescence and phosphorescence spectra were recorded at 15 K with the aid of a closed cycle helium refrigerator using a 0.64m spectrometer coupled to a photomultiplier tube and a photodiode array for single-channel and multichannel detection, respectively. The targeted isomers were BbN12T, BbN21T and BbN23T. On the bases of their fluorescence spectra, the three compounds were directly identified in a solvent refined coal (SRC-II) sample, a petroleum crude oil extract (Standard Reference Material 1582) and a carbon black sample. Their phosphorescence spectra showed weak intensity for analytical use. Although limits of detection were not reported, the successful identification of these three benzonaphthothiophenes demonstrated the analytical potential of Laser-Excited Shpol'skii Spectroscopy for the determination of $C_{16}H_{10}S$ isomers in complex extracts without previous chromatographic separation.

Herein, we report on the 77 K and 4.2 K fluorescence and phosphorescence characteristics of anthra[1,2-*b*]thiophene (A12T), anthra [2,1-*b*]thiophene (A21T), anthra[2,3-*b*]thiophene (A23T, BbN12T, BbN21T and BbN23T in n-octane. To the extent of our literature search, this is the first report on the 4.2 K fluorescence and phosphorescence spectra of anthrathiophenes and benzonaphthothiophenes, and the 77 K and 4.2 K phosphorescence lifetimes of benzonaphthothiophenes. 77 K and 4.2 K analytical figures of merit (AFOM)) revealed the possibility to detect the studied compounds at the parts-*per*-billion ($ng\ mL^{-1}$) concentration levels. The spectral and lifetime data gathered in this article provides the required information to choose an appropriate photoluminescence technique for the analysis of four-ring PASHs in complex environmental extracts.

2. Experimental

2.1. Chemicals and reagents

All chemicals were analytical-reagent grade and used without further purification. HPLC grade n-heptane and n-octane were acquired from Acros Organics. HPLC grade n-nonane was acquired from Sigma-Aldrich. A12T, A21T, A23T, BbN12T, BbN21T and BbN23T were obtained from National Institute of Standards and Technology (NIST) and used as received. The standard for instrumental performance was purchased from Photon Technology International (PTI). It consists of a single crystal of dysprosium-activated yttrium aluminum garnet mounted in a cuvette-sized holder with well-characterized quasi-line excitation and emission spectra.

2.2. Preparation of stock solutions

Stock solutions of PASHs were prepared by dissolving pure standards in an appropriate solvent and kept in the dark at 4 °C. PASHs working solutions were prepared using serial dilution of stock solutions prior to data collection. Possible PASHs degradation was monitored via room-temperature fluorescence spectroscopy.

2.3. Instrumentation

Fluorescence and phosphorescence measurements were carried out with a FluoroMax – P (Horiba Jobin-Yvon) equipped with a continuous xenon arc source for fluorescence measurements and a pulsed xenon arc source for phosphorescence measurements. The flash rate range of the pulse source was 0.05–25 Hz. The duration of the pulse was 3 μs at full-width half maximum. The 1200 grooves/mm gratings in the single excitation and emission monochromators were blazed at 330 nm and 500 nm, respectively. Their reciprocal linear dispersion was equal to 4.25 nm/mm. The uncooled photomultiplier tube (Hamamatsu, Model R928) detector was operated in the photon-counting mode. The gating circuitry for time-resolved measurements provided delays after the flash ranging from 50 μs to 10 s (in increments of 1 μs) and sample windows (gate times) ranging from 10 μs to 10 s (in increments of 1 μs). Commercial software (DataMax version 2.20, Horiba Jobin-Yvon) was used for automated scanning and fluorescence data acquisition. Origin software (version 5, Micronal Software, Inc.) was used for curve fitting of phosphorescence decays. Fitted decay curves were obtained by fixing x_0 and y_0 at a value of zero in Eq. (1).

$$y = y_0 + A_1 e^{[-(x - x_0)t_1]} \quad (1)$$

2.4. Fiber optic probes

Cryogenic fiber optic probes (FOPs) 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. Position alignment of each end of the FOP with the respective focusing mirror was facilitated by commercially available adapters (Horiba Jobin-Yvon). All fibers were 2m long and 500 μm core diameter, silica-clad silica with polyimide buffer coating (Polymicro Technologies, Inc.). The fibers were fed into a 1.2m long section of copper tubing that provided mechanical support for lowering the probe into the liquid cryogen. At the sample end, the fibers were arranged either in a six-around-one or an eight-around-two configuration with the excitation fiber (s) in the center. At the instrument end, the emission fibers were positioned in a slit configuration. At both ends, vacuum epoxy was used to hold the fibers in place, which were then fed into metal sleeves for mechanical support and polished with a diamond rotating disk. At the sample end, the copper tubing was flared stopping a phenolic screw cap threaded for a 0.75 mL propylene sample vial.

2.5. Sample Procedures

All measurements with the PTI standard were performed at RT and in the dark by placing the sample end of the FOP on the surface of the standard. Solution measurements were made as follows: for each sample, a 100 μ L aliquot of un-degassed sample solution was pipetted into a 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 three weeks of daily use, averaging 15–20 samples per day. At both 77 K and 4.2 K, complete sample freezing took less than 90 s. The $\approx$ 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.

2.6. Parallel factor analysis of phosphorescence EEMs

All calculations were done using MATLAB 7.10 (MATLAB 7.10 (2010) The MathWorks Inc., Natick, Massachusetts, USA). PARAFAC was applied with the MVC2 graphical user interface written in MATLAB and available on the Internet [32].

3. Results and discussion

3.1. Improvement of fiber optic probe

Preliminary studies with the original FOP (six-around-one configuration) [33] revealed unsatisfactory signal-to-background ratios for spectral collection. As a tentative means of improving the signal-to-background ratio of our measurements, a new FOP was devised consisting of two excitation fibers and eight emission fibers (eight-around-two configuration). The performance of the two FOPs were then compared with the aid of a commercial standard (PTI standard) often used in our lab for instrument calibration purposes and a PAH (chrysene) with known spectral features at room-temperature (RT), 77 K and 4.2 K.

Fig. S1 compares the excitation and emission spectra of the PTI standard recorded at RT with the six-around-one (original FOP) and the eight-around-two (new FOP) configuration. Since both sets of spectra were recorded with the same excitation (2 nm) and emission (2 nm) band-pass, the higher spectral intensity observed with the new FOP results from the eight-around-two configuration. The average signal intensities and their corresponding standard deviations ($N = 3$) are summarized in Table S1. The intensity ratios ($I_{\text{NewFOP}}/I_{\text{OriginalFOP}}$) remained approximately constant within the wavelength ranges of the experiments. The lowest (4.1 ± 0.07) and the highest (5.7 ± 0.002) ratios were obtained at 327 nm and 296 nm, respectively.

Figs. S2 and S3 compare the 77 K and the 4.2 K excitation and fluorescence spectra of chrysene recorded from standard solutions in n-octane using the original and the new FOP.

Chrysene concentrations were adjusted to fit within the linear dynamic ranges of the calibration curves recorded with each probe at 77 K and 4.2 K. No attempts were made to optimize spectral resolution, nor were the spectra corrected for instrumental response. Previous work in our lab via laser-excited time-resolved Shpol'skii spectroscopy revealed three crystallographic sites for chrysene in n-octane with maximum fluorescence wavelengths at 359.2 nm, 359.6 nm and 360.6 nm [34]. Since site-selective excitation was not attempted, all the spectra in Figs. S2 and S3 were recorded under multi-site excitation conditions and compile the spectral contributions of the three crystallographic sites. Independent of the sample temperature and the concentration of chrysene, the highest intensities were obtained with the new FOP.

3.2. Selection of Shpol'skii solvent

The molecular structures of the studied compounds are shown in Fig. 1. The selection of a matching solvent for the Shpol'skii Spectroscopy followed the same criterion as the one often employed for PAHs [35]. The molecular dimensions of anthra-thiophenes and benzonaphtho-thiophenes were calculated at NIST using commercial modeling programs (PC-Model and MMX, Serena Software, Bloomington) [36]. The best PASH/n-alkane matchings are summarized in Table 1. All compounds were dissolved in the calculated n-alkanes and used without further solvent optimization studies.

3.3. Steady-state photoluminescence spectroscopy at RT, 77 K and 4.2 K

The RT, 77 K and 4.2 K photoluminescence properties of the anthrathiophenes and benzonaphthothiophenes was first investigated under steady-state conditions with the aid of a continuous wave (CW) xenon excitation source. Photoluminescence spectra were recorded from pure standards at the maximum excitation and emission wave-length of each compound. If necessary, cut off filters were used to remove the contribution of second order emission due to scattering of excitation radiation. Datasets with steady-state spectra of anthrathiophenes and benzonaphthothiophenes are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) [37–39].

Anthrathiophenes and benzonaphthothiophenes showed fluorescence at RT with vibrational resolution in excitation and emission spectra. In comparison to the excitation spectra of benzonaphthothiophenes, anthra-thiophenes showed additional excitation bands between 300 nm and 400 nm. In comparison to the emission spectra of benzonaphthothiophenes, anthrathiophenes showed red-shifted spectral profiles with slightly broader emission bands. All the RT fluorescence spectra are shown in Fig. 2, [40]. No phosphorescence was observed from any of the studied compounds at RT. The long lifetime of phosphorescence provides ample opportunity for collisional deactivation of the first triplet state without the emission of radiation. Although oxygen is known to be a strong phosphorescence quencher in liquid solutions at RT [41], no de-gassing was attempted to remove the presence of oxygen from the sample.

The 77 K and 4.2 K excitation and fluorescence spectra of anthrathiophenes are shown in Fig. 3, [40]. The spectral features of three compounds showed the characteristic narrowing that is often observed when lowering the temperature to 77 K. No additional narrowing was

observed from A12T and A21T at 4.2 K. The same is not true for A23T, which showed its narrowest spectrum at 4.2 K. While no significant shifts were observed in the vibrational maxima of A12T and A21T, the maximum wavelengths of A23T presented 5–7 nm red-shifts at 77 K and 4.2 K. None of the anthrathiophenes showed phosphorescence at 77 K and 4.2 K. This is probably due to inefficient intersystem crossing between the first singlet excited state and triplet state manifold of the studied compounds.

Fig. 4, [40] depicts the spectral features of benzonaphthothiophenes at 77 K and 4.2 K. The contribution of phosphorescence to the emission spectra of the three studied compounds indicates efficient intersystem crossing conversion between the singlet and triplet state manifolds. No significant narrowing was observed in the fluorescence of benzonaphthothiophenes as a function of temperature. The same is true for their maximum emission wavelengths, which remained within the reproducibility of measurements at the three studied temperatures. In all cases, the resolution of fluorescence spectra was better than the resolution of phosphorescence spectra. No improvement in spectral resolution was observed from any of the studied compounds at 4.2 K. Although BbN12T provided the best resolution in the benzonaphthothiophene fluorescence spectra, the resolution of its phosphorescence spectrum was rather poor. Better spectral resolution was observed from the phosphorescence of BbN21T and BbN23T, which showed vibrational features at both 77 K and 4.2 K.

Table S2 summarizes the temperature effect on the fluorescence intensities of anthrathiophenes and benzonaphthothiophenes. As expected, lowering the temperature to 77 K or 4.2 K enhanced the fluorescence emission of all the studied compounds. Frozen matrixes are known to inhibit non-radiative processes related to thermal agitation - such as collisions with solvent molecules and intramolecular vibrations - that compete with the deactivation of the first singlet excited state. With the exception of A21T, which presented statistically equivalent intensities at both cryogenic temperatures ($P = 95\%$; $N_1 = N_2 = 3$), all the studied compounds emitted stronger fluorescence at 4.2 K than 77 K.

Table S3 compares the fluorescence and phosphorescence intensities of benzonaphthothiophenes at 77 K and 4.2 K. With the exception of BbN21T at 4.2 K, which showed statistically equivalent signal intensities ($P = 95\%$, $N_1 = N_2 = 3$), all the studied compounds showed stronger fluorescence than phosphorescence emission at both temperatures. This is probably due to the spin-allowed nature of the S_1 to S_0 transition. BbN12T and BbN21T showed stronger phosphorescence at 4.2 K than at 77 K. The same is not true for BbN23T; its phosphorescence signal at 77 K was statistically similar to the one at 4.2 K ($P = 95\%$; $N_1 = N_2 = 3$).

Examination of the molecular structures in Fig. 1 indicates that there is no clear trend in the position of the sulfur atom that could help the analyst to predict the relative intensities of fluorescence and phosphorescence at 77 K and 4.2 K. A better understanding of the photoluminescence properties of PASHs requires additional experiments that are beyond the scope of the present study. The measurement of quantum yields and lifetimes at RT, 77 K and 4.2 K should provide relevant information on the non-radiative and radiative rate constants that govern the observation of fluorescence and phosphorescence from PASHs.

3.4. Steady-state AFOMs at RT, 77 K and 4.2 K

Tables 2 and 3 summarize the AFOMs of anthrathiophenes and benzonaphthothiophenes recorded under steady-state (CW lamp) conditions at RT, 77 K and 4.2 K. Calibration curves were built from serial dilutions of pure standard solutions using the solvents listed in Table 1. All measurements were made at the maximum excitation and emission wavelengths of each compound. Each calibration curve was built with a minimum of five standard concentrations. For each concentration plotted in the calibration graph, the signal intensity was the average of three determinations taken from three sample aliquots ($N = 3$). No efforts were made to experimentally obtain the upper concentration limit of the calibration curve. The best linear fitting for each plot of experimental data points was obtained via the least squares method. The limits of detection (LOD) and the limits of quantitation (LOQ) were calculated with the following equations: $LOD = 3 \times S_B/m$ and $LOQ = 10 \times S_B/m$; where S_B is the standard deviation of 16 blank determinations and m is the slope of the calibration curve. Independent of temperature, fluorescence and phosphorescence measurements provided LODs at the low-parts-*per*-billion level ($ng\ mL^{-1}$) for all the studied compounds

Table S4 summarizes the slopes, the blank signals and the standard deviations of the blanks used to calculate the fluorescence LODs and LOQs of Anthrathiophenes at RT, 77 K and 4.2 K. For each of the studied compounds, the slope of the calibration curves increases with lowering the temperature to 4.2 K. The improvements in the slopes – which reflect the fluorescence enhancements observed at lower temperatures – should have improved the LODs and the LOQs at 77 K and 4.2 K. The lack of improvements, however, can be attributed to the higher blank signals and blank standard deviations at 77 K and 4.2 K. The enhancements in the blank signals could be attributed to the presence of unknown fluorescence impurities in the n-alkane solvents. Although HPLC grade solvents were used for these studies, better LODs could have been obtained with further purification of n-alkane solvents via distillation steps [42]. Similar considerations can be made for the fluorescence and phosphorescence LODs and LOQs of benzonaphthothiophenes (see Tables S5 and S6).

3.5. 77 K and 4.2 K time-resolved phosphorimetry of benzonaphthothiophenes under Shpol'skii conditions

Time-resolved phosphorimetry provides the possibility to time-discriminate spectral overlapping from fluorescence concomitants in the sample matrix. One possible way to time-resolve fluorescence interference is to use a spectrometer equipped with a pulsed excitation source and gated detection. The latter refers to the ability of the detector to be “off” during the duration of the excitation pulse (delay time) and “on” during the emission of phosphorescence (gate time). An appropriate delay time (D) should remove the contribution of fluorescence from the spectrum of the sample. The choice of an appropriate D depends on the full-width at half maximum of the excitation pulse - which is usually in μs - and the duration of the fluorescence decay, which varies from ns to μs . A suitable gate time (G) should record the total emission of the phosphor and avoid the collection of background noise. Since this condition is best accomplished with a G approximately equal to 3 phosphorescence lifetimes [43], the appropriate choice of G requires previous knowledge of phosphorescence decays.

Table 4 summarizes the 77 K and 4.2 K phosphorescence lifetimes of the studied compounds. All measurements were made at the maximum excitation and phosphorescence wavelength of each compound from standard solutions with medium linear concentrations. Excitation/ emission band-passes were set at 4 nm. Shpol'skii solvents were selected according to Table 1. The D values correspond to the delay times that remove completely the fluorescence contribution of the compounds from their own time-resolved spectra. The G values were experimentally determined by monitoring the time it took the phosphorescence signal of each compound to reach the intensity of the background signal. All phosphorescence decays showed well-behaved single exponential decays. BbN23T was the only compound to show a significantly shorter phosphorescence lifetime at 4.2 K than at 77 K ($P = 95\%$; $N_1 = N_2 = 3$). The observed difference could be attributed to an enhancement of the radiative rate constant for the deactivation of the triplet state. Although the average lifetimes of BbN12T and BbN23T are also shorter at 4.2 K than at 77 K, the observed differences fall within the reproducibility of measurements of our experiments.

The time it takes to record a spectrum with a gated phosphorimeter depends on the number of excitation pulses per spectral data point, the time between excitation pulses and the number of data points per spectrum. In the case of the benzonaphthothiophenes in Table 4, 10 excitation pulses per data point were sufficient to obtain satisfactory spectral intensities with 4 nm excitation and emission band-passes. Under optimum delay (0.04 ms) and gate ($G = 3\tau = 684.6$ ms) times, the time it takes to record the excitation (200–350 nm) and phosphorescence (425–700 nm) spectra of BbN23T with acceptable vibrational resolution – i.e. at 1 nm monochromator steps – is approximately 58 min.

Such a long time for recording a set of excitation and emission spectra is impractical. A gate reduction study was then conducted to reach a compromise between phosphorescence intensity and recording time. All the other parameters were kept constant; i.e. 4 nm excitation and emission band-pass, 10 pulses per data point and 1 nm monochromator steps. Fig. 5 compares the spectral intensities of BbN23T recorded under 684.6 ms and 9 ms gate times. The time to record one set of excitation and emission spectra with a 9 ms gate dropped to approximately 4 min. Although the phosphorescence intensity is drastically reduced with the shorter gate time, a 9 ms gate time provides sufficient phosphorescence intensity to reach limits of detection at the parts-per-billion concentration level. Table 5 summarizes the AFOM of the three studied benzonaphtho-thiophenes via 77 K and 4.2 K time-resolved phosphorimetry (TRP). Complete datasets with time-resolved phosphorescence spectra of benzonaphthothiophenes are publicly available through the Gulf of Mexico Research Initiative Information & Data Cooperative (GRIIDC) [44,45].

3.6. Qualitative analysis of BbN21T via time-resolved phosphorimetry and parallel factor analysis

In order to illustrate the potential of TRP for the analysis of PASHs in complex environmental samples, we refer to previous chromatographic work on SRM 1597a recently published by Wilson and co-workers [28,29]. Based on the GC-MS analysis of NPLC fractions, the authors [28,29] were able to identify the presence of 35 PASHs, 59 alkyl-PASHs, 146 PAHs and 173 methyl-PAHs. BbN12T and BbN21T co-elute in NPLC fraction

5 along with fluoranthene (Fluor), acephenanthrene (AcePhe), 1-methylfluoranthene (1-MeFluor), 3-methylfluoranthene (3-MeFluor), 7-methylfluoranthene (7-MeFluor), 8-methylfluoranthene (8-MeFluor) and 1-methylpyrene (1-MePyr).

The advantage of using TRP for the analysis of fraction 5 is to discriminate the fluorescence interference of the two co-eluting PAHs and the five co-eluting methyl-PAHs. Figs. S4–S6 show the spectral features of Fluor, 1-MeFluor, 3-MeFluor and 1-MePyr in n-octane. None of the studied compounds showed phosphorescence at RT, 77 K or 4.2 K. Unfortunately, the lack of commercial standards prevented us from investigating the fluorescence and phosphorescence properties of AcePhe, 7-MeFluor and 8-MeFluor.

Fig. 6 compares the 77 K phosphorescence excitation-emission matrices (EEMs) recorded from a binary mixture of BbN12T and BbN21T in the absence and the presence of Fluor, 1-MeFluor, 3-MeFluor and 1-MePyr in n-octane. EEMs capture the total emission of the sample under the fluorescence or the phosphorescence time regimes [46,47]. The application of a 0.04 ms delay removes the fluorescence contribution of the four PAHs from the total emission of the sample.

Fig. S7 shows the strong overlapping between BbN12T and BbN21T in both the spectral and time domains. One possible way to achieve spectral identification of these two compounds in the co-eluted NPLC fraction is to process phosphorescence EEMs with parallel factor analysis (PARAFAC) [48]. Calibration experiments for qualitative purposes were performed with pure standard solutions of BbN12T and BbN21T in n-octane containing each compound at parts-per-billion concentration level. Sample preparation for validation studies included the binary mixtures in Table S7 and test samples consisting of the synthetic mixtures shown in Table S8. All concentrations were adjusted to fit within the ranges often encountered in SRMs [28,29].

77 K phosphorescence EEMs were recorded with a delay of 40 μ s and a gate of 9 ms using 4 nm excitation and emission band-pass. The excitation range extended from 200 nm to 325 nm and the emission range from 475 nm to 700 nm. Ten pulses per data point were taken at 3 nm monochromator steps. Fig. 7, [49] summarizes the results obtained with the binary mixture 1 and the synthetic mixture 6. The spectral profiles extracted with PARAFAC from the phosphorescence EEMs visually match the experimental spectra recorded from pure standard solutions of BbN21T and BbN12T. Similar results were obtained with binary mixtures 2, 3, 4 and synthetic mixture 5. The visual similarities are confirmed with core consistency values summarized in Table 6. The accurate prediction of BbN21T and BbN12T spectra provides strong evidence on the capability of PARAFAC to handle strong overlapping of phosphorescence spectra and phosphorescence time decays as well as potential inner filter effects of fluorescence concomitants in the sample matrix. Future studies will expand the application of this approach to the quantitative analysis of benzonaphthothiophenes in NPLC fractions of SRMs.

4. Conclusion

This article presents an alternative approach for the analysis of PASHs isomers of MM 234 g mol⁻¹ based on photoluminescence spectroscopy. The lack of phosphorescence at RT, limits the analysis of anthrathiophenes and benzonaphthothiophenes to fluorescence spectroscopy. The same is true for the analysis of anthra-thiophenes at 77 K and 4.2 K. The emission of both fluorescence and phosphorescence provides additional information for the analysis of benzonaphthothiophenes at 77 K and 4.2 K. Independent of the measuring temperature and the excitation source, i.e. CW or pulsed lamp, fluorescence and phosphorescence measurements provided LODs at the low-parts-*per*-billion level (ng mL⁻¹) for all the studied compounds.

A distinct advantage of time-resolved phosphorimetry over steady-state fluorimetry relates to the analysis of benzonaphtho-thiophenes in the presence of strong fluorescence interference. The potential of time-resolved phosphorimetry was demonstrated for the analysis of BbN12T and BbN21T in the presence of fluorescence concomitants usually present in NPLC fractions. Time-discrimination of potential co-eluting fluorescence interference was accomplished with the choice of appropriate delay and gate times. The strong overlapping BbN12T and BbN21T present in the wavelength and time domains was resolved with PARAFAC and the collection of phosphorescence EEMs at 77 K.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.
Molecular structures of anthra-thiophenes and benzonaphthothiophenes.

Fig. 2.

Steady-state room-temperature excitation and emission spectra of (A) 100 ng mL⁻¹ A12T in n-octane; (B) 150 ng mL⁻¹ A23T in n-nonane; (C) 150 ng mL⁻¹ A21T in n-octane; (D) 625 ng mL⁻¹ BbN12T in n-octane; (E) 625 ng mL⁻¹ BbN21T in n-octane; (F) 375 ng mL⁻¹ BbN23T in n-nonane. All spectra were recorded at the maximum excitation and emission wavelengths using an excitation and emission band-pass = 2 nm; all emission spectra were recorded with the aid of a 310 nm cut-off filter.

Fig. 3.

Steady-state excitation and emission spectra of 100 ng mL⁻¹ A12T in n-octane at 77 K (A) and 4.2 K (D); 7.5 ng mL⁻¹ A23T in n-nonane at 77 K (B) and 4.2 K (E); 37.5 ng mL⁻¹ A21T in n-octane at 77 K (C) and 4.2 K (F). All spectra were recorded at the maximum excitation and emission wavelengths using an excitation and emission band-pass = 2 nm; all emission spectra were recorded with the aid of a 310 nm cut-off filter.

Fig. 4. Steady-state excitation and emission spectra of 100 ng mL⁻¹ BbN12T in n-octane at 77 K (A) and 4.2 K (D); 50 ng mL⁻¹ BbN21T in n-octane at 77 K (B) and 4.2 K (E); 500 ng mL⁻¹ BbN23T in n-nonane at 77 K (C) and 4.2 K (F). All spectra were recorded at the maximum excitation and emission wavelengths using an excitation and emission band-pass = 2 nm; all emission spectra were recorded with the aid of a 310 nm cut-off filter.

Fig. 5.

4.2 K excitation and phosphorescence spectra recorded from a $500 \mu\text{g mL}^{-1}$ solution of BbN23T in n-nonane. Instrumental parameters were as follows: cutoff filter with $\lambda_c = 310$ nm; excitation and emission band-pass = 4 nm; delay = 0.04 ms; number of excitation pulses per data point = 10; 1 nm monochromator step; gate = 687 ms (black trace) or 9 ms (red trace). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 6.

77 K excitation and emission matrices of (A) binary mixture of 500 ng mL⁻¹ BbN12T and 500 ng mL⁻¹ BbN21T in n-octane using CW lamp; (B) synthetic mixture of 430 ng mL⁻¹ BbN12T, 310 ng mL⁻¹ BbN21T, 50 ng mL⁻¹ Fluor, 50 ng mL⁻¹ 1-MeFluor, 50 ng mL⁻¹ 3-MeFluor and 20 ng mL⁻¹ 1-MePyr in n-octane using CW lamp; (C) binary mixture of 500 ng mL⁻¹ BbN12T and 500 ng mL⁻¹ BbN21T in n-octane using pulsed lamp; (D) synthetic mixture of 430 ng mL⁻¹ BbN12T, 310 ng mL⁻¹ BbN21T, 50 ng mL⁻¹ Fluor, 50 ng mL⁻¹ 1-MeFluor, 50 ng mL⁻¹ 3-MeFluor and 20 ng mL⁻¹ 1-MePyr in n-octane using pulsed lamp.

Fig. 7.

Summary of results obtained with PARAFAC for (A) binary mixture of 300 ng mL^{-1} BbN12T and 700 ng mL^{-1} BbN21T in n-octane at 77 K. BbN21T-77K Phosphorescence and BbN12T-77K Phosphorescence correspond to 77 K spectra recorded from pure standard solutions of BbN21T and BbN12T in n-octane. BbN21T-PARAFAC and BbN12T-PARAFAC correspond to spectral profiles extracted with PARAFAC from the 77 K Phosphorescence EEM of the binary mixture. (B) Synthetic mixture of 430 ng mL^{-1} BbN12T, 310 ng mL^{-1} BbN21T, 50 ng mL^{-1} K. BbN21T-77K Phosphorescence and BbN12T-77K Phosphorescence correspond to 77 K spectra recorded from pure standard solutions of BbN21T and BbN12T in n-octane. BbN21T-PARAFAC and BbN12T-PARAFAC correspond to spectral profiles extracted with PARAFAC from the 77 K Phosphorescence EEM of the synthetic mixture.

Table 1

Shpol'skii solvents for spectral collection at RT, 77 K and 4.2 K.

PASH	Length of PASH (Å)	Matching Solvent	Length of Solvent (Å)
A12T	13.39	n-octane	13.14
A21T	13.51	n-octane	13.14
A23T	14.12	n-nonane	14.42
BbN12T	12.72	n-octane	13.14
BbN21T	13.66	n-octane	13.14
BbN23T	13.90	n-nonane	14.42

Table 2

Fluorescence analytical figures of merit of anthrathiophenes recorded with the CW lamp.

PASH	Temperature	$\lambda_{\text{ex/em}}^a$ (nm)	LDR ^b (ng mL ⁻¹)	LOQ ^c (ng mL ⁻¹)	LOD ^d (ng mL ⁻¹)	RSD ^e (%)	RSD ^f (%)
A12T	RT	280/410	9.8–350	9.8	3	1.8	1.5
	77 K	288/388	11.7–350	11.7	3.5	3.1	5.1
	4.2 K	282/388	5.8–250	5.8	1.7	1.1	5.1
A21T	RT	284/404	2.9–350	2.9	0.8	2.5	2.1
	77 K	363/383	2.1–100	2.1	0.7	0.6	4.7
	4.2 K	363/383	1.5–75	1.5	0.5	2.1	4.7
A23T	RT	276/434	7.3–350	7.3	2	2.3	2.1
	77 K	280/441	2.1–15	2.1	0.6	2	3.1
	4.2 K	280/441	3.5–10	3.5	1	5.2	3.1

^aExcitation (λ_{ex}) and emission (λ_{em}) wavelengths.

^bLDR = linear dynamic range in ng mL⁻¹ extending from the limit of quantification (LOQ) to an arbitrarily chosen upper linear concentration.

^cLOQs were calculated using LOQ = 10SB/m.

^dLODs were calculated using LOD = 3SB/m, where SB is the standard deviation of 16 blank measurements and m is the slope of the calibration curve.

^eRelative standard deviation (RSD) = $S_F/I_F \times 100$, where S_F is the standard deviation of the average calculated from three emission measurements at medium linear PASH concentrations within same day.

^fRelative standard deviation (RSD) = $S_F/I_F \times 100$, where S_F is the standard deviation of the average calculated from three emission measurements at medium linear PASH concentrations in three different days.

Table 3

Fluorescence analytical figures of merit of benzonaphthothiophenes recorded with the CW Lamp.

PASH	Temperature	$\lambda_{\text{ex/em}}^a$ (nm)	LDR ^b (ng mL ⁻¹)	LOQ ^c (ng mL ⁻¹)	LOD ^d (ng mL ⁻¹)	RSD ^e (%)	RSD ^f (%)
BbN12T	RT	321/352	16–1250	16	5	1.4	3.1
	77 K	323/353	18–250	18	6	8.7	9.9
	4.2 K	324/353	6–250	6	2	9.5	9.9
BbN21T	RT	302/349	18–1250	18	5	0.1	2.9
	77 K	304/350	4–100	4	1	0.5	5.8
	4.2 K	304/350	4–100	4	1	1.6	5.8
BbN23T	RT	274/376	166–750	166	50	0.6	1.5
	77 K	277/377	169–1000	169	50	9	3.6
	4.2 K	331/378	25–750	25	7	1.1	1.2

^aExcitation (λ_{ex}) and emission (λ_{em}) wavelengths.

^bLDR = linear dynamic range in ng mL⁻¹ extending from the limit of quantification (LOQ) to an arbitrarily chosen upper linear concentration.

^cLOQs were calculated using LOQ = 10SB/m.

^dLODs were calculated using LOD = 3SB/m, where SB is the standard deviation of 16 blank measurements and m is the slope of the calibration curve.

^eRelative standard deviation (RSD) = $S_F/|F| \times 100$, where S_F is the standard deviation of the average calculated from three emission measurements at medium linear PASH concentrations within same day.

^fRelative standard deviation (RSD) = $S_F/|F| \times 100$, where S_F is the standard deviation of the average calculated from three emission measurements at medium linear PASH concentrations in three different days.

Table 4

Phosphorescence lifetimes of benzonaphthothiophenes in Shpol' skii matrixes.

PASH	$\lambda_{\text{exc/em}}$ (nm)	D^b (ms)	G^c (ms)	77 K Lifetime ^d (ms)	4.2 K Lifetime ^d (ms)	Statistical Equivalence ^e (t_{exp})
BbN12T	262/513	0.2	3000	746.8 ± 56.3	741.9 ± 18.1	Yes (0.1)
BbN21T	279/482	0.04	2000	935.3 ± 15.0	847.7 ± 18.1	No (4.4)
BbN23T	276/563	0.04	1000	228.2 ± 2.8	221 ± 4.8	Yes (2.3)

^aExcitation (λ_{exc}) and emission (λ_{em}) wavelengths.

^bDelay time.

^cGate time needed to reach background signal intensity.

^dAverage phosphorescence lifetimes ($N = 3$) recorded at the maximum excitation (λ_{exc}) and emission (λ_{em}) wavelengths.

^e $t_{\text{critical}} = 2.78$ ($\alpha = 0.05$; $N_1 = N_2 = 3$).

Table 5

Phosphorescence analytical figures of merit of benzonaphthothiophenes recorded with the CW lamp.

PASH	Temperature	$\lambda_{\text{ex/em}}$ (nm)	LDR ^b (ng mL ⁻¹)	LOQ ^c (ng mL ⁻¹)	LOD ^d (ng mL ⁻¹)	RSD ^e (%)	RSD ^f (%)
BbN12T	77 K	323/513	11.4–250	11.4	3.4	7.6	9.9
	4.2 K	323/513	9.5–250	9.5	2.8	0.5	9.9
BbN21T	77 K	304/481	3.2–100	3.2	0.9	0.6	5.7
	4.2 K	304/481	2.5–100	2.5	0.7	0.8	5.7
BbN23T	77 K	277/562	57.8–1000	57.8	17.3	4.9	5.1
	4.2 K	277/562	32.7–750	32.7	9.8	1.7	2.3

^aExcitation (λ_{ex}) and emission (λ_{em}) wavelengths.

^bLDR = linear dynamic range in ng mL⁻¹ extending from the limit of quantification (LOQ) to an arbitrarily chosen upper linear concentration.

^cLOQs were calculated using LOQ = 10SB/m.

^dLODs were calculated using LOD = 3SB/m, where SB is the standard deviation of 16 blank measurements and m is the slope of the calibration curve.

^eRelative standard deviation (RSD) = $\text{SF}/\text{IF} \times 100$, where SF is the standard deviation of the average calculated from three emission measurements at medium linear PASH concentrations within same day.

^fRelative standard deviation (RSD) = $\text{SF}/\text{IF} \times 100$, where SF is the standard deviation of the average calculated from three emission measurements at medium linear PASH concentrations in three different days.

Table 6

Core consistencies of qualitative analysis of BbN12T and BbN21T in synthetic mixtures obtained by PARAFAC Analysis at 77 K.

Mixture	[BbN12T] ng mL ⁻¹	[BbN21T] ng mL ⁻¹	[Fluor] ng mL ⁻¹	[1-MeFluor] ng mL ⁻¹	[3-MeFluor] ng mL ⁻¹	[1-MePyr] ng mL ⁻¹	Core consistency of [BbN12T] %	Core consistency of [BbN21T] %
Mixture 1	300	700	0	0	0	0	99.93	99.96
Mixture 2	700	300	0	0	0	0	100	100
Mixture 3	500	500	0	0	0	0	100	100
Mixture 4	430	310	0	0	0	0	99.94	99.94
Mixture 5	200	200	100	100	100	50	99.93	99.96
Mixture 6	430	310	50	50	50	20	100	100