Two-photon absorption cross section for Coumarins 102, 153 and 307

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Abstract. Two-photon absorption cross sections of Coumarins 102, 153, 307 solutions in methanol in the wavelength range of 730-870 nm were measured. The maximum cross section for two-photon absorption in this wavelength range were 26±7, 36±9, 28±7 GM for Coumarins 102, 153, 307, respectively. The absolute value of the cross sections was determined by comparison with a fluorescein (43 GM at a wavelength of 790 nm). The dependence of the two-photon absorption cross section on the excitation wavelength coincides with the shape of the single-photon absorption cross section.

1. Introduction

Many novel research methods based on two-photon absorption (TPA), in particular multiphoton fluorescence microscopy, photodynamic therapy with TPA are currently being intensively developed. These methods use fluorescent probes, which are usually organic phosphors. Coumarins as a class of compounds are among the most versatile and widely used fluorescent probes. In biological preparations, coumarins show a high degree of sensitivity to the environment [1]. The photophysical properties of coumarins are well understood, however data on the TPA of coumarins are poor. The aim of this work was to study TPA of Coumarins 102, 153 and 307 in methanol solutions.

It was found in paper [2] that the shape of the TPA cross section coincides with the one-photon absorption for the Coumarin 102 solution in methanol. This effect is possible in the absence of a central symmetry in the molecule and was observed earlier for certain organic phosphors [3]. The use of this type of phosphors as standards makes it possible to simplify the relative measurements of the TPA cross section. The Coumarins 102, 153 and 307 with a single-photon absorption maximum in the 375-435 nm region were chosen as the object of measurements, since their two-photon absorption corresponds to the widely used 730-870 nm range.

2. Experimental setup

A Ti:Sa laser was used for dyes excitation. Parameters of laser radiation were: pulse width - 50 fs, repetition rate – 80 MHz, power - 0.45 W, wavelength tuning range - 730-870 nm. To adjust the intensity of the laser radiation, a λ/2 plate and a Glan-Taylor prism were used. The photodiode was used to monitor the laser intensity.

The laser beam was focused into a quartz 10x10 mm cuvette. The cuvette was placed in such a way that the distance between beam caustic and the cuvette surface did not exceed 0.5 mm to lower reabsorption. Diameter of the caustic (FWHM) was approximately 20 microns. Liquid in the cuvette was stirred vigorously to eliminate any thermal effects. Concentration of the solutions did not exceed 100µmol/L. Emission spectra were recorded using miniature spectrometer (Ocean Optics HR2000).
Halogen lamp LS-1-CAL (Ocean Optics) and deuterium lamp DH2000 (Ocean Optics) were used to calibrate the absolute spectral response of the spectral system in the 200-1100 nm range.

The following phosphors were used to prepare the solutions: Coumarin 307 (hereinafter referred to C307, C13H22NO2F3, MW = 271.24, manufactured by Exciton), Coumarin 153 (C153, C16H14NO2F3, MW = 309.29, manufactured by LambdaChrom), Coumarin 102 (C102, C16H12NO3, MW = 255.32, manufactured by Lambdachrom), Fluorescein (Fl, C26H22O3, MW = 332.31, manufactured by Sigma-Aldrich), Rhodamine B (RhB, C32H31N2O3Cl, MW = 479.02, manufactured by Exciton).

To measure the TPA cross section the two-photon-induced fluorescence method was used. The number of photons (R) emitted after the laser pulse from the volume dldxdy can be written in the form [4]:

$$ R = \frac{1}{2} \frac{1}{(h\nu)^2} N\sigma_{\text{TPF}} I^2 \tau dldxdy $$

where $I$ is the laser radiation intensity, $\tau$ is the pulse length, $h\nu$ is the quantum energy, $N$ is the concentration of molecules, $dl$ is the length of the absorbing layer, and $\sigma_{\text{TPF}}$ is the cross-section of two-photon luminescence. The last is related to cross section of two-photon absorption ($\sigma_{\text{TPA}}$) as:

$$ \sigma_{\text{TPF}} = q_2 \sigma_{\text{TPA}} $$

where $q_2$ is the quantum yield of two-photon luminescence. As a rule, the quantum yield of two-photon luminescence will coincide with the quantum yield of single-photon luminescence $q$ [4]. In the present paper we assume that $q_2 = q$. The absolute emission quantum yield was determined by comparison with the solution of Rhodamine 6G in methanol as a standard.

3. Results and discussion

Figure 1 shows the dependence of the luminescence intensity on the pump power for the investigated phosphors (the pump power square is plotted along the abscissa axis). The luminescence intensity for two-photon excitation depends quadratically on the pump power and in this scale should be expressed in direct proportionality. As a rule, deviations from the quadratic dependence are observed at a high radiation density and clearly indicate the presence of other nonlinear processes. It can be seen that deviations from the quadratic dependence are absent taking into account the experimental error.

As mentioned above, it was found in paper [2] that the shape of the TPA cross section coincides with the one-photon absorption shape for the C102 solution in methanol. We measured the shape of the TPA cross sections of solutions C307 and C153 in methanol using C102 as a reference. It was found that the shape of the TPA cross sections for C153 and C307 in the wavelength range 730-870 nm coincides with single-photon absorption cross sections within the error of the experiment. Thus, C102, C153 and C307 can be used as a reference for relative measurements of the TPA cross section in the range of 730-870 nm.

It is more convenient to carry out relative measurements of the TPA cross sections using C307 solution in methanol as a standard, since at $\lambda > 800$ nm its TPA cross section decreases more slowly upon increasing wavelength than that of C102. Figure 2 shows the relative TPA cross sections for C102 and C153 in methanol, while C307 was used as a reference. It supports that good agreement between the shape of one- and two- photon absorption cross sections takes place.
Figure 1. Dependencies of luminescence intensity on pump power. C102, C153, C307 – solution in methanol, Fl – solution in water (pH = 11), excitation wavelength 790 nm.

Figure 2. Dependencies of the single-photon absorption cross-section (line) and the TPA cross-section (symbols) on the excitation wavelength for C102, C153 solutions in methanol. Solution C307 in methanol was used as a reference.

Fluorescein and Rhodamine B were used for absolute calibration as the most studied luminescent substances. We carried out relative measurements of the TPA cross sections of an aqueous solution of fluorescein (pH = 11), as a reference we used a solution of C307 in methanol. Figure 3 presents the...
results of this work, as well as the results of [5, 6]. It can be seen that the data of [5, 6] is consistent well with our data in the regions 740-750 nm and 800-840 nm. Outside this wavelength range, the differences between the values of [5] and [6] reach 1.5 times. For absolute normalization of the data of this work, the value \( \sigma_{\text{TPA}} = 43 \text{ GM} \) was chosen at the maximum of the cross section (wavelength 790 nm). With this normalization in the spectral regions 740-750 and 820-840 nm, the results of this work are in good agreement with the results of [5, 6]. The maximum difference with the results of [5] is observed in the wavelength region 760-800 nm and does not exceed 16%, which agrees with the magnitude of the error in the work [5]. In the range 750-840 nm, the differences between the values obtained in this work and in [6] do not exceed 15%, in the 850-870 nm region our data differ from the [6] by not more than 50%. Results of earlier works can be found in [5].

**Figure 3.** Dependencies of the TPA cross section on the wavelength in water solution of Fl (pH = 11), curves 1 - this work, 2 – [6], 3 – [5];

Using the above-described normalization of the fluorescein cross-section (\( \sigma_{\text{TPA}}(790 \text{ nm}) = 43 \text{ GM} \)), the C307 cross-section at the maximum at \( \lambda = 790 \text{ nm} \) is 28±7GM. This normalization of the TPA cross section for the C307 solution in methanol was used for all subsequent measurements.

Figure 4 shows the results of measurements of the TPA cross-section in a solution of Rhodamine B in methanol obtained in this paper and in papers [5, 7]. Note that in the work [5] there is a discrepancy between the data shown in the graph and in the table, so here we give both results. It can be seen that the results of this paper are in good agreement with the data given in the graph in [5]. The maximum discrepancy is observed in the region of 770-780 nm and reaches 20%. In the region of 730-840 nm, the data of this paper are in good agreement with the work of [7]. In the 850-870 nm area, the values of work [7] are below the values of this paper and paper [5].

Thus, the TPA cross-sections of the Fluorescein and Rhodamine B measured using a solution of C307 in methanol as a standard with an absolute value 28 ± 7 GM at maximum and the shape of TPA cross-section coinciding with single-photon absorption show good agreement with the most reliable results obtained in the works [5, 7]. It should be emphasized that using the proposed calibration, the TPA cross-sections for C102, C153, RhB and Fl are smooth and do not contain local peaks of width ~ 10 nm on the wavelength dependencies. Such peaks appear, for example, on the dependences of the TPA cross sections for C102 and C153 when using the data from [5] for fluorescein as reference.
Figure 4. Dependencies of the TPA cross section on the wavelength in Rhodamine B methanol solution, curves 1 - this work, 2 – paper [7], 3 - [5] (Fig.4), 4 - [5] (Table 4).

The TPA cross sections at the maximum for solutions of C102, C153, C307, RhB in methanol and Fl in water are shown in the Table 1. The values of the quantum yields measured in the present work were used to calculate the TPA cross-section, see Table 1. The value q = 0.925 obtained in the work [8] was used for fluorescein.

| Substance | Solvent | q       | λmax, nm | σTPA (λmax), GM |
|-----------|---------|---------|----------|-----------------|
| C102      | MeOH    | 0.88±0.06 | 780      | 26±7            |
| C153      | MeOH    | 0.47±0.05 | 850      | 36±9            |
| C307      | MeOH    | 0.81±0.06 | 790      | 28±7            |
| RhB       | MeOH    | 0.64±0.05 | 840      | 210±30          |

Next, we compare the results of this paper with the previous publications for coumarins. As far as we know, the absolute value of TPA cross-section for C102 in methanol was evaluated only in one work [2]. In work [2], a comparison with bis-MSB at a wavelength of 725 nm was used to estimate the absolute value of the C102 cross-section. The TPA cross-section for bis-MSB at 725 nm was calculated by extrapolation from data from paper [9], resulting in a σTPA(λ = 725 nm) = 4.8 GM. To calculate the cross section for two-photon absorption, C102, the quantum yield of both molecules was assumed to be 1, and the spectral sensitivity of the recording system was not taken into account. As a result, for the TPA cross-section, 160 GM was obtained at the maximum (λ = 781 nm). This value is more than five times higher than value obtained in the present study. However, in paper [7] a direct measurement of the TPA cross section for bis-MSB was carried out. It was shown that σTPA = 1.0 GM at the wavelength of 725 nm. If we recalculate the results of [2] with the results of [7],
we get \( \sigma_{TPA} = 33 \) GM at \( \lambda = 780 \) nm. This value is consistent with that obtained in this paper. In paper [10], as a result of calculation, the maximum value of 21.7 GM at 725 nm was obtained, which agrees well with the data of the present work.

The two-photon emission cross sections for C307 in methanol reported in [7] is \( \sigma_{TPE} = 19.5 \) GM at \( \lambda = 776 \). Taking into account the quantum yield of 0.81, the TPA cross-section is \( \sigma_{TPA} = 24 \) GM, which coincides with the results of this paper within the experimental error. Note that in the [7], the data from the table are contradictory to the figure from the same paper. The maximum cross-section in Figure 2 in [7] is 16 GM. In the paper [10], as a result of the calculation, a value of 25.1 GM was obtained, a maximum position of 782 nm, which agrees well with our results.

We could not find the results of the measurement of TPA cross-section C153 in methanol. In the work [5], TPA cross-section was measured in a solution of C153 in CCl\(_4\). The maximum cross section of the C153 solution in CCl\(_4\) is 45 GM at a wavelength of 800 nm. Since there is significant change of the spectra of single- and two-photon absorption of C153 in methanol and CCl\(_4\), it can be assumed that there is good agreement between the results of this work and the results of [5].

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