

## Fluorescent standards for photodynamic therapy

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**Abstract.** Photodynamic therapy is an evolving technique for treatment of various oncological diseases. This method employs photosensitizers – species that lead to death of tumor cells after the photoactivation. For further development and novel applications of photodynamic therapy new photosensitizers are required. After synthesis of a new photosensitizer it is important to know its concentration in different biological tissues after its administration and distribution. The concentration is frequently measured by the extraction method, which has some disadvantages, e.g. it requires many biological test subjects that are euthanized during the measurement. We propose to measure the photosensitizer concentration in tissue by its fluorescence. For this purpose fluorescent standards were developed. The standards are robust and simple to produce; their fluorescence signal does not change with time. The fluorescence intensity of fluorescent standards seems to depend linearly on the dye concentration. A set of standards thus allow the calibration of a spectrometer. Finally, the photosensitizer concentration can be determined by the fluorescence intensity after comparing the corresponding spectrum with spectra of the set of fluorescent standards. A biological test subject is not euthanized during this kind of experiment. We hope this more humane technique can be used in future instead of the extraction method.

### 1. Introduction

Photodynamic therapy (PDT) is a clinically approved, minimally invasive technique for cancer treatment [1]. This technique requires a photosensitizer (PS) – a compound that absorbs the light and, consequently, initiates a photophysical reaction. This reaction results in the production of highly reactive species that damage nearby cells. A photosensitizer should thus accumulate selectively in malignant cells and have an absorption band in the optical window of tissue (i.e. in the region between 600 and 1200 nm, where tissue is transparent).

However, there is not an ideal PS for PDT. For this reason chemists are searching for new PSs that have better properties. Before some newly synthesized PS is clinically approved, its properties must be investigated in numerous experiments on biological objects (e.g. mice, rats). Specifically, it is important to know the concentration of PS in various biological tissues. The concentration is conventionally measured by the extraction method, which is time demanding and requires plenty of biological test subjects.

Alternatively, the concentration of PS can be measured by its fluorescence signal, since all PSs are fluorescent species. We call this technique for the concentration measurement the fluorescent method.

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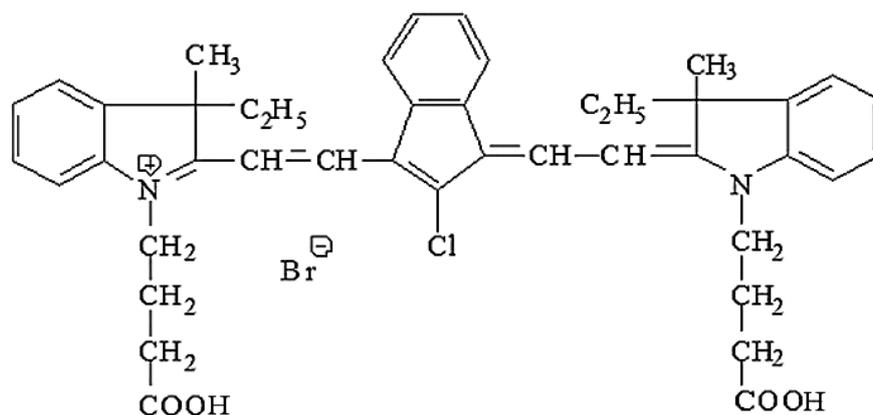
Although the fluorescence of PSs is commonly used for tumor imaging [2], exact values of the concentration are not important in this case.

In contrast, the fluorescent method allows us to determine the exact value of the PS concentration in tissue by its fluorescence, i.e. by using a spectrometer. All spectrometers are plagued by instabilities and should be calibrated before experiments. Consequently, we need objects with the fluorescence signal that does not change over a long period of time. This kind of objects we call fluorescent standards.

Our goal is to create fluorescent standards that allow us to measure the concentration of PS in biological tissue by a spectrometer (i.e. without extraction). The fluorescence of these objects is required to be similar to the fluorescence of the studied PS in tissue. In addition, fluorescent standards should be stable and simple to produce.

## 2. Methods

A tricyanocyanine dye (TD) was chosen as a fluorescent component of fluorescent standards (see the structural formula in figure 1). The dye belongs to the class of polymethine dyes, which are promising for use as PSs for PDT [3]. The fluorescence of TD can be efficiently excited at 680 nm, whereas widely used for PDT porphyrins should be excited at 630 nm [1]. TD has therefore an advantage over porphyrins when treating tumors of high thickness, since light with a longer wavelength penetrates more deeply through tissue.



**Figure 1.** The structural formula of the utilized tricyanocyanine dye.

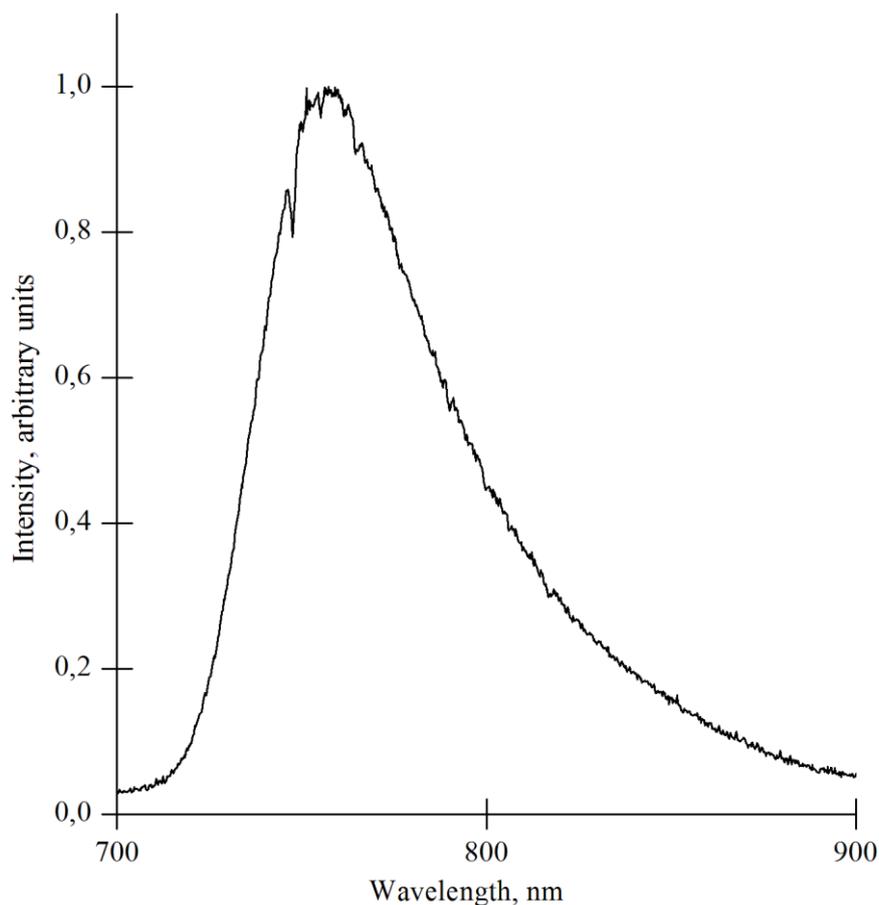
Disks of filter paper, which consists of cellulose, were chosen as a substrate for the dye. Dye solution was dropped on filter paper (the volume of droplets was 20 microliters), and then solvent evaporated at room temperature. Consequently, dye molecules were in nonpolar environment of cellulose fibers. Ethanol and acetone were used as solvents. The utilized dye is water-insoluble to prevent effects of air humidity.

The fluorescence was excited by a semiconductor laser at 680 nm corresponding to the mentioned above absorption peak of TD. To eliminate scattered laser light a colored glass filter was used when registering the fluorescence of the dye. The fluorescence signal was registered by a spectrometer with the Toshiba TCD1304A CCD linear image sensor.

## 3. Results and Discussion

The fluorescence of developed fluorescent standards can be observed in the range ca 700-900 nm, the peak of the spectrum is located at 757 nm (figure 2). The fluorescence spectrum has the same shape, when the dye concentration in the initial solution belongs to the range from  $10^{-8}$  to  $10^{-6}$  M. Lower dye concentration results in too low signal-to-noise ratio, whereas higher concentration leads to the

deformation of the fluorescence spectrum due to the reabsorption. The spectrum does not seem to change with time.



**Figure 2.** The fluorescence spectrum (normalized) of one of fluorescent standards.

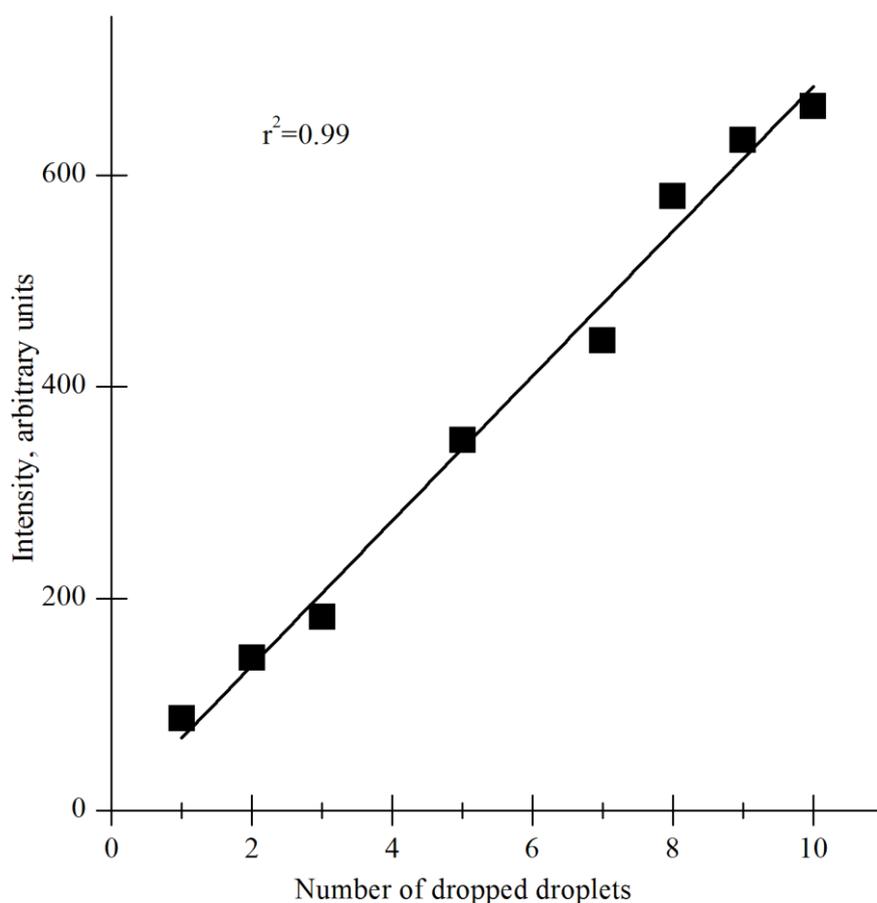
The dye is distributed over fluorescent standards uniformly after the evaporation of solvent. The standard deviation of the peak intensity in the fluorescence spectra of different points of a fluorescent standard was 5 %.

The dye concentration on fluorescent standards can be multiplied by dropping of several solution droplets (after drying of previous droplets). It means the spectrum of a fluorescent standard with  $n$  droplets of dye solution with the concentration  $c_0$  is almost identical to the spectrum of a fluorescent standard with one droplet of dye solution with the concentration  $nc_0$ . It was proved for  $n=2$ ,  $n=5$ ,  $n=10$ . For example, the maximal deviation between two corresponding spectra for  $n=2$  was only 2 %.

The fluorescence intensity of fluorescent standards appears to depend linearly on the number of dropped dye droplets (figure 3), i.e. on the concentration of TD according to the previous result. Deviations from the linear dependence can be explained by the incomplete uniformity of the dye distribution and filter paper.

We believe this result can be useful when calibrating a spectrometer. For the correctly calibrated spectrometer the dependence of the fluorescence intensity on the dye concentration must be linear. Taking this fact into account, the calibration can be conducted after measuring of this dependence by the utilized spectrometer. For convenient calibration a set of fluorescent standards with different dye concentrations is required.

The spectrum of fluorescent standards is similar to the spectra of TD in different tissues of a rat (figure 4). The TD concentration in some tissue of a living biological test subject can therefore be measured by the fluorescence signal of the dye in the tissue. To do this the corresponding spectrum is compared with the spectra of a set of fluorescent standards. In this case the biological test subject is not euthanized. The fluorescence of tissues of the living test subject can be excited and registered by optical fibers after administration and distribution of TD.



**Figure 3.** The dependence of the fluorescence intensity of fluorescent standards on the tricarboyanine dye concentration.

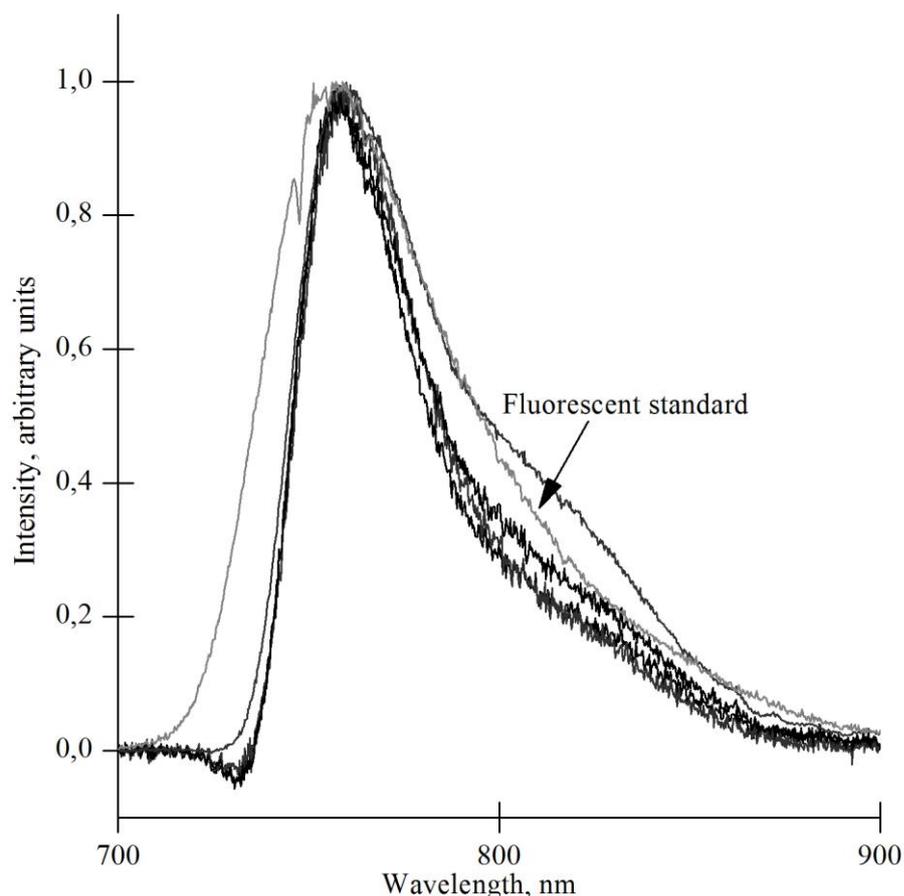
This technique for concentration measurements is significantly simpler than the conventional extraction method, it requires less time and few biological objects. Moreover, the micro-environment of dye molecules does not change during measurements, whereas it does change when using the extraction method. The extraction method has therefore a disadvantage, since the change of the micro-environment can probably bias the measured values.

#### 4. Summary

Photodynamic therapy is an efficient method for cancer treatment. A key component of photodynamic therapy is the photosensitizer. However, no photosensitizer is ideal, and new species are steadily synthesized. Properties of new photosensitizers must be carefully studied in experiments on biological object before clinical approval. In particular, it is important to know the distribution of photosensitizer in different biological tissues.

Robust, simply producible fluorescent standards for photodynamic therapy were developed. The standards can be used for the measurements of the photosensitizer concentration in various tissues by

its fluorescence, i.e. by a spectrometer. A set of fluorescent standards allows the calibration of the spectrometer, which is important, since spectrometric facilities are subject to instabilities. Consequently, the photosensitizer concentration can be determined by comparing the spectrum of the tissue with spectra of the set of fluorescent standards.



**Figure 4.** Normalized fluorescence spectra of tricarbo-cyanine dye in tissues of a rat (skin, brain, heart, liver, and kidney) and of a fluorescent standard.

This technique, i.e. the fluorescent method, is simple, not time-demanding, requires a little amount of biological objects, and thus can be efficiently used instead of the extraction method. We hope the fluorescence method will be widely employed in the future, since few biological objects are required and are not euthanized during the measurement.

#### References

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