

Singlet Oxygen Quantum Yield Determination for a Fluorene-Based Two-Photon Photosensitizer

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The quantum yield, Φ_{Δ} , of singlet oxygen generation under two-photon excitation has been determined for a fluorene derivative. A photochemical method was developed using 1,3-diphenylisobenzofuran (DPBF), a chemical quencher of $^1\text{O}_2$, and 2-(9,9-didecyl-7-nitrofluoren-2-yl)benzothiazole (**1**) as a two-photon photosensitizer (PS). The photochemical kinetics of the quencher was measured by two different fluorescence methods. Fluorene **1** exhibited relatively high singlet oxygen quantum yield, $\Phi_{\Delta} \approx 0.4 \pm 0.1$, and had a two-photon absorption cross-section of $28 \pm 5 \text{ GM}$. Thus, **1** may have potential for use as a two-photon PS in the near-IR spectral region for biomedical applications.

KEY WORDS: Singlet oxygen; photosensitizer; two photon; fluorene.

INTRODUCTION

Photosensitized generation of singlet oxygen, $^1\text{O}_2$, remains a subject of great interest for a wide variety of disciplines, ranging from polymer science to clinical medicine. The high chemical reactivity of $^1\text{O}_2$ has been utilized for the oxidation of different biological constituents with important practical applications (for example, photodynamic therapy [1,2]) and also for numerous photooxidative processes in organic chemistry [3,4]. The key role of $^1\text{O}_2$ in these photochemical processes mandates the necessity to determine the quantum yield of singlet oxygen generation (Φ_{Δ}) for different photosensitizers (PS). The PS can generate singlet oxygen under one- [5,6] or two-photon excitation [7–9] via energy transfer from its excited triplet state to triplet ground-state oxygen, $^3\text{O}_2$. Efficient photosensitizers for one-photon excitation include porphyrins [10], phthalocyanines [11], hypocrellins [12],

and other compounds. Singlet oxygen quantum yields can be determined by luminescence [13] or photochemical [14] methods.

With the advent of two-photon induced fluorescence and photochemical processes, e.g. in three-dimensional imaging, the use of more benign near-IR radiation for excitation of photosensitizers in photodynamic therapy is intriguing. In pioneering studies by Ogilby *et al.*, two-photon absorptivity and singlet oxygen quantum yields were reported for several compounds [8,9]. Data from such studies have far reaching impact ranging from photodynamic therapy to biological imaging. Thus, methods to determine $^1\text{O}_2$ quantum yields under two-photon excitation are of broad interest. We have developed two methods for singlet oxygen quantum yield determination under near-IR two-photon excitation, and present these results for a new fluorene-based photosensitizer. The main goal of this paper is to determine a value of singlet oxygen quantum yield of a new fluorene derivative, 2-(9,9-didecyl-7-nitrofluoren-2-yl)benzothiazole (**1**), under two-photon excitation. It is anticipated that the same mechanism of $^1\text{O}_2$ generation occurs under both one- and two-photon excitation. This expectation is reasonable due to the very short duration of the femtosecond laser pulse used for two-photon excitation. Thus, it can be expected

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that all photochemical processes start from the same relaxed first electronic excited state of **1**. The methods employed in this study are based on an established photochemical method involving the reaction of $^1\text{O}_2$ with a singlet oxygen-sensitive compound (quencher) [14] in combination with accurate, time-dependent spectrofluorimetric determination of quencher concentration. 1,3-Diphenylisobenzofuran (DPBF) was used as an efficient chemical quencher for the two-photon singlet oxygen quantum yield measurements.

EXPERIMENTAL SECTION

The fluorene derivative 2-(9,9-didecyl-7-nitrofluorene-2-yl)benzothiazole (**1**) was synthesized previously [15] and exhibited efficient $^1\text{O}_2$ generation ($\Phi_\Delta \approx 0.53$) [16] under one-photon excitation. The chemical structures of **1** and DPBF are presented in Fig. 1. The solvent, absolute ethanol, and DPBF were used as received. The steady-state absorption and fluorescence spectra of **1** and DPBF in ethanol were measured with Cary-3 UV-visible spectrophotometer and a PTI Quantmaster spectrofluorimeter, respectively. The fluorescence quantum yield of **1** was measured relative to Rhodamine 6G in ethanol [17]. Two-photon excitation of compound **1** was carried out with a Clark-MXR 2001 Ti:Sapphire amplified, second harmonic of erbium-doped fibre ring oscillator system (output 775 nm), pumping two optical parametric generator/amplifiers (TOPAS, Light Conversion), providing 120 fs (FWHM) pulses at a 1 kHz repetition rate with independently tunable wavelengths from 560–2100 nm and up to 20 $\mu\text{J}/\text{pulse}$. All two-photon measurements were performed using a custom quartz microcuvette having dimensions of 0.1 cm \times 0.1 cm \times 1 cm (Fig. 2), irradiating the entire volume of the cuvette simultaneously. The transverse

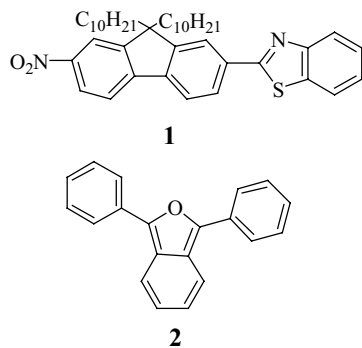
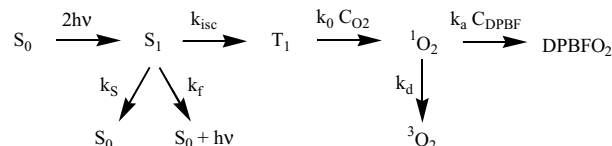


Fig. 1. Structures of 2-(9,9-didecyl-7-nitrofluorene-2-yl) benzothiazole (**1**) and 1,3-diphenylisobenzofuran (**2**).

space distribution of the laser beam intensity in the microcuvette was measured with an Electronics Micron Viewer model 7290A, and corresponded to the Gaussian distribution with correlation coefficient 85% and FWHM, $d_0 \approx 0.075$ cm. Ethanol solutions of **1** and DPBF were prepared on the day of use and stored in the dark.

Though beyond the scope of this paper to discuss mechanisms of singlet oxygen photosensitization, a simplistic scheme is presented below, while many excellent reports on the subject have been reported [18–24]:



where S_0 , S_1 , T_1 are the ground, first excited, and lowest triplet electronic states of PS (compound **1**), respectively; k_s and k_f are the rate constants of radiationless and fluorescence transitions of PS; k_{isc} corresponds to the intersystem crossing rate constant; k_0 is the rate constant of energy transfer from T_1 state of PS to molecular oxygen $^3\text{O}_2$; C_{O_2} and C_{DPBF} are the concentrations of $^3\text{O}_2$ and DPBF, respectively; k_a is the rate constant of the reaction of $^1\text{O}_2$ with DPBF, resulting in the production of the photoproduct DPBFO₂; k_d corresponds to the rate constant for the decay of $^1\text{O}_2$. The values of C_{DPBF} were chosen in the range 10^{-4} – 10^{-6} M. It is anticipated that the same mechanism of $^1\text{O}_2$ sensitization occurs under both one- and two-photon excitation, particularly considering the very short duration of the laser pulse (≈ 120 fs) used for two-photon excitation (minimizing the likelihood of any other reactions [18]), an aspect to be demonstrated herein.

The DPBF-photooxidative product quantum yield, Φ_{DPBFO_2} , and singlet oxygen quantum yield of the PS, Φ_Δ , are related according to [18]

$$1/\Phi_{\text{DPBFO}_2} = 1/\Phi_\Delta + (1/\Phi_\Delta)(\beta/C_{\text{DPBF}}), \quad (1)$$

where $\beta = k_d/k_a$ is constant for the DPBF decomposition in certain solvent [18]. Equation (1) was used to obtain the value of Φ_Δ and β from the Stern–Volmer plot, $(1/\Phi_{\text{DPBFO}_2})$ versus $(1/C_{\text{DPBF}})$.

The photochemical quantum yield, Φ_{DPBFO_2} , was determined as

$$\Phi_{\text{DPBFO}_2} = \Delta N/\Delta Q, \quad (2)$$

where ΔN is the number of decomposed molecules of DPBF during the unit time of irradiation and ΔQ is the corresponding number of absorbed photons. In order to determine ΔN , we used the fluorescence method previously described [25] based on the detection of

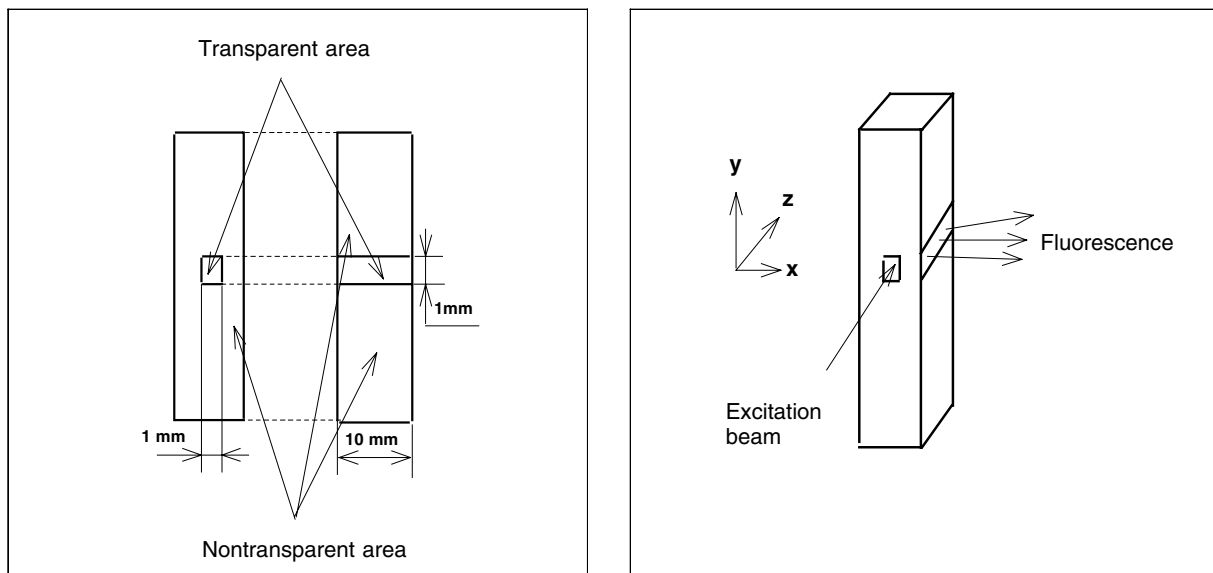


Fig. 2. Illustration of the fluorescence microcuvette.

the fluorescence intensity of DPBF under two-photon excitation. For this method we assume the following:

1. Laser pulse irradiance has a Gaussian distribution in space and time

$$I(t, x, y) = I_0 \exp[-\alpha t^2 - \gamma(x^2 + y^2)], \quad (3)$$

where I_0 is the maximum pulse irradiance; $\alpha = \ln 2/(\tau_p/2)^2$, $\gamma = \ln 2/(d_0/2)^2$, $\tau_p = 120$ fs and $d_0 = 0.075$ cm are the pulse duration and size of the beam (FWHM), respectively;

2. the entire volume of the microcuvette was simultaneously irradiated and $I(t, x, y) \approx$ constant in the z -direction in the microcuvette (see Fig. 2);
3. the concentration of PS (fluorene **1**) in solution remains approximately constant under two-photon excitation and the changes in DPBF concentration do not exceed 10%;
4. the reabsorption of the DPBF fluorescence in x -direction in the microcuvette (1 mm pass) is negligible (absorbance of DPBF in this direction, $A \leq 0.1$); and
5. the absorbance and fluorescence of the photochemical products of DPBF are negligible in the spectral region of DPBF fluorescence.

Under these conditions, the number of photoreacted DPBF molecules per unit time, ΔN , can be expressed as [25]

$$\Delta N = C_{\text{DPBF}} L S_0 [\Delta F / (F t_{\text{irr}})], \quad (4)$$

where L and S_0 are the length and cross-section of the microcuvette, respectively; $\Delta F / (F t_{\text{irr}})$ is the relative change in the fluorescence intensity of DPBF, F , per unit time; and t_{irr} is the irradiation time (concentration, C_{DPBF} , in cm^{-3}).

The number of photons absorbed in the microcuvette during one pulse of two-photon excitation, Q_P , will be equal to twice the number of PS molecules excited to the first electronic state, S_1 , and can be written as

$$Q_P = 2L \int_{-x_0}^{x_0} \int_{-y_0}^{y_0} C_1(\tau_p, x, y) dx dy, \quad (5)$$

Where $2x_0 = 2y_0 = 0.1$ cm are the x - and y -dimensions of microcuvette, and $C_1(\tau_p, x, y)$ is the concentration of PS molecules at the first excited electronic state, S_1 , after one pulse of irradiation. The value of $C_1(\tau_p, x, y)$ can be expressed as [25]

$$C_1(\tau_p, x, y) = C_{\text{PS}} \left\{ 1 - \exp \left[-\sigma_{\text{TPA}} \int_{-\infty}^{+\infty} I^2(t, x, y) dt \right] \right\}, \quad (6)$$

where C_{PS} is the total concentration of the PS molecules in the solution, and σ_{TPA} is the two-photon absorption cross-section of PS. The substitution of Eq. (3) into Eq. (6) gives

$$C_1(\tau_p, x, y) = C_{\text{PS}} \left\{ 1 - \exp \left[-\sigma_{\text{TPA}} I_0^2 \eta \exp[-2\gamma(x^2 + y^2)] \right] \right\}, \quad (7)$$

where $\eta = \int_{-\infty}^{+\infty} \exp(-2\alpha t^2) dt$. For $\tau_p = 120$ fs, $\eta \approx 9 \times 10^{-14}$ s. In the case $\sigma_{\text{TPA}} I_0^2 \eta \ll 1$, Eq. (7) can be written as

$$C_1(\tau_p, x, y) \approx C_{\text{PS}} \sigma_{\text{TPA}} I_0^2 \eta \exp[-2\gamma(x^2 + y^2)]. \quad (8)$$

For pulse excitation with repetition rate, f (in Hz), the number of absorbed photons per unit time, $\Delta Q = Q_p f$. Thus, after the substitution of Eq. (8) into Eq. (5) we obtain

$$\Delta Q \approx 2LC_{\text{PS}} \sigma_{\text{TPA}} I_0^2 \eta S f, \quad (9)$$

where $S = \int_{-x_0}^{+x_0} \int_{-y_0}^{+y_0} \exp[-2\gamma(x^2 + y^2)] dx dy \approx 2.7 \times 10^{-3} \text{ cm}^2$.

The average power of the laser beam, P_0 (in W), can be expressed through the maximum irradiance, I_0 (in photon $\text{cm}^{-2} \text{ s}^{-1}$) as

$$P_0 = (hc/\lambda) f I_0 \chi S', \quad (10)$$

where h , c , and λ are the Planck's constant, the velocity of the light, and excitation wavelength, respectively; $\chi = \int_{-\infty}^{+\infty} \exp(-\alpha t^2) dt$ and $S' = \int_{-x_0}^{+x_0} \int_{-y_0}^{+y_0} \exp[-\gamma(x^2 + y^2)] dx dy$. For $\tau_p = 120$ fs, $\chi \approx 1.28 \times 10^{-13}$ s, and for $d_0 = 0.075$ cm $S' \approx 4.5 \times 10^{-3} \text{ cm}^2$. Taking into account Eq. (10), the substitution of Eqs. (9) and (4) in Eq. (2) gives

$$\Phi_{\text{DPBFO}_2} \approx [C_{\text{DPBF}} f S_0 (\Delta F/F) \times (hc\chi S')^2] / [2t_{\text{irr}} C_{\text{PS}} \sigma_{\text{TPA}} \eta S (P_0 \lambda)^2] \quad (11)$$

Hence, from the experimental measurements of $\Delta F/(F t_{\text{irr}})$, the photochemical quantum yield of DPBF at different concentrations, C_{DPBF} , can be determined, then Φ_{Δ} and β -values can be calculated using Eq. (1).

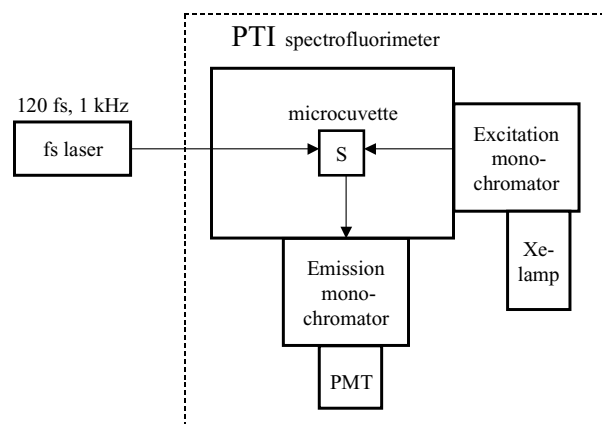


Fig. 3. Schematic diagram of the experimental setup.

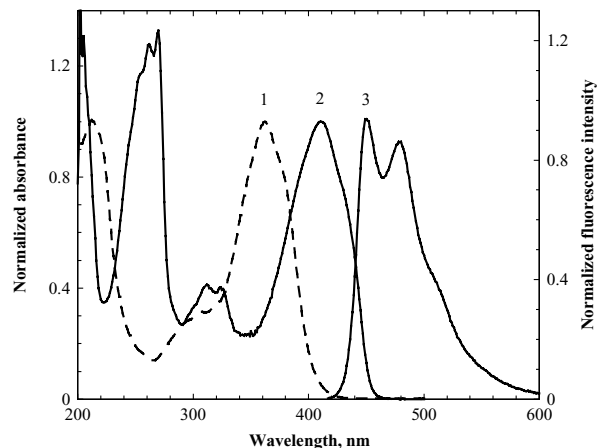


Fig. 4. Normalized absorption spectrum of fluorene **1** in ethanol (1); normalized absorption (2), and fluorescence (3) spectra of DPBF in ethanol.

The schematic diagram of the experimental setup is shown in Fig. 3. The changes in the concentration of DPBF under two-photon excitation of the PS were measured by two methods. The first method involved periodic two-photon irradiation of the solution. In this case, the excitation laser beam was blocked after a certain period of time (~ 50 s) and the fluorescence intensity of DPBF was measured under one-photon excitation using the weak Xe lamp of the spectrofluorimeter as a probe source and the photon-counting regime of the PMT. The irradiance of the Xe lamp in the microcuvette was $\leq 10 \mu\text{W}/\text{cm}^2$, too weak to cause photochemical decomposition of DPBF during the measurements. The average power of the Xe lamp was measured with an Ophir Laserstar powermeter with sensitivity in nW range. The excitation wavelengths of the Xe lamp, λ_{exc} , were varied and corresponded to the condition $A(\lambda_{\text{exc}}) \leq 0.1$. The high stability of the Xe lamp and high sensitivity of the photon-counting regime of the PMT provided accurate photochemical kinetic data.

The second method used to determine Φ_{Δ} was based on temporal measurements of up-converted fluorescence of DPBF in the analog regime of the PMT under strong two-photon excitation. In this case, the PS and DPBF simultaneously underwent two-photon excitation (due to the partial overlap of their absorption spectra (Fig. 4)). Two-photon absorption of the PS resulted in singlet oxygen generation. Weak two-photon absorption of DPBF provided up-converted fluorescence for the determination of the photochemical kinetics and did not effect the two-photon absorption of the PS. The values of σ_{TPA} were measured for PS **1** and DPBF by a z-scan method [26].

RESULTS AND DISCUSSION

The absorption spectrum of fluorene PS **1**, along with steady-state absorption and fluorescence spectra of DPBF in ethanol, are shown in Fig. 4.

The production of singlet oxygen, $^1\text{O}_2$, was performed by two-photon excitation of the PS at an energy corresponding to the absorption maximum ($\lambda_{\text{max}} \approx 360$ nm) with excitation wavelength $\lambda_{\text{exc}} \approx 720$ nm. The two-photon absorption cross-sections of PS **1** and DPBF at this wavelength were 28 ± 5 GM and ≤ 4 GM, respectively. The values of C_{PS} in all ethanol solutions were kept nearly the same $(2.5\text{--}3) \times 10^{-5}$ M and C_{DPBF} was varied over the range of $(0.05\text{--}1) \times 10^{-4}$ M. The fluorescence quantum yield of PS **1** in ethanol was $\leq 5 \times 10^{-4}$ and, therefore, its fluorescence intensity was too weak to effect the measurements of the photochemical kinetics. Different spectral positions of the DPBF fluorescence spectrum (Fig. 4, curve 3) and the absorption spectrum of PS **1** (curve 1) ruled out the direct energy transfer from DPBF to PS molecules by the Förster mechanism [27]. Under strong two-photon excitation at 720 nm, with maximum energy per pulse $E_P \approx 15$ μJ , the changes in the initial PS concentration (with DPBF) were negligible during the total irradiation time, $t_{\text{irr}} \leq 300$ s. The concentration of DPBF in microcuvette, $C_{\text{DPBF}} \leq 10^{-4}$ M, (without PS) exhibited changes corresponding to less than 5% under the same irradiation conditions (owing to its small two-photon absorption at $\lambda_{\text{exc}} \approx 720$ nm), and were taken into account. Solutions containing both the PS and DPBF led to dramatic photobleaching of DPBF (up to 80–90% of the initial concentration) under the same two-photon excitation conditions.

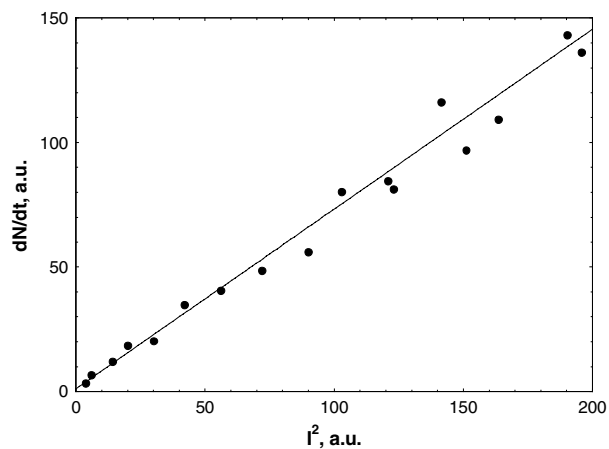


Fig. 5. Dependence of the photodecomposition rate of DPBF, dN/dt , on the square of the irradiation intensity, I^2 , at $\lambda_{\text{exc}} \approx 720$ nm.

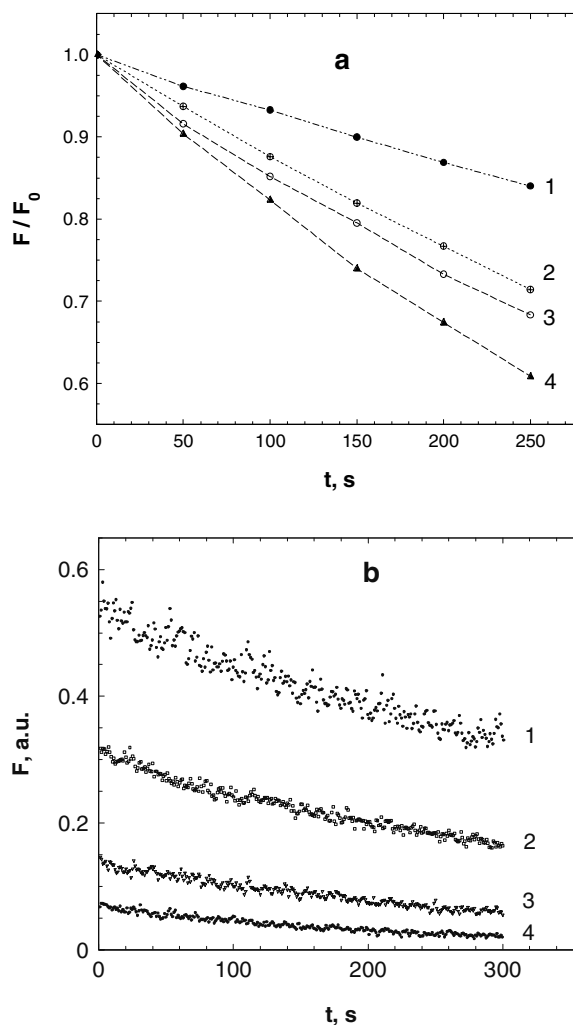


Fig. 6. (a) The temporal dependences of the fluorescence intensities of DPBF (probed by Xe lamp excitation) at different concentrations of DPBF: (1) 9.4×10^{-5} M; (2) 2.2×10^{-5} M; (3) 8.5×10^{-6} M; (4) 5.0×10^{-6} M. (b) Up-converted fluorescence intensities of DPBF ($\lambda_{\text{exc}} \approx 720$ nm, DPBF concentrations: (1) 5.0×10^{-5} M; (2) 2.2×10^{-5} M; (3) 1.2×10^{-5} M; and (4) 5.7×10^{-6} M).

The rate of photodecomposition of DPBF in ethanol with PS **1** exhibited a linear dependence on the square of two-photon irradiance (Fig. 5), characteristic of two-photon sensitization of $^1\text{O}_2$ by PS **1**.

Typical curves of the photochemical kinetic data under two-photon excitation for the DPBF solutions at different concentrations, C_{DPBF} , are shown in Fig. 6. The dependences in Fig. 6(a) were obtained using the method that employed weak one-photon Xe lamp probe excitation, as described above. The direct measurements of up-converted DPBF fluorescence intensity are presented in Fig. 6(b). From the initial slopes of these curves, the values

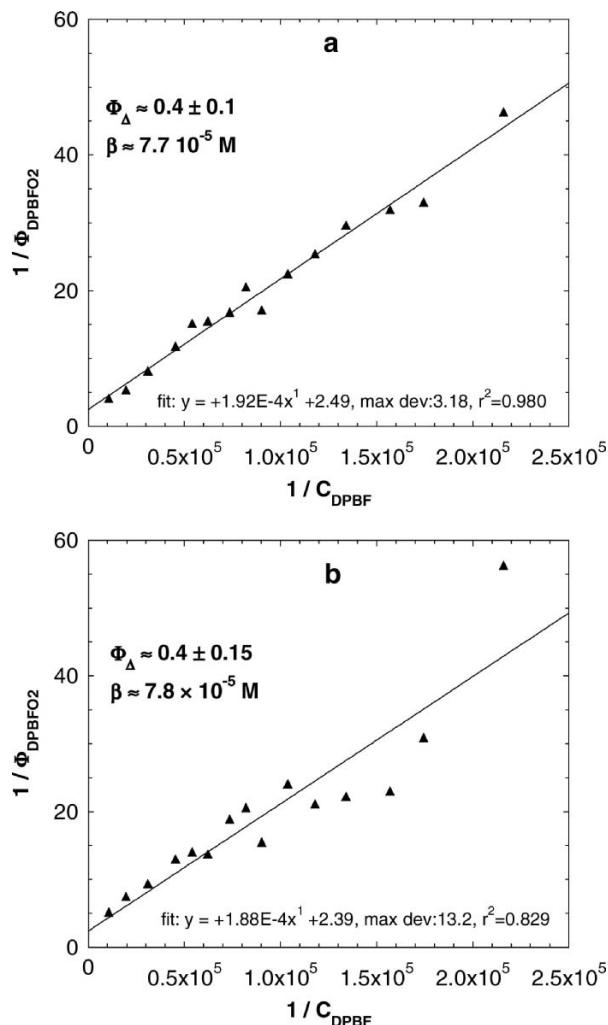


Fig. 7. Stern–Volmer plots according to Eq. (1) for the photooxidation of DPBF using two-photon excitation of fluorene **1**: (a) Xe lamp probe method; (b) two-photon up-converted fluorescence method.

for $\Delta F/(F_{t_{\text{irr}}})$ were obtained. Stern–Volmer plots for the whole range of DPBF concentrations are shown in Fig. 7.

Analysis of these data, obtained by the two different fluorescence methods, afforded the same value of singlet oxygen quantum yield, $\Phi_{\Delta} \approx 0.4$, for fluorene **1** in ethanol. It should be noted that the method with Xe lamp probe excitation afforded a more accurate value of $\Phi_{\Delta} \approx 0.4 \pm 0.1$ (as can be seen from Fig. 7), relative to the up-converted fluorescence method ($\Phi_{\Delta} \approx 0.4 \pm 0.15$). The accuracy of ± 0.1 is primarily determined by the relatively large experimental error associated with the two-photon absorption cross-section of PS **1**, $28 \pm 5 \text{ GM}$, (typical for two-photon absorption measurements [28,29]). Within experimental error, $\Phi_{\Delta} \approx 0.4 \pm 0.1$ coincides with

the corresponding singlet oxygen quantum yield determined under one-photon excitation, $\Phi_{\Delta} \approx 0.53 \pm 0.05$ [16]. The close agreement of one- and two-photon singlet oxygen quantum yield values suggests that a similar mechanism for one- and two-photon singlet oxygen generation prevail, supporting one of our assumptions.

The β -values determined from Eq. (1), $\beta \approx (7.6\text{--}7.8) \times 10^{-5} \text{ M}$, are in a good agreement with literature data [14]: Thus, fluorene **1** is a relatively efficient two-photon singlet oxygen photosensitizer.

CONCLUSION

The singlet oxygen quantum yield determination for the near-IR two-photon excitation of a fluorene-based photosensitizer has been determined using a photochemical method with DPBF as a chemical quencher of generated singlet oxygen. Two different fluorescence methods were used to obtain time-dependent DPBF concentration data during the excitation processes, consistently providing similar values of singlet oxygen quantum yields, $\Phi_{\Delta} \approx 0.4 \pm 0.1$, and reasonable β -values, $(7.6\text{--}7.8) \times 10^{-5} \text{ M}$. Fluorene **1** exhibited a relatively high singlet oxygen quantum yield, with a sufficient two-photon absorption cross-section, $\sigma_{\text{TPA}} \approx 28 \pm 5 \text{ GM}$. Thus, PS **1** is potentially valuable as an efficient two-photon photosensitizer of $^1\text{O}_2$ in the near-IR spectral region. Photodynamic therapy investigations with this and related derivatives are currently underway.

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