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PAPER

# Triplet quantum yields in light-scattering powder samples measured by laser-induced optoacoustic spectroscopy (LIOAS)<sup>†</sup>

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In recent years, different methods and techniques have been applied to study the primary photophysical processes occurring in dye-loaded light-scattering powdered samples. In spite of this, there are still no reliable methods for the determination of triplet quantum yields for this kind of systems. Laser-induced optoacoustic spectroscopy (LIOAS) has been extensively used for the determination of triplet quantum yields of dyes in solution. In a previous work, LIOAS was applied to the measurement of absolute emission quantum yields of highly fluorescent powdered samples. Excellent agreement was found with values obtained from reflectance data. In this work, we apply the same technique for the determination of triplet quantum yields of Rose Bengal and Erythrosine B adsorbed on microcrystalline cellulose. In contrast to water and other solvents, internal conversion cannot be neglected in the cellulose environment. The triplet quantum yield for both dyes is around 0.55 and does not change with dye concentration.

# Introduction

The photophysics of dyes in microheterogeneous media (micelles, liposomes, membranes, polymers, *etc.*) impacts on various fields, such as photomedicine,<sup>1–3</sup> inactivation of microorganisms,<sup>4,5</sup> abatement of organic pollutants,<sup>6,7</sup> and so forth. Applications based on organized systems composed of a dye anchored to nanoparticles or incorporated into a solid matrix received particular attention in connection to photodynamic therapy,<sup>8–10</sup> heterogeneous photocatalysis<sup>11–13</sup> and the development of sensors, transducers and solar cells.<sup>14,15</sup> With the battery of techniques already available, the photophysical characterization of this kind of systems is in most cases straightforward. This is not the case when light scattering comes into play, as it happens when characteristic lengths are in the order or greater than wavelength.

The interest in light-scattering heterogeneous systems involving photoactive species was accompanied by the development of specific methods and techniques.<sup>16–19</sup> They allowed the understanding of the effect of the solid matrix on the properties of dyes and the role of intermolecular interactions at large local concentrations on energy transfer and trapping processes. Our group has developed different techniques, methods and models useful for the study of primary photophysical processes in solid particulate systems, especially those taking place from the singlet state of the dye, including energy transfer to different substrates.<sup>20–27</sup> In such cases, the determination of the fraction of incident radiation absorbed by the system depends on its geometry. For moderate scatterers with arbitrary geometry the problem becomes rather involved, whereas, for optically thick samples, phenomenological treatments, such as the Kubelka–Munk theory,<sup>28,29</sup> may be easily applied. Reabsorption of emitted light can equally be acquainted for using well established models.<sup>18</sup> However, in spite of the intense work dedicated by several research groups to the determination of quantum yields in particulate light scattering systems, the determination of triplet quantum yields,  $\Phi_{\rm T}$  remains elusive and eventually based on uncertain assumptions, such as neglecting internal conversion.<sup>30</sup>

In addition to its well known application to solution systems,<sup>31–33</sup> laser-induced optoacoustic spectroscopy (LIOAS) has been successfully applied to the study of non-radiative processes in turbid suspensions,<sup>34–36</sup> thin films,<sup>37,38</sup> intact leaves,<sup>39,40</sup> and polymeric matrices,<sup>41</sup> among others. Regarding dyed powders, application was restricted to the measurement of absorption spectra due to the difficulty in achieving reproducible acoustic contact.<sup>42</sup> The presence of multiple solid–air interfaces constitutes a severe drawback, discouraging further studies. Recently, we developed a cell suited to this kind of measurement and thoroughly analyzed the conditions within which reliable measurements can be made. The method, based on the contact of the powder with the piezoelectric transducer (PZT) under reproducible conditions, was successfully applied to the determination of fluorescence quantum yields in optically thick light-scattering solid samples.<sup>43</sup>

The present work deals with the determination of triplet quantum yields of Rose Bengal (RB) and Erythrosine B (EB) adsorbed on microcrystalline cellulose (MC) as a function of the

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concentration of the dye. These xanthene dyes are good triplet sensitizers due to the presence of four iodine atoms in the xanthene moiety, together with four chlorine atoms in the pendant carboxyphenyl group in RB, which drive intersystem crossing through spin–orbit coupling.<sup>44–50</sup> RB has been used in photodynamic therapy and selective inactivation of microorganisms, <sup>51–54</sup> exploiting its high  $\Phi_{\rm T}$  values in different media. It has a moderate absorption at 532 nm, the LIOAS excitation wavelength in this work. Some years ago, we investigated the photophysical properties of RB on MC and showed that this molecule forms fluorescent aggregates in the solid.<sup>23</sup> EB absorbs strongly at 532 nm and also has high  $\Phi_{\rm T}$  values in common solvents. It is commonly used as a fluorescence marker and phosphorescence environmental sensor. Some papers report on the photophysical properties of these dyes in solid media.<sup>45,55–58</sup>

# **Experimental**

#### Materials and sample preparation

Erythrosine B (Sigma-Aldrich, laser grade), Rose Bengal (Aldrich, disodic salt, 93%), Brilliant Blue G (BBG, Sigma-Aldrich) – the reference selected for LIOAS measurements – and Rhodamine 101 inner salt (R101, Kodak) – the fluorescence reference – were used without further purification. Ethanol (Cicarelli, ACS grade) and microgranular cellulose powder (Sigma, pH 5–7, average particle size 20  $\mu$ m) were used also as received.

Dye and reference samples were prepared by suspending weighed amounts of cellulose (1.5 g), previously dried under vacuum at 40 °C for 48 h, in known amounts of a dye stock solution in ethanol, adding solvent to attain a final volume of 30 cm<sup>3</sup>. The suspension was shaken for 5 min and the solvent evaporated at low pressure in a Rotavap at 40 °C. The vacuum was regulated to attain solvent evaporation in *ca*. 15 min. In this way, samples bearing 0.023–0.41 µmol RB, 0.045–0.45 µmol EB and 0.36–2.9 µmol BBG/g MC were prepared. Samples were dried in a vacuum at 40 °C for 48 h and maintained in the dark. Drying was repeated before reflectance and LIOAS measurements. Relative fluorescence quantum yields were determined against samples bearing R101 on cellulose prepared in the same way.

Measurements were performed at room temperature on optically thick and thin layers of each sample. A layer depth of 0.2 cm ensures optical thickness (no light transmission). Thin layers were prepared by spreading a small amount of sample on a double-sided sticky tape.

# Methods

Total and diffuse reflectance spectra of optically thick solid layers were measured in a Shimadzu UV-3101 scanning spectrophotometer equipped with an integrating sphere. Barium sulfate was used as the reflectance reference. Measurements were performed by packing the sample into a suitable holder with a Plexiglas stab and releasing pressure before scanning.

Steady-state emission spectra of optically thick layers were recorded on a PTI model QM-1 spectrofluorometer. Samples were measured in front face, placing a suitable optical filter before the detector to block excitation light. Spectra were corrected according to the dependence of the detection channel responsivity on wavelength obtained from the manufacturer and checked in our laboratory. The fluorescence quantum yield,  $\Phi_{\rm F}$ , is defined for a single chromophore as the number of emitted photons per photon absorbed by the chromophore. In solid samples, the supporting material may contribute slightly to the absorption and fluorescence can be partly reabsorbed by the sample. Therefore, the measurable quantity is the observed fluorescence quantum yield,  $\Phi_{\rm obs}$ , defined as the number of emitted photons leaving the sample per photon absorbed by any present species. Values were determined against R101 using the following equation:

$$\Phi_{\rm obs} = \Phi_{\rm obs}^{\rm r} \frac{J(I - R_{\lambda_0}^{\rm r})I_0^{\rm r}}{J^{\rm r}(I - R_{\lambda_0})I_0} \tag{1}$$

where  $\Phi_{obs}^{r}$  is the reference fluorescence quantum yield, measured on an absolute basis as already described;<sup>19</sup> *J* and *J*<sup>r</sup> are the areas under the sample and reference spectra, respectively, *R* and *R*<sup>r</sup> are the respective reflectances measured at the excitation wavelengths  $\lambda_0$  and  $\lambda_{0r}$ , respectively; and  $I_0$  and  $I_0^{r}$  the respective excitation photon fluxes, the ratio of which is obtained from the known relative excitation spectra.

In contrast to fluorescence, phosphorescence is not reabsorbed by the emitting dye and, if the solid support is transparent at the absorption and emission wavelengths, the observed phosphorescence quantum yield does not differ from the molecular property,  $\Phi_{\rm P}$ .

LIOAS probes were prepared by pressing a weighed amount of dry solid samples (typ. 60 mg) into a specially designed aluminum holder at a definite pressure (typ. 25.5 bar) for a given time (typ. 120 s). Unless otherwise stated, before LIOAS measurements the probes were allowed to relax at atmospheric pressure in a desiccator for at least 24 h. On conditioning samples for reflectance measurements, no special care other than drying was needed.

The LIOAS cell was already described in ref. 43. A 2 mm thick probe contained in the aluminum holder is illuminated from above with pulses from a Nd-YAG laser (Spectron, 8 ns @ 532 nm). The laser beam passes through a set of three IR filters (Schott, KG5, 0.2 cm thickness) to avoid unwanted heating of the sample and spurious LIOAS signals, a gray wedge filter, a pinhole and a prism. The diameter of the laser beam at the sample surface is ca. 3 mm. Light scattered by the sample is driven away by a reflector to avoid its reentrance into the probe. A 0.8 cm thick quartz plate is placed between the holder and the PZT assuring acoustic contact with a small amount of silicon grease. To afford reproducibility, the probe is pressed against the holder with a Plexiglas window and pressure is manually regulated with a specially designed screw system. The PZT and the quartz plate are kept fixed during every set of experiments, whereas the window, the reflector and other mechanical parts are adjusted after probe exchange. Dye samples and calorimetric references are measured under similar conditions during the same measurement session to afford reproducible signals. Negligible signals are obtained when an unloaded cellulose sample is placed in the holder. For a thorough description of the LIOAS setup, see ref. 43.

The following energy balance equation applies in our LIOAS experiments:

$$\frac{H}{E} = A(1-R) \\ \times \left(1 - \Phi_{\text{obs}} \frac{\langle \bar{v}_{\text{F}} \rangle}{\bar{v}_0} - \Phi_{\text{P}} \frac{\langle \bar{v}_{\text{P}} \rangle}{\bar{v}_0} - \Phi_{\text{T}} \frac{E_{\text{T}}}{hc \bar{v}_0}\right) \exp(-\mu d) \quad (2)$$

where H is the amplitude of the first maximum of the optoacoustic signal, E is the laser pulse energy, A is an instrumental constant, R is the total reflectance of the sample,  $\langle \bar{v}_{\rm F} \rangle = /\bar{v}_{\rm F} f(\bar{v}_{\rm F}) d\bar{v}_{\rm F}$ is the fluorescence average wavenumber,  $f(\bar{v}_{\rm F})$  being the area normalized fluorescence spectrum,  $\langle \bar{\nu}_{\rm P} \rangle$  is the phosphorescence average wavenumber defined in an analogous way,  $\bar{v}_0$  is the incident radiation wavenumber,  $E_{\rm T}$  is the relaxed triplet energy,<sup>31</sup> h is the Planck constant, c the velocity of light in vacuum,  $\mu$  is the napierian sound absorption coefficient of the sample, and d its thickness. Eqn (2) is the generalization of eqn (1) in ref. 43 when a long lived triplet is the only species storing energy after laser excitation of the dye. The exponential term reflects sound loss within the sample and is a constant factor for samples prepared under identical conditions, particularly equal thickness and compaction. The value of A  $exp(-\mu d)$  is obtained by plotting H/E vs. (1 - R) for the calorimetric reference, characterized by  $\Phi_{\rm obs} = 0$  and  $\Phi_{\rm T} = 0$  (accordingly  $\Phi_{\rm P} = 0$ ).

As it has been demonstrated, MC samples fulfill the conditions needed for eqn (2) to be valid,<sup>43</sup> among them: a) the sample is optically thick; this is the case for d = 0.2 cm, b) multiphoton absorption and ground state depletion are negligible; in this case *H* is proportional to *E*, valid for  $E < 300 \mu$ J, and c) sound waves originate from the sample outer surface and are attenuated to the same extent while traversing the sample; this condition is fulfilled if the time spread of the acoustic waves reaching the detector is lower that the reciprocal bandwidth of the PZT and the decay of the illumination into the sample is faster than the decay of the amplitude of the acoustic wave due to sound absorption.

The calorimetric reference required for optoacoustic measurements should be characterized by very fast radiationless deactivation, assuring essentially no fluorescence or phosphorescence emission and no formation of photoproducts or any long-lived intermediate. In this work we used BBG, a dye with a broad absorption around 600 nm.<sup>59</sup>

### Results

#### Reflectance and luminescence spectra

Spectroscopic properties of RB and EB on MC obtained on optically thick layers are summarized in Fig. 1. Absorption spectra are calculated in terms of the remission function,



**Fig. 1** Normalized remission function (left) and luminescence spectra (right) are displayed for RB (upper panel) and EB (lower panel) on MC at different dye concentrations,  $C_0$  (not to scale). In each case the spectrum of the most dilute sample is brought to the front (broken lines, indicated by the arrows) to appreciate the effect of concentration. Insets show remission functions at maximum (left) and observed fluorescence quantum yields (right) as a function of  $C_0$  (µmol g<sup>-1</sup>). Excitation wavelength: RB, 532 nm; EB, 505 nm.

 $F(R_{\rm d}) = (1 - R_{\rm d})^2 / 2R_{\rm d}$ , where  $R_{\rm d}$  is the diffuse reflectance. Both dyes display similar spectral features. The main differences lay in the position of absorption bands (564 and 525 nm for RB and 542 nm with a shoulder at ca. 510 nm for EB) and fluorescence bands (575 nm for RB and 560 nm for EB), yielding a larger Stokes shift for EB. The slight increase in the low wavelength band relative to the absorption maximum and the widening of absorption spectra as concentration increases may be taken as signs of dye aggregation. At the same time, hypochromism, apparent as a downward curvature in the plot of remission function maxima against concentration (see insets at the left side of Fig. 1), is observed. Fluorescence spectra show typical effects of light reabsorption, namely red shift and larger relative amplitude of the secondary maximum or shoulder as concentration increases. Phosphorescence is observed at longer wavelengths (at ca. 687 nm for RB and 675 nm for EB). No shift of phosphorescence spectra with concentration is noticed owing to the lack of reabsorption in this spectral range.

Fig. 2 shows thin and optically thick layer fluorescence spectra for the most concentrated RB and EB layers, respectively, together with thick layer spectra corrected for reabsorption according to ref. 18. The quantitative agreement between thin layer and corrected thick layer spectra demonstrates that reabsorption is the source of differences found on thick layer spectra as a function of concentration.

Observed fluorescence quantum yields were calculated against R101 (see insets at the right side of Fig. 1). The fluorescence spectrum, corrected by the detection channel response function, was integrated from the fluorescence onset to 680 nm for RB and to 650 nm for EB.  $\Phi_{\rm obs}$  decreases with concentration, the effect being greater for EB. Phosphorescence quantum yields were not calculated. On one side, the detector channel responsivity could not be determined accurately at the longest wavelengths. On the other side, the effect of the fluorescence term in eqn (2) is small, as will be demonstrated below (see Laser induced optoacoustic spectroscopy) and, as it may be seen from Fig. 1, energy loss by phosphorescence should be much smaller.

The photophysical behavior of RB on MC has been described elsewhere,<sup>23</sup> with emphasis on fluorescence properties. Results were consistent with the formation of fluorescing dimers as concentration increases. Upon correction of  $\Phi_{\rm obs}$  by reabsorption,  $\varPhi_{\rm F}$  values were estimated as 0.120  $\pm$  0.004 and 0.070  $\pm$  0.006 for monomers and dimers, respectively. Similar calculations performed in this work for samples ranging from  $2.3 \times 10^{-8}$  to 4.1  $\times 10^{-7}$  mol RB (g MC)<sup>-1</sup> yielded 0.13 and 0.08 for the same quantities, respectively, in accordance with previous results. The fact that  $\Phi_{obs}$  is generally smaller than 0.08 and decreases steadily with concentration (see Fig. 1) is ascribed to fluorescence reabsorption. A similar calculation has been performed for EB but, though aggregation takes also place in this case, no evidence of fluorescing dimers was found. This might be the reason why  $\Phi_{\rm obs}$  decreases for EB much more rapidly with concentration than for RB, though fluorescence reabsorption is less probable for EB due to the larger Stokes shift and lower remission function values for the same concentration range (see insets at the left of the figure). A rough calculation yielded  $\Phi_{\rm F} \cong 0.17$  for the monomer.



Fig. 2 Normalized fluorescence spectra of 0.41  $\mu$ mol Rose Bengal (top) and 0.45  $\mu$ mol Erythrosine B (bottom) per g microcrystalline cellulose: thin layers (full line), optically thick layers (broken line) and corrected thick layers (dotted line). Excitation wavelength: RB, 532 nm; EB, 505 nm. See text.

#### Laser induced optoacoustic spectroscopy

Samples characterized spectroscopically, as detailed in the last section, were conditioned for LIOAS measurements as described in the experimental section. Each sample was measured, exchanging the probe several times. A minimum of three measurements of H and E were measured for each probe. The optoacoustic signal was registered for several microseconds and its shape inspected. A constant shape assures equally good contact between sample and holder and a good transmission of the optoacoustic signal. Therefore, outliers can easily be discovered.

Fig. 3 shows plots according to eqn (2) for BBG samples, characterized by  $\Phi_{obs} = 0$  and  $\Phi_T = 0$ , used as references for RB and EB. The slope of the straight lines is  $A \exp(-\mu d)$ . Though reference samples used for both dyes were the same, the slope differs because the instrumental constant A depends on the actual conditions of the experiment, which are slightly different for both dyes. In the scale used in Fig. 3, slopes are 1.473 for RB and 1.520 for EB. From these slopes,  $\Phi_T$  values displayed in Table 1 are calculated for RB and EB samples. For that purpose,  $\Phi_{obs}$  and  $\bar{v}_F$  values, also given in Table 1, are determined as outlined above (see Methods).

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For the calculation, eqn (3) is used. The fluorescence term amounts from 0.04 to 0.07. Compared to fluorescence, the phosphorescence term included in eqn (2) is much less important, as shown by the corresponding areas below the emission spectra (see Fig. 1), and is therefore neglected. By far, the most important term is the one including H/E, also the most relevant factor in defining the error. As shown in Table 1, a constant uncertainty deduced from the overall reproducibility of the measurements, is considered. Another source of uncertainty refers to  $E_{\rm T}$ , which is calculated from the maxima of the phosphorescence spectra (740 nm for RB and 700 nm for EB). These values are not the relaxed triplet energies as required in eqn (2), which cannot be easily obtained. Inspection of Fig. 1 shows that in both cases,  $E_{\rm T}$ might be underestimated by less than 5% and the effect of this uncertainty in  $\Phi_{\rm T}$  should be much less than the error derived from inaccuracies in H/E values.

$$\Phi_{\rm T} = \frac{hc\bar{v}_0}{E_{\rm T}} \left( 1 - \frac{H/E}{A\exp(-\mu d)(1-R)} - \Phi_{\rm obs} \frac{\langle \tilde{v}_{\rm f} \rangle}{\bar{v}_0} \right)$$
(3)



**Fig. 3** Optoacoustic signal divided by the laser pulse energy as a function of 1 - R for BBG references, from left to right: 0.18; 0.36; 0.72; 1.1; 1.8; and 2.9 µmol g<sup>-1</sup>. Values for RB (circles) and EB (squares) are separately fitted by least squares (lower line, RB; upper line, EB).

Within the experimental error,  $\Phi_{\rm T}$  is independent of the dye concentration both for RB and EB (Table 1), with average values of  $0.57 \pm 0.12$  and  $0.55 \pm 0.15$ , respectively.

# Discussion

The following questions, which apply to both dyes, will be addressed in what follows: 1) why is the triplet quantum yield obtained in cellulose lower than in other media? 2) can the sum of monomer fluorescence and triplet quantum yields be less than one?; 3) why do the triplet quantum yields not decrease with concentration?

#### Monomer triplet quantum yield

As it was pointed out, both dyes aggregate as concentration increases. Spectroscopic results are consistent with a formal monomer-dimer equilibrium and point to a weak interaction between monomers within the aggregate. Otherwise, changes in absorption spectra (Fig. 1) would be far more important. As  $\Phi_{\rm T}$ does not depend on concentration, the average values should be attributed both to the monomeric and to the dimeric states of both dyes. Let us concentrate first on the monomeric state. The monomer fluorescence quantum yield has been calculated in this work as  $\Phi_{\rm F} = 0.13$  for RB and = 0.17 for EB. Taking into account quoted  $\Phi_{\rm T}$  values, the sum of monomer quantum yields is  $\Phi_{\rm F} + \Phi_{\rm T} = 0.70 \pm 0.13$  for RB and  $0.72 \pm 0.18$  for EB. A relatively large error was assigned to the fluorescence quantum yield for EB monomers. The uncertainty for RB has been set to a smaller value because our measurements confirm previous results.<sup>23</sup> Two points appear clear from these results:  $\Phi_{\rm T}$  values are low in contrast to the same dyes in water (see below) and  $arPsi_{
m F}$ +  $\Phi_{\rm T}$  values are well below unity, so that internal conversion cannot be neglected.

Fleming *et al.*,<sup>60</sup> based on their own as well as Martin's<sup>61</sup> results, point out that xanthene dye S<sub>0</sub>, S<sub>1</sub> and T<sub>1</sub> states are stabilized in proton donating solvents in such a way that  $\Delta E_{S0} > \Delta E_{S1} > \Delta E_{T1}$ . This means that, on passing from isopropyl alcohol to ethanol, methanol and water, the S<sub>1</sub>–T<sub>1</sub> energy gap becomes smaller and the S<sub>1</sub>–S<sub>0</sub> energy gap increases. Accordingly, absorption energies and triplet quantum yields increase

Table 1 Photophysical and LIOAS data for samples of Rose Bengal (RB) and Erythrosine B (EB) adsorbed on microcrystalline cellulose

Sample	Concentration ( $\mu$ mol g <sup>-1</sup> )	R	$\Phi_{ m obs}$	$\bar{v}_{\rm F}~({\rm cm}^{-1})$	<i>H/E</i> (a.u.)	$arPsi_{ m T}$
RB-1	0.023	0.90	0.078	16750	$0.073 \pm 0.02$	$0.61 \pm 0.1$
RB-2	0.047	0.86	0.073	16 640	$0.098 \pm 0.02$	$0.64 \pm 0.1$
RB-3	0.069	0.83	0.082	16 620	$0.112 \pm 0.02$	$0.67 \pm 0.1$
RB-4	0.120	0.80	0.070	16 600	$0.190 \pm 0.02$	$0.41 \pm 0.1$
RB-5	0.210	0.77	0.069	16 570	$0.180 \pm 0.02$	$0.57 \pm 0.1$
RB-6	0.310	0.74	0.060	16 580	$0.221 \pm 0.02$	$0.52 \pm 0.1$
RB-7	0.410	0.68	0.057	16510	$0.255\pm0.02$	$0.57\pm0.1$
EB-1	0.045	0.78	0.063	17 270	$0.178 \pm 0.02$	$0.54 \pm 0.1$
EB-2	0.067	0.77	0.064	17 290	$0.186 \pm 0.02$	$0.54 \pm 0.1$
EB-3	0.090	0.72	0.068	17 240	$0.192 \pm 0.02$	$0.64 \pm 0.1$
EB-4	0.170	0.69	0.048	17210	$0.323 \pm 0.02$	$0.36 \pm 0.1$
EB-5	0.230	0.58	0.062	17 180	$0.264 \pm 0.02$	$0.70 \pm 0.1$
EB-6	0.330	0.59	0.043	17 160	$0.388 \pm 0.02$	$0.45 \pm 0.1$
EB-7	0.450	0.57	0.043	17 130	$0.296\pm0.02$	$0.67\pm0.1$

and fluorescence quantum yields decrease in that order. In cellulose, a weak proton donating medium,  $\Phi_{\rm T}$  should be smaller, whereas  $\Phi_{\rm F}$  should be larger than in water. This is in fact the case as it will be corroborated below. According to Fleming et al.,<sup>60</sup> absorption maxima are  $\lambda_{max} = 561, 558, 556, and$ 548 nm for RB and 532, 532, 526, and 521 nm for EB for the solvents indicated above, respectively. In cellulose,  $\lambda_{max}$  = 564 nm for RB and 542 nm for EB, implying that the proton donating capacity of cellulose is lower than that of isopropanol. It should be noticed that, in all cases, spectra correspond to the dianionic form of the dyes. Regarding the rule  $\Phi_{\rm F} + \Phi_{\rm T} \cong 1$ , considered valid for xanthene dyes by various authors, 45,47,60 it has to be expected to break down in poor proton donating media. In fact, for halogenated xanthenes, the same internal conversion decay constant,  $k_{ic}$ , should be much lower than the intersystem crossing decay constant,  $k_{ics}$ , for strong proton donors but in the same order for poor donors, as it might occur in the case of cellulose.

The formation of triplet states of halogen substituted xanthene dyes has been investigated for a long time. In the study by Fleming et al.<sup>60</sup> on Eosin, EB and RB, unfortunately, only the nonradiative singlet decay constant  $k_{nr} = k_{ic} + k_{ics}$  was given but not the individual constants, together with the radiative constant  $k_{\rm r}$ . For each dye,  $k_{\rm r}$  is almost independent of the solvent. Values are similar for all dyes, between  $1.2 \times 10^8 \text{ s}^{-1}$  and  $2 \times 10^8 \text{ s}^{-1}$ . In contrast,  $k_{nr}$  increases from isopropanol to water, being dramatically larger in water than in the rest of the solvents. Encinas et al.<sup>50</sup> recently measured the triplet quantum yield of several xanthene dyes in water at pH 9.5 by laser flash photolysis and LIOAS. As both methods yield the product  $\Phi_{\rm T}E_{\rm T}$  triplet energies were obtained from the literature. Results were consistent with previously measured values. In contrast,  $\Phi_{\rm T}$  values obtained in other solvents by several authors show very large differences. As an example, Reindl and Penzkofer report values from 0.52 and 0.96 for RB and 0.58 to 0.94 for EB, most of them being estimations.62

Knowledge of  $\Phi_{\rm F}$  and  $\Phi_{\rm T}$  allows calculation of relative values of  $k_{\rm r}$ ,  $k_{\rm ic}$  and  $k_{\rm isc}$  in water and cellulose (Table 2). The same relative internal conversion rate constant adjusts experimental quantum yields for each dye within experimental error. Accordingly, medium independent  $k_{\rm r}$  and  $k_{\rm ic}$  reproduce very well the  $\Phi_{\rm F}$ and  $\Phi_{\rm T}$  values in water and cellulose and, adjusting  $k_{\rm isc}$ , also fit the  $\Phi_{\rm F}$  values in methanol. The estimation of  $\Phi_{\rm T}$  for both dyes in methanol falls within the large range of reported values.

**Table 2** Comparison of monomer quantum yields fluorescence andtriplet quantum yields of Rose Bengal (RB) and Erythrosine B (EB) invarious media

		Relative values		Calculated		Experimental		
		$k_{\rm r}$	$k_{\rm ic}$	kisc	$arPhi_{ m F}$	$\Phi_{\mathrm{T}}$	$arPhi_{ m F}$	$arPhi_{ m T}$
RB	Water	1	2	46	0.02	0.94	$0.02^{a}$	$0.93^{b}$
	Methanol	1	2	6	0.11	0.66	$0.11^{a}$	?
	Cellulose	1	2	4	0.14	0.57	$0.13^{c}$	$0.57^{c}$
EB	Water	1	2	49	0.02	0.94	$0.02^{a}$	$0.97^{b}$
	Methanol	1	2	9	0.08	0.75	$0.08^{a}$	?
	Cellulose	1	2	3	0.17	0.50	0.17 <sup>c</sup>	0.55 <sup>c</sup>

<sup>a</sup> Ref. 60. <sup>b</sup> Ref. 50. <sup>c</sup> This work.

Results also predict the breakdown of the  $\Phi_{\rm F} + \Phi_{\rm T} \cong 1$  rule for poor proton donors and a dramatic increase of  $k_{isc}$  in water. Regarding the validity of the independence of  $k_{ic}$  on the medium, no evidence exists for xanthene dyes undergoing principally intersystem crossing. For obvious reasons, there is data only for fluorescing xanthenes. In contrast, the analysis of the possible dependence of  $k_r$  on the medium is well documented for fluorescent, as well as for triplet forming, xanthenes. As an example, Ferreira et al.<sup>63</sup> report the behavior of Rhodamine 3B, for which  $k_{ic} = k_{nr}$ , in a series of solvents and discuss the general properties of rhodamines. The dependence of  $k_{ic}$  on the medium is ascribed to microviscosity and sometimes to micropolarity, the first factor related to the rotation of the dimethylamino groups, with no effect of the pendant carboxyphenyl group. The absence of rotating groups in RB and EB rules out any effect of rigidization imposed by cellulose on the xanthenes. Other factors related to internal charge transfer seem to be nonrelevant for these dyes as well.

#### The effect of concentration

It is not surprising that the  $\Phi_{\rm obs}$  values decrease as concentration increases (Fig. 1). At the highest dye concentrations fluorescence reabsorption takes place (Fig. 2). Moreover, formation of dimers or higher aggregates reduces fluorescence quantum yields. Though fluorescent aggregates are found for RB, the  $\Phi_{\rm F}$  value for the dimer is lower than for the monomer. The effect is more pronounced for EB, which builds non luminescent dimers. It is a rule that H- or quasi H-dimers (sandwich type) are not or only slightly fluorescent because the higher excitonic state undergoes rapid conversion to the lower state, from which radiative decay is forbidden.<sup>64</sup> If the last state has a larger energy than T<sub>1</sub>, rapid intersystem crossing may occur, populating the triplet state. Once the triplet state is formed, excitonic interaction is drastically reduced because the dipole moment of the  $S_0 \leftarrow T_1$  transition is much smaller than that of the  $S_0 \leftarrow S_1$  transition. Triplet monomers become independent of the ground state partner in terms of exciton theory and their radiationless decay is not enhanced in contrast to singlet state dimers.<sup>65</sup>

The fact that  $\Phi_{\rm T}$  is equal for monomers and dimers requires that the ratio between  $k_{\rm r} + k_{\rm ic}$  and  $k_{\rm isc}$  remains unchanged on dimerization. It was pointed out that the spreading out of the singlet levels may enhance  $k_{\rm isc}$  for the aggregate;<sup>65</sup> thus,  $k_{\rm r} + k_{\rm ic}$ must increase accordingly. Of course, the rather large experimental error might obscure any slight dependence of  $\Phi_{\rm T}$  with concentration.

Kamat and Fox<sup>45</sup> have found, in accordance with other authors, that intermolecular charge transfer might cause RB and EB triplet state quenching as concentration increases when dyes are electrostatically attached to poly(4-vinylpyridine), leading to an apparent reduction of  $\Phi_{\rm T}$ . These effects should be absent in our case taking into account the range of intermolecular distances involved. It does not seem either a source of triplet state quenching within the dimers.

Finally, exciton interactions among monomers within aggregates seem to be very weak on grounds of the slight absorption spectrum changes as concentration increases (Fig. 1). Nevertheless, these interactions cannot be neglected as there is evidence on their effect on the fluorescence quantum yield, at least for RB.<sup>23</sup>

We have demonstrated in this work that, using LIOAS and a specially designed sample holder, it is possible to derive the triplet quantum yield of dyes in solid highly scattering samples. For Rose Bengal and Erythrosine B monomers adsorbed on microcrystalline cellulose  $\Phi_{\rm T} = 0.57 \pm 0.12$  and  $0.55 \pm 0.15$ , respectively. Taking into account the reabsorption-corrected monomer fluorescence quantum yields,  $\Phi_{\rm F} + \Phi_{\rm T} = 0.70 \pm 0.13$ for RB and  $0.72 \pm 0.18$  for EB are obtained. This means that  $\Phi_{\rm F} + \Phi_{\rm T}$  values are below unity beyond the experimental uncertainty, so that internal conversion cannot be neglected in cellulose, a poor proton donating medium, as is the case in aqueous solutions or other strong proton donating solvents. For both dyes, triplet quantum yields do not depend on concentration. Thus, the obtained average values should be attributed both to the monomeric as well as to the dimeric states. This result might arise from a the parallel increase of  $k_{isc}$  and  $k_r + k_{ic}$  in the dimeric state. The relatively high and concentration independent  $\Phi_{\rm T}$  value obtained for both dyes should be of importance for the usage of supported dyes as heterogeneous photosensitizers.

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