

# Fast and slow processes of thermal deactivation of excited stilbazolium merocyanine dyes

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## Abstract

The long living triplet states play important role in sensitizing action in all photochemical reactions. The yield of generation of triplet states of dyes can be evaluated on the basis of measurements of their slow (microsecond) thermal deactivation (TD). All experiments were carried out in the oxygen presence, it means under quenching dye triplets. The pulse dye laser generates in the investigated solution pressure signal. The high of the amplitudes of first maximum of this pressure wave-form signal in the solution of the investigated dye and in the reference sample were measured. Reference sample exhibits only fast processes of TD. The comparison of the first maximum of wave-form photothermal signal of sample and of reference enable to calculate part of energy exchanged into heat in time longer than time resolution of arrangement. The fluorescence yields of investigated dyes were also established. On the basis of such data, using procedure described in literature, the yield of singlet–triplet intersystem crossing (ISC) was evaluated. It was shown that this yield depends on the length of stilbazolium merocyanine chain. The product of triplet state yield and energy was lower for merocyanines with longer chains. At lower temperatures the yield of fluorescence increases and amount of excitation exchanged in short time into heat decreases. The slow TD process increases in low temperature because of the decrease in the quenching of the dyes triplet states by oxygen. The amount of energy exchanged into heat in a time longer than time resolution of apparatus is due predominantly through TD of the dye triplet states. © 2001 Elsevier Science B.V. All rights reserved.

*Keywords:* Laser induced optoacoustic spectroscopy; Stilbazolium merocyanines; Yield of triplet states

## 1. Introduction

The triplet state lifetime is important for the efficiency of the sensitizers used in all photochemical reactions among other in photodynamic therapy of cancer. The dyes with short living triplets are not efficient in both mechanisms of sensitizing: type I by direct interaction of sensitizer in a triplet state with tissue neither by type II — production of the singlet oxygen [1–5]. The most important precursor of singlet oxygen is the triplet state of sensitizer [1,2]. Stilbazolium merocyanines can be used as the sensitizers in photodynamic therapy, [3,6–8] and as an optical probe of microenvironment in natural and model [9], membranes. First one of this applications need high yield of singlet–triplet intersystem crossing (ISC) ( $\phi_{ISC}$ ). The information about the yield of triplet state formation can be drawn from photothermal measurements [10–13]. Fig. 1 shows the Jablonski diagram in which the measured triplet thermal deactivation (TD) process is denoted as TTD. The phosphorescence (Ph) yield for investigated dyes is very low, therefore, it can be neglected. Competing with TTD delayed fluorescence (DF) is also

very low, therefore, the TTD can be taken in approximation as the yield of triplet formation by ISC. In air triplets of dye molecules are partially quenched forming triplet oxygen by triplet energy transfer to oxygen (TETO) in Fig. 1. At given condition (pressure, temperature, dye concentration) TETO process is in approximation similar for the various merocyanine dyes. Therefore, on the basis of slow photothermal signal the yield of triplet states of various dyes can be compared.

The fast processes such as fluorescence emission, prompt TD from excited singlet state ( $S_1$ ) and also fast ISC process are competing between them self. The slower processes are representing, for air saturated samples, by two competing deactivation channels due to TD in Fig. 1 and to triplet deactivation associated to dye TETO in Fig. 1. The last, sequential to TETO event, which can take part in slow TD is the heat related to decay of singlet oxygen [10]. The measurements of the high of first thermal signal maximum (related to “prompt” exchange of excitation into heat) due to pulse laser illumination of the sample and reference dye solution, as well as of the yield of fluorescence of investigated dye gives the opportunity, using methods described in [14,15], to evaluate and compare yields of the formation of the triplet states of dyes ( $\phi_{ISC}$ ).

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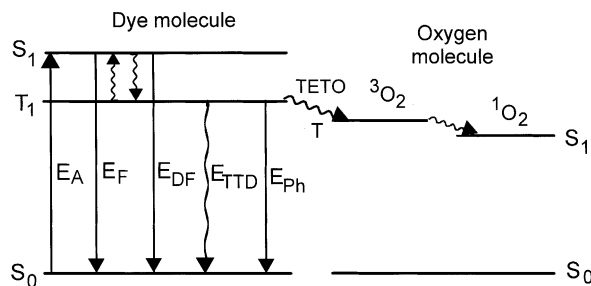


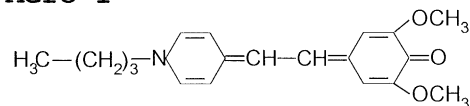
Fig. 1. Jablonski diagram.  $S_0$ : ground singlet state;  $S_1$ : excited singlet state;  $T$  and  $T_1$ : triplet states;  $E_A$ : absorption energy;  $E_F$ : fluorescence energy;  $E_{DF}$ : delayed fluorescence energy;  $E_{TTD}$ : triplet thermal deactivation;  $E_{Ph}$ : phosphorescence energy;  $TETO$ : triplet energy transfer to oxygen;  $^3O_2$  and  $^1O_2$ : oxygen molecule in triplet and in singlet state, respectively; wavy-shape lines: non radiative transitions.

In present work, such evaluation method was applied to two stilbazolium merocyanines with different length of their alkyl chains. The effect was investigated at several temperatures. All experiments were carried out in the oxygen presence. The illumination can form, in such conditions, intermediates, predominantly singlet oxygen, which can also take part in slow processes of TD.

## 2. Material and methods

The formula of investigated stilbazolium merocyanines are presented in Fig. 2. The dyes will be denoted as Mero 1 and as Mero 2, respectively. The dyes were kindly provided by Dr. I. Gruda (Université du Québec, Trois Rivières, Canada). Merocyanines were synthesized and characterized as described earlier [16–18]. As a reference dye for photothermal measurements the bromocresol purple (BCP) ( $C_{21}H_{16}Br_2O_5S$ ) from RdH Laborchemikalien (Germany) was used without further purification.

### Mero 1



### Mero 2

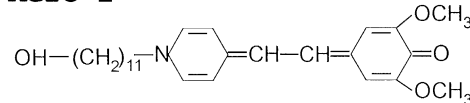


Fig. 2. Structures of merocyanines Mero 1 (1-butyl-4[(3,5 dimethoxy-4-oxocyclohexa-2,5-dienylidene)ethylidene]-1,4-dihydro pyridine) and Mero 2 (2-(11-hydroxyundecyl)-4 [(3,5-dimethoxy-4-oxocyclohexa-2,5-dienylidene)ethylidene]-1,4-dihydro pyridine).

The scheme of arrangement for time resolved photo-thermal signal measurements is shown in Fig. 3. The arrangement is typical for all laser induced optoacoustic spectroscopy (LIOAS) measurements [11,12]. Sample was illuminated by flash from dye nitrogen laser from Photon Technology International, Canada (model GL-3300/GL 301). Pulse duration was 0.2 ns. Laser light energy was registered by means of the Laser Probe INC (USA) energy radiometer model Rj 7620 with RjP-736 pyroelectric probe. The sample was located in the temperature-controlled cuvette-holder (produced by Quantum Northwest Co., USA). The piezoelectric transducer (Panametric, Model V1030), with 1 MHz frequency resolution, was attached to the cuvette wall. The signal from transducer was memorized using Gold Star model 0S3060 (60 MHz) digital oscilloscope. The time resolution of apparatus at used slit (0.8 mm) was about 0.6  $\mu$ s [11].

The absorption spectra were taken using Specord M40 spectrometer (Carl Zeiss, Jena, Germany), while the fluorescence spectra by means a spectrofluorimeter constructed in our laboratory [8].

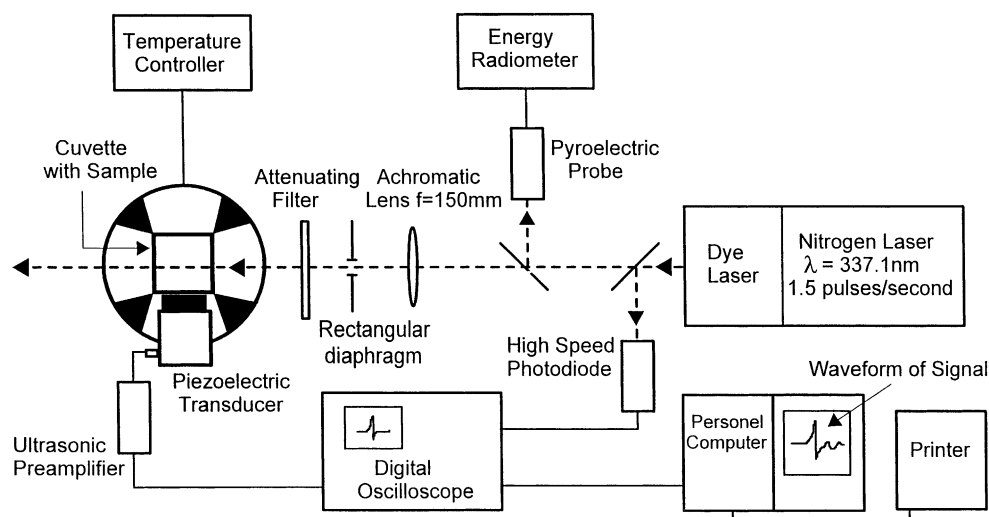


Fig. 3. Apparatus for laser induced optoacoustic spectroscopy (LIOAS).

### 3. Results and discussion

The laser energy, at applied wavelength 380nm, was calculated as  $E_{\text{las}} = hcN/\lambda$ , where  $h$  is the Planck constant,  $c$  the velocity of light,  $N$  the Avogadro number and  $\lambda$  is the wavelength of light. It was obtained  $E_{\text{las}} = 315 \text{ kJ/mol}$ .

Absorption spectra of reference BCP and investigated merocyanines dyes are shown in Fig. 4. As it follows from this figure the spectra of reference are changing with the dye concentration. These changes influence, as we have established, BCP thermal properties. For each experiment the concentrations of measured dye and reference have been chosen such that the absorptions of both dyes for wavelength of laser light (380 nm) were the same. The wave-form LIOAS signal for measured dye and reference were registered at four intensities of light — changed by proper gray filters. The examples of such signals for dye and reference are shown in Fig. 5. It is seen from Fig. 5 that the reference sample has always higher first maximum of wave-shaped curve ( $H_{\text{max}}$ , Fig. 5) then the measured samples. This maximum is predominantly responsible for excitation promptly exchanged into heat, it means exchanged in times shorter than time-resolution of our apparatus ( $0.6 \mu\text{s}$ ). As it follows from Fig. 5 the first maximum for the measured sample is shifted towards longer times with respect to the first maximum of the reference signal. It shows that merocyanines exhibit in this short time region the components slightly slower than BCP thermal decay. The wave shape signal can be analyzed on the exponential components [11,12]. We apply simpler procedure proposed in [14] and [15]. According to this procedure the high of the maximal amplitude of first signal  $H_{\text{max}}$  (Fig. 5) was taken into further elaboration. Such sig-

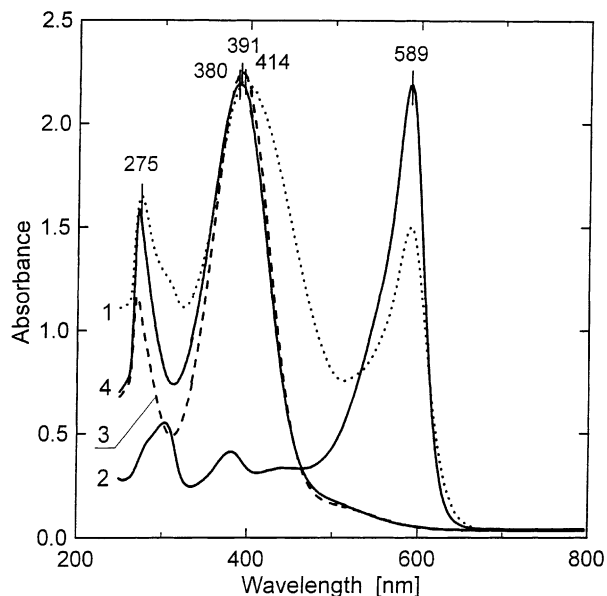


Fig. 4. Absorption spectra of dyes in phosphate buffer (pH 7.2). BCP for two concentrations  $c_1$  and  $c_2$ : curves 1 and 2, respectively; Mero 1: curve 3; Mero 2: curve 4.

nal was measured for four intensities of laser light, obtained by application of the calibrated gray filters. The dependence of  $H_{\text{max}}$  on laser light intensity ( $E_{\text{las}}$ ) was in all cases linear (Fig. 6). Such dependence was drawn for investigated dyes as well as for BCP solution. All dyes were investigated in water solutions. In the absence of photo changes in the sample volume due to its structural changes the first maximum is

$$H_{\text{max}} = k\alpha E_{\text{las}}(1 - 10^{-A}) \quad (1)$$

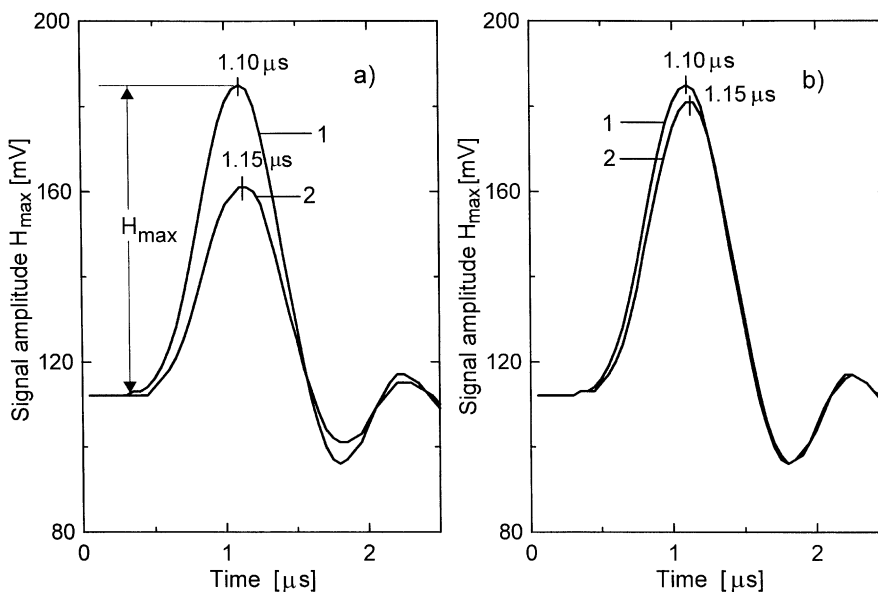


Fig. 5. The wave-shape LIOAS signals. (a) Curve 1: BCP; curve 2: Mero 1 and (b) curve 1: BCP; curve 2: Mero 2.

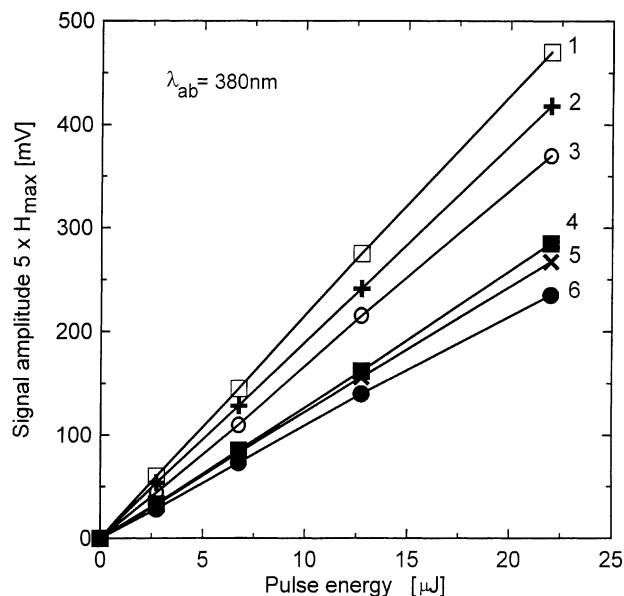


Fig. 6. First maximum ( $H_{\max}$ ) of LIOAS signal vs. laser pulse energy. Lines 1, 4: BCP; lines 2, 5: Mero 1; lines 3, 6: Mero 2. Lines 1–3: concentration  $c_1$  ( $A = 3.7$ ). Lines 4–6: concentration  $c_2$  ( $A = 2.2$ ). Both absorbance values  $A$  were measured at wavelength of laser line ( $\lambda = 380$  nm).

where  $k$  is the coefficient related to apparatus optical geometry, electronic impedance of the system and thermoelastic properties of solvent,  $\alpha$  the part of energy exchanged promptly into heat,  $A$  the absorbance of the sample at 380 nm and  $E_{\text{las}}$  is the energy of laser light measured by radiometer.

The second set of straight lines (Fig. 7) were obtained by drawing the ratio  $dH_{\max}/dE_{\text{las}}$  versus  $(1 - 10^{-A})$  where  $A$  is absorbance of two samples having two different dye

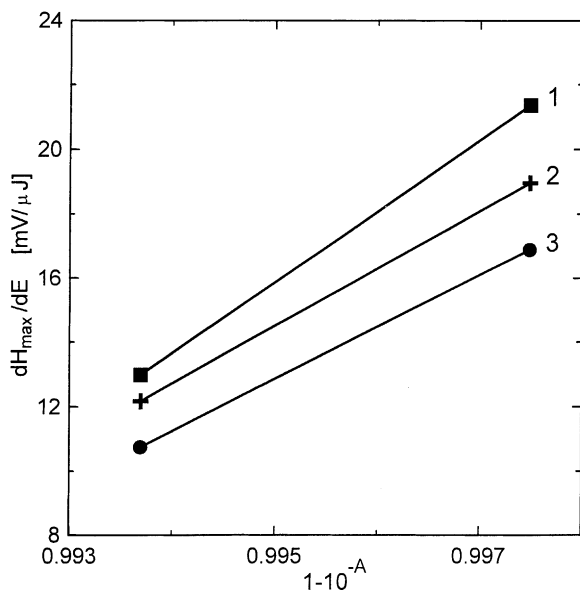


Fig. 7. The dependence of  $dH_{\max}/dE_{\text{las}}$  vs. absorption presented as  $(1 - 10^{-A})$  ( $A$ , absorbance). Curve 1: PCB; curve 2: Mero 1; curve 3: Mero 2.

Table 1

The results of calculation of the part of energies deactivated promptly ( $\alpha$ ) and the product of the yield of triplet state formation ( $\phi_T$ ) and triplet energy ( $E_T$ ) of merocyanines dyes<sup>a</sup>

Dye	BCP	Mero 1	Mero 2
$\alpha$	1	$0.71 \pm 0.07$	$0.81 \pm 0.08$
$\phi_T E_T$ (kJ/mol)	0	$92 \pm 23$ (20°C), $257 \pm 10$ (2°C)	$59 \pm 25$ (20°C)

<sup>a</sup> Measurements was done with laser light pulses at  $\lambda = 380 \pm 1$  nm (energy of  $N_A$  photons  $E_{\text{las}} = 315.0 \pm 0.9$  kJ;  $N_A$ , Avogadro number).

concentrations. In both cases the sample and reference absorptions in a region of laser light wave length ( $\lambda = 380$  nm) were the same. The reference sample was not fluorescent and exchanged into heat whole excitation energy in time shorter than time resolution of apparatus. The yield of merocyanines fluorescence was established according to the methods described in [19] using Rhodamine 6G as a reference. The prompt TD of merocyanine (occurring in time shorter than time resolution of apparatus (Fig. 3)) was denoted as  $\alpha_1$  (Table 1). It is included in Eq. (2) as

$$\phi_T E_T = (1 - \alpha_1) E_{\text{las}} - \phi_F E_F \quad (2)$$

where  $\phi_T$  and  $\phi_F$  are the yields of triplet and fluorescence,  $E_T$  and  $E_F$  the respective energies explained in Fig. 1,  $E_{\text{las}}$  was given in kJ/mol.

The merocyanines fluorescence quantum yields establish at 20°C by the comparison with Rhodamine 6G were  $0.006 \pm 0.001$  for Mero 1 and  $0.005 \pm 0.001$  for Mero 2. Fluorescence spectra of Mero 1, Mero 2 and Rhodamine 6G are shown in Fig. 8. The BCP was practically not fluorescent, merocyanines exhibit much lower fluorescence than Rhodamine. The prompt deactivation of reference  $\alpha_{\text{ref}}$  in good approximation can be taken as equal 1. The ratio

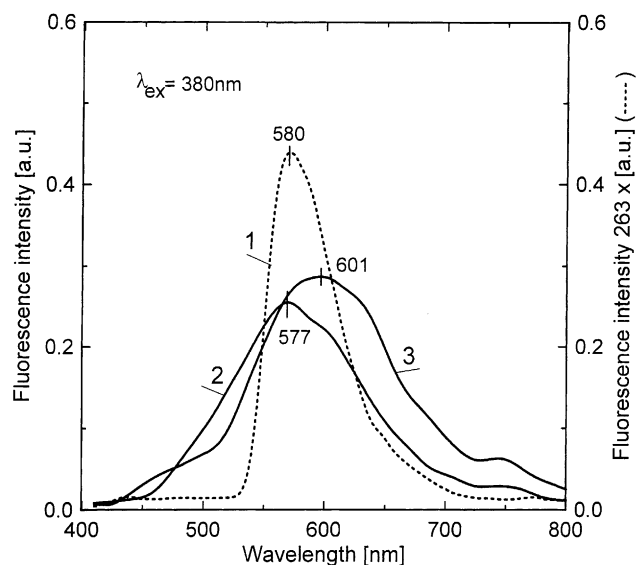


Fig. 8. Fluorescence spectra: 1: Rhodamine (right scale); 2 and 3: Mero 1 and Mero 2 (left scale), respectively.

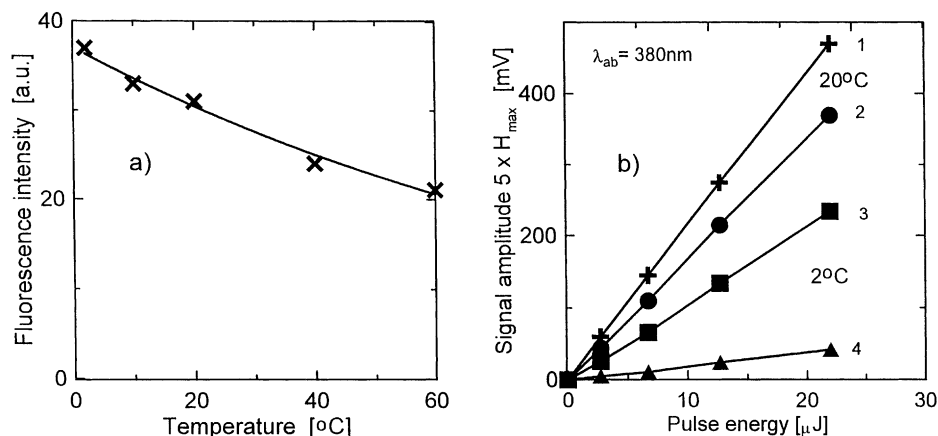


Fig. 9. (a) The dependence of the Mero 1 fluorescence intensity on temperature and (b) the  $H_{\max}$  vs. laser energy measured at 20°C for BCP (curve 1) and Mero 1 (curve 2); at 2°C BCP (curve 3) and Mero 1 (curve 4).

of the slopes of sample and reference lines gives value of  $\alpha_1$  which after introducing to Eq. (2) enable us to calculate  $E_T\phi_T$ , i.e. the product of triplet state energy ( $E_T$ , Fig. 1) and the yield  $\phi_T$ . The obtained values for  $\alpha_1$  was  $0.73 \pm 0.07$  for Mero 1 and  $0.81 \pm 0.08$  for Mero 2. It means that in room temperature most of the energy is deactivated in very short time. The calculated  $E_T\phi_T$  for Mero 1 and Mero 2 were  $92 \pm 23$  and  $59 \pm 25$  kJ/mol, respectively (Table 1).

The lowering in temperature to 2°C causes some increase in dye fluorescence (Fig. 9a) and strong decrease in prompt process of deactivation (Fig. 9b). Evaluated for Mero 1 at 2°C the product  $\Phi_T E_T$  is about  $257 \pm 10$  kJ/mol. It means that some lowering in temperature can encourage the merocyanine sensitizers action. The increase in the fluorescence intensity causes the decrease in prompt TD. This effect is included in Eq. (2). The increase in the slow processes of TD with the decrease in temperature is probably due to the decrease in the quenching of dye triplets by oxygen.

In condition of our experiments slow signal responsible for TD can be due by direct deactivation of triplet states TTD or by TD of photo-reactions products. We plan to carry in a future the measurements in the presence and in the absence of oxygen. Such experiments can deliver us more information about long-living photoreactions products.

It is possible also from the analysis of wave-form LIOAS signal to obtain the lifetimes of long living intermediates as well as of the triplet states [12]. From the comparison of the LIOAS signal for the sample and reference one can also obtain additional information. The shift of first maximum of LIOAS signal towards longer times region in respect to reference signal shows that short time TD of investigated dyes possess slightly slower component than that of reference.

#### 4. Conclusions

1. The merocyanine with shorter chain (Mero 1, Fig. 1) exchanges less excitation into heat in short time than mero-

cyanine with longer alkyl chain (Mero2) (Table 1). The last one loses most excitation promptly to heat almost in similar way as the reference BCP molecules.

2. With the lowering in the sample temperature fluorescence intensity of merocyanines increases and prompt TD decreases. The slow deactivation process increases. Last effect shows that merocyanines at lower temperature are more effective as sensitizers.
3. Slow TTD through triplet states in room temperature for both merocyanines is rather low, but experiments were carried out with oxygen presence, therefore, the triplet of merocyanines can be quenched and some oxygen intermediates can be formed. These products of photoreactions can also deactivate part of their energy as a heat.

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