

Photodynamic Action in Thin Sensitized Layers: Estimating the Utilization of Light Energy

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Abstract. The result of photodynamic action significantly depends on the density of the light dose absorbed by the photosensitizer. The efficiency of using light to excite photosensitizer molecules and minimization of its loss plays an important role in ensuring the overall success of the process. When carrying out photodynamic treatment of thin sensitized layers (such as inactivation of surface pathogens or *in vitro* screening studies of photosensitizers), only a part of the light dose is absorbed in the layer, while a significant part is lost, especially at low concentrations of the photosensitizer. In this work, we evaluate the decrease in absorbed light dose depending on the extinction and concentration of the photosensitizer in a thin sensitized layer, the shape of its absorption spectrum, and the shape of the excitation light source spectrum. It was found out that a significant loss of the absorbed dose occurs upon excitation of photosensitizers, especially with low extinction, when using light sources with a broad emission spectrum. This loss must be taken into consideration when predicting the results of photodynamic exposure and optimizing its tactics. © 2021 Journal of Biomedical Photonics & Engineering.

Keywords: photodynamic action; relative absorbed photodynamic dose; laser diode; photosensitizer.

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1 Introduction

Photodynamic action (PDA) is widely used for the therapy of neoplasms [1, 2], infected wounds and other pathological foci of various etiologies [3–5], disinfection of biological fluids, blood components, and water [6]. The photodynamic effect is the photooxidative destruction of molecular structures that ensure the functioning of tumor cells and pathogens (bacteria,

viruses) by reactive oxygen species (ROS). Molecules of a photosensitizer (PS) that sensitize the environment in the affected area catalyze the generation of ROS in their vicinity upon excitation with light. The light transfers the energy necessary for this process to the medium containing the photosensitizer. The effect of PDA increases with the volume density of the energy absorbed by PS, which depends on the light intensity, the concentration and extinction of PS.

Light-emitting diodes (LED) and lasers, primarily on laser diodes (LD), are widely used as light sources for PDA. These light sources provide radiation with high intensity, and their emission spectrum is narrower ($\Delta\lambda_{LED} = 20 - 30$ nm, $\Delta\lambda_{LD} = 4$ nm) than the absorption bands of PS. Such light sources can be created, in general, with practically any wavelength (at least in the red and near-infrared spectral ranges) suitable for the excitation of PS. Broadband light sources with a spectral band selected by a bandpass filter in the PS absorption region can also be used for the excitation of PSs [7]. However, only the part of the light energy that lies in the spectral region of the PS absorption band is absorbed. The rest of the light passes through the sensitized medium.

Many PDA problems require the PS excitation in thin (≤ 5 mm) layers of sensitized aqueous compositions. Such problems include photodynamic inactivation of pathogens – viruses or bacteria, on various surfaces [8, 9], *in vitro* screening studies of PS properties [10, 11]. For small layer thicknesses and low (less than 10 μ M) PS concentrations used for such a PDA, a significant part of the light in the spectral range of the PS absorption band can pass through the layer without participating in the PDA process. This loss, which can be described as a decrease of relative absorbed photodynamic dose (RAPD) [12], results in decreased PDA efficiency. It can also lead to errors in screening studies of PSs with various extinction and concentration since both the losses and the amount of the absorbed energy in the sensitized medium for different PSs will be different.

2 Materials and Methods

In this work, such an assessment was carried out for aqueous solutions of cationic PS, which are distinguished by high values of extinction and quantum yield of ROS generation: methylene blue, $C_{16}H_{18}ClN_3S$ ($\epsilon_{664} = 0.61 \times 10^5$ $M^{-1}cm^{-1}$); zinc octakis(cholanyl)phthalocyanine, $ZnPcChol_8$ ($\epsilon_{682} = 1.7 \times 10^5$ $M^{-1}cm^{-1}$); meso-tetrakis[1-(2'-bromoethyl)-3-pyridyl]-bacteriochlorin tetra-bromide, $(3-PyBrE)_4BCBr_4$ ($\epsilon_{762} = 1 \times 10^5$ $M^{-1}cm^{-1}$). These PS do not aggregate in a wide range of concentrations and are promising both for photodynamic inactivation of pathogens [6, 8, 9, 13] and for antitumor photodynamic therapy [10, 14, 15]. Fig. 1 shows the normalized spectral contours $A(\lambda)$ of the absorption bands of these PSs (the absorption spectrum at low concentration divided by its maximum value) and the normalized emission spectra $I_{norm}(\lambda)$ of LEDs (mLED-664, mLED-684, mLED-763, Biospec, Russia), which can be used to excite these PSs (light intensity spectral density divided by the integral intensity).

The estimates of the RAPD in a non-scattering layer with the thickness L , obtained in the approximation that the spectral characteristics of PS absorption are independent of its concentration, lead to the following relation characterizing RAPD parameter η , equal to a ratio between the light energy absorbed in the layer and

total light energy illuminating the layer during the PDA process:

$$\eta = 1 - \frac{\int_{\lambda_1}^{\lambda_2} I(\lambda) \times e^{-2.3 D(\lambda)} d\lambda}{\int_{\lambda_1}^{\lambda_2} I(\lambda) d\lambda}, \quad (1)$$

where

$$D(\lambda) = \epsilon A(\lambda) CL. \quad (2)$$

Here $D(\lambda)$ is the optical density of the layer containing PS, ϵ and C – extinction and molar concentration of PS, λ_1 and λ_2 are the boundaries of the spectral range in which the spectrum of the radiation source lies, $I(\lambda)$ is the spectral density of the light intensity.

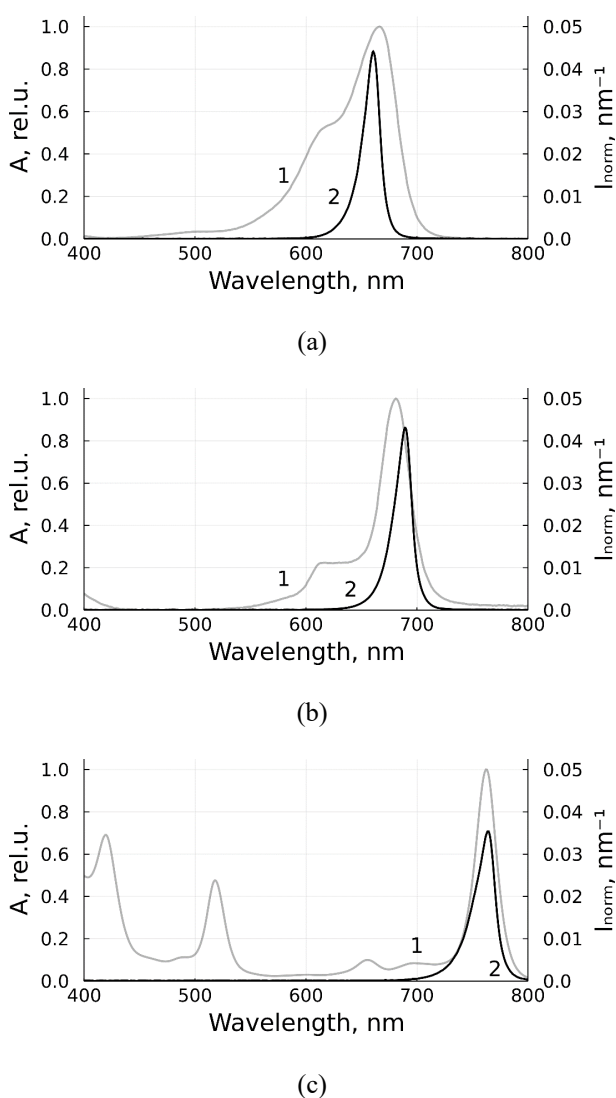


Fig. 1 Normalized spectral contours (1) of the absorption bands of photosensitizers (a – $C_{16}H_{18}ClN_3S$, b – $ZnPcChol_8$, c – $(3-PyBrE)_4BCBr_4$) and normalized emission spectra (2) of LEDs for their excitation.

For a broadband light source with a bandpass filter that illuminates in the range from λ_1 to λ_2 , the Eq. (1) becomes

$$\eta = 1 - \frac{\int_{\lambda_1}^{\lambda_2} e^{-2.3 D(\lambda)} d\lambda}{\lambda_2 - \lambda_1}. \quad (3)$$

For a monochromatic or narrow-band source, the spectral half-width of which is much smaller than the absorption spectrum half-width, the Eq. (1) is simplified to

$$\eta = 1 - e^{-2.3 D(\lambda)}. \quad (4)$$

3 Results

The estimates obtained using these formulas show that when thin layers of the studied PS with a concentration

below 10 μM are irradiated, a significant part of the light energy is not absorbed. Instead, it passes through the sensitized layer. RAPD depends on the PS's extinction, its concentration, the spectral shapes of the PS's absorption and the source emission (Figs. 2, 3). The highest values of RAPD are achieved upon monochromatic excitation at a wavelength coinciding with the spectral absorption maximum of the PS. When using LEDs with optimally selected radiation wavelengths, the difference in the value of RAPD is small. However, when using a source whose radiation wavelength is noticeably different from the wavelength of the spectral maximum of the absorption band of the PS (even within the boundaries of the band), RAPD decreases significantly, especially for PS with a narrow absorption band. RAPD of broadband sources with transmission bandpass filters in thin layers is much lower. The values of RAPD for light from such sources can be 1.5–2 times lower than from narrow-band sources.

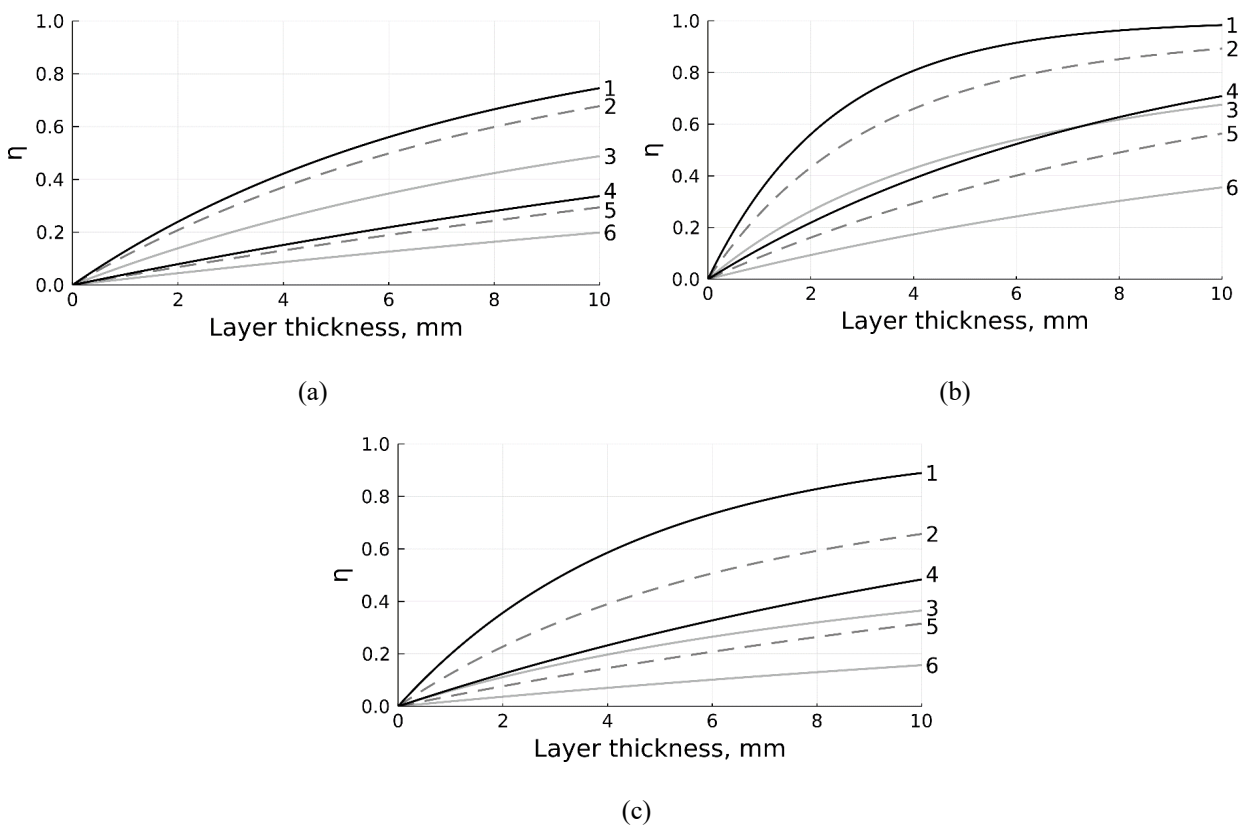


Fig. 2 Dependence on the layer thickness of RAPD for sources with the wavelength of the maximum radiation coinciding with the wavelength of the spectral maximum of absorption of PS with two concentrations: (a) $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S}$, (b) ZnPcChol_8 ; (c) $(3\text{-PyBrE})_4\text{BCBr}_4$; 1, 2, 3 – concentration of 10 μM , 4, 5, 6 – concentration of 3 μM ; 1, 4 – laser; 2, 5 – LED; 3, 6 – broadband radiation source with a bandwidth of 100 nm.

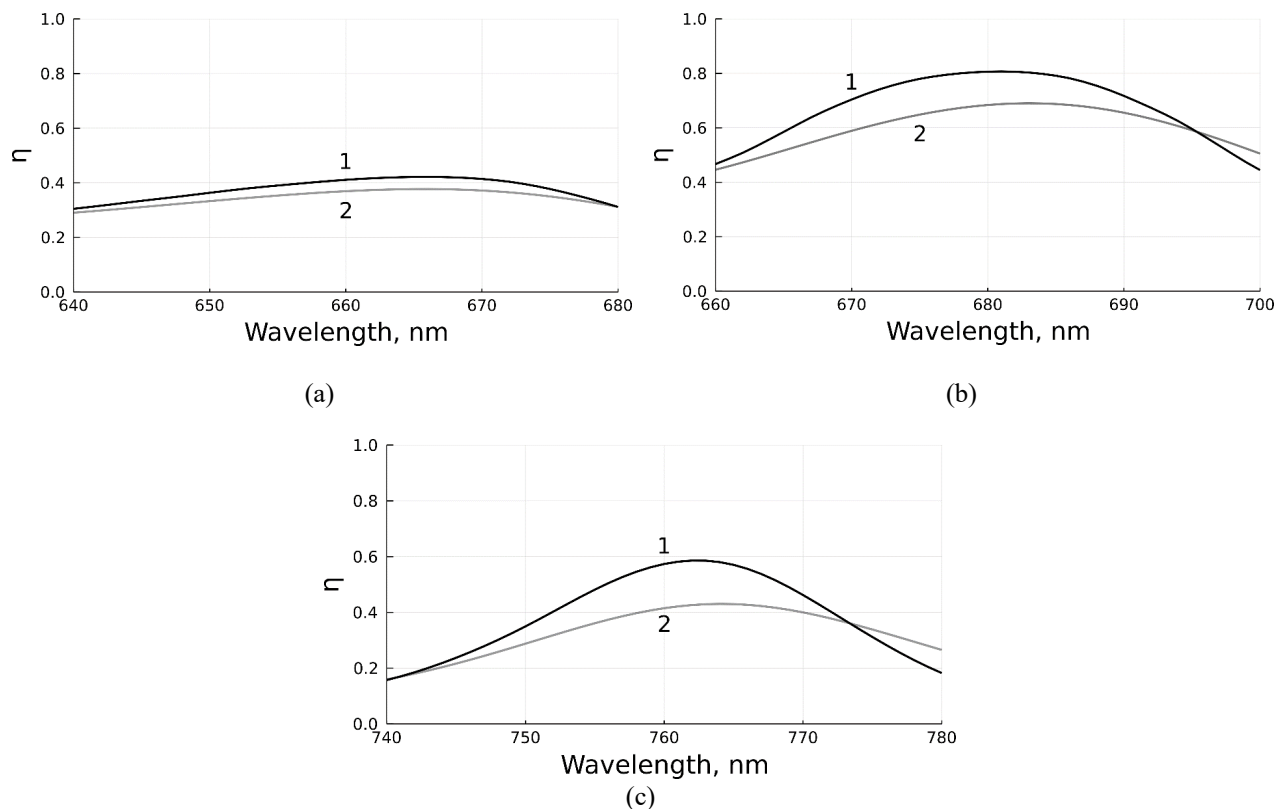


Fig. 3 RAPD of the sensitized PS layer with a thickness of 5 mm depending on the wavelength of the spectral maximum of the light source (1 – laser, 2 – LED). PS concentration is 10 μM : (a) $\text{C}_{16}\text{H}_{18}\text{ClN}_3\text{S}$, (b) ZnPcChol_8 ; (c) $(3\text{-PyBrE})_4\text{BCBr}_4$.

4 Conclusions

The estimates demonstrate that during PDA in thin sensitized layers, a significant part of the exciting light leaves the layer without being absorbed, and RAPD depends on the layer thickness, PS extinction and concentration, as well as the shape of the exciting light and absorption spectral bands of PS. The loss of a part of the light energy reduces the efficiency of the PDT as a whole. This loss must be taken into account when predicting PDA results and optimizing its tactics, particularly when choosing light sources and parameters of irradiation for photodynamic disinfection of surfaces from viral and bacterial infections [2, 5], or clinical photodynamic treatment to local virus-infected foci [5, 6]. The obtained results are also important for *in vitro* studies and comparing the photodynamic efficiency of PSs [10, 14] since the values of RAPD are very different for PSs with different extinction, especially when using broadband light sources. However, it should be noted that for *in vivo* studies of antitumor PDA, it becomes necessary to consider the scattering properties

of the medium for evaluating RAPD. Photodynamic effectiveness also becomes more dependent not only on RAPD but on photochemical and photobiological factors as well. High absorption and scattering would also result in greater depth inhomogeneity of PDA in thick sensitized layers or solid tumors, reducing the overall effectiveness of the treatment.

Disclosures

All authors declare that there is no conflict of interests in this paper.

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