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Curcuma Longa Extract as a Sensitizer for Singlet Oxygen Generation

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Abstract

In this work, the spectral study of aqueous extract of Curcuma Longa (Turmeric) to determine the photodynamic properties. It is established that due to the absorption extract light of wavelength 400-450 nm and intensive fluorescence in the red region of the spectrum, this extract can be used as a sensitizer of singlet oxygen ($^1\Delta_g$) at excitation wavelength 430 nm when the source photoexcitation in a pulsed mode. According to a kinetic study of the lifetime of emission of oxygen in $^1\Delta_g$ state is 0,043 ms. The work was carried out research on toxicity of Curcuma Longa extract against human breast cancer cells.

Keywords: singlet oxygen, lifetime fluorescence, sensitizer, Curcuma Longa, extract

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1. Introduction

Photodynamic therapy (PDT) is a promising procedure for cancer treatment applying red light on the aerobic cancer cells [1, 2]. PDT employs three components: photon of red light (600-800 nm), molecular oxygen and a photosensitizer. An important role is played by a photon that activates oxygen, turning it into a strongly oxidizing agent and, thereby, initiating oxidative stress inside the target cell (for example, a cancer cell). Energy levels of the excited singlet oxygen states are 22.5 and 37.5 kcal/mol, while the red light energy (700 nm) is 2 eV or 46 kcal / mol. Therefore, the PDT is realized when the target cells are illuminated with red light leading to the formation of $^1O_2$, responsible for 90% of the cell damage caused by oxygen activation.

Another problem of the PDT is that both participants of this event are extremely small, the cross section (probability) of collision is negligible. To increase it, a chemical dye of a large size is used, which has levels of excitation of an electron of the outer orbitals close in energy to those of molecular oxygen.

The process of interaction of the triplet states of the sensitizer with triplet oxygen causes a number of chemical processes and leads to the formation of reactive oxygen species[3, 4].

Thus a Singlet oxygen playing a main role in the process of photodynamic therapy, is hard register, because its generation requires certain physico-chemical conditions and specialized optical detection system of luminescence at the wavelength of radiation of singlet oxygen (1272 nm) [5, 6]. At this time, scientists around of the world are working with problems of singlet oxygen generation in different media and continue to look for optimal non-toxic sensitizers [7-10].

In the present work, we develop a search for new photosensitizers for PDT based on extracts from plants. The goal is to expand the class of photosensitizers to replace existing synthetic drugs with natural ones, improve their quality and reduce the cost of PDT. One of the main selection criteria is the candidate absorption spectrum with peaks in the red spectral region (600-800 nm). Here we present the result obtained with a water extract from Curcuma. The turmeric (Curcuma longa), also known as curcuma, turmeric, sun-root, saffron, safflower and yellow ginger, is a herbaceous plant of the ginger family (Zingiberaceae) originating in Asia (India and Indonesia). From its dried and ground root the powder is extracted used as condiment in the cooking and the preparation of medicines.

Turmeric - Long is a medicinal plant that can prevent and cure more than 175 diseases. This plant is the most studied of all time [11, 12]. And yet, although it has been shown to have therapeutic value in more than 500 disease states in animals and in vitro, it still has little clinical study in humans.

2. Materials and Methods

The extracts of Curcuma Longa were prepared by dissolving 0.0066 g of the powder extract in 10 ml distilled water. The absorption spectra of extracts solution were recorded on spectrophotometer (Shimadzu, Japan). The fluorescence steady-state and time-resolved measurements were carried out with Fluorolog-3 optical set (Horiba, Japan-France). This device is equipped with two detectors for working in visible and infrared regions.The radiation source of this device is a Xe lamp. The picosecond diodes (NanoLed) wavelength λ = 405 nm was used to record the extract fluorescence lifetimes in visible region. To record spectrum and the emission lifetime of the singlet oxygen in Curcuma Longa aqueous solution was used pulsed Xe lamp as a source and a cooled Solid State IR-detector with a PS/TC-1 controller.

3. Results and discussion

The optical properties of aqueous extract in the first part of the experiment was investigated. A water extract from the dried roots of turmeric growing in the Amazon was prepared and the UV-Visible spectrum studied. The results of absorbance spectrum is shown in Fig. 1a, indicate that, according to the absorption criterion in the red region of the spectrum, this extract may be a candidate for photosensitizers for PDT. This is evidenced by the presence of a peak near 660 nm. Fluorescence spectra of aqueous extract are presented in Fig.1b.

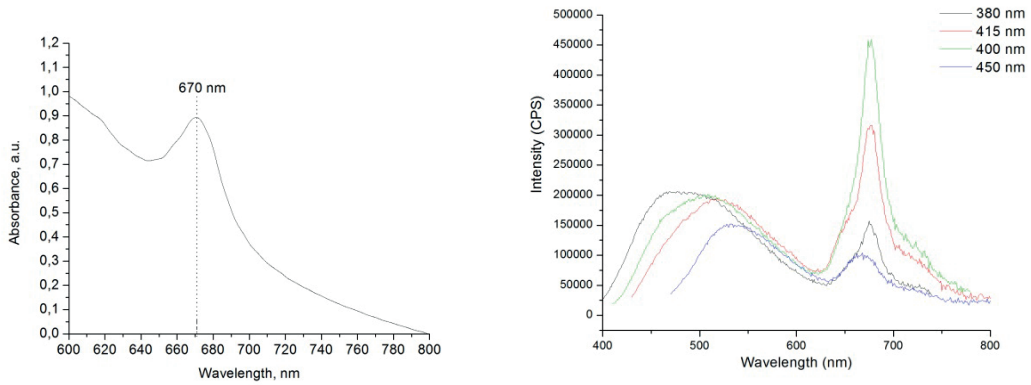

Figure 1: Absorbance spectrum (a) and fluorescence spectra (b) of aqueous extract from the roots of Curcuma Longa.

According to Fig.1b the fluorescence spectra of Curcuma Longa are containing two peaks. The location of the first peak depends on excitation wavelength. The location of second maximum is λ = 677 nm. The most intensive peak is under λ = 400 nm excitation. With increasing of the excitation wavelength the first maximum is shifted to the longwave region. The location of second maximum is λ = 677 nm. The most intensity peak according to excitation wavelength λ = 400 nm. Further, the lifetimes of the fluorescence at wavelengths of peaks at the excitation by picosecond NanoLed-405nm were measured. Kinetic curves shown on Figure 2.

There were calculated a fluorescence kinetics decay curves and average lifetimes (eq. 2) to get some knowledge about possibility of singlet oxygen generation in Curcuma solution. The biexponential model was used for experimental curves:

$$I(t) = A + B_1 \cdot \exp(t/T_1) + B_2 \cdot \exp(t/T_2) \quad (1)$$

$$< t >= \frac{B_1 \cdot T_1 + B_2 \cdot T_2}{B_1 + B_2}; \quad (2)$$

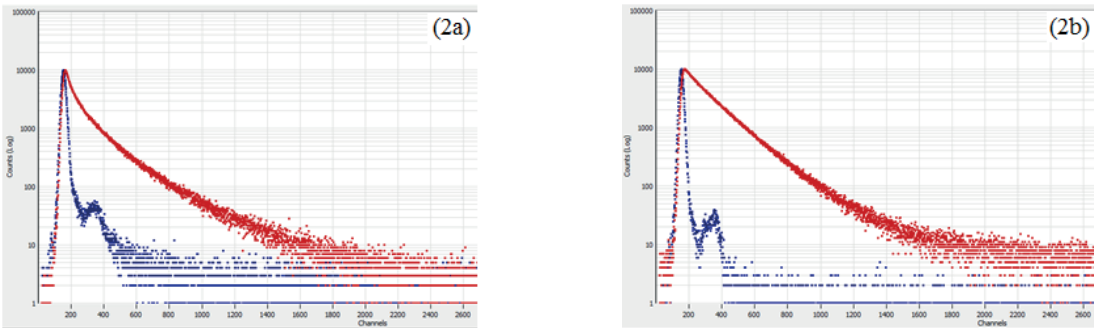

Figure 2: Fluorescence kinetics decay of Curcuma molecules water solution at wavelengths 500 nm (a) and 677 nm (b) detection.

where A, B_1, B_2 – kinetic parameters.

The results of kinetic measurements were presented in Table 1.

Table 1: Approximation parameters of fluorescence kinetics decay of Curcuma as biexponential function and average lifetime of fast fluorescence for two detection wavelengths.

Kinetic parameters	λ_{em} = 500 nm	λ_{em} = 677 nm
T_1, ns	0.86	1.76
T_2, ns	4.96	5.03
A	17.52	29.20
B_1	0.05	0.03
B_2	0.01	0.02
<t>, ns	1.54	3.07

We also observed delay fluorescence spectrum on λ = 680 nm at excitation wavelength 430 nm.This fact shows that there are a triplet states in extract molecule. Due to the presence of long-lived States of Curcuma extract in the red region of the spectrum, experiments were conducted on the generation of singlet oxygen at 1272 nm.

The luminescence spectrum of singlet oxygen under the pulsed excitation wavelength λ = 430 nm was detected from the extract solution (Fig.3).

Singlet oxygen deactivation time was calculated according to the expression:

$$y = A_1 \cdot e^{-x/t} + y_0; \quad (3)$$

where parameters A_1, x, y *and* y_0 could be determined from experimental data (Fig. 3, insert).

According to calculated kinetics data of oxygen emission at wavelength 1272 nm, oxygen $^1\Delta_g$ lifetime turned out to be t = (0.030±0.002) ms.

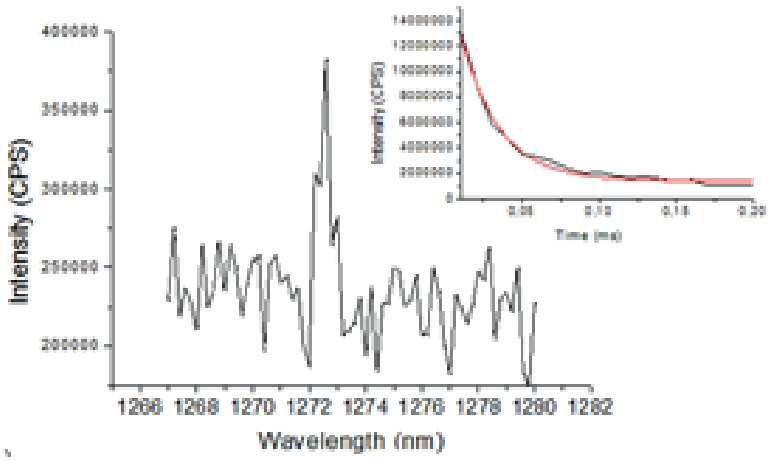

Figure 3: The emission spectrum of singlet oxygen in $^1\Delta_g$ state on the wavelength of ≈1272 nm under pulsed photoexcitation λ = 430 nm. The deactivation kinetics of the luminescence of singlet oxygen is presented in the inset: Black curve – experimental data; red curve – exponential approximation curve.

Further studies have been conducted on the toxicity of this extract against some malignant tumors. The first tests for anti-cancer activity were performed with human breast cancer cells of the MFC7 line [13]. The toxicity of the extract was measured by the residual survival of the cells after treatment with extract, using the MTT method [14]. The control experiment was carried out in the dark. Cells were incubated for 3 hours in the dark. Another portion of the cells was irradiated with red lamps for 5 to 60 minutes. After all the procedures, the number of surviving cells was measured by the MTT method. The results presented in Fig. 4 indicate the realization of the classical photodynamic effect: the extract studied is not toxic in the dark, but effectively kills cancer cells when irradiated with red light.

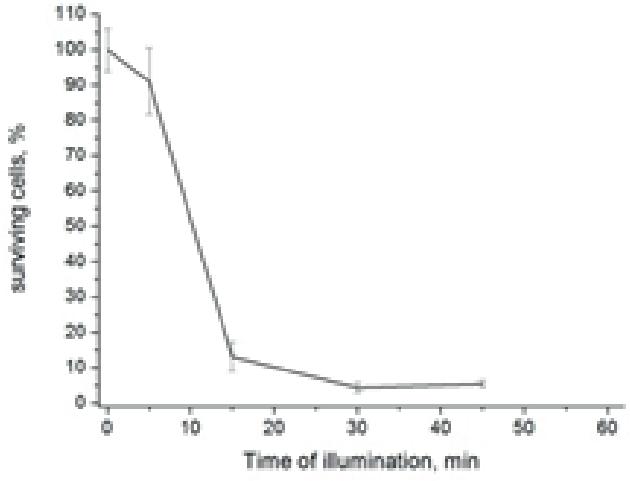

Figure 4: A test for phototoxicity of Curcuma Longa extract in relation to cells of human breast cancer. A zero point (100%) is obtained after a 3-hour incubation of cells with an extract in the dark.

4. Conclusions

This paper revealed that triplet state accumulation could be realized under pulse 430 nm excitation in curcuma longa extract solution. In addition it was measured that 677 nm fluorescence lifetime is approximately 3 ns, and it is more than 500 nm fluorescence one. It was experimentally proved that oxygen $^1\mathbf{\Delta}_g$ emission 1272 nm occurred under pulse 430 nm excitation and duration of this process is around 0,03 ms. Phototoxic tests of curcuma extract to breast cancer cells showed cancerous cells perished after 3 hours interaction with curcuma extract in the dark.

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