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Interference filter tilting to detect a polycyclic aromatic hydrocarbon at the second harmonic of wavelength modulation frequency

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Abstract

We present a practical implementation of the wavelength-modulation spectroscopy technique with second-harmonic detection (WMS-2f) for selective detection and concentration measurements of polycyclic aromatic hydrocarbons (PAHs) - common fossil fuel pollutants. The method is based on excitation light wavelength modulation around the maximum of a narrow peak in the absorption spectrum of a PAH and lock-in detection of fluorescence at the second harmonic of the excitation wavelength modulation frequency. A violet LED is used as the excitation source and wavelength modulation is performed via narrow-band interference filter tilting. Feasibility is demonstrated with a carcinogenic PAH, benzo(k)fluoranthene. Model computations are presented to estimate the influence of background signal on the sensitivity of the method.

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1. Introduction

For several decades, it has been generally accepted that polycyclic aromatic hydrocarbons (PAHs) have mutagenic and carcinogenic effects [1]. They have received considerable attention and presently different restrictions and contamination standards imposed on PAH contamination in food [2], consumer products [3], water [4] and air [5] have been established. However, due to forest fires, combustion of fossil fuels and wide usage of other fossil products that are the main sources of PAHs [6], PAH pollution is constantly increasing and negatively affects our environment and health [7–9]. Therefore, there is a great need for accurate PAH detection and concentration measurements.

Traditionally, chromatography and mass-spectrometry [10, 11] techniques are used for PAH contamination analysis and concentration determination, which provide results of high

precision and sensitivity but require complex instruments, an expensive laboratory environment, and long processing times. Therefore, these techniques are not suited for real-time measurements that are necessary for effective PAH contamination monitoring and fast pollution-prevention actions. In the present work, we propose an in-solution (methanol, water) PAH detection method based on wavelength modulation spectroscopy (WMS) techniques.

The method utilizes narrow absorption peaks of PAHs and their natural fluorescence. We excite the sample around certain PAH absorption peaks and measure fluorescence with a photodiode. The excitation light wavelength is modulated around a PAH absorption peak at known frequency via tilting of an interference filter. The photodiode signal is fed to a lock-in amplifier in order to extract fluorescence signal at the second harmonic of excitation light wavelength modulation frequency, which is high only in the presence of PAH. This enables detection of PAHs and estimation of their concentrations.

Our approach has two major advantages. Firstly, high sensitivity even to weak signals results from lock-in detection that removes most background and noise, allowing detection of low concentrations of studied substances. Secondly, implementation is simple, relying on filter tilting for modulation. As true for WMS methods in general, one must simply pass this wavelength-modulated light through the sample and measure the signal at the frequency of interest. Coupled with fast electronics, this method is ideally suited for real-time PAH detection, as demonstrated herein for benzo(k)fluoranthene (BKF), a typical PAH.

2. Theory

A. Dependence of fluorescence on absorption spectrum and concentration

WMS for detection of PAHs in solution is made possible by their intrinsic fluorescence and relatively narrow absorption peaks. The fluorescence intensity I_{fl} depends linearly on the amount of absorbed light energy. This can be calculated in terms of the PAH molecules' absorbance A by:

$$I_{fl} = \varphi_0 I_0 (1 - 10^{-A}) \quad (1)$$

Here I_0 is incident light intensity and φ_0 is quantum efficiency.

In practice PAHs are relatively insoluble in polar solutions, resulting in very small absorbance. Thus, only the first Taylor component of (1) contributes appreciably and the fluorescent signal is directly proportional to absorption:

$$I_{fl} = \varphi_0 I_0 A. \quad (2)$$

Detecting the fluorescence signal is greatly advantageous, as it provides intrinsically higher signal-to-noise ratio (SNR) than absorption measurements. In particular, fluorescence+background depends only on the part of background absorption that also results in fluorescence, which is typically minimal.

Quantitative information about PAH concentration in homogeneous solutions can be obtained with the Beer-Lambert law:

$$A = \varepsilon(\lambda)cl \quad (3)$$

where $\varepsilon(\lambda)$ is the extinction coefficient at wavelength λ , l is the excitation light optical path length within the sample and c is the concentration of PAH. Substituting this relation into (2) we obtain equation

$$I_{fl} = \varphi_0 I_0 \varepsilon(\lambda) cl \quad (4)$$

which reveals the fluorescent signal dependence on concentration of absorbing molecules. We can use this direct proportionality to conduct a quantitative analysis on PAH contamination.

B. WMS signal near and away from absorption peaks

Fig. 1 shows an absorption peak of BKF in methanol as a function of wavelength, which can be locally approximated by a Gaussian:

$$A(\lambda) = A_0 e^{-\frac{(\lambda - \lambda_0)^2}{2\sigma^2}} \quad (5)$$

A_0 is absorbance at absorption spectrum maximum λ_0 . Constant σ is related to the peak full-width-half-maximum (FWHM) by $\text{FWHM} = 2\sqrt{2\ln(2)}\sigma$.

On the upslope of the PAH absorption peak (Fig 1, case 1), curvature is negligible, and the absorbance can be approximated linearly with slope k :

$$A(\lambda) = A_0 + k\lambda \quad (6)$$

This describes the situation where nonlinearities, such as absorption maxima, are insignificant within the wavelength modulation range. Though this assumption may not always hold, it is true in the case of broad absorption backgrounds in natural waters [12].

The excitation light wavelength modulation can be described by Fourier series:

$$\lambda(t) = \sum_{n=-N}^N \Delta\lambda_n e^{i2n\pi f_0 t} \quad (7)$$

Here t is time, $\Delta\lambda_n$ is the amplitude of n -th Fourier component, N is number of modulation components and f_0 is the fundamental frequency of modulation. We employ the simplest case of $N = 1$ - a pure sine wave modulated around the peak center λ_0 with modulation amplitude $\Delta\lambda$ at angular frequency $\omega_0 = 2\pi f_0$:

$$\lambda(t) = \lambda_0 + \Delta\lambda \sin(\omega_0 t) \quad (8)$$

Accordingly, the time-dependent absorbance of BKF in the presence of symmetric wavelength modulation around the absorption peak is:

$$A(t) = A_0 e^{-\frac{(\Delta\lambda \sin(\omega_0 t))^2}{2\sigma^2}} \quad (9)$$

with corresponding fluorescence per (4):

$$I_{fl}(t) = \varphi_0 I_0 A_0 e^{-\frac{(\Delta\lambda \sin(\omega_0 t))^2}{2\sigma^2}} \quad (10)$$

By contrast, away from a peak the fluoresce intensity is:

$$I_{fl}(t) = \varphi_0 I_0 (A_0 + k\lambda_0 + k\Delta\lambda \sin(\omega t)) \quad (11)$$

Figure (2) shows the fluorescence power spectrum for wavelength modulation frequency $f_0 = 1 \text{ Hz}$ calculated by a numerical Fast Fourier Transform (FFT) of equations (10) and (11). Near the peak (Fig. 2.A), only even Fourier components appear in the output, with the most significant component at the second harmonic of the wavelength modulation frequency (2 Hz). By contrast, the power spectrum of fluorescence in the linear regime away from a peak (Fig. 2.B) contains only one component, which is the first harmonic of the fundamental excitation light wavelength modulation frequency ($f_0 = 1 \text{ Hz}$).

Accordingly, lock-in measurement of fluorescence intensity at the second harmonic of the fundamental will yield a significant signal only when an absorption maximum is present within the modulated wavelength range. If other substances in the sample fluoresce into the detector, the second harmonic signal 2ω , will be negligible as long as their absorption features are broad enough to change linearly in the wavelength modulation range λ .

C. Fourier and Taylor generalization of WMS

As a generalization of the WMS scheme described above, consider the fluorescence intensity signal $I_{fl}(t)$ that is the overlap integral over λ in each time instant of absorbance $A(\lambda)$ and the excitation spectrum $E(\lambda, t)$.

$$I_{fl}(t) = \int d\lambda A(\lambda) E(\lambda, t) \quad (12)$$

We expand the absorption spectrum around the peak λ_0 as a Taylor series with constants A_n (calculated in the usual way from derivatives):

$$A(\lambda) = \sum_n A_n (\lambda - \lambda_0)^n \quad (13)$$

Now expand the excitation light spectrum with periodicity ω_0 as a Fourier series with coefficient functions $E_m(\lambda)$ (calculated in the usual way from one period):

$$E(\lambda, t) = \sum_m E_m(\lambda) e^{im\omega_0 t} \quad (14)$$

Accordingly, let us decompose $I_{fl}(t)$ into the same Fourier components in terms of harmonics of ω_0 :

$$I_{fl}(t) = \sum_m I_m(\lambda) e^{im\omega_0 t} \quad (15)$$

Comparing this to (12), (13), and (14), it is evident that I_m is simply the summed mathematical correlation (at λ_0) of functions λ^n and E_m :

$$I_m = \sum_n A_n \int d\lambda (\lambda - \lambda_0)^n E_m(\lambda) \quad (16)$$

Therefore, in the case when the excitation spectrum is symmetric around λ_0 , it becomes clear that for each even harmonic m (in particular $m = 2$, the second harmonic), there is no contribution from any odd components n of the absorption spectrum (in particular for $n = 1$ in the case of linear component of absorption spectrum) due to cancellation of the integral's negative and positive halves. In the case of broken symmetry (as for example if $E(\lambda, t)$ is detuned from λ_0), this may not in general hold.

C. Interference filter incidence angle adjustment for wavelength modulation

Traditional options for wavelength modulation methods typically complicate experimental setups with additional optical components. For example, modulation can be achieved via oscillation of a slit, mirror, diffraction gratings or prism of a monochromator [13]. In the present work, we add the approach of interference filter tilting, which has the advantage of relying on an optical component necessary in most excitation-detection schemes.

Interference filter transmission central wavelength is known to depend nonlinearly on angle of incidence θ according to:

$$\lambda(\theta) = \lambda_c \sqrt{1 - \frac{\sin^2 \theta}{K^2}} \quad (19)$$

where λ_c is the central transmission wavelength at normal incidence and K is the effective refraction index of the interference filter. However, our detection scheme requires wavelength modulation with only the first Fourier component (i.e. a pure sinusoid) to avoid second harmonic signals of background fluorescence.

In order to create the desired wavelength modulation waveform expressed in equation (8), we must accordingly tilt the filter with angle time dependence:

$$\theta(t) = \arcsin \left[K \sqrt{1 - \left(\frac{\Delta\lambda \sin(\omega_0 t) + \lambda_0}{\lambda_c} \right)^2} \right] \quad (20)$$

This was used to calculate the control voltage waveform for the rotating mechanism, which has a linear voltage-angle dependence. In order to obtain a clean second harmonic signal, the modulation amplitude $\Delta\lambda$ in equation (8) is selected as broad as permitted by the filter transmission bandwidth and range of PAH absorption band symmetry about λ_0 .

2. Experimental designs

A. Optical setup

To experimentally test the proposed method, we selected benzo(k)fluoranthene (BKF) as a representative PAH because of its high fluorescence quantum yield and narrow absorption peaks. We select the longest wavelength absorption maximum of BKF, which occurs at 399.5 nm.

Fig. 6 shows the experimental setup. An excitation light beam from a near-UV LED (UV5TZ-400-30, Bivar Inc., USA) is collimated onto a narrow band interference filter (Omega Optics, 405BP3, effective refractive index $K = 2.223$, FWHM = 3 nm, and normal center wavelength $\lambda_c = 404.6$ nm) that is nearly matched to the BKF sample peak $\lambda_0 = 399.5$ nm. This combination permits a modulation amplitude $\Delta\lambda = 3.5$ nm (Fig. 1).

LED is powered by laser-diode controller (LDC501, Stanford Research Systems, USA) that is electronically regulated with an external voltage applied to its modulation input. For wavelength modulation of the transmission band, the interference filter is tilted with a high-speed (20 kilopoints per second (Kpps) galvo-scanner (PT-20K, TE-LIGHTING, Guangzhou, China).

The galvo-scanner control voltage waveform was calculated using the galvo-scanner linear transmission function and the equation (20). The obtained waveform was applied via digital-analog converter (NI USB-6251, National Instruments, Austin, TX, USA) controlled by a LabVIEW (National Instruments, Austin, TX, USA) custom program. For the full range of excitation wavelength from $\lambda_{min} = 396$ nm to $\lambda_{max} = 403$ nm, the corresponding excitation light angles of incidence extrema are $\theta_{min} = 11.5^\circ$ to $\theta_{max} = 27.3^\circ$.

Wavelength modulation sinusoidality was checked with spectrometer (FSD-9 mini, Art Photonics, Berlin, Germany) by recording interference filter transmission spectra at 10s time

interval during 1.75 periods ($T=6\text{min}$) of interference filter tilting. The result, depicted in Fig. 3, confirms high accuracy sinusoidal modulation of wavelength.

As interference filter oscillated the LED current was regulated to compensate excitation light intensity changes. The regulation was done by another LabVIEW program (described in next section below) that automatically found the required control voltage and provided it to the LED power supply (SRS LDC501) through DAC of NI USB-6251 analog-digital converter.

The resultant wavelength modulated and intensity stabilized excitation light was directed onto the sample within a quartz cuvette with a $10 \times 10 \text{ mm}^2$ cross section. Fluorescence was gathered at 90 degrees focused with a lens onto the preamplified photodiode (ODA-6WB-500M, Opto Diode, Camarillo, California, USA) through a bandpass interference filter (D430/30M, Edmund Optics, Barrington, NJ, USA). The photodiode signal was measured at the second harmonic of excitation light wavelength modulation frequency with a lock-in amplifier (SR850, Stanford Research Systems, USA).

B. Compensation of intensity changes

Although interference filter tilting allows to achieve an accurate sinusoidal wavelength modulation, variation in the excitation light intensity, reaching the sample, can generate unwanted second harmonic signals of background fluorescence. Two major potential sources of this variation in our setup are the well-known [14] phenomenon of transmissivity changes during interference filter tilting (Fig. 4.A) and the wavelength-dependent spectrum of excitation light source emission spectrum (Fig. 4.B). Therefore, it is essential to compensate excitation light intensity changes during wavelength modulation.

Excitation light intensity diminished 63% as we changed incidence angle from $\theta_{min} = 11.5^\circ$ to $\theta_{max} = 27.3^\circ$ (narrow line in Fig. 5). We compensated this variation by regulating the output current of the excitation source power supply via a control voltage waveform. This waveform was automatically determined by a LabVIEW program. Firstly, it recorded raw excitation intensities (I_{raw}) during excitation filter tilting, as measured at the detecting photodiode without the emission bandpass filter (narrow line in figure 5). For each angle of interference filter tilt, the LED power supply modulation input voltage was adjusted to a constant photodiode signal, yielding the initial LED control voltage (U_{init}) waveform. Proportional–integral–derivative controller (PID) principles were used to optimize and accelerate this process. However, stability of excitation intensity during application of initial control voltage was insufficient. Therefore, a simple correction algorithm was developed using the following equation:

$$U_{corr} = \frac{U_{init} * (I_{perfect} - I_{raw})}{(I_{stab} - I_{raw})} \quad (21)$$

where U_{corr} is corrected control voltage, $I_{perfect}$ is the theoretically perfectly constant intensity and I_{stab} is measured intensity stabilized by U_{init} control voltage.

We found that the time dependence of LED supply control voltage was bell-shaped and could be fitted by a Voigt function. We used it to eliminate the statistical noise of corrected control voltage. Thus we achieved excitation light stability up to 0.5% (bold line in Fig. 5) that we used in further experiments with PAH samples.

C. Preparation of PAH dilution series

To estimate WMS techniques suitability for PAH detection we prepared a series of BKF solutions from $1 \cdot 10^{-9} M$ to $2.30 \cdot 10^{-5} M$ by serially diluting an initial solution with concentration of $2.30 \cdot 10^{-5} M$. Initial solution was prepared from pure, crystalline benzo(k)fluoranthene dissolved in methanol. The concentration of initial solution was determined using benzo(k)fluoranthene extinction coefficient and absorption spectrum measurements conducted with a spectrophotometer (V-570, Jasco Inc., Easton, MD, USA). Dilution was performed with the use of variable pipettes (Genex beta, Genexlab, Northampton, UK) with volume ranges of 20–200 μL and 100–1000 μL .

4. Results

Several measurements were conducted using the $2.30 \cdot 10^{-5} M$ BKF solution. The time-dependent fluorescence signal was visualized by digital oscilloscope (TDS 524A, Tektronix Inc., Beaverton, Oregon, USA) and compared with the galvo-scanner control voltage (which varies at the modulation frequency ω). For excitation light wavelength modulation around the absorption maximum, the fluorescence signal was, as predicted, at the second harmonic 2ω (panel A in Fig. 7). When wavelength modulation is centered at the upslope of the absorption peak (panel B in Fig. 7), both signals have the same first harmonic frequency. The finite response rate of the photodiode preamplifier (cutoff frequency = 130 Hz) results in the visible phase shift in the fluorescence signals. Lock-in measurements confirmed the second harmonic fluorescence signal prevailing over first harmonic with ratio 6:1 during wavelength modulation around the absorption maximum, whereas first harmonic signal prevailed during upslope modulation.

To estimate the sensitivity of WMS techniques for PAH detection we recorded the second harmonic signal by SR850 lock-in amplifier for a series of concentrations ($1 \cdot 10^{-9} M$ to $2.30 \cdot 10^{-5} M$ mol/L) of benzo(k)fluoranthene solutions (Fig. 8).

Due to direct proportionality of concentration and fluorescence intensity, we expected also a linear relation between lock-in amplifier signal and benzo(k)fluoranthene concentration changes. To check this hypothesis, we applied a linear regression to acquired data. We used a weighted linear regression because of concentration heteroscedasticity. For the weighting factor, we chose a $1/x^2$ as was suggested by Huidong Gu et al. in the case of direct proportionality between variable and its standard deviation [15]. The obtained linear approximation (Fig. 8) goes through almost all the points, and lies within concentration uncertainties. Only one point deviates significantly from the linear regression line, what is most probably the result of the limited precision in sample preparation. The minimum detectable concentration, we achieved, was $5 \cdot 10^{-9} M$. Lock-in amplifier signal for smaller concentrations was indistinguishable from background signal.

The uncertainty of the measurements stem from two basic sources: the sample preparation uncertainty and fluorescence intensity measurement uncertainty. The sample preparation uncertainty originates from Jasco spectrophotometer instrumental error of optical density measurements and from volume measurement uncertainty of Genex variable pipettes. These uncertainties were taken into account and are depicted in Fig 7.

The fluorescence intensity measurement uncertainty results from the finite optical density ($OD = 5$) of the fluorescence emission filter, which permitted some excitation light bleed-through onto the detector. This resulted in a background signal about $50 \mu V$ during lock-in measurements (while smallest measured fluorescence signal is $200 \mu V$), that distorted the fluorescence signal at concentrations smaller than $5 \cdot 10^{-9} M$. The SR850 lock-in amplifier instrumental uncertainty is quoted by the manufacturer in the range of 0.2 nV , and therefore should not significantly affect these measurements.

5. Discussion

The results of the conducted experiments indicate that wavelength modulation spectroscopy can be successfully applied for sensitive PAH detection. Our experience shows that the PAH detection at the limit concentration of $5 \cdot 10^{-9} M$ can be achieved. However, we don't see any conceptual obstacles for diminishing this detection limit even further.

The detection limit concentration corresponds to a signal of 0.2 mV . The minimum signal, which can be measured with SR850 lock-in amplifier, is 0.2 nV at low-pass filter bandwidth of 10 mHz (corresponding to a time constant of 10 s). This indicates that, in respect of sensitivity of electronic equipment, several orders of magnitude lower concentrations could be detected.

In our case it couldn't be achieved due to imperfection of our experimental system. As we found out, used fluorescence filter optical density at excitation light wavelengths wasn't high enough and caused excitation light leakage. This significantly contributed to background during lock-in measurements and distorted the fluorescence signal at concentrations smaller than $5 \cdot 10^{-9} M$. Also due to excitation light residual intensity modulation (remained after intensity stabilization) at level of $2\text{--}4\%$ contained higher harmonics, we weren't able to filter excitation light leakage with the lock-in amplifier at second harmonic frequency. In principal, the intensity stabilization of higher accuracy could be achieved, if we would build a feedback system to control LED current instead of calculating control voltages. Or if we could implement normalization of second harmonic signal (S_2) by first harmonic signal (S_2/S_1) within the lock-in amplifier as it was done by G.B.Rieker et al. [16]. In our case, this was not possible because our lock-in amplifier (SR850 DSP) could not simultaneously measure two harmonics. Our method has the advantage of a constant signal to noise ratio during wavelength modulation.

In the real world pollution incidents the oil contaminants certainly contain several PAHs. In the case of overlapping absorption bands the selectivity for single PAH detection could be insufficient. Nevertheless, there is a possibility to enhance our method to further improve the selectivity. In addition to excitation wavelength modulation we can selectively filter out the

fluorescence band with a narrowband interference filter. To further increase the selectivity, we can modulate its transmission wavelength around the fluorescence peak center λ_{fl} while registering the fluorescence signal at the second harmonic of the difference of the excitation and fluorescence modulation frequencies $2(\omega_0 - \omega_{fl})$. This excitation/emission double selection method allows the selectivity to be greatly enhanced.

6. Conclusion

Sensitive detection of the polycyclic aromatic hydrocarbon benzo(k)fluoranthene by application of the WMS method has been realized. The sensitivity and selectivity is achieved by the lock-in detection of the second harmonic 2ω of the fluorescence signal under sinusoidal modulation (at frequency 1ω) of excitation wavelength around the narrow BKF absorption peak. The sinusoidal excitation wavelength modulation was achieved by precisely tilting a narrowband interference filter in the excitation path. Variation of the filter transmission was compensated by controlling the LED current, achieving an excitation intensity variation below 4%. We detected benzo(k)fluoranthene to a concentration limit of $5 \cdot 10^{-9} M$.

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Fig. 1.

Drawing showing part of the benzo(k)fluoranthene absorption spectrum and two possible cases that can occur during PAH detection. Nr 1. describes the case when the wavelength modulation is performed symmetrically about the absorption maximum. Nr 2. corresponds to the case when maxima are not present in the selected wavelength modulation range $\Delta\lambda$. Here, sinusoids under the absorption peak depict excitation light wavelength modulation with period T . Sinusoids in the right upper corner show fluorescence intensity waveforms in case of presence (2) or absence (1) of absorption maximum.

Fig. 2. Power spectra of excitation light wavelength modulation obtained from equation (7) in the case of an absorption maximum (A) and equation (9) in the case of a linear absorption background (B).

Fig. 3. Excitation light wavelength modulation waveform measured (dots) and approximated with sinusoid (line).

Fig. 4. Experimentally measured interference filter (Omega Optics, 405BP3) transmission dependence on angle (A) and excitation light source (UV5TZ-400–30, near-UV LED, Bivar Inc.) spectrum (B).

Fig. 5. Excitation light intensity changes during one period of wavelength modulation without (narrow dash line) and with (bold line) LED current adjustment.

Fig. 6.
Experimental setup schematics.

Fig. 7. Waveforms of fluorescence intensity signals during wavelength modulation around absorption maximum (A) and at the downslope of absorption peak (B).

Fig. 8.

Measured SR850 lock-in amplifier signal vs benzo-(k)fluoranthene probe concentrations, approximated by weighted linear regression with weighting of $1/x^2$. Data is expressed in log-log scale due to the broad range of concentrations. Error bars show uncertainty of concentrations at a level of confidence of 95% (see text for details).