

163. Determination of the Quantum Yield of Intersystem Crossing of Rose Bengal

by Patricia Murasecco-Suardi¹⁾, Ernst Gassmann²⁾, André M. Braun*, and Esther Oliveros³⁾*

Institut de chimie physique, Ecole Polytechnique Fédérale de Lausanne, CH-1015 Lausanne

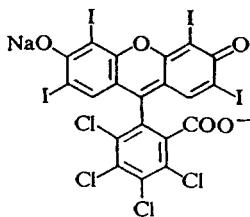
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The quantum yield of intersystem crossing (Φ_{isc}) of a sensitizer is related to the quantum yield of singlet-oxygen production ($\Phi(^1O_2)$) by the efficiency of the energy transfer (φ_{et}) and is an important parameter in the evaluation of potential applications of sensitized photo-oxidations. Using two different laser photolysis techniques, the energy-transfer method and the partial saturation method, Φ_{isc} of rose bengal has been determined in MeOH and in aqueous solutions. The results confirm that with $\Phi_{isc}(H_2O) = 1.05 (\pm 0.06)$ and $\Phi_{isc}(MeOH) = 0.90 (\pm 0.08)$, the generally assumed relation $\Phi_{isc} \cdot \varphi_{et} = \Phi(^1O_2)$, with $\varphi_{et} = 1$, cannot be maintained any longer ($\Phi(^1O_2, H_2O) = 0.75$ and $\Phi(^1O_2, MeOH) = 0.76$). During these experiments, a second intermediate has been observed which is produced from the triplet state of rose bengal and, stabilized in a anionic micellar solution, has been shown to be the radical cation of the sensitizer. The efficiency of the electron transfer has been evaluated from transient absorption and bleaching recordings, and it seems conclusive to attribute the results to the difference between Φ_{isc} and $\Phi(^1O_2)$.

Introduction. – In the domains of photophysics and photochemistry, dye-sensitized photo-oxidations [1–6] are increasingly investigated because of their many potential applications in photobiochemistry, photobiology, and photomedicine [7–10]. Most often, singlet oxygen is claimed to be the reactive species; it is produced by energy transfer from the triplet state of the sensitizer (dye) to ground-state molecular oxygen (*Reaction 1*, type-II photo-oxidation).



Several xanthene dyes are good singlet-oxygen sensitizers, and among them, rose bengal (**RB**) is certainly one of the most efficient and most widely used in preparative



Disodium salt of 3,4,5,6-tetrachloro-2',4',5',7'-tetraiodofluorescein or rose bengal (**RB**)

¹⁾ Part of the thesis of P. M.-S., EPF Lausanne, 1988.

²⁾ Part of the thesis No. 519 of E.G., EPF Lausanne, 1984. Present address: Ciba-Geigy AG, CH-4002 Basel.

³⁾ Permanent address: Laboratoire IMRCP, UA au CNRS n° 470, Université Paul Sabatier, F-31062 Toulouse Cedex.

photo-oxidations, as well as in studies of the reactivity of singlet oxygen towards biological substrates [8–10].

The quantum yield of singlet-oxygen production $\Phi(^1\text{O}_2)$ is directly related to the quantum yield of intersystem crossing of the sensitizer (Φ_{isc} , Eqn. 2), since the triplet state of the latter sensitizes the formation of singlet oxygen (cf. Reaction 1),

$$\Phi(^1\text{O}_2) = \Phi_{\text{isc}} \cdot \varphi_{\text{et}} \quad (2)$$

where φ_{et} is the efficiency of energy transfer.

RB exhibits a higher $\Phi(^1\text{O}_2)$ than other fluorescein dyes. This might be due to an increased Φ_{isc} , since **RB** contains a higher number of halogen substituents (heavy atom effect) [11–13]. In fact, Φ_{isc} of **RB** has never been determined by direct analysis, although investigations of primary photophysical processes are providing important parameters for the evaluation of the photochemical reactivity of dyes, including singlet-oxygen sensitization. For some xanthene dyes (fluorescein, dibromofluorescein, eosin, erythrosin), Φ_{isc} and molar absorption coefficients of the triplet state (ϵ_T) have been determined by flash (or laser-flash) photolysis experiments [14–17]. For **RB**, however, the values of Φ_{isc} cited in the literature have been estimated from measurements of quantum yields of sensitized photo-oxidation (Φ_{ox}) of specific acceptors of singlet oxygen [13] [18]. In this case, the relation between Φ_{ox} and Φ_{isc} is given by Eqn. 3

$$\Phi_{\text{ox}} = \Phi_{\text{isc}} \cdot \varphi_{\text{et}} \cdot \varphi_r \quad (3)$$

where φ_r is the efficiency of the chemical reaction of singlet oxygen with the acceptor (A) (Reaction 4).



Only if the conditions are such that φ_{et} and φ_r are equal to unity, the experimentally determined Φ_{ox} is equal to the values of $\Phi(^1\text{O}_2)$ and Φ_{isc} of the sensitizer. For example, Gollnick and Schenck [18], using 2,5-dimethylfuran at high concentration as a singlet-oxygen acceptor, and assuming φ_{et} and $\varphi_r = 1$, published a value of $\Phi_{\text{isc}} = 0.76$ for **RB** in MeOH solution, a result generally referred to in the literature. In a recent paper, Gandin *et al.* [13] established a relative scale of $\Phi(^1\text{O}_2)$ for a series of fluorescein derivatives, using a simplified kinetic model of the sensitized photo-oxidation of 1,3-diphenylisobenzofuran (DPBF). Taking eosin as a standard, and assuming that for this compound as well as for all substituted fluoresceins investigated, $\Phi_{\text{isc}} = 1 - \Phi_f$ (Φ_f : quantum yield of fluorescence, $\Phi_f(\text{eosin}) = 0.68$ [19] [15] and assuming $\varphi_{\text{et}} = 1$, the authors could calculate Φ_{isc} of **RB** to be 0.86 in EtOH ($\Phi_f(\text{RB}) = 0.11$ in EtOH [19] [20]).

The hypothesis of unit efficiency of energy transfer based on a concentration of dissolved oxygen considered to be sufficient can be erroneous. In fact, electron transfer between the sensitizer triplet and oxygen-producing superoxide anion (O_2^-) and the radical cation of the sensitizer (Reaction 5), cannot be excluded.



Different authors have shown that, along with singlet oxygen, superoxide is indeed generated to some extent in fluorescein- or **RB**-sensitized photo-oxidations [10] [21–23].

Moreover, a possible physical quenching of singlet oxygen by the sensitizer (Reaction 6) must also be taken into account. However, it has been shown that in the case of **RB** the rate constant of Reaction 6 is smaller than $6 \cdot 10^4 \text{ l} \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$ [24].



As long as *Reactions 5* and *6* cannot be excluded, Φ_{ox} , $\Phi({}^1\text{O}_2)$, and Φ_{isc} of a given sensitized photo-oxidation cannot be related as simply as done in the literature cited above. Φ_{et} can, in fact, be determined from independent analysis of Φ_{isc} and $\Phi({}^1\text{O}_2)$.

In this paper, we present the results of laser-flash-photolysis experiments for the determination of the quantum yield of intersystem crossing (Φ_{isc}) and the absorption coefficient of the triplet state of **RB** in MeOH and in H₂O, using two techniques: the energy-transfer method [25–28] and the partial-saturation method [29].

Description of Methods. – 1. *Partial-Saturation Method.* This method [9] had been originally developed as a two-state model for a monophotonic photo-ionization process and was later applied to triplet-triplet absorption measurements to calculate molar-absorption coefficients of triplet states (ϵ_{T}) and triplet quantum yields (Φ_{isc}) [30]. In the latter application of this simple model, only the ground state and the triplet state of the light-absorbing species are considered, the intermediary of the excited singlet state being neglected. Recently, *Carmichael* and *Hug* [31–33] showed the limitations of this approach. They developed the equations for a three-state model and established the necessary conditions for the use of the simpler two-state model.

Based on the well known scheme of the photophysical processes of an electronically excited molecule, the triplet quantum yield (Φ_{isc}) is given by

$$\Phi_{\text{isc}} = k_{\text{isc}}/(k_{\text{isc}} + k_{\text{F}} + k_{\text{ic}}) = k_{\text{isc}}/k_{\text{S}} \quad (7)$$

where k_{isc} , k_{F} , and k_{ic} are the rate constants of S₁–T₁ intersystem crossing, S₁–S₀ fluorescence, and S₁–S₀ internal conversion, respectively.

In laser-flash photolysis, the triplet state is monitored by the analyzing beam of a suitable frequency, where triplet-triplet absorption is observed. After laser excitation, the change in absorbance at the wavelength of analysis may be written as

$$\Delta A = (\epsilon_{\text{T}} - \epsilon_{\text{O}})[\text{T}_1]l \quad (8)$$

ϵ_{T} , ϵ_{O} molar absorption coefficient of the triplet excited state and of the ground state, respectively, at the wavelength of analysis [$\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$],

l optical path length in the direction of the monitoring beam [cm],

$[\text{T}_1]$ concentration of the first triplet state at time t of observation [$\text{mol} \cdot \text{l}^{-1}$].

To calculate the triplet-state concentration, *Carmichael* and *Hug* [31–33] solved the differential equations for the three-state model involving the triplet state and the ground state as well as the singlet excited state, where the first is assumed not to decay. At all times t , less than or equal to the pulse length of a square excitation pulse, the concentration of the triplet state produced is then given by

$$[\text{T}_1] = [\text{S}_0] \left(1 + \frac{\lambda_3 \exp(-\lambda_2 t)}{\lambda_2 - \lambda_3} - \frac{\lambda_2 \exp(-\lambda_3 t)}{\lambda_2 - \lambda_3} \right) \quad (9)$$

where $[\text{S}_0]$ is the initial concentration of the molecule in its ground state, $\lambda_2 = (X + Y)/2$, $\lambda_3 = (X - Y)/2$, $X = k_{\text{ex}} + k_{\text{S}}$ (k_{ex} being the rate constant of excitation) and $Y = X(1 - (k_{\text{isc}}k_{\text{ex}}/X^2)^{1/2})$.

If the experimental parameters are such that 1) $k_{\text{isc}}k_{\text{ex}}/X^2$ is small and 2) $k_{\text{ex}} \ll k_{\text{S}}$, then *Eqn. 9* may be simplified to *Eqn. 10* [31]:

$$[\text{T}_1] = [\text{S}_0] (1 - \exp(-k_{\text{ex}}\Phi_{\text{isc}}t)) \quad (10)$$

This equation can be developed from the two-state model [29]. In fact, the condition $k_{\text{ex}} \ll k_s$ means that the decay of the excited singlet state is very fast and, thus, this state can be neglected in the model.

k_{ex} depends on the excitation energy and, if optically thin samples are assumed, may be expressed as:

$$k_{\text{ex}} = 2303 \varepsilon_0^{\text{ex}} E_p(t) \quad (11)$$

$\varepsilon_0^{\text{ex}}$ molar absorption coefficient of the ground state at the exciting (laser) wavelength ($\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$),
 $E_p(t)$ photonic irradiance [$\text{einstein} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}$].

Combining *Eqns. 8, 10, and 11* and taking the integrated laser photonic irradiance during the pulse (E), we obtain at the end of the pulse:

$$\Delta A = a(1 - \exp(-bE)) \quad (12)$$

$a = (\varepsilon_T - \varepsilon_0)[S_0]I$, $b = 2303 \varepsilon_0^{\text{ex}} \Phi_{\text{isc}} [\text{cm}^2 \cdot \text{einstein}^{-1}]$, $E [\text{einstein} \cdot \text{cm}^{-2}]$.

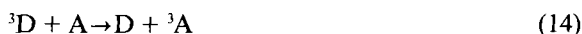
In plotting the measured ΔA (extrapolated value at the end of the laser pulse) *vs.* E until a partial saturation region is observed, and in fitting the obtained curve, ε_T and Φ_{isc} can be calculated from the fitted values of a and b , if the above mentioned conditions for the use of the two-state model are met. If bE is relatively small, no partial saturation can be reached. The exponential in *Eqn. 12* can be expanded in a power series leading to a linear relationship between ΔA and E

$$\Delta A = abE \quad (13)$$

from which ε_T and Φ_{isc} cannot be obtained independently. Only their product can be calculated from the slope (ab) of the linear function.

2. Energy-Transfer Method. This method is widely used for the determination of molar absorption coefficients of triplet states [25–29] [33]. It may also be useful to determine the triplet-quantum yield [34] of a compound **D** if the molar absorption coefficient of the triplet state of a reference compound **A**, acting as an acceptor, is known.

Given the conditions of an efficient (exothermic) energy transfer (*Eqn. 14*),



the molar absorption coefficient of the triplet state of one of the molecules involved in the transfer ($\varepsilon({}^3\text{D})$) or $\varepsilon({}^3\text{A})$) may be determined from *Eqn. 15*, if the molar absorption coefficient of the other triplet state is known.

$$\varepsilon({}^3\text{D}) = \varepsilon({}^3\text{A}) \frac{\Delta A_{\text{D}}}{\Delta A_{\text{A}}} \varphi_{\text{et}} \quad (15)$$

ΔA_{A} change in absorbance of the triplet-triplet absorption of **A**, set equal to the ΔA_{A} at infinite time,

ΔA_{D} change in absorbance of the triplet-triplet absorption of **D**, extrapolated to t_0 (end of the laser pulse).

The efficiency of energy transfer φ_{et} can be obtained from *Eqn. 16*

$$\varphi_{\text{et}} = \frac{k_{\text{et}}[\text{A}]}{k_{\text{et}}[\text{A}] + k_{\text{d}}} = \frac{\tau_1 - \tau_2}{\tau_1} \quad (16)$$

$\tau_1 = 1/k_{\text{d}}$,

$\tau_2 = 1/(k_{\text{et}}[\text{A}] + k_{\text{d}})$,

k_{d} unimolecular deactivation of ${}^3\text{D}$ (mainly intersystem crossing to the ground state).

A more precise evaluation of k_{et} and, thus, of φ_{et} can be achieved by a *Stern-Volmer* analysis measuring the actual lifetime of ${}^3\text{D}$ in solutions of different concentrations of **A**.

Corrections for an inner filter effect may be avoided, if the absorbance of the acceptor at the excitation wavelength is much smaller than the optical density of the donor at the same wavelength [33].

If the triplet state 3D is only formed by intersystem crossing from the excited singlet state of the same molecule D, the quantum yield of intersystem crossing is defined as:

$$\Phi_{isc} = \frac{n(^3D)}{N_{abs}} \quad (17)$$

where $n(^3D)$ is the maximum number of 3D states resulting from the laser pulse, and N_{abs} is the number of absorbed photons during the laser pulse. If the molar absorption coefficient of 3D ($\varepsilon(^3D)$) is known, $n(^3D)$ can be directly calculated from ΔA_D by extrapolating to the end of the laser pulse, and Φ_{isc} can be determined by an independent measurement of the excitation energy. If $\varepsilon(^3D)$ is unknown, the method of energy transfer can be used to determine Φ_{isc} (without measuring $\varepsilon(^3D)$) provided a convenient acceptor of known $\varepsilon(^3A)$ can be found. $n(^3D)$ being directly related to the measured ΔA_A , Φ_{isc} is given for optically thin samples by

$$\Phi_{isc} = \frac{n(^3D)}{N_{abs}} = \frac{\mathcal{N} \cdot v \cdot \Delta A_A}{10^3 \cdot l \cdot \varphi_{et} \cdot \varepsilon(^3A)} \cdot \frac{hc}{2.303 \lambda_{ex} E_{ex} A_{O,D}^{ex}} \quad (18)$$

h	Planck constant [$6.62 \cdot 10^{-34} \text{ J} \cdot \text{s} \cdot \text{photon}^{-1}$],
c	velocity of light [$3 \cdot 10^8 \text{ m} \cdot \text{s}^{-1}$],
\mathcal{N}	Avogadro constant [$6.023 \cdot 10^{23} \text{ mol}^{-1}$],
v	irradiated volume [cm^3],
l	optical path length in the direction of the monitoring beam [cm],
λ_{ex}	excitation wavelength [m],
E_{ex}	excitation energy [J],
$A_{O,D}^{ex}$	absorbance of the solution of D at the excitation wavelength.

Results. – 1. *Ground State and Transient Absorption Spectra of Rose Bengal.* The absorption spectrum of **RB** shows a maximum in the VIS region at 556.5 nm in MeOH and at 548.5 nm in neutral aqueous solution [13] [20]. The molar absorption coefficients at the wavelength of the laser beam (532 nm) have been determined from the linear plots of absorbance *vs.* concentration to be 30300 (± 300) $\text{l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ in MeOH and 44000 (± 400) in H_2O . Optically thin samples at the wavelength of excitation have been prepared, using **RB** at concentrations of about $1.2 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1}$.

Fig. 1 shows the difference-absorption spectrum and the calculated triplet spectrum (see *Discussion*) of **RB** in MeOH in the range of 580–750 nm obtained by using two different energies of the laser pulse. The spectra in H_2O (*Fig. 2*) are similar to those in MeOH. Below 600 nm **RB** absorbs the monitoring light and a strong depletion of the ground state is observed, compensating the triplet-triplet absorption. The difference spectrum in H_2O is very similar to the one published very recently by *Lee and Rodgers* [10], although these authors were not using optically thin samples. In the entire spectral range of 580 nm to 750 nm, a mono-exponential decay of the signals with rate constants of $6.3 (\pm 0.4) \cdot 10^3 \text{ s}^{-1}$ ($\tau_T = 160 (\pm 10) \mu\text{s}$; $\tau_T = 154 \mu\text{s}$ [18]) in H_2O and of $7.7 (\pm 0.3) \cdot 10^3 \text{ s}^{-1}$ ($\tau_T = 130 (\pm 5) \mu\text{s}$) in MeOH is observed. The decay kinetics are clearly first-order for energies of the laser pulse smaller than 15 mJ. At higher energies, complex kinetics have been observed which might be due to triplet-triplet annihilation and biphotonic processes.

Fig. 3 shows the transient spectrum in MeOH between 360 nm and 490 nm. In this spectral region, mixed first- and second-order decay kinetics are observed, even at very

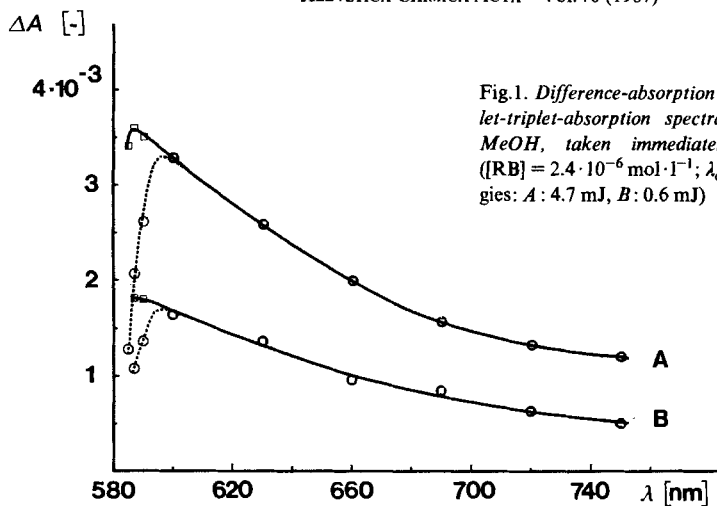


Fig. 1. Difference-absorption spectra (·····) and triplet-triplet-absorption spectra (—) of rose bengal in MeOH, taken immediately after the laser pulse ($[RB] = 2.4 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1}$; $\lambda_{\text{ex}} = 532 \text{ nm}$; excitation energies: A: 4.7 mJ, B: 0.6 mJ)

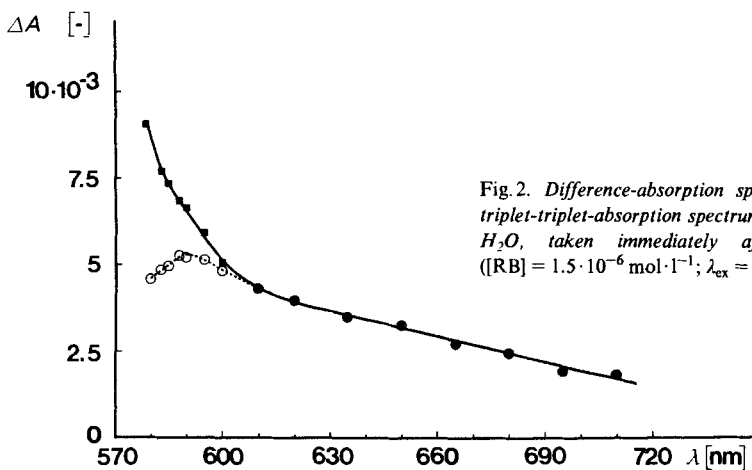


Fig. 2. Difference-absorption spectrum (·····) and triplet-triplet-absorption spectrum (—) of rose bengal in H_2O , taken immediately after the laser pulse ($[RB] = 1.5 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1}$; $\lambda_{\text{ex}} = 532 \text{ nm}$)

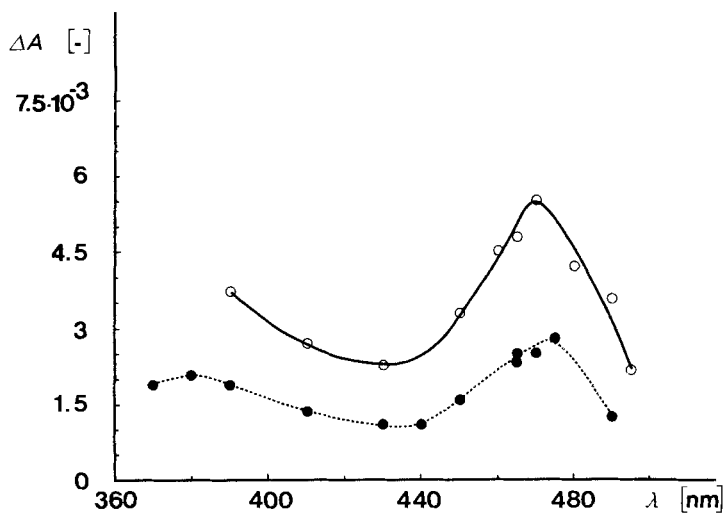


Fig. 3. Difference-absorption spectra of rose bengal in MeOH taken immediately after the laser pulse between 360 and 490 nm ($[RB] = 2.4 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1}$; $\lambda_{\text{ex}} = 532 \text{ nm}$; excitation energies: (—) 4.8 mJ, (·····) 0.57 mJ)

low laser energy. These results can be explained by a simultaneous absorption of the triplet state and of the radical cation of **RB** (see *Discussion*).

2. *Partial-Saturation Method.* We measured the variation of the absorbance at 590 nm (triplet-triplet absorption) in MeOH and in H₂O as a function of the energy of the laser pulse (Figs. 4 and 5). Saturation was obtained for excitation energies less than 5 mJ. To test the validity of the two-state model for the excitation process in the **RB** case, we had to check that the condition $k_{\text{ex}} \ll k_{\text{s}}$ is met for energies of the laser pulse less than or equal to 5 mJ. Using Eqn. 11 to calculate k_{ex} , we find $E_{\text{p}} = 6 \text{ einstein} \cdot \text{s}^{-1} \cdot \text{cm}^{-2}$ for a 5-mJ pulse of

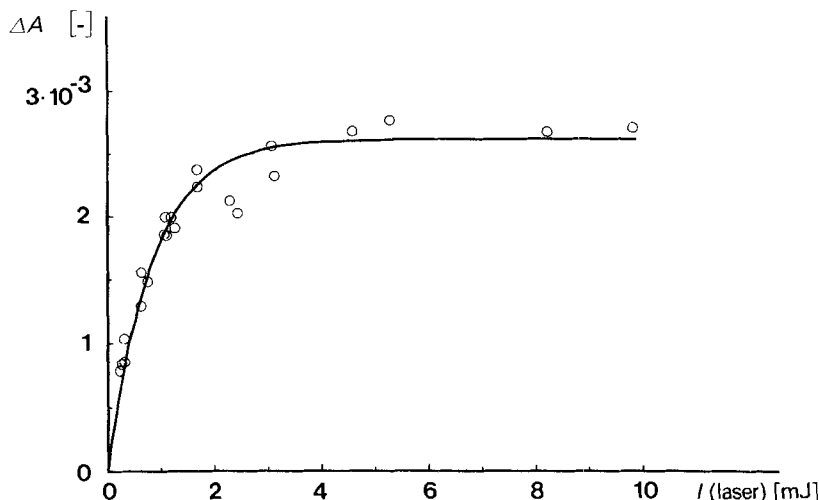


Fig. 4. Experimental points of the variation of the transient optical density at 590 nm of rose bengal in MeOH vs. the energy of the laser pulse and the corresponding calculated curve for the two-state model ($[\text{RB}] = 1.2 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1}$; $\lambda_{\text{ex}} = 532 \text{ nm}$)

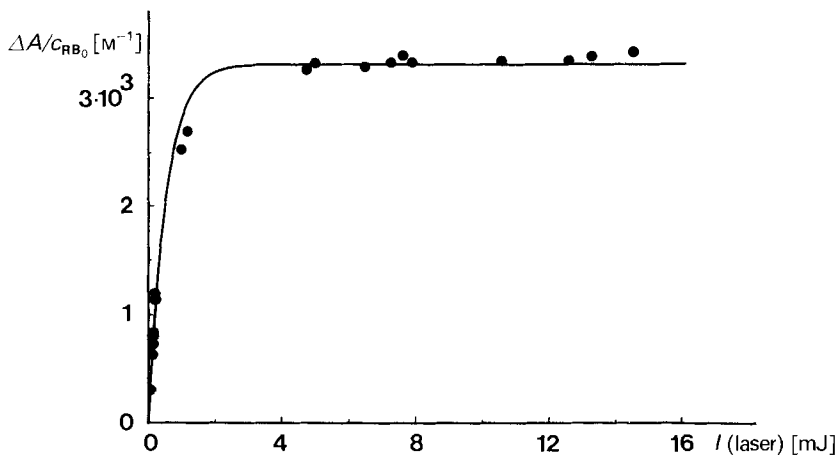


Fig. 5. Experimental points of the variation of the transient optical density at 590 nm of rose bengal in H₂O vs. the energy of the laser pulse and the corresponding calculated curve for the two-state model ($[\text{RB}] = 1.25 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1}$; $\lambda_{\text{ex}} = 532 \text{ nm}$)

15-ns duration on a surface of 0.25 cm², and, thus, $k_{\text{ex}} = 4.0 \cdot 10^8 \text{ s}^{-1}$. The experimental fluorescence lifetime of **RB** is 655 (± 85) ps in MeOH, yielding a k_{S} of $1.5 (\pm 0.2) \cdot 10^9 \text{ s}^{-1}$ [20]. Since partial saturation is already observed for pulses of *ca.* 2.5 mJ, *i.e.* $k_{\text{ex}} = 2.0 \cdot 10^8 \text{ s}^{-1}$, k_{ex} is at least 7.5 times smaller than k_{S} for the laser energies of interest. In H₂O, a value of $3.0 \cdot 10^8 \text{ s}^{-1}$ is found for k_{ex} in the case of a pulse energy of 2.5 mJ. With a k_{S} of $1.05 (\pm 0.15) \cdot 10^{10} \text{ s}^{-1}$ [20], the condition $k_{\text{ex}} \ll k_{\text{S}}$ holds better for **RB** in H₂O than in MeOH. In the latter case, the energy-transfer method was chosen as an alternative evaluation of Φ_{isc} and ε_{T} (see below).

A computer-assisted fit of the parameters a and b of the two-state model (Eqn. 12) with the data reported in Figs. 4 and 5 yields the results summarized in Table 1.

Table 1. Quantum Yields of Triplet-State Production and T-T Molar-Absorption Coefficients for **RB** in MeOH and H₂O. Values obtained by the partial-saturation method (**RB** concentrations: $1.2 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1}$ in MeOH and $1.25 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1}$ in H₂O).

Solvent	$a^a)$	$b^b)$ [mJ ⁻¹] ^{c)}	$\varepsilon_{\text{T}}(590 \text{ nm})^b)$ [l · mol ⁻¹ · cm ⁻¹]	Φ_{isc}
MeOH	$2.62 (\pm 0.15) \cdot 10^{-3}$	1.2 (± 0.1)	5800 (± 300)	0.96 (± 0.08)
H ₂ O	$4.15 (\pm 0.17) \cdot 10^{-3}$	1.9 (± 0.1)	8400 (± 350)	1.05 (± 0.06)

a) Parameters of Eqn. 12.

b) $\varepsilon_{\text{O}} = 1470 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ in MeOH and $\varepsilon_{\text{O}} = 1795 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ in H₂O at 590 nm.

c) To obtain b in cm² · einstein⁻¹ (Eqn. 12), b in mJ⁻¹ must be multiplied by $10^3 \cdot \mathcal{N} \cdot h \cdot c \cdot S / \lambda_{\text{ex}}$ (S : irradiated surface = 0.25 cm²).

3. *Energy-Transfer Method.* To apply this method to determine the molar absorption coefficient of the triplet state and the triplet-quantum yield of **RB**, an acceptor with adequate spectroscopic characteristics must be used. The triplet energy of **RB** had been published to be *ca.* 165–176 kJ · mol⁻¹ [6], hence, we chose tetracene as an acceptor ($E_{\text{T}} = 123 \text{ kJ} \cdot \text{mol}^{-1}$ [35]), in order to assure an exothermic-energy transfer. It must be noted that we could not find any published data on an acceptor of convenient triplet energy, for which the molar absorption coefficient of the triplet state had been measured in MeOH. However, ε_{T} and Φ_{isc} of tetracene in benzene have been published to be 31200 l · mol⁻¹ · cm⁻¹ at 465 nm [36] and 0.60–0.63 [36] [37], respectively. The former value is still recommended as a tentative standard [33].

In changing the solvent from benzene to MeOH, the maximum of the triplet-triplet absorption spectrum of tetracene is shifted bathochromically to 480 nm. Under the given experimental conditions, only slight partial saturation has been observed (Nd YAG, $\lambda_{\text{ex}} = 353 \text{ nm}$), which is probably due to the short laser pulse [33]. We restricted, therefore, our experiments to a domain where the variation of the absorbance at 480 nm *vs.* the laser energy remains linear (Fig. 6). From the slope of the straight line, we calculated using Eqn. 13 the product of $\varepsilon_{\text{T}} \Phi_{\text{isc}}$ in MeOH to be $1.65 (\pm 0.08) \cdot 10^4 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. The product $\varepsilon_{\text{T}} \Phi_{\text{isc}}$ in benzene is about $1.9 \cdot 10^4 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. If we assume that there is no solvent effect on Φ_{isc} , ε_{T} at 480 nm is then 26600 (± 1900) l · mol⁻¹ · cm⁻¹ in MeOH.

For the energy-transfer experiment, **RB** was excited at 532 nm in presence of a high enough concentration of tetracene in MeOH in order to optimize energy transfer. The difference absorption spectrum of **RB** in the range 580 to 740 nm is not modified by the addition of tetracene. We verified that the rise time of the tetracene absorption was identical to the decay time of **RB** triplet within the limits of error. Using a concentration

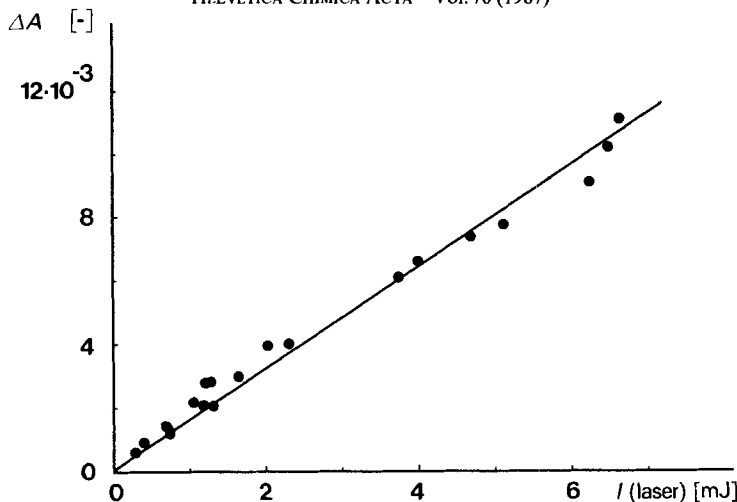


Fig. 6. Variation of the optical density of the tetracene triplet at 480 nm in MeOH vs. the energy of the laser pulse ([tetracene] = $7.5 \cdot 10^{-6}$ mol \cdot l $^{-1}$; λ_{ex} = 353 nm)

of tetracene of $2 \cdot 10^{-5}$ mol \cdot l $^{-1}$, the lifetime of the **RB** triplet decreases from 130 to 10 μ s, and the efficiency of the energy transfer under those conditions is 0.92.

We measured ΔA_D (D = **RB**) at 590 nm and ΔA_A (A = tetracene) at 480 nm for different energies of the laser pulse in the linear region of Fig. 4. The absorption coefficient of the triplet state of **RB** at 590 nm ($\epsilon(^3D)$) was calculated using Eqn. 19

$$(\epsilon(^3D) - \epsilon_{O,D})_{590} = (\epsilon(^3A) - \epsilon_{O,A})_{480} \frac{\Delta A_D^{590}}{\Delta A_A^{480}} \tau_{\text{et}} \quad (19)$$

$\epsilon_{O,D}$ and $\epsilon_{O,A}$ being the molar absorption coefficients of the donor and the acceptor ground states at the respective monitoring wavelengths.

Eqn. 19 is similar to Eqn. 15 but takes into account the absorption of the respective analyzing radiations by the ground states of the donor and of the acceptor. From all experiments, we calculated an average value of 5750 (± 600) l \cdot mol $^{-1}$ \cdot cm $^{-1}$ for the molar absorption coefficient of the **RB** triplet state at 590 nm; this result is in very good agreement with the one obtained from the partial-saturation method (Table 1).

Φ_{isc} for **RB** in MeOH was calculated from Eqn. 18 by measuring ΔA_A and the energy of the laser pulse. We replaced in Eqn. 18 $\epsilon(^3A)$ by $(\epsilon(^3A) - \epsilon_{O,A})$ in order to take into account the absorption of the analyzing light by the acceptor ground state and obtained from all experiments an average value of Φ_{isc} of 0.85 (± 0.10). It should be noted that this method of measuring ϵ_T and Φ_{isc} is less accurate than the partial-saturation method. This is due to the very restricted linear region for the variation of ΔA_A in function of the energy of the laser pulse: in fact, non-linearity already starts at energies as low as 0.5 mJ, as already found in experiments with **RB** in the absence of an acceptor (Fig. 4).

Discussion. – This work describes the means and results of a direct analysis of the triplet state of **RB**, permitting 1) to establish corrected triplet-triplet absorption spectra in MeOH and in aqueous solution and 2) to verify and to quantify some of the conclusions on the efficiency of triplet formation and triplet quenching by oxygen, reached by extrapolation or conclusions in analogy [10] [13] [18] [38].

Table 2. Maxima of the Triplet-Triplet-Absorption Spectra of **RB** in MeOH and H₂O

	λ_{\max} [nm]	ϵ_T [$l \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$]
MeOH	587	6270 (± 300)
H ₂ O	580 ^{a)}	14500 (± 500) ^{b)}

a) Measurements below 580 nm are seriously impaired by the strong absorption of **RB**, consequently, the limits of error implied by these measurements do not allow for a precise determination of λ_{\max} .

b) We found in H₂O $A\epsilon_{600} = 6110 (\pm 250) l \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$; with regard to the result published by Lee and Rodgers ($A\epsilon_{600} = 4900 l \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ [10]), the observed difference is probably due to the fact that these authors have taken a more than ten fold concentration of **RB** and, hence, have used optical densities placing their experiments outside the conditions imposed for the application of the two-state model.

In applying Eqn. 12 under the experimental conditions imposed by the two-state model [31–33], the triplet-quantum yields and the molar absorption coefficients of the **RB** triplet have been established at given wavelengths in MeOH and in H₂O. The average values are in MeOH $\Phi_{\text{isc}} = 0.90 (\pm 0.10)$, ϵ_T (590 nm) = 5800 (± 300) $l \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$, and in H₂O $\Phi_{\text{isc}} = 1.05 (\pm 0.06)$, ϵ_T (590 nm) = 8400 (± 350) $l \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. The knowledge of these parameters allows the transformation of the difference absorption spectra into the corrected triplet-triplet-absorption spectra of **RB** (Figs. 1 and 2).

Taking the most recent results of Φ_f determinations for **RB** (Φ_f (MeOH) = 0.08, Φ_f (H₂O) = 0.018 [20]), our results on Φ_{isc} fit well into the earlier published hypothesis that internal conversion of the excited singlet state of **RB** is even less efficient than fluorescence [15], hence, $\Phi_{\text{isc}} \approx 1 - \Phi_f$. Results on Φ_{isc} and Φ_f determinations for **RB** are in close resemblance to erythrosin B, an analog of **RB**, which also contains four I substituents. For erythrosin B, Φ_f has been determined to be 0.08 [20] and 0.02 [17] in MeOH and H₂O, respectively, and Φ_{isc} in aqueous solution has been measured by flash photolysis to be 1.07 (± 0.13) [17]. Evaluations of Φ_{isc} in MeOH and EtOH vary from 0.62 to 0.69 from photo-oxidation experiments [13] [18] to about unity from time resolved ground-state spectroscopy [16].

The hypothesis of maximum efficiency for both energy transfer to oxygen (Reaction 1) and chemical trapping of ¹O₂ (Reaction 4) led to an evaluation of Φ_{isc} in determining Φ_{ox} to be 0.76 and 0.75 for **RB** in MeOH and H₂O, respectively [18]. Φ_{ox} in MeOH has since been transformed to an average value of $\Phi(^1\text{O}_2)$ of 0.80 (± 0.03) [39]. On the other hand, Gandin *et al.* [13] maintain their hypothesis of $\Phi_{\text{isc}} = \Phi(^1\text{O}_2)$ for EtOH solutions ($\Phi(^1\text{O}_2) = 0.86$), although several authors [21–23] and recently Lee and Rodgers [10] have reported the production of superoxide in irradiated oxygenated solutions of **RB**. It becomes evident that an investigation on the relation between Φ_{isc} and $\Phi(^1\text{O}_2)$ must imply quantitative experiments on the electron transfer (Reaction 5).

Laser-photolysis experiments with deoxygenated solutions of **RB** show mixed first- and second-order kinetics of the decay of the transient absorbing at 470 nm (Fig. 7). The slow decay of the transient absorption is purely second order at observation times > 130 μs ($1/\Delta A = f(t)$ linear), with a rate constant of $2.0(\pm 0.2) \cdot 10^6 l \cdot \text{mol}^{-1} \cdot \text{s}^{-1}$. (The molar absorption coefficient has been determined independently, *vide infra*.) The slowly decaying intermediate could be the radical cation of **RB**. Following the working hypothesis that this intermediate is produced from the **RB** triplet, we assume that at the end of the laser pulse only **RB** triplet is present, the decay of which has been determined independently (*vide supra*). Subtracting the triplet decay from the experimental transient ab-

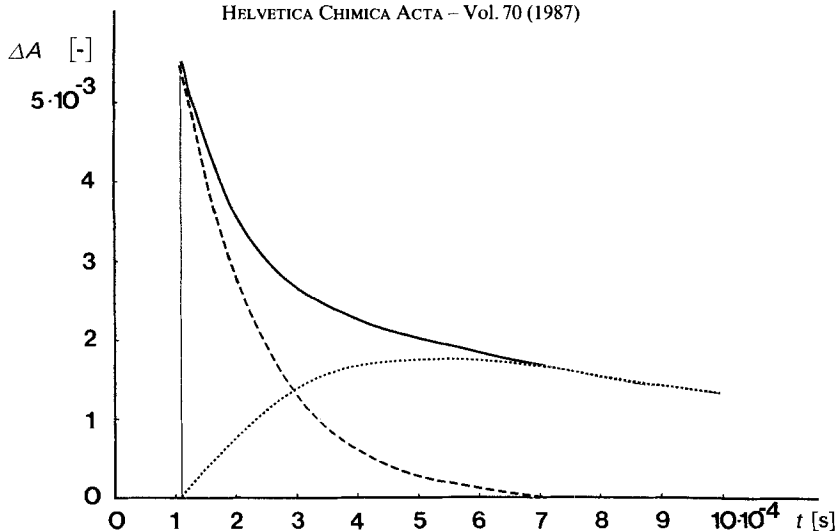


Fig. 7. Difference-absorption spectrum of rose bengal at 470 nm in MeOH (—) and calculated curves of triplet decay (---) and for the formation and decay of the radical cation (·····) ([RB] = $2.4 \cdot 10^{-6}$ mol · l⁻¹; λ_{ex} = 532 nm; excitation energy: 6.5 mJ)

sorption yields a curve representing the formation and the decay of the above mentioned intermediate, its rise time corresponding to the decay time of the **RB** triplet (Fig. 7). This deconvolution has been repeated at different wavelengths between 450 and 490 nm yielding equivalent results. They, thus, confirm that the long-living intermediate is produced from the **RB** triplet. Given the very short lifetime of the excited singlet state of **RB**, it is not conceivable that the intermediate is produced directly from this state. It should be noted that the decay of the bleaching signal at 550 nm follows a similar combination of first- and second-order kinetics as observed for the transient signal at 470 nm, and we

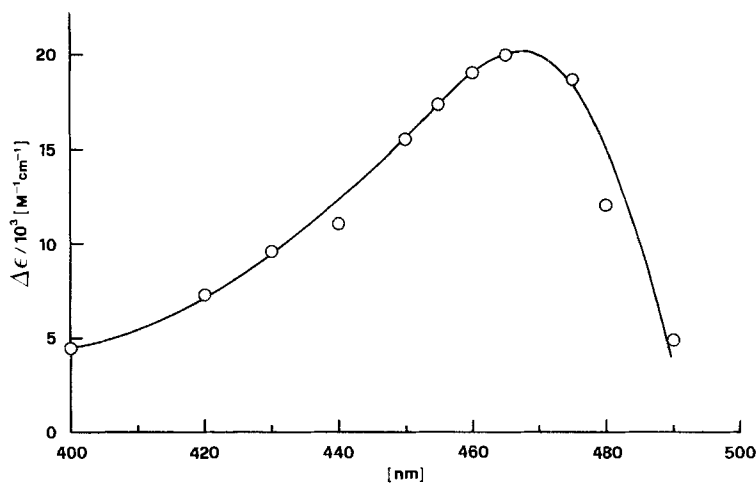


Fig. 8. Difference-absorption spectrum of rose bengal at 470 nm, 80 μ s after the laser pulse, in a micellar solution ([SDS] = 10^{-2} mol · l⁻¹) to which 10^{-4} mol · l⁻¹ of $K_3Fe(CN)_6$ have been added ([RB] = $1.5 \cdot 10^{-6}$ mol · l⁻¹); λ_{ex} = 532 nm)

may assume that neither the triplet nor the intermediate absorb at 550 nm. Similar results have been obtained in aqueous solution and in MeOH.

To obtain experimental evidence that the long-living intermediate is the radical cation, we tried to stabilize it in an anionic micellar solution, to which $K_3Fe(CN)_6$ was added ($10^{-4} \text{ mol} \cdot \text{l}^{-1}$) as an electron acceptor. Under these conditions, a faster decay of the triplet is observed, and the signal of the intermediate remains stable within the millisecond range. The chosen experimental conditions would, in fact, increase the rate of electron transfer from the **RB** triplet and strongly delay the back transfer by coulombic effects of the aggregate [39].

The difference-absorption spectrum of the radical cation has been taken in the micellar solution 80 μs after the laser pulse, when the triplet state is practically depleted, and the radical cation absorption is stable (Fig. 8).

These experimental conditions allow us to evaluate the concentration of the radical cation ([rc]) from the bleaching of the ground state at 550 nm (Eqn. 20) and, hence, its molar absorption coefficients ($\epsilon_{rc,\lambda}$, Eqn. 21).

$$t > 80 \mu\text{s} \quad [\text{rc}] = \frac{\Delta A_{550}}{\epsilon_{0,550} \cdot l} \quad (20)$$

$$\text{and} \quad \Delta \epsilon_{rc,\lambda} = \epsilon_{rc,\lambda} - \epsilon_{O,\lambda} = \frac{\Delta A_{\lambda}}{[\text{rc}] \cdot l} \quad (21)$$

$\epsilon_{rc,470}$ is, thus, found to be $25500 (\pm 1000) \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$.

To evaluate maximum concentrations of the radical cation and, hence, the efficiency of the electron transfer in H_2O and MeOH, either the signal of the transient absorption or of the bleaching of the ground state may be analyzed.

In the first method, we need to know the molar absorption coefficient of the radical cation at the wavelength of analysis, e.g. 470 nm, and we must assume that the value determined in the micellar solution is insensitive to solvent effects within reasonable limits of polarity. For the quantification of the electron transfer, we evaluated the maximum concentration of the radical cation using the deconvolution as given in Fig. 7: we used Eqn. 22 in order to fit the formation of the radical cation, since its decay is slow in comparison.

$$\Delta A_{rc,470}(t) = (\epsilon_{rc,470} - \epsilon_{O,470}) [\text{rc}]_{\text{max}} l (1 - \exp(-t/\tau_r)) \quad (22)$$

The second method of quantification of electron transfer relies only on the bleaching of the ground state observed at 550 nm. Since deconvolution of the observed bleaching signal yields curves corresponding to those obtained for transient absorption, we used Eqn. 23 in order to fit the formation of the radical cation and calculate its maximum concentration.

$$\Delta A_{550}(t) = -\epsilon_{O,550} [\text{rc}]_{\text{max}} l (1 - \exp(-t/\tau_r)) \quad (23)$$

The efficiency of radical cation production from the **RB** triplet may then be calculated using the results of one of the two methods of analysis, after having determined independently, the maximum concentration of the triplet produced under the same experimental conditions. Following the first method of analysis (transient absorption), the efficiency of electron transfer is $\sim 9\%$ in MeOH and $\sim 3\%$ in H_2O . The results of the second method of analysis (bleaching) are ~ 7 and $\sim 5\%$, respectively.

With air-saturated solutions, a higher efficiency of electron transfer has been published, the presence of oxygen apparently enhancing radical cation and superoxide formation (*Reaction 5*) [10]. Within the limits of error of all experiments published, it seems then conclusive to attribute the difference between Φ_{isc} and $\Phi(^1\text{O}_2)$ to the electron-transfer reaction. Published values of $\Phi(^1\text{O}_2)$ or Φ_{ox} , assuming $\varphi_r = 1$ (*Eqn. 5*), are, therefore, not identical with Φ_{isc} in the case of **RB**.

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Experimental Part

A selected sample of rose bengal (*Fluka*) showing the same spectroscopic and photophysical characteristics as a sample of pure rose bengal (Prof. *K. Leismann*) has been used for solns. ($1.5 \cdot 10^{-6} \text{ mol} \cdot \text{l}^{-1}$) in MeOH (*Fluka, puriss.*), in triply distilled H_2O as well as in micellar systems. Tetracene (*Fluka*) has been first recrystallized from MeOH, then further purified by sublimation ($110^\circ/10^{-2}$ Torr). Electron-transfer experiments in micellar systems have been run in $10^{-2} \text{ mol} \cdot \text{l}^{-1}$ aq. solns. of SDS (*Fluka*, recrystallized from EtOH using $\text{K}_3\text{Fe}(\text{CN})_6$ (*Fluka*)).

Solns. have been degassed in 1-cm sample cells by at least three cycles of the freeze-thaw technique using a diffusion pump (*Pfeiffer TPH110*, $2 \cdot 10^{-6}$ Torr).

Ground-state-absorption spectra were recorded on a *UV-260 Shimadzu* spectrophotometer.

For laser-flash photolysis, a Nd-YAG laser (*JK Laser Systems 2000*) with a frequency doubler ($\lambda_{\text{ex}} = 532 \text{ nm}$) or a frequency tripler ($\lambda_{\text{ex}} = 353 \text{ nm}$) and with a pulse width of $\sim 15 \text{ ns}$ has been used. The energy of the laser pulse has been measured by a fast photodiode (*ITT F-4014*) which has been calibrated with a joulemeter (*Laser Instruments Ltd.*, series 14 [40]). The irradiated surface on the sample cell has been determined to be 0.25 cm^2 . In a cross-beam geometry, the monitoring light from a Xe lamp (*Oriel*, 450 W) has been conveniently filtered before and after the passage through the sample cell and then analyzed by means of a monochromator (*Bausch & Lomb*, blaze: 500 nm) and a photomultiplier (*Hamamatsu R928*). The signals have been amplified by a *Tektronix 7A13* and recorded on a digital oscilloscope (*Tektronix 7D20*) controlled by a *HP87* computer. The same computer has been used for the kinetic analysis of the recorded data.

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