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# Two-photon absorption cross section of aluminium phthalocyanine excited by a femtosecond Ti : sapphire laser

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*Abstract*. **The two-photon absorption (TPA) cross section is measured to be 543 ± 16 GM for aluminium phthalocyanine excited by a femtosecond Ti : sapphire laser. The TPA cross section at the Ti : sapphire laser wavelength of 800 nm exceeds by more than 40 times the TPA cross section measured earlier at the Nd : YAG laser wavelength of 1064 nm. The role of the resonance TPA enhancement caused by the presence of the real 14815-cm$^{-1}$ $S_{1u}$ level near the virtual 12594-cm$^{-1}$ level in phthalocyanine is discussed.**

***Keywords***: *aluminium phthalocyanine, two-photon absorption cross section, Ti : sapphire laser, resonance enhancement.*

## 1. Introduction

One of the main problems of laser photodynamic therapy is the transport of optical radiation to photosensitiser molecules selectively accumulated in pathologic cells. Recently, two-photon excitation of photosensitisers by near-IR laser radiation was proposed for the non-invasive transport of radiation inside biological tissues [1 – 4]. It is assumed that upon two-photon excitation of photosensitisers by IR radiation, the photodynamic effect will take place at large depths because near-IR radiation lies within the transparency window of biological tissues with low two- and three-photon absorption cross sections and can interact only with exogenous molecules with high nonlinear absorption cross sections. Such an approach was called in world practice the two-photon photodynamic therapy (PDT), which is now in a stage of pre-clinical tests.

Two-photon excitation of photosensitisers was demonstrated for hematoporphyrin derivatives [1, 2], different psoralens [3, 5], stilbene derivatives [4], protoporphyrin IX [6], aluminium phthalocyanine [7], and hypocriline [8]. The two-photon absorption (TPA) cross sections of a number of photosensitisers were measured at some wavelengths.

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We measured earlier the TPA cross section for one of the most popular photosensitisers used in clinics, aluminium phthalocyanine (Photosense). The TPA cross section measured upon excitation by 200-ns, 1064-nm pulses from a Nd : YAG laser was 12.7 ± 0.20 GM (1 GM = $10^{-50}$ cm$^4$ s phot$^{-1}$mol$^{-1}$), which is close to the TPA cross sections of hematoporphyrin derivatives and 4′-hydroxymetgyl-4,5,8′-trimethylpsoralen equal to 15 GM (at 750 nm) [2] and 20 GM (at 730 nm) [5], respectively. Molecules with the TPA cross section lower than 100 GM are not very efficient sensitisers for two-photon PDT from the point of view of excitation (the singlet-oxygen generation efficiency is not taken into account). The purposeful search for molecules with high TPA cross sections has recently resulted in the synthesis of organic molecules AF-50 (N,N-diphenyl-7-[2-(4-pyridinol) ester]-9,9-di-*n*-decyl-fluorene-2-amino) and PDHF [poly(di-hexyl)fluorene] with record TPA cross sections of 19 400 GM [9] and 20 000 GM [10], respectively. It is obvious that the higher the TPA cross section, the lower-power lasers can be used in two-photon technologies, in particular, in medicine.

Femtosecond Ti : sapphire lasers emitting at 800 nm are widely used in the last years for two-photon excitation of dyes and photosensitisers. These lasers are considered ideal radiation sources for two-photon technologies [11]. They have found applications in medicine and biology in the fields of two-photon laser confocal microscopy [12, 13] and two-photon PDT [3]. In this connection it is necessary to measure the TPA cross sections for photosensitisers at the Ti : sapphire laser wavelength.

In this paper, we measured the cross section for two-photon-excited fluorescence of aluminium phthalocyanine (a product of the TPA cross section by the quantum yield of fluorescence) excited by a 800-nm Ti : sapphire laser and calculated the TPA cross section from these measurements.

## 2. Experimental

We studied aqueous solutions of a mixture of sodium salts of sulfonated aluminium phthalocyanine from di- to tetra-substituted and its ethanol solutions with concentrations $10^{-3}$ M (two-photon excitation) and $10^{-5}$ M (one-photon excitation). Aluminium phthalocyanine was synthesised at the Research Institute of Organic Intermediates and Dyes (Moscow).

The two-photon excitation of aluminium phthalocyanine was performed by a FemtMed Ti : sapphire laser pumped by an INVERsia Ar-5-150 argon laser ('Inversion', Novosibirsk). The FemtoMed femtosecond unit was developed by 'Tekhnoskan' company (Novosibirsk) for medical and bio-

logical studies and was used in the laboratory of laser physics at the Research Institute of Physiology, Siberian Branch, Russian Academy of Medical Sciences for two-photon PDT investigations. The Ti : sapphire laser pumped by 6-W all-line radiation from the cw argon laser generated 50-fs, 800-nm pulses with a pulse repetition rate of 89 MHz.

The average power of the Ti : sapphire laser without any additional amplification was 300 – 450 mW, both in the cw and femtosecond regimes. The output power of the Ti : sapphire laser was measured with a four-probe LP-905 calorimeter (Tekhnoskan). The average output power was changed without changing the pulse duration with a Glan prism. The Ti : sapphire laser radiation was focused by a long-focus lens ($F = 140$ mm) into a 1-cm quartz cell with a sample. The focal spot diameter in the beam waist was $\sim 100$ μm

Fluorescence was detected at an angle of 90° with the help of a fibre connected with an optical analyser (Angstrom, Novosibirsk). The linear absorption spectra of solutions of aluminium phthalocyanine were recorded with and UVIKON-943 spectrophotometer (Kontron Instruments, France), and the linear fluorescence spectra with an Aminco-Bowman Series 2 spectrofluorimeter (Thermo Spectronic, USA) in the 630 – 800-nm range upon excitation at 530 nm.

Two-photon-excited fluorescence (TPF) cross sections were measured by the reference method. A quartz cell with a solution under study was placed in the focal region of a focused optical beam. In this case, the exciting radiation was transmitted through a thin layer of the solution near the front wall of the cell to minimise fluorescence self-absorption. The optical fibre with the 125-μm input aperture was led up with the help of a two-coordinate stage to the fluorescence track to detect the maximum fluorescence signal. After the detection of the TPF spectrum, the cell with the solution under study was replaced by a similar cell with a reference without changing the excitation and detection geometry. A change in the average power of exciting radiation during the measurements of fluorescence of samples and reference did not exceed 5 %.

The TPF cross section $\sigma_{TPF}$ was measured by comparing the TPF intensity ($I$) of a sample with that ($I_0$) of rhodamine 6G, whose TPF cross section is equal to the product of the TPA cross section $\sigma_{TPA}$ at a given wavelength by the quantum yield $\eta_0$ of fluorescence, and was calculated from the expression

$$\sigma_{TPF} = \frac{I\eta_0\sigma_{TPA}C_0}{I_0C},$$

where $C$ and $C_0$ are the molar concentrations of the sample and reference, respectively. All the cross sections were measured in GM. For rhodamine 6G, $\sigma_{TPA} = 134$ GM [14] and the quantum yield of fluorescence is $\eta_0 = 0.94$ [15].

## 3. Experimental results and discussion

Figure 1 shows the absorption spectrum of aluminium phthalocyanine in ethanol ($C = 10^{-5}$ M) [curve (*1*)]. The spectrum exhibits the intense 675-nm band and two lower intensity bands at 607 and 352 nm (the Soret band). The fluorescence spectrum exhibits one band at 681 nm [curve (*2*)]. The TPF spectrum consists of the 689-nm band and the lower intensity 715-nm band [curve (*3*)]. The TPF

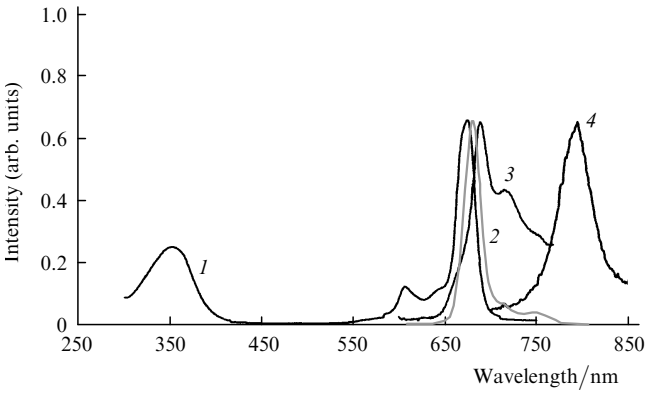


**Figure 1.** Absorption (*1*), fluorescence (*2*), and TPF (*3*) spectra of aluminium phthalocyanine and the emission spectrum of a femtosecond Ti : sapphire laser (*4*).

spectrum is shifted to the red because of reabsorption in highly concentrated solutions ($C = 10^{-3}$ M). One can see that radiation from a Ti : sapphire laser [curve (*4*)] does not fall into the absorption band of aluminium phthalocyanine and, hence, cannot produce one-photon excitation of fluorescence.

The two-photon nature of fluorescence is usually verified by studying the dependence of its intensity on the exciting-radiation intensity (power). This dependence is close to quadratic for two-photon processes. Because the generation of femtosecond radiation is very critical to the pump power, the intensity of femtosecond radiation can be varied only in a narrow dynamic range by varying the pump power.

It is inconvenient to vary the intensity of femtosecond radiation with the help of a stack of glass filters because of uncontrollable increase in the pulse duration. Since the Ti : sapphire laser radiation is linearly vertically polarised, the radiation intensity can be varied in a broad dynamic range with the help of a polariser (Glan prism).

Figure 2 shows the dependences of the average power of the Ti : sapphire laser at the output of the Glan prism on its angle of rotation for the femtosecond and cw lasing regimes. The initial power of the laser was 338 and 340 mW in the femtosecond and cw regimes, respectively. The losses in the Glan prism were 20 % and 23 % in the femtosecond and cw regimes, respectively. The difference in the losses can be probably explained by the presence of two-photon interac-

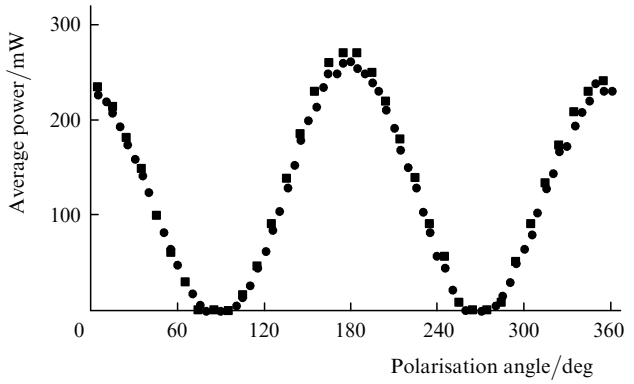


**Figure 2.** Polarisation dependences of the average output power of a Ti : sapphire laser in the cw (●) and femtosecond (■) regimes.

tion on a power meter. As the angle of rotation of the Glan prism was varied from 95° to 175°, the radiation intensity at the prism output was change almost linearly from zero to its maximum value.

Figure 3 shows the dependence of the fluorescence intensity logarithm on the average excitation power logarithm. The slope of this dependence is 1.88, reliably indicating to the two-photon excitation mechanism. (The deviation of the slope from 2 is within the measurement error of the radiation power.)

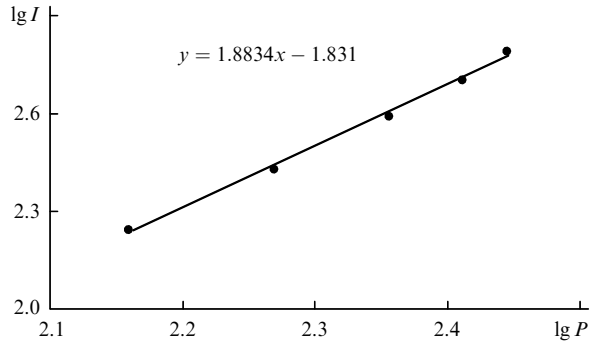


**Figure 3.** Dependence of the TPF intensity $I$ of aluminium phthalocyanine on the average excitation power $P$.

The TPF cross section for aluminium phthalocyanine in ethanol measured by the reference method is $380 \pm 11$ GM. By assuming that the quantum yield of fluorescence of aluminium phthalocyanine is 0.7 [7], we obtained the TPA cross section equal to $543 \pm 16$ GM. This value proved to be almost 43 times greater than the TPA cross section at 1064 nm [7].

A significant increase in the TPA cross section upon the blue shift from the doubled wavelength of the absorption maximum was observed for a number of dyes [16]. Thus, the absorption maximum of rhodamine B is located at 526 nm [17]. Therefore, one can expect that the TPA cross section will be maximal upon excitation at the wavelength close to the doubled wavelength of the absorption maximum, for example, by a 1064-nm Nd : YAG laser. At the same time, the TPA cross section at this wavelength is $14.3 \pm 7$ GM [18] and increases irregularly with decreasing wavelength (Table 1). The nature of the blue shift is not clear so far for most dyes.

In the case of aluminium phthalocyanine, the blue shift can be explained by the resonance TPA enhancement. It is known [20] that the resonance enhancement takes place when a real level is located near the virtual level through which TPA occurs. Two-photon excitation of phthalocyanine by a Ti : sapphire laser results in the population of its higher singlet state corresponding to the Soret band (Fig. 4). In this case, the virtual level lies by $\sim 2220$ cm$^{-1}$ ($\Delta E_1$) lower than the 16474-cm$^{-1}$ excited singlet $S_{1u}$ level.

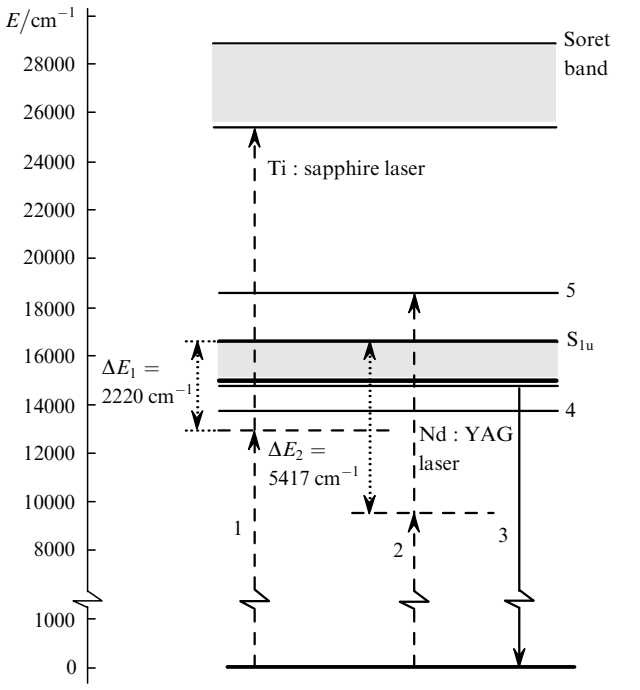


**Figure 4.** Energy level diagram and two-photon transitions in aluminium phthalocyanine: (1, 2) two-photon excitation by the Ti : sapphire laser and Nd : YAG lasers, respectively; (3) TPF; (4, 5) two-photon-transition allowed levels, which are not observed in the absorption spectrum and are excited by the Nd : YAG and Ti : sapphire lasers, respectively.

Irrespective of the excitation mechanism and whether the higher or first singlet state is excited, fluorescence is emitted from the first excited singlet state ($S_{1u} \rightarrow S_0$). However, the TPF efficiency and, therefore, its cross section is higher upon two-photon excitation of aluminium phthalocyanine to the higher singlet state.

Therefore, due to the resonance enhancement, the TPA cross section for aluminium phthalocyanine at the Ti : sapphire laser wavelength proves to be quite high ($593 \pm 16$ GM), which allows the use of this photosensitiser

**Table 1.** Blue shift of the TPA cross section for rhodamine B.

| Solvent | Concentration/M | Excitation radiation wavelength/nm | Excitation pulse duration | TPA cross section/GM | References |
|---|---|---|---|---|---|
| Methanol | $10^{-4}$ | 691 | 100 fs | $194 \pm 50$ | [19] |
| Ethanol | – | 694.3 | nanosecond pulses | $140 \pm 70$ | [18] |
| Ethanol | – | 694.3 | –"– | 170 | [17] |
| Methanol | $10^{-4}$ | 800 | 100 fs | 150 | [19] |
| Methanol | $10^{-4}$ | 840 | 100 fs | $210 \pm 55$ | [19] |
| Methanol | $10^{-4}$ | 1050 | 100 fs | $20 \pm 6$ | [19] |
| Ethanol | $10^{-2}$ | 1064 | nanosecond pulses | $14.3 \pm 7$ | [18] |
| Ethanol | $10^{-2}$ | 1064 | picosecond pulses | $12 \pm 2$ | [17] |

in the two-photon PDT in conjunction with a femtosecond Ti : sapphire laser.

The resonance TPA enhancement was also recently observed for some derivatives of azoporphyrin [21].

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