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Research Article

Effect of Solvents on the Fluorescent Spectroscopy of BODIPY-520 Derivative

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Photodynamic therapy (PDT) is a selective and minimally invasive technique for the treatment of tumors. It includes three components such as photosensitizer, light, and molecular oxygen. The purpose of this work is to investigate the effect of the solvents such as methanol, ethanol, acetone, and water on fluorescent spectroscopy produced by one of the BODIPY derivatives in turbid media. A 520 nm laser diode is used for exciting one of the BODIPY derivatives as a photosensitizer in tissue-like optical phantoms. Results show that the photosensitizer studied without absorption and scattering components in the methanol and ethanol solvent has a prominent fluorescence peak at 600 nm, whereas acetone solvent has a prominent fluorescence peak at 546 nm. Experimental results reveal that when absorption and scattering components are present in addition to the studied solvents, the characteristic fluorescence intensity peak is red-shifted to 678 nm.

1. Introduction

In the past decade, photodynamic therapy (PDT) has appeared as a treatment method that offers an attractive new direction for treating different types of cancers and non-cancerous diseases [1]. PDT is a minimally invasive therapeutic procedure. It provides a safe, selective, and effective method for eradicating cancerous cells [2]. PDT is based on three basic components (photosensitizer, light, and oxygen). These three ingredients are harmless when alone. However, when combined, it causes tissue damage. After 48 hours of photosensitizers (PS) injection, it accumulates preferentially in the tumor cells as compared to normal cells. When photosensitizers are excited by light with specific wavelengths and in the presence of molecular oxygen, reactive oxygen species ROS is generated and thus induces tumor cell death in two ways, either apoptosis or necrosis [3, 4]. The most important advantages of PDT over other traditional cancer treatments are reduced PS systemic toxicity in the absence of a light source, selectivity in destroying cancer cells, and the ability to apply it alone or in combination with other traditional treatments such as radiotherapy, chemotherapy, or surgery [5]. Over the past three decades, chemists have attempted to synthesize and discover molecules that could act as improved PSs [6, 7]. Designing and making better drug molecules is still an ongoing field of research to improve drug delivery systems and targeting. New techniques rely on smart systems such as nanoparticles for drug delivery, encasing therapeutic agents, and imaging agents and in addition, by the use of new particles such as

diketopyrrolopyrrole (DPP) or tuning of chemical structure by inserting heavy atoms [8, 9]. An effective photosensitizer must have an absorption wavelength that falls within the treatment window, a high intersystem spin-crossing probability, a good singlet oxygen quantum yield, and other properties [10]. At present, there are many natural and artificial PSs; development of new PS aims to improve the pharmacological properties and tumor selectivity [1]. Recent studies focus on porphyrin, chlorin, phthalocyanine, and BODIPY derivatives to obtain the best factors affecting the effectiveness of PS molecules [11].

In medicinal chemistry, compounds containing a fluorine atom are useful in the pharmaceutical industry, and the chemical composition of the BODIPY structure is unique and suitable for therapeutic applications [12].

Thus, BODIPY derivatives have gained great interest due to their featured optical physical properties [13]. Moreover, the physical properties can be controlled by modifying the chemical composition of chromophore BODIPY; as a result, it covers a wide band of the absorption spectrum [14]. Thus, it has many potential applications such as bioimaging, artificial antennae, photosensitizers in solar cells, and photosensitizers in photodynamic therapy [15, 16]. The difference in oxygen levels between cancer cells and normal cells gives oxygen an important role in the development and treatment of cancer, as hypoxia in the environment causes cancer cells not to respond to treatment [17]. New treatment strategies aimed at counteracting hypoxia in the tumor environment by designing drugs that selectively target cancer cells and activate them in hypoxia [18]. Therefore, the fluorescence signal contains more information than color and intensity. In addition, it provides information about the molecular structure of the sample and the surrounding environment. It is considered a valuable marker in biomedical applications [19]. Fluorescence spectroscopy proved to be an attractive technique for the noninvasive, early diagnosis of cancer. Since the fluorescence spectra contain important biochemical information about tissue, it has been involved in many medical optical applications such as photodynamic therapy and the detection of cancerous or dysplastic lesions [20].

However, solvent polarity and local environment have profound effects on the spectral emission properties of fluorophore molecules. The effect of solvent polarity is stemmed from the origin of the Stokes shift, which is one of the observations in the fluorescence spectrum [21]. Whereas, the change in emission characteristics is a response to cellular conditions and sensitivity to the surrounding environment such as polarity, pH, and hypoxia [22].

Local environmental factors have profound influence on the emission spectral properties of fluorophores such as solvent polarity, organic and inorganic solvents, the concentration of the fluorophores, temperature, and pH. One of the main sources of the Stokes shift is the effect of the polarity of the solvent. A study was conducted on curcumin which has potential biological applications to see how it behaves within thirteen different solvents and the effect of the polarity of the solvent on the absorption and fluorescence spectrum [23]. Another study was conducted on chalcone derivatives to observe how the fluorescence and absorption characteristics change within a different number of solvents. A bathochromic shift was found from polar solvents to nonpolar solvents [24]. Filarowski et al. studied the spectroscopic and optical properties characteristics of difluoroboron dipyrromethene- (BODIPY-) based fluorescent dyes in various solvents; they found the fluorescence properties of dye very sensitive to the solvent polarity [25].

Therefore, the main objective of the present work is to experimentally investigate the effect of the solvents such as methanol, ethanol, acetone, and water on the spectroscopic properties of intratissue fluorescence in simulating optical phantoms containing BODIPY-520 as a potential photosensitizer for photodynamic therapy. These investigations were conducted in the presence of and the absence of absorption and scattering components.

2. Materials and Methods

2.1. Sample Preparation. 2,8-Diethyl-1,3,5,7-tetramethyl-9-phenylbipyrromethene difluoroborate (BODIPY-520) was used as a photosensitizer model (795526, Sigma-Aldrich Inc., USA). The chemical structure of BODIPY-520 is shown in Figure 1. It was dissolved in four different separated solvents (methanol, ethanol, acetone, and ultra distilled water). The concentration of the BODIPY-520 photosensitizers was set to 2000 μM in initial fluorescent measurements.

The detection system including optical instrumentation for the measurement of fluorescence is shown in Figure 2. A continuous semiconductor laser of 520 nm (L520P50, Thorlabs Inc., USA) was used as an exciting light source. The temperature and the current intensity were set at 40°C and 150 mA using a temperature control unit (TED200C, Thorlabs Inc., USA) and a current control unit (LDC205C, Thorlabs Inc., USA). The laser source provides power up to 50 mW. The laser power used to excite the sample is set at 5 mW using a proper attenuator followed by a mirror (PF05-03-M01, Thorlabs Inc., USA). A lens was used to expand the excitation light that illuminate the entire sample. The sample was placed in a standard quartz cuvette (12.5 × 12.5 mm, with a 10 mm light path) mounted on a cuvette holder (CVH100/M, Thorlabs Inc., USA). The cuvette was tightly sealed with rubber caps. The detector-source-sample geometry was maintained at the same position during the measurements.

Then, for collecting the emitted fluorescence signal, a collection fiber (Ocean Optics Inc.) with a diameter of 400 μm was used. The fiber was coupled to a miniature spectrometer (USB4000 FL, Ocean Optics Inc. USA). The spectrometer was connected via the USB cable to a computer for acquiring fluorescence spectra. Recoding data and signal processing of the collected spectra were controlled by the SpectraSuite software (Ocean Optics Inc., 2011).

2.2. Optical Phantom. Optical phantoms were made of scattering and absorbing materials with selected coefficients that represent the optical properties of biological tissues. The optical phantom was made of India ink as an absorber and

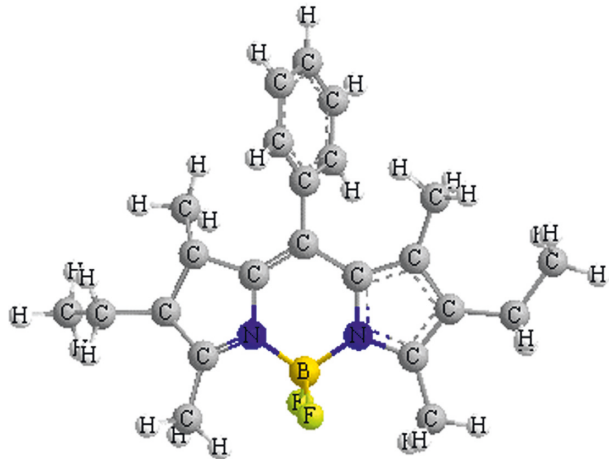
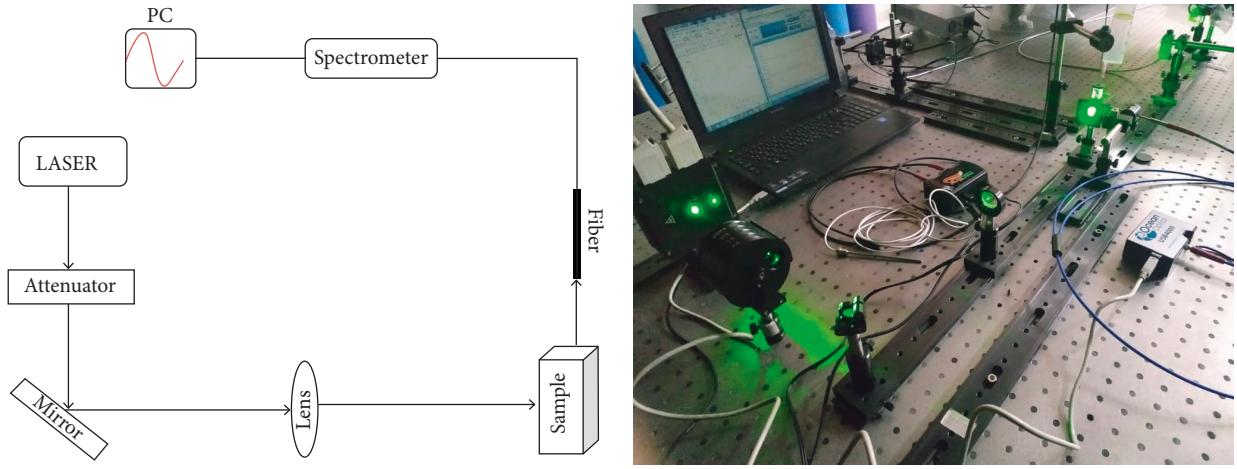


Figure 1: The chemical structure of 2,8-diethyl-1,3,5,7-tetramethyl-9-phenylbipyrromethene difluoroborate (BODIPY-520).



(a) (b)

Figure 2: (a) Schematic layout of the optical setup used for recording fluorescence spectra resulting from BODIPY-520 photosensitizer in tissue-like optical phantoms. (b) Overview of the optical setup.

intralipid as a scatterer. The developed optical phantoms were used to study the effect of four different solvents, namely, methanol, ethanol, acetone, and water, on the fluorescence spectrum of the photosensitizer. The concentration of BODIPY-520 in phantom is 50 μM. The size of the phantom sample used in the work was 3 ml, prepared in air-saturated phosphate-buffered saline (PBS, pH 7.5) and stored at 4°C in the dark.

Optical phantoms were constructed in this work with constant absorption and scattering coefficients for all samples where the absorption coefficient was set at the value of 0.90 mm^{-1} and the scattering coefficient was set at the value of 5 mm^{-1}. All measurements were performed in a dark laboratory environment at room temperature.

3. Results

Photosensitizer fluorescence was investigated for each one of the following solvents separately: methanol, ethanol, acetone, and water. The excitation was performed with a 520 nm laser source. Figure 3 shows the fluorescence signal of the photosensitizer in solvents such as methanol, ethanol, acetone, and water. An initial measurement in the wavelength ranges from 500 to 800 nm was conducted. One can notice a prominent peak around 600 nm corresponding to the emission of the photosensitizer in solvents i.e., methanol and ethanol. However, for acetone, a prominent peak around 546 nm can be noticed. There is a shift in the fluorescence peak of about 45 nm. However, in a water solvent, no clear fluorescence peak was observed.

3.1. Effect of Solvent on the Spectral Features. To investigate the effect of solvent on the spectral features of the measured fluorescence spectra of the BODIPY-520 photosensitizer, Intralipid 20% (I141, Sigma-Aldrich, USA) was used as a scatterer with a constant scattering coefficient set at 5 mm^{-1} and India ink as an absorber with a constant absorption coefficient set at 0.9 mm^{-1}. Figure 4 shows the measured fluorescence intensity of the BODIPY-520 photosensitizer

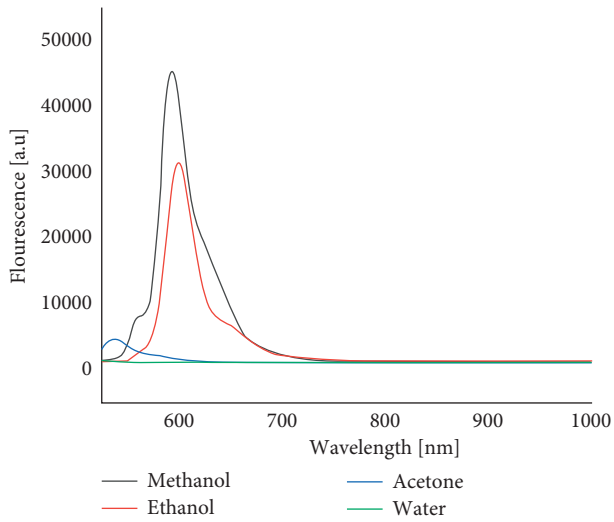


Figure 3: Fluorescence spectra for photosensitizer for each solvent, methanol, ethanol, acetone, and water, in the absence of absorption and scattering components.

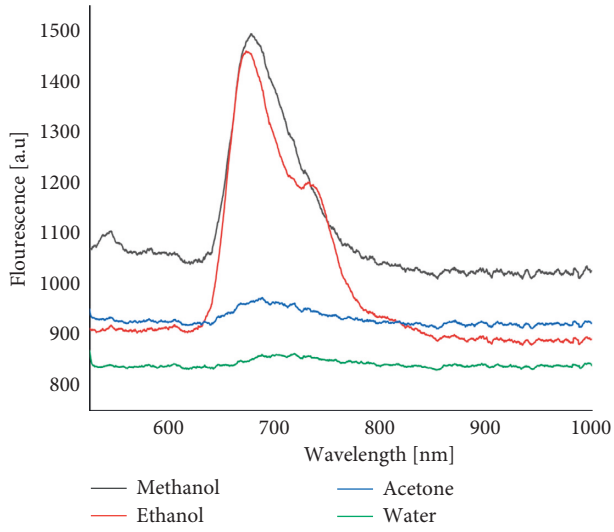


Figure 4: Fluorescence spectra for photosensitizer for each solvent, methanol, ethanol, acetone, and water, with the presence of absorption and scattering components.

with constant scattering and absorption coefficients. One can also notice the spectral shift of the fluorescence peak to 678 nm in all solvents used. Methanol and ethanol show higher fluorescence intensity than acetone and water.

3.2. Effect of Concentration on Photosensitizer Fluorescence. Since the solvent of methanol and ethanol gave the greatest fluorescence intensity, we studied the effect of changing the concentration of the photosensitizer within the absorption and scattering components. The concentration of the photosensitizer varied within the following ranges (150 μM, 100 μM, 50 μM, 20 μM, and 0 μM). One can see that the fluorescence intensity of the photosensitizer is directly proportional to photosensitizer concentration as shown in Figures 5 and 6.

4. Discussion

The results presented in this work demonstrated the significant influence of solvents on the detected spectroscopic properties of fluorescence of one of the BODIPY derivatives as a photosensitizer. Determination of the effect of solvent on the spectral properties of the photosensitizer is of great interest before, during, and after photodynamic therapy of neoplastic tissues. One of the important factors affecting optical properties of the photosensitizer is the type of solvent used, as the fluorescence efficiency changes according to the nature of the solvent. Therefore, fluorescence measurements of the photosensitizer in different solvents can provide valuable feedback on the emission properties.

It can be said that the fluorescence spectrum of the studied photosensitizer without the presence of absorption and scattering components shows that the carbonyl group in the acetone solvent led to fluorescence at the wavelength of 564 nm, while the hydroxyl group in both methanol and ethanol solvents led to fluorescence at the wavelength of 600 nm. This is consistent with a study by Banakova et al. of BODIPY derivatives, in which under the influence of different solvents, they noticed a wavelength shift in the fluorescence due to the change of the solvent [13]. In another study of BODIPY derivatives conducted by Donnelly et al., the fluorescence wavelength shifts depending on the nature of the solvent [26]. It is also in agreement with the study by Filarowski et al. on BODIPY dyes [25].

Furthermore, the findings of this study have shown that the fluorescence spectrum of the studied photosensitizer with the presence of absorption and scattering components led to bathochromic shifts towards the wavelength around 678 nm. That is a shift in fluorescence intensity peak by 78 nm. This redshift is due to introducing absorption and scattering components in optical phantoms in all the studied solvents. The presence of absorbing and scattering materials canceled the effect of solvents on the fluorescence spectrum of the studied photosensitizer and thus canceled the effect of the type n-π^* and type π-π^* transitions of the carbonyl group, while the effect of the type n-σ^* transitions of the oxygen atom in ethanol and methanol.

We noted that the fluorescence intensity in solvents did not differ in the presence or absence of absorption and scattering components. The highest peaks remained for methanol, then ethanol, acetone, and distilled water, respectively. The high fluorescence intensity in methanol and ethanol solvents is due to the convergence between the polarity of the studied photosensitizer and the polarity of methanol and ethanol. In addition, the low fluorescence intensity in acetone is due to the polarity difference between the photosensitizer and acetone. Also, highly polar distilled water did not give a clear fluorescence spectrum because it is far different from the polarity of the photosensitizer.

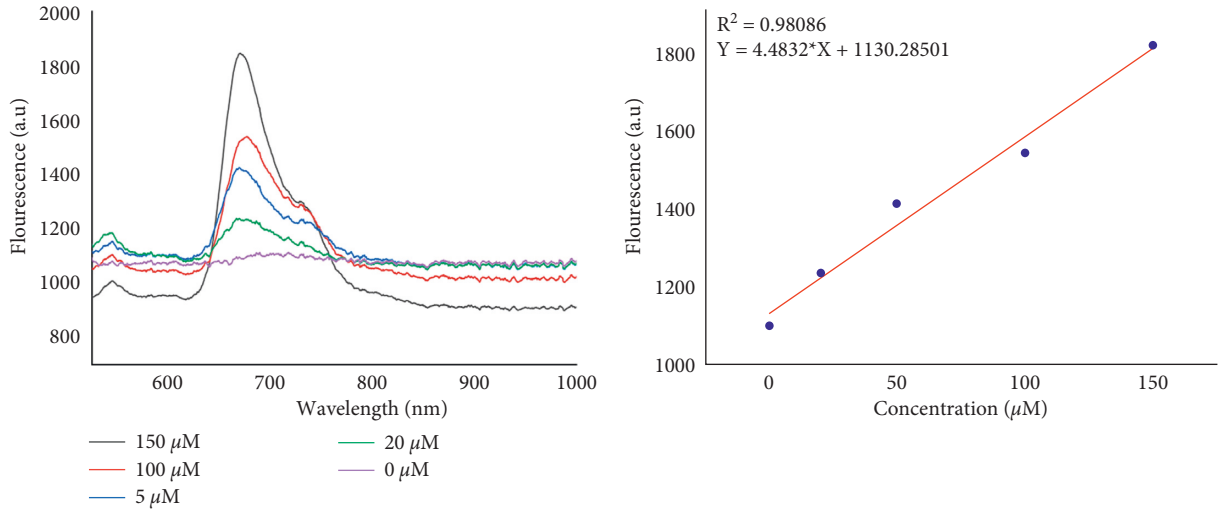


Figure 5: An example of the fluorescence spectra acquired for different concentrations of the photosensitizer in the ethanol solvent.

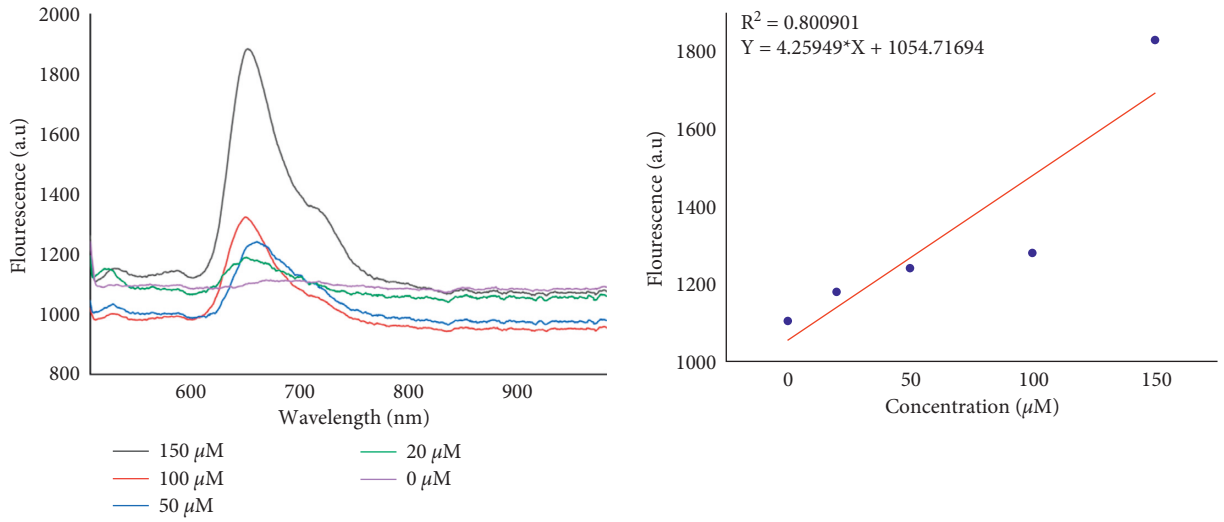


Figure 6: An example of the fluorescence spectra acquired for different concentrations of the photosensitizer in the methanol solvent.

5. Conclusion

In this contribution, we investigated the effect of four solvents methanol, ethanol, acetone, and water on fluorescent spectroscopy produced by one of the BODIPY derivatives in turbid media. Our results show that photosensitizer studied without absorption and scattering components in the methanol and ethanol solvent have a prominent fluorescence peak at 600 nm. Acetone solvent has a prominent fluorescence peak at 546 nm.

Interestingly, in the presence of absorption and scattering components and all the studied solvents, the characteristic fluorescence intensity peak is red-shifted to 678 nm. The findings also revealed that both concentration of the photosensitizer and the type of the solvent have a profound impact on the intensity of fluorescence.

This has in turn potential applications in biomedical optical measurements related to the fluorescence measurements during photodynamic therapy. The study can be further extended to include the effect of mixed solvents of different natures on the fluorescence of the photosensitizer in the absence of absorption or scattering components, in addition to the effect of solvent viscosity.

Furthermore, the presented experimental setup can be modified to explore the effect of solvent on the fluorescence lifetime of BODIPY-520.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

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