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Spectral properties of 4-methylumbelliferone in PVA films; long-lived room temperature phosphorescence

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Methods and Applications in Fluorescence



PAPER

Spectral properties of 4-methylumbelliferone in PVA films; long-lived room temperature phosphorescence

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Bong Lee¹ , Emma Alexander¹ , Danh Pham¹ , Mariusz Gagoś², Arkadiusz Matwijczuk^{1,3}, Zygmunt Gryczynski¹ and Ignacy Gryczynski¹

¹ Department of Physics and Astronomy, Texas Christian University, Fort Worth, TX, 76129, United States of America

² Department of Biochemistry and Molecular Biology, Medical University of Lublin, Chodźki 1, 20-093 Lublin, Poland

³ Department of Biophysics, Faculty of Environmental Biology, University of Life Sciences in Lublin, Poland

E-mail: bong.lee@tcu.edu

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Supplementary material for this article is available [online](#)

Abstract

We studied absorption and fluorescence as well as room temperature phosphorescence (RTP) of 4-methylumbelliferone (4MU) in poly (vinyl alcohol) (PVA) films. We focused our study on the long-wavelength basic form of 4MU with absorption centered at 375 nm. The strong fluorescence with a quantum yield of above 70% appears at ~430 nm. The fluorescence anisotropy of 4MU-doped PVA film is high, reaching a value of about 0.3. The emission with gated detection shows a broad phosphorescence spectrum with a peak at ~510 nm and a residual delayed fluorescence at 430 nm. The excitation spectra for fluorescence and phosphorescence roughly follows 4MU absorption. The phosphorescence lifetime of 4MU is remarkably long, almost 3 s. 4MU excitation and emission phosphorescence anisotropies are low, very close to zero.

1. Introduction

4-methylumbelliferone (4MU, also known as Coumarin 4) has been proposed as a valuable pH sensor already in the 1960s [1], and later, in the 1980s, as an efficient laser dye in a blue spectral region [2]. In the 1990s, the phosphorescence of 4MU on filter paper was reported [3]. However, only phosphorescence signal from a crystalline phase of 4MU has been reported without a spectral analysis. Recently, there has been growing interest in this coumarin dye. As a fluorescent labeling agent, 4MU has been extensively used for intracellular pH measurements because of its low toxicity, pKa value, and relatively large fluorescence intensity changes as a function of pH [4–6]. Furthermore, it has also been routinely utilized for pH-dependent enzymatic assays [7–10] and for the highly sensitive determination of tyrosinase [4]. 4MU is one of the main precursor molecules from the biosynthetic pathway of cinnamic acid that engenders other complex coumarin molecules.

Room temperature phosphorescence (RTP) recently became a ‘hot topic’ [11, 12]. This is because of its potential applications like antiforgery [13, 14], encryption/decryption of information [15], sensing (temperature, pH, chemical analytes) [16], bioimaging/

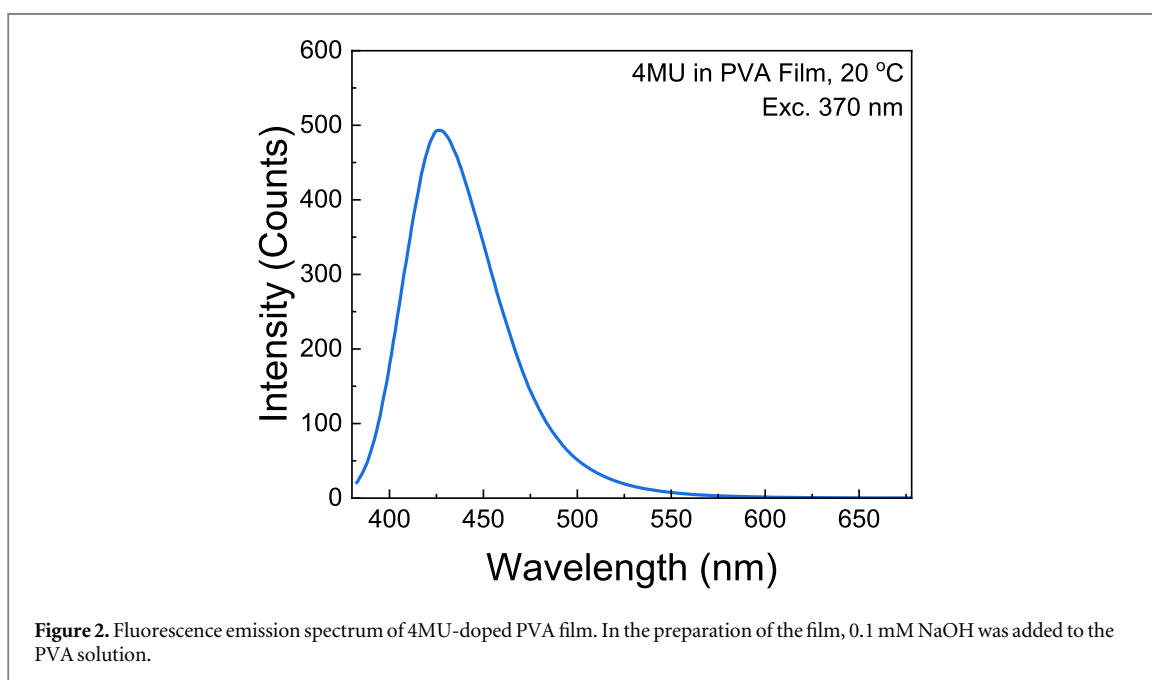
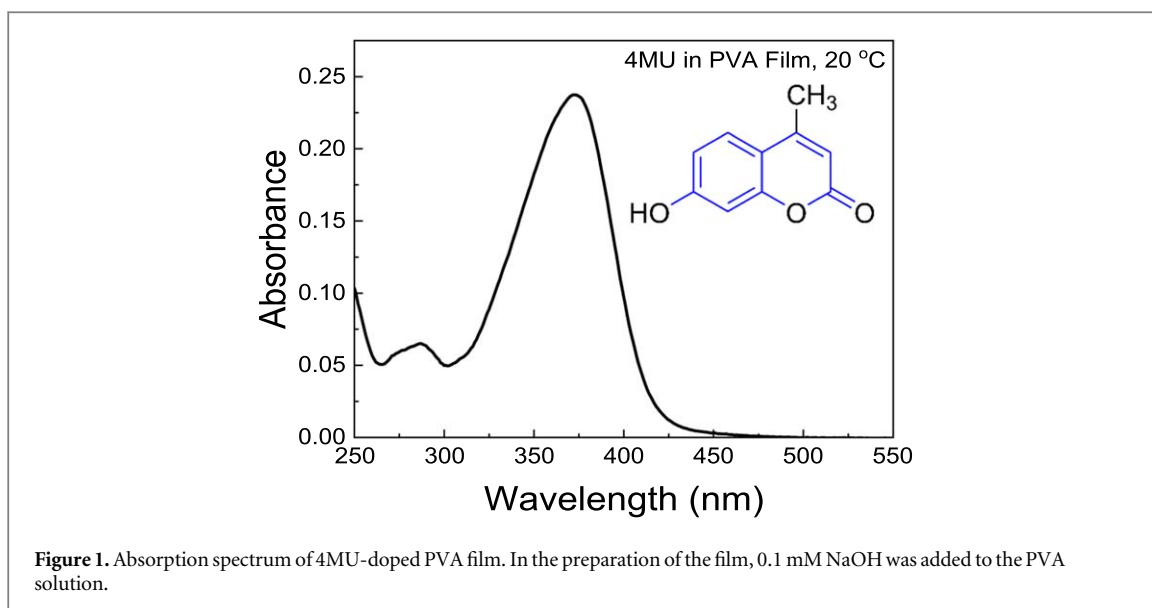
diagnostics [17], and organic light-emitting diodes [18]. Other applications of RTP include but are not limited to visualization of hidden fingerprints in forensic sciences and printing [19]. Recent works have directed their attention on the RTP afterglow from natural resources [20–22], and polymer-based materials [13, 14, 23–26]. In this report, we studied fluorescence and phosphorescence properties of 4-methylumbelliferone (4MU) doped in poly (vinyl alcohol) (PVA) films.

2. Materials and methods

The 4-methylumbelliferone (4MU, Coumarin 4) was from Kodak, laser dye grade. 98% hydrolyzed poly (vinyl alcohol) (PVA) with a molecular weight of 130,000 has been purchased from Sigma Aldrich.

2.1. Preparation of film

PVA films were prepared as described in detail in our previous works [24, 27]. The average thickness of each film was about 200 μm. In order to achieve the 4MU basic form, we added 0.1 mM NaOH to the PVA solution. The estimated concentration of NaOH in a



dried film was about 1 mM. Identical amount of NaOH was added to the reference PVA without 4MU.

2.2. Measurements

2.2.1. Absorption

Absorption measurements were done using the Cary 60 spectrophotometer (Agilent Technologies, Inc.). The PVA background was subtracted. Even so, the PVA background was negligible at the excitation wavelength.

2.3. Fluorescence spectra

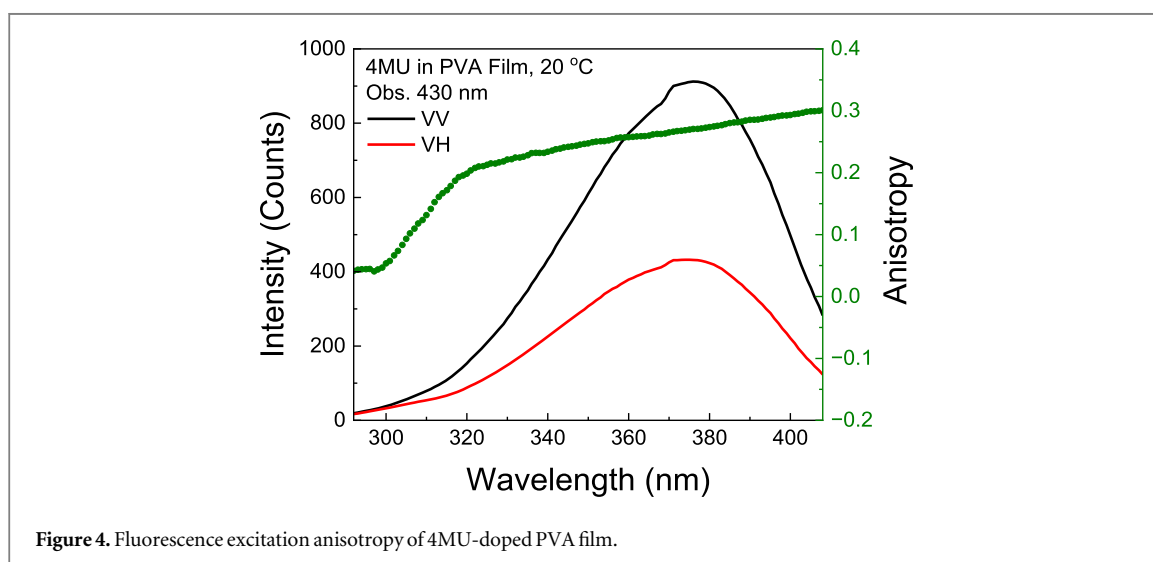
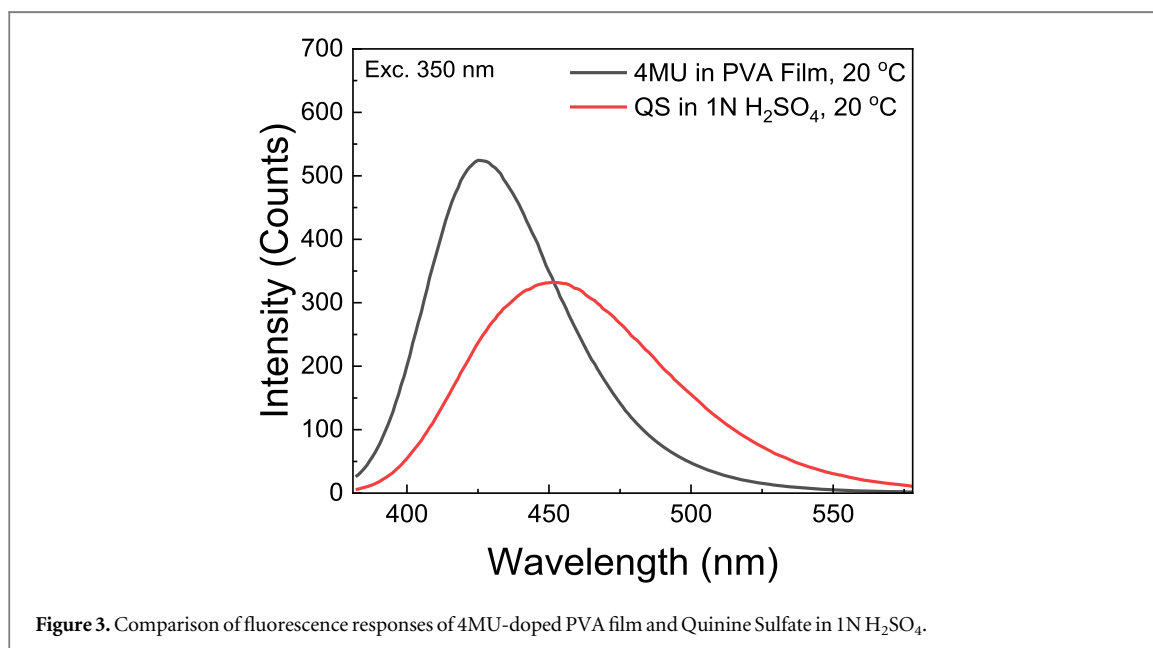
Using a front-face configuration, the Cary Eclipse spectrofluorometer (Agilent Technologies, Inc.) was used for fluorescence measurements with a UV wire grid linear polarizer and linear plastic polarizer for excitation and emission sides, respectively [27].

2.4. Fluorescence quantum yield

Fluorescence quantum yield (QY) was determined by using a solution of quinine sulfate (QS) with 1N H₂SO₄ (QY = 0.54) as the standard [28]. Measurements were done in 1 mm pathlength micro cuvettes. The cuvette with 4MU-doped PVA film was submerged in benzene that is nonreactive to PVA and has a refractive index matching that of PVA. For the QY calculation, the refractive indexes were taken into account ($n = 1.35$ for QS in 1N H₂SO₄ and 1.48 for 4MU-doped PVA film and benzene).

2.5. Fluorescence lifetime

Time-domain FT200 fluorometer (PicoQuant GmbH) has been used for lifetime measurements using a 375 nm pulsed laser diode. The repetition of 90 ps



pulses was 4MHz and the time resolution was 4 ps. The time-dependent data were analyzed with FluoFit4 software (PicoQuant GmbH).

2.6. Phosphorescence spectra measurements

Phosphorescence spectra were measured on the Varian Cary Eclipse instrument using phosphorescence mode, which is based on a time gating mode to remove shorter lived emission components such as scattering and background from fluorescence. The following parameters were used for this mode: number of flashes of 10, delay time of 0.5 ms, gate time of 5 ms, and total decay time of 1.0 s. Anisotropies for phosphorescence excitation and emission spectra were calculated using the following equation:

$$r = \frac{I_{VV} - I_{VH} * G}{I_{VV} + 2I_{VH} * G} \quad (1)$$

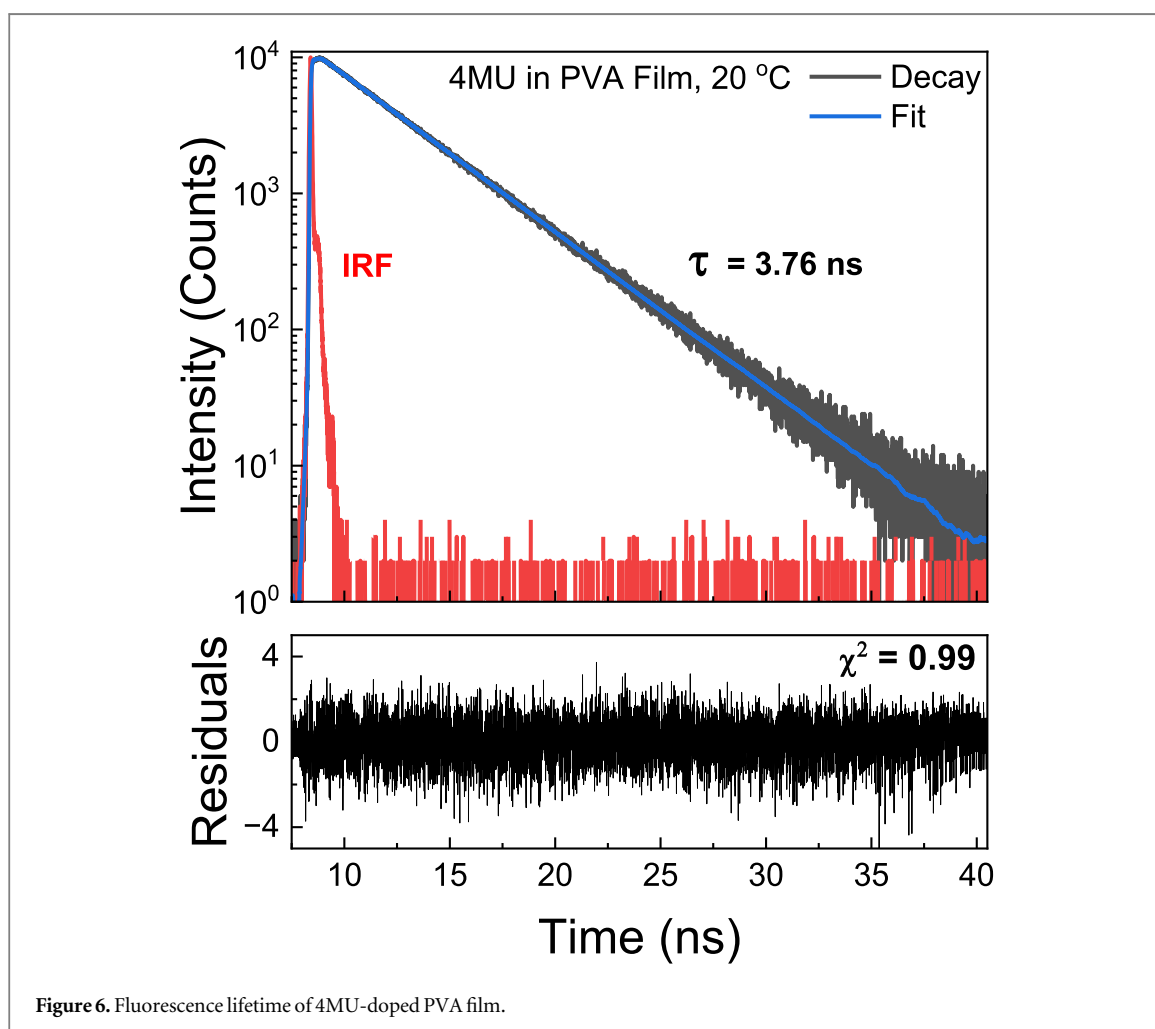
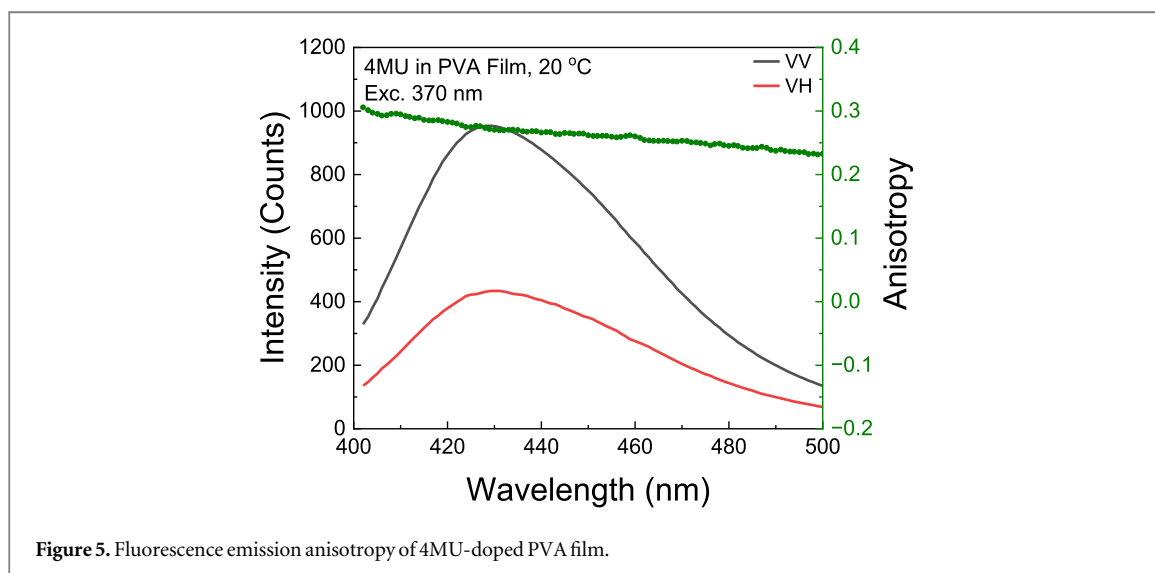
where the I_{VV} and I_{VH} are polarized phosphorescence intensity components obtained from a vertically

oriented polarizer for excitation and vertically or horizontally oriented polarizer for observation, respectively. G-Factor, G , was measured in the front-face configuration and used as a correction factor for uneven transmissions from I_{VV} and I_{VH} [27]. Equation (1) was used for both fluorescence and phosphorescence emissions.

2.7. Phosphorescence lifetime estimation

For phosphorescence lifetime estimates, a smartphone was used to capture a video, ensuring that the entire film was visible within the frame. The video was subsequently processed using ImageJ to tabularize the average brightness, which served as a proxy for intensity across frames.

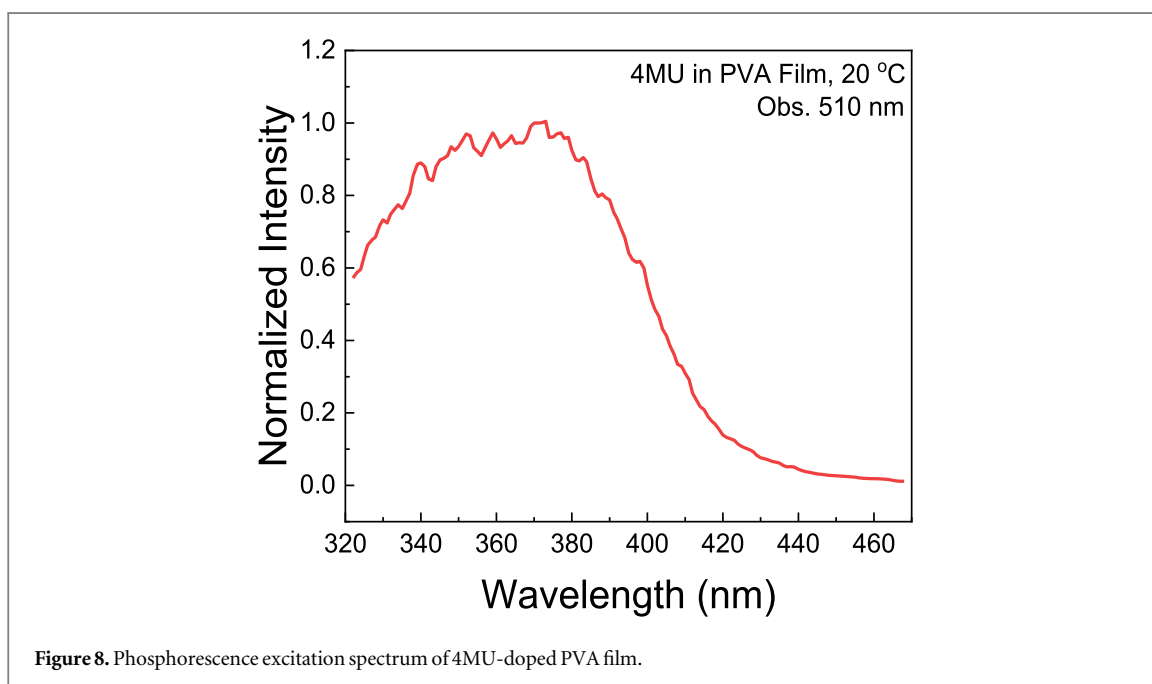
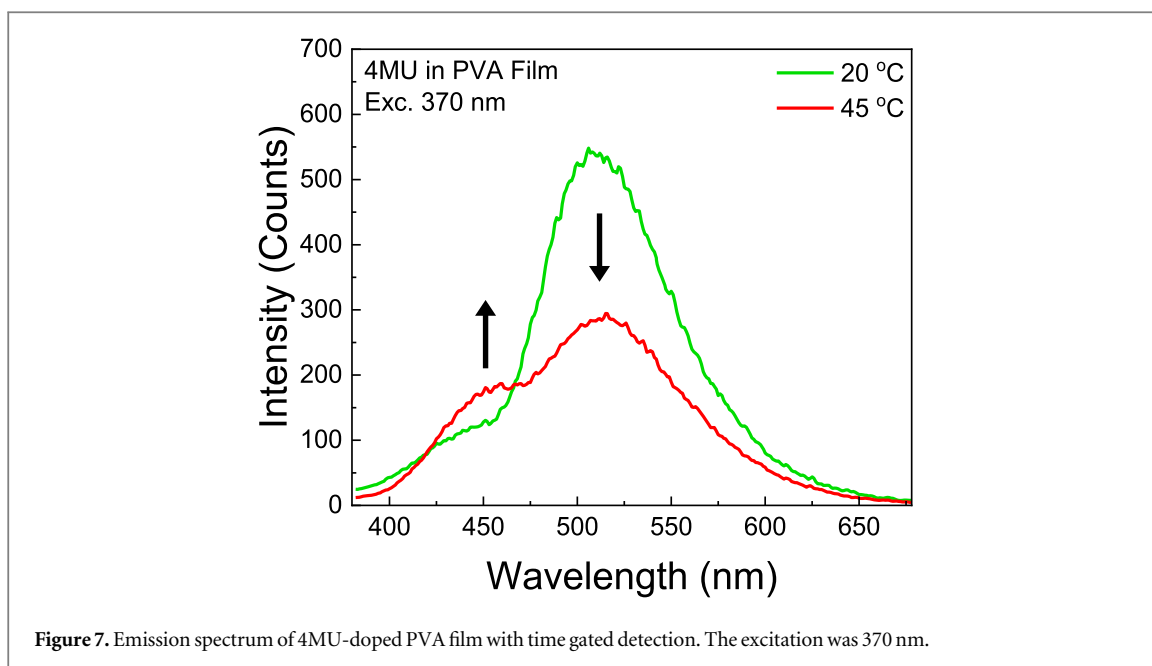
The images in the plot are the frames used for analysis. Due to the size of the images, it was only possible to fit one image per second onto the plot. The number of frames is entirely arbitrary and



dependent on the phosphorescence lifetime of the sample at a given temperature. The lifetime calculation used 3600 frames as data points for curve fitting. For the smartphone phosphorescence lifetime estimations, the exposure time for the camera was

16.67 milliseconds per frame at a framerate of 60 frames per second.

The frame rate was then used to determine the time in between frames, and the resulting data were fitted using a single-exponential model:



$$I(t) = I_0 e^{-\frac{t}{\tau}} \quad (2)$$

where I_0 term represents the amplitude for the decay and τ represents the lifetime. Equation (2) was used for both fluorescence and phosphorescence lifetimes.

3. Results and discussion

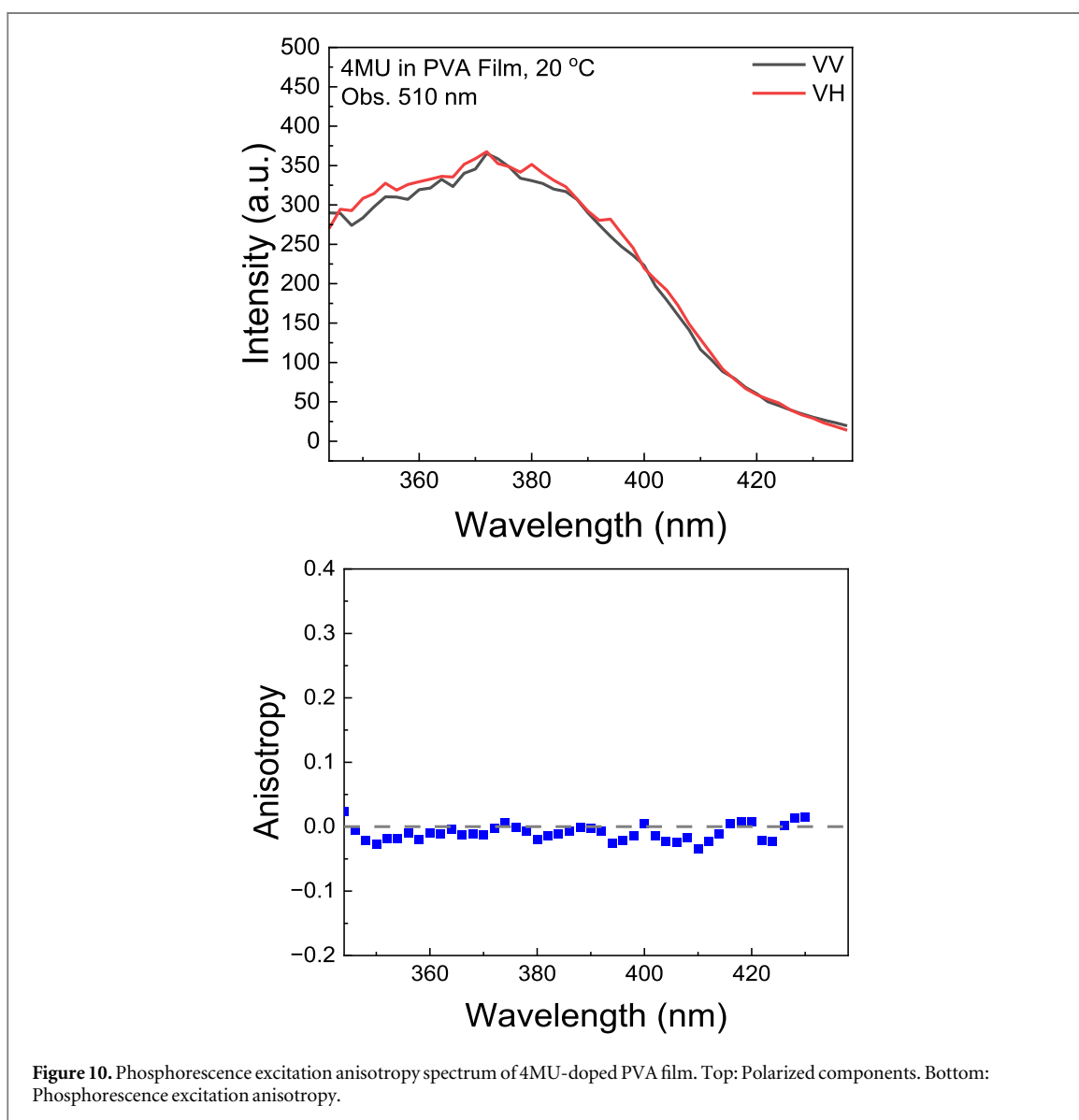
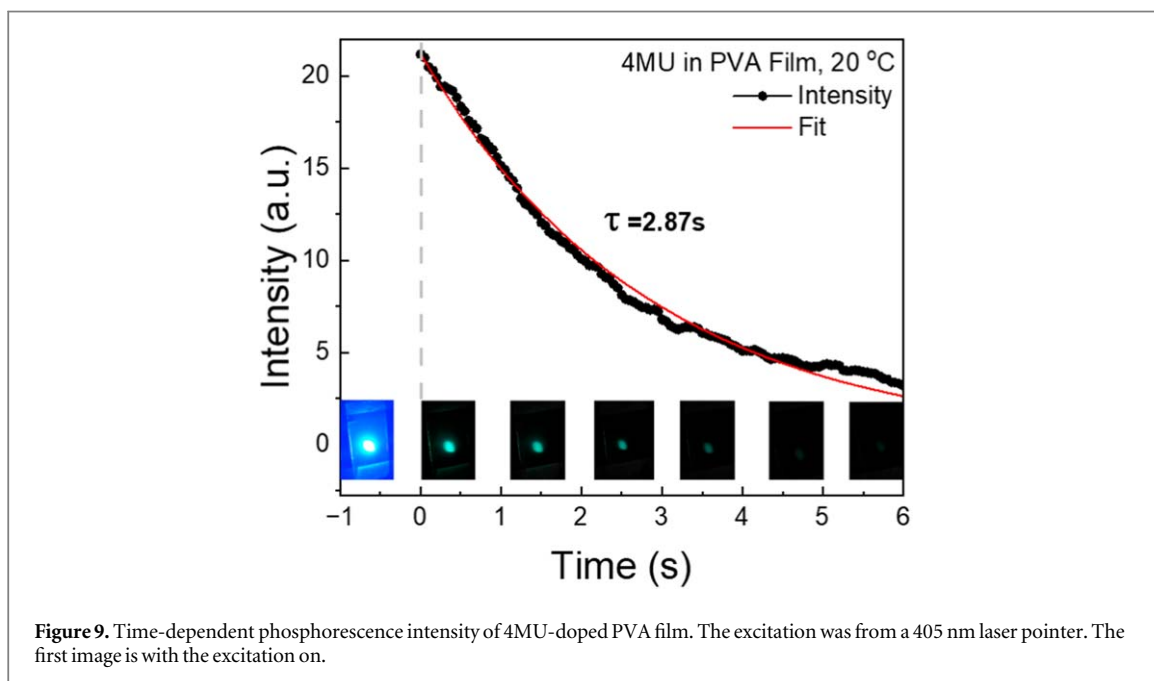
3.1. Fluorescence

3.1.1. Absorption and fluorescence spectra

4MU spectral properties depend strongly on pH. In neutral PVA, the absorption spectrum of 4MU clearly shows two distinguishing peaks; see figure 1SM in *Supplementary Materials*. However, the addition of

NaOH to the PVA solution during film preparation results in the absorption of a single long-wavelength peak, similar to the 4MU absorption at pH higher than 11 [1, 2]; see figure 1. It should be noted that the films containing NaOH are very stable, and their spectral properties did not change over a period of a few months.

We used 370 nm excitation to study 4MU-doped PVA emissions. Taking into account the thickness of the PVA film (0.2 mm) and the extinction coefficient of 4MU anionic form ($36,600\text{ l mol}^{-1}\text{ cm}^{-1}$), we estimate the concentration to be about 0.35 mM. The strong blue fluorescence is centered at 430 nm and goes to about 600 nm; see figure 2.



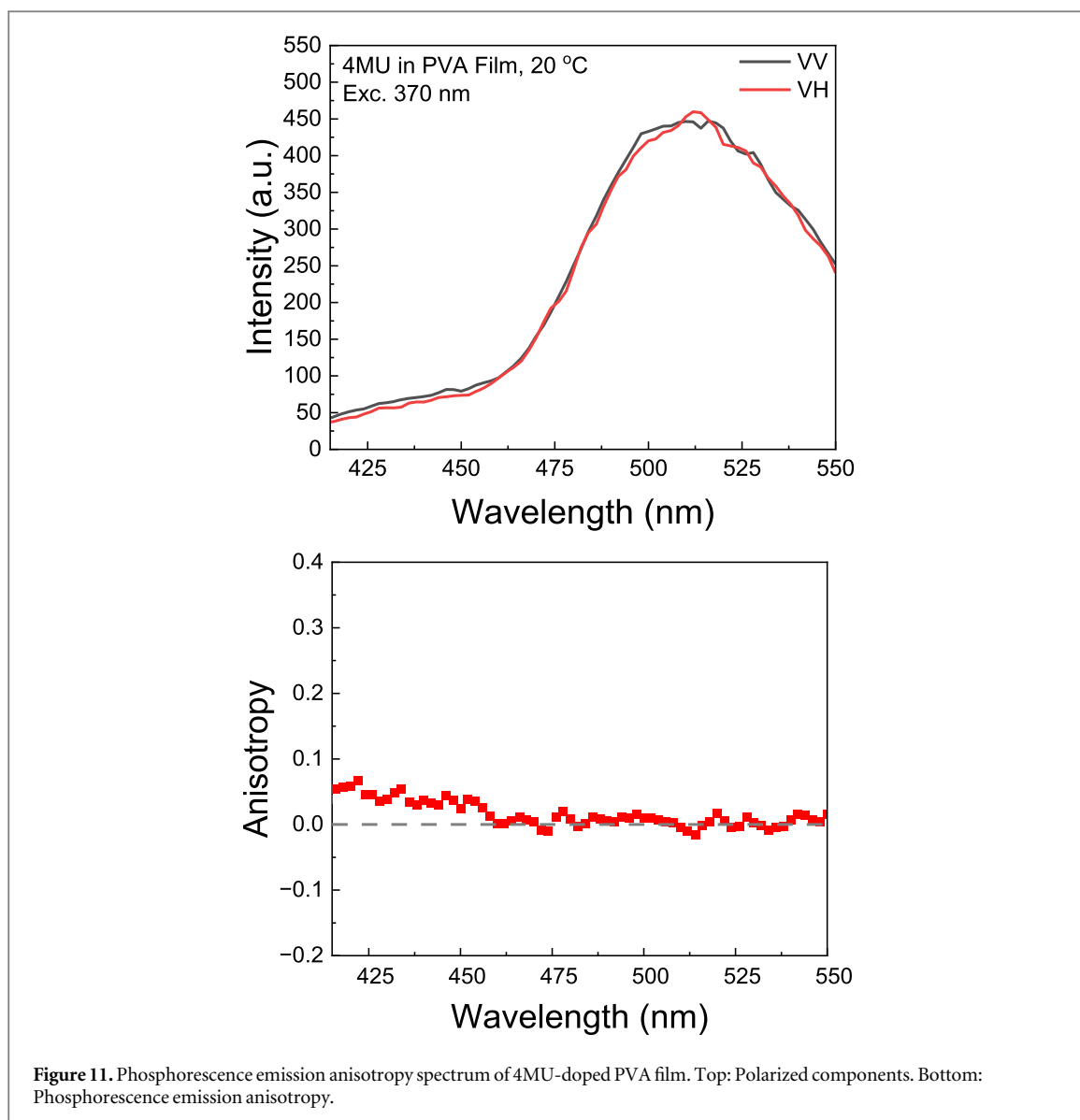


Figure 11. Phosphorescence emission anisotropy spectrum of 4MU-doped PVA film. Top: Polarized components. Bottom: Phosphorescence emission anisotropy.

The fluorescence emission of 4MU is very strong, as shown in figure 3, compared to quinine sulfate (QS), a known fluorescence standard.

3.1.2. Fluorescence quantum yield

For this comparison, the absorption of QS in 1N H_2SO_4 was adjusted to match the absorption of 4MU-doped PVA film. See figure 2SM in *Supplementary Materials* for absorption spectra and QY calculation. After considering the correction for refractive indices, the QY of 4MU-doped PVA has been estimated to be as high as 0.73, which is not a surprise for a laser dye.

3.1.3. Fluorescence anisotropy

The PVA matrix is rigid and effectively hinders molecular rotations. Therefore, it is expected that the observed fluorescence anisotropy will be high. In fact, it reaches the value of 0.3 at longer wavelengths, see figure 4. The excitation anisotropy drops at shorter

wavelengths, which suggests the excitation to the second single excited state.

The fluorescence emission anisotropy is almost constant within the fluorescence spectrum, see figure 5. However, at longer wavelengths, it decreases with a stepwise decrease at about 530 nm, see figure 3SM in *Supplementary Materials*.

3.1.4. Fluorescence lifetime

Fluorescence intensity decay of 4MU-doped PVA is shown in figure 6. It is very homogeneous (see randomly distributed residuals in figure 6) and fitted with a single exponential, revealing a lifetime of 3.76 ns with an accuracy of ± 0.02 ns.

3.2. Phosphorescence

Usually, phosphorescence is detected at low temperatures. It was a pleasant surprise to observe a green/yellow afterglow lasted up to ten seconds when the excitation of 4MU-doped PVA went off.

3.2.1. Phosphorescence spectra

The spectrum of 4MU-doped PVA measured with gated detection spans from 400 nm to 650 nm. The phosphorescence is centered at about 510 nm, and a small amount of delayed fluorescence appears, which can be seen at shorter wavelengths, see figure 7. At the same experimental condition, we repeated the measurement at 45 °C. The phosphorescence decreased while delayed fluorescence increased. This implies that the reverse inter-system crossing process increased with temperature and eosin-type delayed fluorescence increased.

The phosphorescence excitation spectrum roughly resembles absorption and/or fluorescence excitation spectra; see figure 8 and compare it to figures 1 and 4. The comparison of red-edge excitations of fluorescence and phosphorescence suggests a very low probability of direct triplet excitation, see figure 4SM in *Supplementary Materials*.

3.2.2. Phosphorescence lifetime

The phosphorescence glow after switching off the excitation is very impressive and lasts several seconds; see time-lapse in figure 9. The phosphorescence intensity decays exponentially with a mean time of about 3 s. For RTP, it is extremely long since a few hundred milliseconds are considered ultralong [15, 19, 23].

3.2.3. Phosphorescence anisotropy

A transition moment in the phosphorescence process ($T_1 \rightarrow S_0$) is usually orthogonal to the absorption transition ($S_0 \rightarrow S_1$), which results in a negative or very low fundamental anisotropy.

Polarized components of the phosphorescence in both the excitation and emission spectra are almost equal, which suggests almost zero anisotropy; see figures 10 and 11, top. In fact, the excitation and emission phosphorescence anisotropy are slightly negative, very close to zero; see figures 10 and 11, bottom. We consider this to be an advantage in potential applications of depolarized RTP of 4MU-doped PVA.

4. Conclusions

The flexible 4MU-doped PVA films are fully transparent. Their strong, radiant blue fluorescence is highly polarized. We prepared and tested several films with various dye concentrations. The spectral properties of 4MU-doped PVA films do not depend on the concentrations in the range from 0.1 mM to 1.0 mM. We were pleasantly surprised by the possibility of pH adjustment in PVA films. The NaOH-doped PVA films behave just as normal (neutral) films. They are stable and did not change their properties over several months. The fluorescence/phosphorescence of 4MU-doped films containing NaOH remained unchanged after a two-month period. The phosphorescence of 4MU-doped PVA film is almost fully depolarized. This could be

important in their practical applications because it provides independence from the excitation/observation polarization conditions. It should be noted that the excitation of 4MU is possible in a spectral region (above 370 nm) where strong excitation sources (like lasers) are available. The most important finding of this study is the relatively strong room temperature phosphorescence of 4MU-doped PVA with remarkably long lifetime. Usually, RTP lifetimes of organic compounds are in the micro/millisecond scale.

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Data availability statement

The data cannot be made publicly available upon publication because they are not available in a format that is sufficiently accessible or reusable by other researchers. The data that support the findings of this study are available upon reasonable request from the authors.

Supporting information description

Absorption spectrum of 4MU-doped PVA film; Absorption spectra of 4MU-doped PVA film and QS in 1N H₂SO₄ for quantum yield measurements; Fluorescence emission anisotropy of 4MU-doped PVA film; Phosphorescence excitation spectrum of 4MU-doped PVA film for fluorescence and phosphorescence excitation spectra at red edge.

Notes

The author(s) declare no competing interests.

ORCID iDs

Bong Lee  <https://orcid.org/0009-0003-7936-5327>

Emma Alexander  <https://orcid.org/0000-0002-9226-2432>

Danh Pham  <https://orcid.org/0000-0003-0274-9640>

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