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BODIPY dye with 4-benzyloxystyryl groups at the 3,5-positions embedded electrospun polystyrene nanofibers: transient spectroscopic and Antibacterial studies

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ABSTRACT

The 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY was used as photosensitizer for photodynamic inactivation of two types of selected bacteria strains namely *Staphylococcus aureus* and *Escherichia Coli*. Ground state absorption spectra has shown a maximum band at 670 nm in DMF and transient absorption spectra showing the triplet state maximum bands around 472 nm and 503 nm with lifetime of $(1.42\pm0.04)\times 10^{8}$ s^{-1}. Electrospinning method was used to incorporate the dye in polystyrene matrix prior irradiation in dipped contaminated water.

Keyword: Transient spectroscopy, Electrospinning, nanofibers, Energy dispersive X-ray spectra.

INTRODUCTION

Contamination of water by several dyes from the textile industry, by bacteria and other pollutants from breweries, petrochemical industries is a real environmental problem that requires an effective means of remediation. In Africa in general and Democratic Republic of Congo in particular latrines are built at distances that are not long enough from wells or sources for drinking water.This results in an obvious transfer of microorganisms from fecal wastes to well water and thus contamination in terms of bacteria load. Physicochemical dye removal [1], oxidation mechanisms [2], photocatalytic oxidation approaches are used to solve this problem. The last approach includes photosensitization where singlet oxygen generated by photosensitizers in water plays a role of inactivating or destroying microorganism cells is used in this work. Photosensitization is one of the alternative reactions used nowadays by researchers for photodégradation of organic pollutants [3-4], for antimicrobial photodynamic therapy and for purification of contaminated water [5-6]. In this work two types of bacteria among those found in contaminated water namely *Staphylococcus aureus* and *Escherichia Coli* were selected to evaluate the antibacterial activity of the 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY(Fig.1) used as photosensitizer. To avoid direct contact of the used BODIPY and water, electrospinning method was used to incorporate the photosensitizer in polystyrene nanofibers. Incorporation of dyes in a polymeric matrix has been also used by many authors dealing with antimicrobial aspect [7-10]. In the other and nanosecond laser flash photolysis of the 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY was investigated in order to characterize the transient spectra and the lifetime of the triplet state.

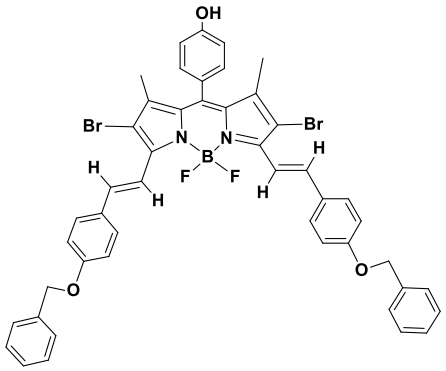

Figure 1. The molecular structure of 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY

Experimental

Materials

Polystyrene (PS, Mw = 192,000 g/mol) were purchased from Sigma-Aldrich while tetrahydrofuran (THF) and dimethylformamide (DMF) were purchased from Saarchem. 1,7-dimethyl-4-hydroxyphenyl-BODIPY core was synthesized, characterized and published by Ngoy *et al* [11] and recently used to make BODIPY-Metallophthalocyanine-graphene quantum dots[12] and BODIPY -Metallophthalocyanine-nanodiamond[13] hybrids for assessing PDT activity. *Staphylococcus aureus* and *Escherichia coli* cells were purchased from the department of bacteriology at University of Kinshasa Hospital in Democratic Republic of Congo. Water collected from milli-Q water (Millipore corp., Bedford, MA, USA) was used for the preparation of all aqueous solutions.

Equipment

Ground state electronic absorption spectra were performed on a Shimadzu UV-2550 spectrophotometer at room temperature. 1HNMR spectra were recorded in deuterated chloroform on a Bruker spectrometer operating at 600 MHz. The morphology of the electrospun nanofibers was determined using a scanning

electron microscope (SEM, JOEL JSM 840) operating at an accelerating voltage of 20 kV. The average nanofiber diameters together with standard deviations were determined from 70 measurements by using the Cell D software package from Olympus. An Inca PentaFET Precision coupled to a Vega Tescam operating at a 20 kV accelerating voltage was used to collect the energy dispersive X-ray spectroscopy (EDX) data.

Electrospinning

The solvent system containing 50 mL of dimethylformamide and tetrahydrofuran (4:1) was used to dissolve polystyrene pellets by stirring at room temperature until formation of 20 Wt % polymer solutions. 1,7-dimethyl-4-hydroxyphenyl-BODIPY (25 mg) was added into the polymer solution and the reaction mixture was stirred for another 24 h. Functionalized solution was prepared in a 20 mL syringe coupled with a needle at 0.1 mL per hour. The solution was electrospun at around 20 kV and collected as solid at ambient temperature with 45 % humidity.

Laser Flash Photolysis

Nanosecond laser flash photolysis kinetics and transient spectra were carried out using an EKSPLA SL334 Nd-YAG laser, with pulses of ≤ 700 ps duration as reported by Ngoy *et al* [14]. The output energy at 355 nm excitation was around 175 mJ. In a 10 mm cuvette the absorbance of samples were adjusted between 0.5–0.7 at 355 nm. wavelngths of monitoring kinetic decays were fixed using a photomultiplier and the Andor iSTAR incorporated camera was used to record transient absorption spectra. Samples were degassed by 3 freeze-thaw cycles under reduced pressure (5 Pa). Kinetic traces were obtained by fitting data with flashfit 0.11.

Evaluation of antibacterial activity

Preparation of the bacterial suspension

Using a sterile platinum loop, an aliquot of each clinical isolate was taken from the preservation media to be seeded on chapman and Mac Conkey agar for *Staphylococcus aureus* and *Escherichia coli* respectively. After 24 hours of incubation, the opaque and whitish colonies characterized *Staphylococcus aureus* on the Chapman agar while, the red colonies indicated the growth of *Escherichia coli* on Mac Conkey agar. The bacterial suspension was prepared by taking three isolated colonies of each strain to be tested on the selective media using a sterile loop and placing them into 2 mL of sterilized physiological water contained in two different tubes. The turbidity of the suspension has been adjusted in the nutrient broth to obtain a density equivalent to that of the standard 0.5 Mac Farland ($1.5x10^8$ UFC. mL^{-1}).

Dilution method and irradiation of solution containing nanofibers

A batch of five sterile test tubes was prepared for each strain and 4.5 mL of culture medium was added to each tube. From the stock solution of each strain 0.5 mL was inoculated in all first tubes followed by a decimal dilution up to 10^{-5} then 10 mg of nanofibers were dipped into each tube containing a concentration of 10^{-5} M of the inoculums for each strain. Irradiation of each solution was conducted using LED shinning at 632 nm with light dose 77 J/cm^2 at different times starting from zero minute to 25 minutes and compared with dark solutions in one hand and with the strain irradiated without nanofibers in the other hand. After irradiation all the cultures (0.1 mL) were transferred to different solid media (Mac Conkey et Muller Hinton) and incubated for 24 hours at 37° C. Results (appearance of colonies) are read in Petri dishes containing the solid media.

RESULTS AND DISCUSSION

Optical spectroscopy

Ground state absorption spectra of 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY in DMF given in Fig.2 from which it can be seen that the maximum absorption lies around 670 nm. The UV spectra of the same compound in DMSO was reported by Ngoy *et al* to have a maximum absorption around 675-677 nm and the destabilization of the HOMO upon styrylation was demonstrated through TD-DFT calculations [11].

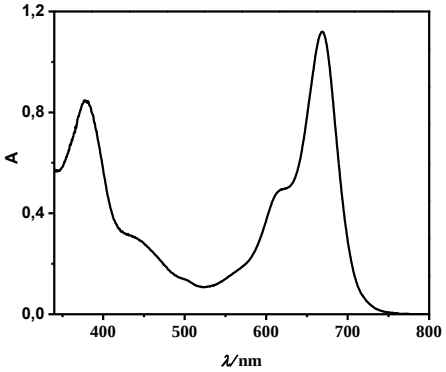

Figure 2. Ground state absorption spectra of 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY in dimethylformamide.

Laser Flash Photolysis

Nanosecond laser flash photolysis of 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY gave a transient absorption spectra with a strong maximum around 472 nm followed by a shoulder at 503 nm shown in Figure 3a with a rate constant of (1.42±0.04)× 10^8 s^{-1} in degassed solution. The transient absorption spectra and the rate constant given by the kinetic trace in Figure 3b were attributed to the triplet state of the studied compound. Triplet state absorption spectra and lifetime for the brominated BODIPY without conjugation at 3,5-position was reported to be in the range of microsecond and millisecond in aerated and degassed solution respectively[15]. Short lived triplets are found after styrylation.

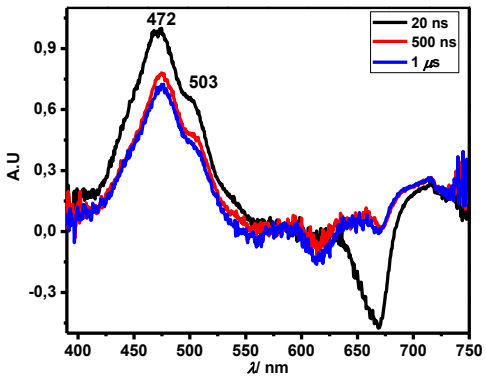

Figure 3a. Transient absorption spectra of 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY in degassed DCM obtained with nanosecond laser after 20 ns, 500 ns, and 1 microsecond after the flash.

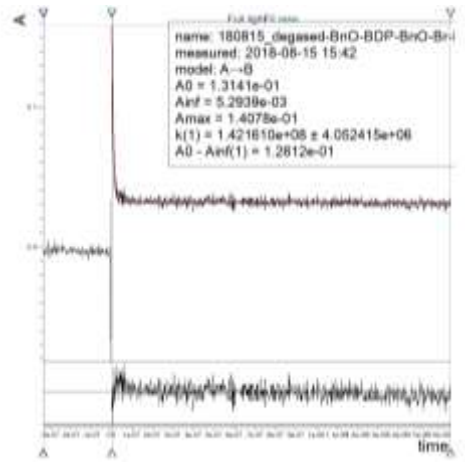

Figure 3b. Kinetic traces of 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY in degassed DCM, observation wavelength 475 nm, excitation at 355 nm

Electrospun nanofibers

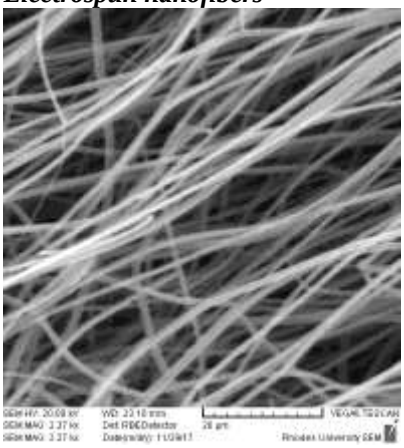

Figure 4. SEM micrograph of the functionalized nanofiber mats 25 mg of BODIPY.

Characterization of the prepared nanofibers was performed using scanning electron microscopy (SEM) combined with energy dispersive X-ray spectrometry (EDS). Parameters such as voltage (20 kV), TCD (13 cm), flow rate (0.1 $mL \cdot h^{-1}$), and viscosity of the polymer solution (20 wt % polymer), were adjusted in order to have uniformed nanofibers without beads. Micrograph showing 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY embedded electrospun polystyrene nanofibers is shown in Fig. 4. However, EDS spectra confirming the presence of the compound in nanofibers are shown in Fig.5 where Carbon, boron, bromine, fluorine, and oxygen are well seen. The same results were reported before when 2,6-dibromo-8-pyrenyl-1,3,5,7-tetramethylBODIPY was used to prepare nanofibers [4].

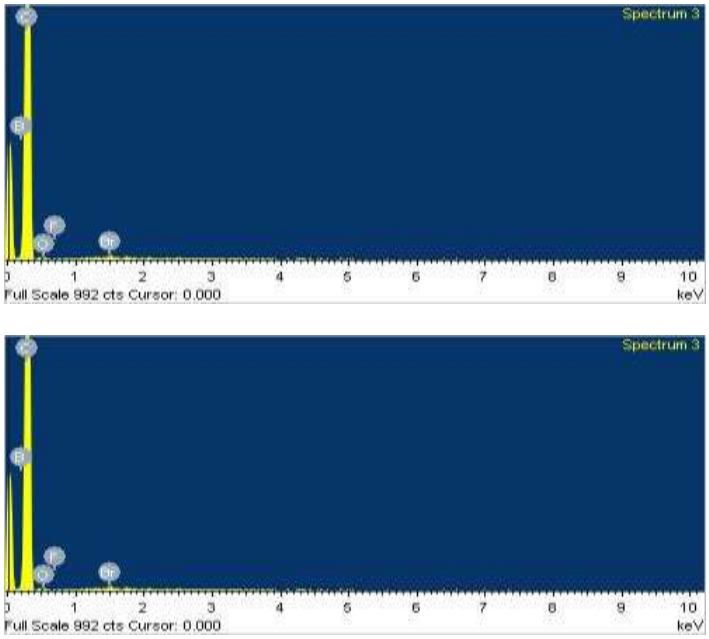

Figure 5. EDX spectra of functionalized BODIPY embedded polystyrene nanofibers mats.

Evaluation of antibactérial activity of nanofibers

Antibacterial investigations were performed using 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY incorporated in polystyrene and both dipped in contaminated water and irradiated for 5, 10, 15, 20, 25 minutes. The table 1 summarizes results showing the capacity of nanofibers to inactivate bacteria after irradiation at different times. From these results it can be seen that the heavy atom effect did play a role along with substitution or styrylation at position 3 and 5. A value of singlet oxygen quantum yield around 0.38 was reported [11]. And we strongly think that the reactive oxygen species responsible of the inactivation of S. Aureus and E. Coli is the singlet oxygen. However, the easy inactivation of S. Aureus compared to E. Coli was reported by several previous researchers [16-20] and confirmed by our recent work where BODIPY in solution at different concentrations was used [6]. From this Table 1 it was also noticed that the mixture of strain and nanofibers kept in dark including strains without nanofibers irradiated for the same times as other solutions did not show any inhibition.

Table 1. Antibacterial activity of nanofibers after irradiation

Treatment of bacterial isolates	**Irradiation time**				
	5 munites	10 munites	15 munites	20 munites	25 munites
E. Coli + Nanofibers + $h\nu$	+	+	+	+	+
E. Coli + Nanofibers in dark	-	-	-	-	-
S. Aureus+ Nanofibers + $h\nu$	+	+	+	+	+
S.Aureus+ Nanofibers in dark	-	-	-	-	-
E. Coli + $h\nu$	-	-	-	-	-
S. Aureus + $h\nu$	-	-	-	-	-

CONCLUSION

This work has shown that 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY was incorporated in polystyrene matrix successfully by electrospinning. Transient absorption spectra showing the triplet state of the used BODIPY was characterized and the maximum absorption wavelength including the lifetime are known. Results from antibacterial activity demonstrate that 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY is able to inactivate Staphylococcus aureus and Escherichia coli after irradiation at 632 nm because of the decrease of number of colonies during photosensitization of 2,6-dibromo-1,7-dimethyl-4-hydroxyphenyl-BODIPY embedded electrospun polystyrene nanofibers in both strain solutions.

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