Photophysical behavior of new acridine(1,8)dione dyes

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The photophysical behavior of five acridine(1,8)dione dyes of biological interest was studied by absorption and fluorescence spectroscopy, photoacoustics and time resolved phosphorescence techniques. The results obtained in ethanol and acetonitrile solutions show that the main spectroscopic and photophysical parameters of these compounds depend strongly on both the solvent and oxygen concentrations. Oxygen completely quenched the triplet state of all dyes. In nitrogen-saturated solutions, quantum efficiencies of triplet formation in ethanol were lower than those in acetonitrile.

1. Introduction

Acridine(1,8)dione dyes have received increasing attention due to their unique photophysical and photochemical properties. The structure of these dyes is similar to that of 1,4-dihydropyridine which has been extensively studied because of its structural similarity to reduced nicotinamide adenine dinucleotide (NADH), which acts as a coenzyme in biological systems.[1,2] There are several drugs of this family, such as nimoldipine, nisoldipine and nifedipine, which are used as calcium antagonist, antihypertensive agents and anti-inflammatory drugs.[2,3] Acridinedione derivatives exhibit a wide range of biological activities, especially mutagenic, antitumour and antiamoebic properties.[4–10] Acridinedione dyes have been reported to be good photoinitiators for photopolymerization of acrylates and methacrylates,[11] and are used as photosensitizers in onium salt decomposition.[12] These dyes have been reported to be a class of laser dyes operating in the blue–green region and they have lasing yields which are comparable to those of the well-known standard coumarin-102.[13] Their bichromophoric structure, with the heterocyclic nitrogen and carbonyl groups, enables these compounds to act as both electron donors and acceptors.[14,15]

By using spectroscopic, luminescence, photoacoustic and time resolved phosphorescence techniques, we report now on the photophysical characterization of five new compounds of the acridinedione family in ethanol and acetonitrile neutral and acidified solutions in the presence and in the absence of O_2.

2. Results and discussion

2.1 Absorption and emission spectra

In non-acidified acetonitrile and ethanol solutions the absorption spectra of the acridinedione dyes show 2 peaks around 243 and 360 nm and around 245 and 370 nm, respectively. The highest wavelength band has been assigned to the charge transfer from nitrogen to oxygen.[13]

In acidified solutions spectral changes were observed as a function of the acid concentration. Dyes **1**, **2**, **3** and **5** show a red shift in the characteristic bands in both solvents. In contrast, dye **4** shows a blue shift of the short-wavelength band and a red shift of the long-wavelength band.

Changing from acetonitrile to ethanol in neutral solutions results in a hypsochromic effect in dye **4**, whereas for dyes **1–3** a bathochromic effect is observed. This difference in the behavior of dye **4** may be attributed to the opposite location of the two N (pyridinic nitrogen almost in a mirror position to the secondary amine) *vs.* the protonation of the pyridine. This must generate in the excited electronic state a very different dipole than the ground state, which interacts with the environment in a different manner than the ground electronic state. Note that at acidic pH, again the shift observed for compound **4** is different from the shift for dyes **1–3** when passing from one solvent to another.

The fluorescence spectra of the acridinediones dyes show a structureless behavior and a red shift is observed when the polarity and protic character of the solvent are increased.

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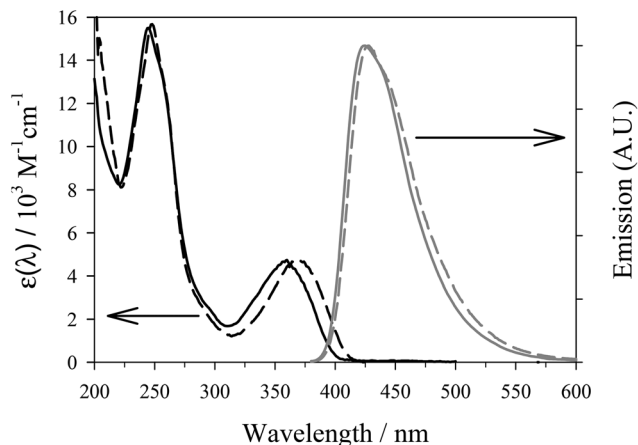
Fig. 1 Absorption coefficient and fluorescence normalized spectra of dye **1** in acetonitrile (solid line) and ethanol (dotted line) air saturated neutral solutions.

As a representative example of this behavior, Fig. 1 shows the absorption and fluorescence spectra of dye **1** in two solvents of differing polarity and proticity.

The spectroscopic data for the five dyes in acetonitrile and in ethanol are summarized in Table 1.

A comparison between absorption and emission spectra of acridinedione dyes **1–5** in acetonitrile and ethanol shows that the emission spectrum of each compound presents higher shifts with the solvent than the absorption spectrum. This behavior is characteristic of compounds that have π–π^* transitions like in this case.[16] The fluorescence quantum yield of the acridinedione dyes depends on solvent and oxygen concentrations. When the solution was saturated with O_2, the values of Φ_f were 40% lower than those determined in air. In contrast,

in N_2-saturated solutions, the value of Φ_f increased 15% with respect to the air value. O_2 might be inducing intersystem crossing (ISC), and thus increasing triplet yield, as shown below. Similar results were reported for other aromatic compounds such as anthracene derivatives, in which O_2 induces ISC.[17] The value of Φ_f quoted for the protonated form is a weighed value of the fluorescence yields for the neutral and protonated forms. In any case, probably the protonated form does not emit because the value of Φ_f is very low under acidic conditions.
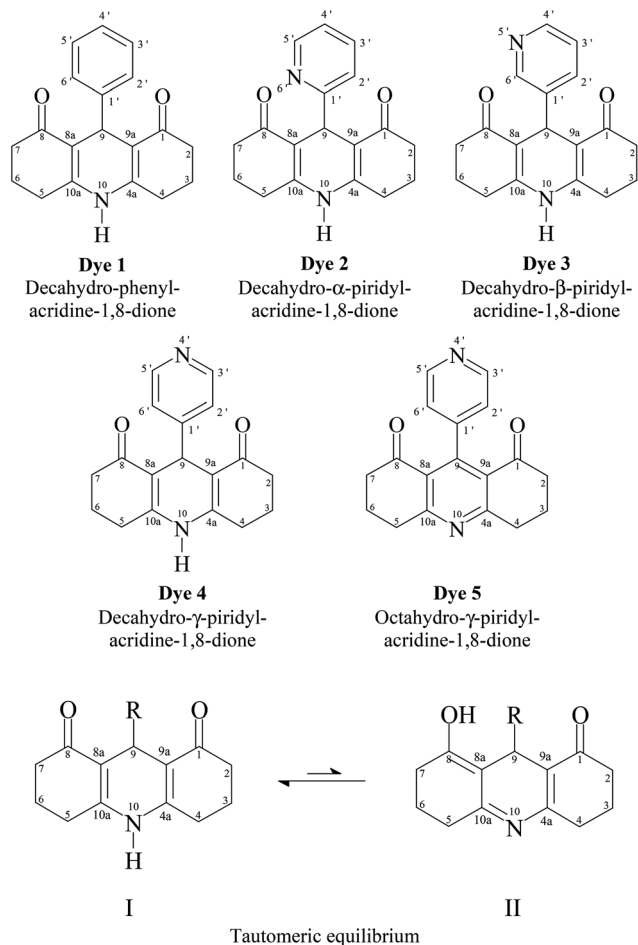
The fact that in EtOH the spectra show the same shift with respect to those in acetonitrile as that obtained by acidification of the neutral acetonitrile solutions can be explained by taking into account that EtOH is a proton donor. Ethanol increases the value of Φ_f (expect for compound **5**, see below) and reduces the triplet quantum yield and, consequently, the singlet oxygen quantum yield. This suggests that the hydrogen bonding provided by ethanol may increase the singlet lifetime.

Dye **5** is the only one that has two aromatic pyridine rings in its structure, one of them in the condensed tricyclic unit. For this reason, the tautomeric equilibrium shown in Scheme 1 is not important in the ground state. A tautomeric equilibrium may occur in the excited electronic state, but of a keto–enol type, similar to other aromatic carbonyl compounds such as acetophenone, benzophenone, and other calorimetric references (CR).[18] In EtOH, this equilibrium is affected by the bimolecular H-bridge between the solvent and the compound, which decreases its Φ_f value. In acetonitrile, another way of deactivation would be favored without protonation of C=O or pyridinic N. The shift of the absorption and emission maxima in acidic pH observed for this dye is characteristic of the protonation of the pyridinic nitrogen observed in β-carbolines and

Table 1 Spectroscopic data of the acridinediones dyes, determined in this study

Sample	Solvent	Neutral solution								Acidified solutions (200 µl H_2SO_4)				
		$\lambda_{abs.}$ (nm)	Log ε^a	λ_{exc} (nm)	λ_{emis} (nm)	ϕ_f Air	ϕ_f O_2	ϕ_f N_2	St. Sh^b (cm^{-1})	λ_{abs} (nm)	$\lambda_{exc.}$ (nm)	λ_{emis} (nm)	ϕ_f Air	St. Sh^b (cm^{-1})
Dye 1	EtOH	373	3.67	368	426	0.53	0.40	0.60	3335	382	382	498	0.02	6098
		248	4.19											
	CH$_3$CN	356	3.67	358	415	0.14	0.10	0.15	3877	414	414	498	0.03	4074
		244	4.20											
Dye 2	EtOH	375	3.71	384	430	0.74	0.50	0.80	3410	384	384	498	0.03	5717
		246	4.19											
	CH$_3$CN	362	3.68	362	418	0.26	0.18	0.32	3701	396	394	498	0.05	4927
		243	4.21											
Dye 3	EtOH	369	3.81	369	426	0.56	0.40	0.65	3681	382	382	498	0.02	6057
		244	4.27											
	CH$_3$CN	359	3.78	359	415	0.20	0.17	0.22	3816	390	390	498	0.07	4721
		242	4.28											
Dye 4	EtOH	369	3.15	365	426	0.57	0.45	0.65	3736	391	391	498	0.03	5455
		259	4.28											
	CH$_3$CN	381	3.18	355	415	0.20	0.16	0.25	2266	385	394	498	0.04	5607
		247	4.23											
Dye 5	EtOH	384	3.38	384	435	0.01	0.01	0.01	3053	400	400	487	0.01	4466
		249	4.15											
	CH$_3$CN	372	3.40	369	454	0.53	0.35	0.60	4855	400	400	512	0.03	5469
		247	4.20											

$^a \varepsilon$ in (M^{-1} cm^{-1}). ^{b} St. Sh: Stokes shift.

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Dye 1
Decahydro-phenyl-acridine-1,8-dione

Dye 2
Decahydro-α-piridyl-acridine-1,8-dione

Dye 3
Decahydro-β-piridyl-acridine-1,8-dione

Dye 4
Decahydro-γ-piridyl-acridine-1,8-dione

Dye 5
Octahydro-γ-piridyl-acridine-1,8-dione

I

II

Tautomeric equilibrium

Scheme 1 Structure and tautomeric equilibrium of the studied dyes.
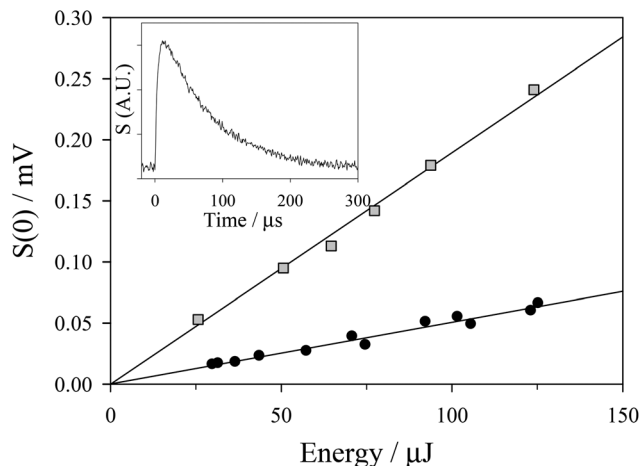

Fig. 2 Dependence of NIR emission of $O_2(^1\Delta_g)$ at zero time, $S(0)$, as a function of laser energy for dye **1** (circles) and the phenalenone reference (squares) in acetonitrile with matched absorbance (0.197 ± 0.001). The inset shows a typical $O_2(^1\Delta_g)$ phosphorescence rise and decay.

Table 2 Photophysical data of neutral solutions of acridinedione dyes, determined in this study

Dye	Solvent	Φ_Δ (± 0.01)	α_{O_2} (± 0.03)	α_{N_2} (± 0.02) α_1	α_2	τ_T N_2 (μs)	Φ_T N_2 (± 0.03)
1	EtOH	0.20	0.60	0.27	0.24	1	0.40
2		0.10	0.60	0.30	0.15	1	0.07
3		0.10	0.65	0.25	0.25	1	0.38
4		<0.01	0.60	0.25	0.20	2	0.30
5		<0.01	1	1	—	<0.2	—
1	CH$_3$CN	0.25	0.85	0.55	0.30	0.4	0.57
2		0.20	0.80	0.32	0.40	0.4	0.70
3		0.20	0.70	0.37	0.43	0.6	0.80
4		<0.01	0.60	0.30	0.50	1	0.90
5		0.10	0.65	0.43	0.10	3	0.18

pyridoindolic compounds. The sp3 character of the lone electron pair of the pyridine allows greater interaction with the π orbitals of the aromatic ring and thus the shifts are produced.[19]

2.2. Time resolved phosphorescence measurements

Singlet oxygen phosphorescence measurements of dyes **1–4** in both ethanol and acetonitrile solutions and dye **5** in acetonitrile show clear evidence of singlet oxygen formation under our experimental conditions. Phosphorescence measurements in ethanol solutions of dye **5** did not show any evidence of singlet oxygen formation.

Fig. 2 shows the dependence of the singlet oxygen phosphorescence intensity emission at zero time, $S(0)$, as a function of the laser energy, for dye **1** and the reference. The inset shows a typical $O_2(^1\Delta_g)$ phosphorescence intensity rise and decay. Similar linear correlations and excellent reproducibility were obtained also for the other dyes in both solvents, working with fluences below 2.8 J m^{-2} (that means laser energies lower than 150 μJ).

From these slopes and the usual procedure described elsewhere[20–23] the quantum yield Φ_Δ of $O_2(^1\Delta_g)$ production was determined and the values are shown in Table 2.

2.3. Photoacoustic measurements

Fig. 3 shows typical energy-normalized photoacoustic signals detected by the piezoelectric transducer for the reference (dashed line), and dye **1** in oxygen (solid line) and nitrogen (dotted line) in acetonitrile solutions of the same absorbance.

Under oxygen all the dyes showed in both solvents photoacoustic signals similar to the signals obtained for the CR. In contrast, under N$_2$ and in both solvents, the signals obtained with dyes **1–4** were phase shifted with respect to the reference signal (see Fig. 4). This is also the case for dye **5** in acetonitrile. In ethanol and under N$_2$, dye **5** signals showed no shift with respect to the reference.

As was discussed by several authors,[18,24,25] prompt heat means the heat integrated by the transducer in processes faster than roughly $\tau_R/5$, where τ_R is the transit acoustic time of the experiment. In the case of the CR, all the deactivation processes take place in a time shorter than $\tau_R/5$. Then, when deactivation of the sample is in a time $t < \tau_R/5$, no temporal shift between the sample and the reference should be expected. In this case, the peak to peak amplitude of the first
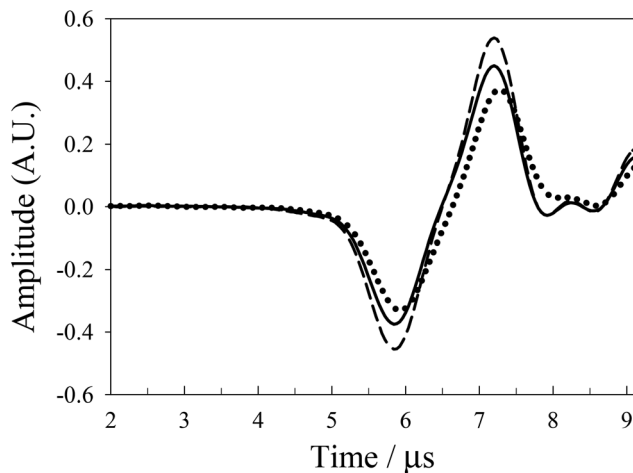
Fig. 3 Photoacoustic signals of 2-HBP (dashed line: air, N$_2$ and O$_2$) and dye **1** (solid line: with O$_2$, dotted line: with N$_2$) measured in acetonitrile solutions of matched absorbance (0.105 ± 0.001).
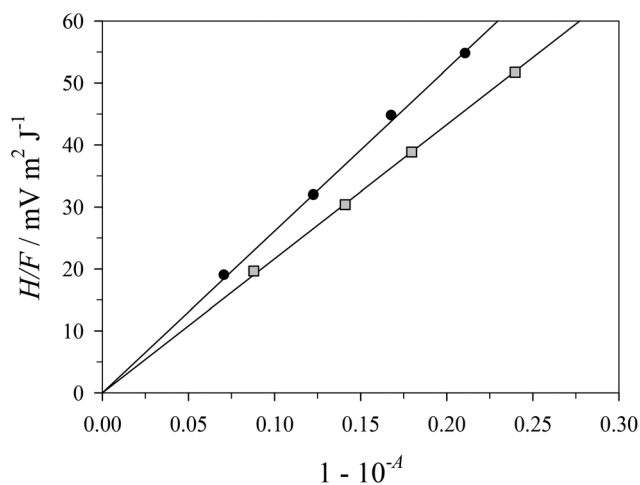

Fig. 4 Amplitude of the fluence-normalized photoacoustic signals as a function of the fraction of absorbed energy for acetonitrile solutions of dye **1** in O$_2$ (squares) and of the reference (circles).

acoustic pulse (H) was used to measure the prompt heat (α) release to the medium by the sample within the time resolution of the experiment (Amplitude method).[23]

Plots of H as a function of the excitation fluence (F) for the reference (2-HBP) and for all the dyes in both solvents in O$_2$, as well as for dye **5** in ethanol in the presence of N$_2$ show linear relationships and excellent reproducibility at different absorbances for fluences lower than 25 J m^{-2}.

These results can be interpreted by using eqn (1)[24]

$$\frac{H}{F} = K\alpha(1 - 10^{-A}) \qquad (1)$$

where K is an experimental constant containing the thermoelastic properties of the solution and instrumental factors, A is the sample absorbance at 355 nm, and α is the fraction of energy released to the medium as prompt heat.

Fig. 4 shows linear plots of H/F as a function of the fraction of absorbed energy for the reference and dye **1** in solutions with O$_2$ and with A = 0.03–0.14. The H/F values for each solution were obtained from the corresponding linear plots of H vs. F.

Taking into account that α = 1 for 2-HBP and that the measurements for the sample and the reference were performed under the same experimental conditions, the ratio of the H/F values obtained for the sample and the reference yielded the sample α value. The results are shown in Table 2.

On the other hand, if deactivation processes for the sample take place in a time between $\tau_R/5$ and $5\tau_R$, the amplitude and time dependence of the acoustic signal will depend on the decay time processes, and a time shift between the sample and reference signals is expected. In that case, α cannot be determined by the Amplitude method and other procedures such as deconvolution of the signals must be used. For dyes **1–4** in both solvents and dye **5** in acetonitrile under nitrogen, a convolution method was used in order to determine the α_{N2} values and the lifetime (τ) of the transient species.[26,27] In those cases, the fluence normalized acoustic wave was a convolution of the system response, determined experimentally by using the CR, and a function describing the pressure evolution after excitation. Assuming a single experimental fast decay with one storing species and performing the convolution by an iterative program based on the Levenberg–Marquardt χ^2 minimization procedure,[26] τ and α values were calculated.

Fig. 5 shows, as representative examples, acoustic waveforms of dye **1** and the reference in acetonitrile under N$_2$ and the best-fit convolution of the measured signal with its
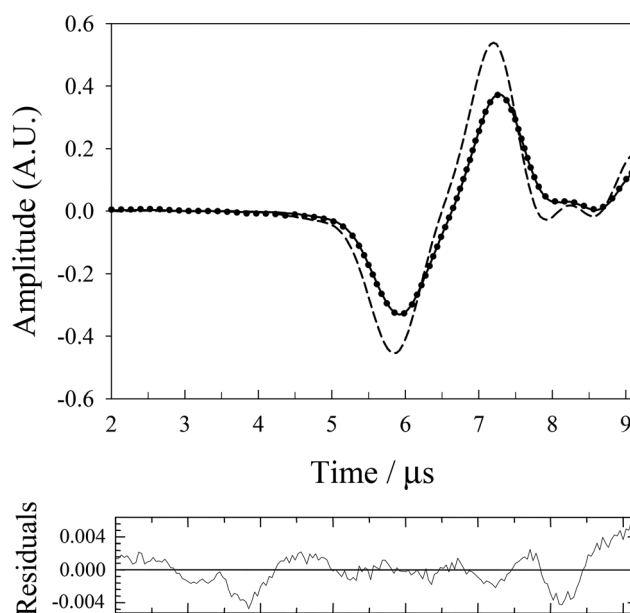

Fig. 5 Acoustic waveform for the reference (dashed line: air, N$_2$ and O$_2$) and dye **1** (solid line: with N$_2$) and dye **1** acetonitrile solutions of matched absorbance (0.105 ± 0.001). The best fit simulation (dotted line) and residual are also shown.

residual distribution. Similar plots were obtained for the rest of the dyes under N$_2$. The calculated values of α are listed in Table 2, where α_1 is the "prompt heat", the fraction of energy release to the medium by fast processes with lifetimes shorter than a few nanoseconds, and α_2 the fraction of energy release to the medium by slow processes (*i.e.*, the partial deactivation of the triplet state). The fast processes involve a combination of vibrational relaxation plus internal conversion in singlet excited states and intersystem crossing S$_1$–T$_1$. The slow processes are mainly the deactivation process T$_1$–S$_0$. Table 2 also reports the triplet lifetime, τ_T, estimated by fitting the signals with the convolution method. The lifetimes obtained for these dyes are similar to those reported by Srividya *et al.* for other compounds of the acridinedione family.[14] The authors also observed by laser flash photolysis a transient whose lifetime is 2 μs, and assigned it to the radical anion of the dye.[28] However, the presence of oxygen does not influence the kinetics of this radical, unlike that observed by us.

By using the data of the photophysical parameters of the dyes in combination with simple energy balance considerations, the quantum yields of triplet state formation and possible quenching effects can be estimated.

In N$_2$ saturation, eqn (2) can be used:

$$E_a = \Phi_f E_f + \alpha_1 E_a + \Phi_T E_T \qquad (2)$$

where Φ_f is the fluorescence quantum yield; $E_a = hc/\lambda_a$, $E_f = hc/\lambda_f$ where λ_a and λ_f are the absorption and fluorescence maximum wavelengths measured experimentally; α_1 is the fraction of energy released as heat obtained by the convolution method in N$_2$, and Φ_T and E_T are the formation quantum yield and the energy content of the triplet state, respectively. From eqn (2), the product $\Phi_T E_T$ is calculated. The value of E_T for the group of our studied dyes was determined by several authors as $E_T = 184 \pm 6$ kJ mol^{-1}.[12,28–31] With these data, Φ_T values in N$_2$ were determined for the five acridinedione dyes listed in Table 2.

To determine whether the triplet state is totally or partially quenched in O$_2$, eqn (3) can be used taking into account that now the long lifetime species is O$_2$($^1\Delta_g$):

$$E_a = \Phi_f E_f + \alpha_{O_2} E_a + \Phi_\Delta E_\Delta \qquad (3)$$

This equation corresponds to the case in which the triplet state is totally quenched. By using the data obtained from the experiments performed in O$_2$, singlet oxygen results and $E_\Delta = 94.2$ kJ mol^{-1},[23] we found that eqn (3) fits correctly within less than 5% uncertainty for all the dyes. Then it can be assumed that O$_2$ completely quenches the triplet state of these compounds in a time shorter than $\tau = 200$ ns. In both the solvents ACN and EtOH, the [O$_2$] for oxygen saturated solutions is *ca.* 10^{-2} M.[32] A simple calculation, if the quenching process is diffusionally controlled, gives a lifetime value of the triplet state shorter than 20 ns in both solvents.

3. Experimental

Spectrograde and HPLC grade ethanol (EtOH), dimethyl sulphoxide, chloroform (Merck) and acetonitrile (Fischer Scientific) were used without further purification. 2-Hydroxybenzophenone (2-HBP) and phenalenone were purchased from Aldrich and were used without further purification. The dyes were synthesized according to published methods.[33,34]

The structures of the dyes were identified by ^{1}H NMR using a Bruker ACP 300 MHz in deuterated chloroform (CDCl$_3$) or dimethylsulfoxide (d$_6$-DMSO). The sample concentration was adjusted to 5% w/v to obtain a better signal to noise ratio. Chemical shifts are reported in ppm, using tetramethylsilane as the internal standard. The acridinedione dyes **2** and **4** show in solution a tautomeric equilibrium (Scheme 1), with a strong shift of the equilibrium towards the enamine form (tautomeric form **I**), whereas for dyes **1** and **3** the imine form (tautomeric form II) was not detected at all by ^{1}H NMR spectroscopy in solution.

^{1}H NMR data, (solvent) δ ppm: compound **1** (CDCl$_3$), 12.29 (s, 1H, N–H); 7.30–6.90 (m, 5H, arom); 5.41 (s, 1H, H(9)); 2.70–2.15 (m, 8H, 2H(2), 2H(4), 2H(5), 2H(7)); 2.10–1.80 (m, 4H, 2H(3), 2H(6)); compound **2** (d$_6$-DMSO), *I* (64%): 7.58 (s, 1H, N–H); 8.20 (d, 1H, H(5′)); 7.44 (td, 1H, H(3′)); 7.20 (d, 1H, H(2′)); 6.93 (dd, 1H, H(4′)); 4.89 (s, 1H, H(9)); 2.31–1.92 (m, 8H, 2H(2), 2H(4), 2H(5), 2H(7)); 1.89–1.70 (m, 4H, 2H(3), 2H(6)) and II (36%): 8.21 (d, 1H, H(5′)); 7.51 (td, 1H, H(3′)); 7.30 (d, 1H, H(2′)); 6.84 (dd, 1H, H(4′)); 3.88 (d, 1H, H(9)); 3.04(d, 1H, H(8a); 2.61–2.44 (m, 2H, H(7), H(5)); 2.44–2.23 (m, 2H, H(2)); 2.40–2.28 (m, 2H, H(7), H(5)); 2.21–2.10 (m, 2H, H(4)); 1.98–1.86 (m, 2H, H(6)); 1.70–1.40 (m, 2H, H(3)); compound **3**, (d$_6$-DMSO), 9.44 (s, 1H, N–H); 8.264 (s, 1H, H(6′)); 8.146 (s, 1H, H(4′)); 7.41–7.33 (m, 1H, H(2′)); 7.09 (dd, 1H, H(3′)); 4.77 (s, 1H, H(9)); 2.2–2.0 (m, 6H, 1H(4), 1H(5), 2H(2), 2H(7)); 1.88–1.58 (m, 6H, 1H(4), 1H(5), 2H(3), 2H(6)); compound **4**, (d$_6$-DMSO), *I* (55.4%): 8.34 (dd, 2H, H(3′), H(5′)); 7.25–7.0 (m, 2H, H(2′), H(6′)); 5.06 (s, 1H, H(9)); 2.91–2.03 (m, 8H, 2H(2), 2H(4), 2H(5), 2H(7)); 2.03–1.68 (m, 4H, 2H(3), 2H(6)); and *II* (44.6%): 8.28 (dd, 2H, H(3′), H(5′)); 7.45–7.15 (m, 2H, H(2′), H(6′)); 4.87(s, 1H, H(9)); 2.95–2.10 (m, 8H, 2H(2), 2H(4), 2H(5), 2H(7)); 2.10–1.80 (m, 4H, 2H(3), 2H(6)); compound **5**, (d$_6$-DMSO, 8.34 (dd, 2H, H(3′), H(5′)); 7.25–7.0 (m, 2H, H(2′), H(6′)); 5.06 (s, 1H, H(9)); 2.91–2.03 (m, 8H, 2H(2), 2H(4), 2H(5), 2H(7)); 2.03–1.68 (m, 4H, 2H(3), 2H(6)).

Absorption measurements were performed using a UV-Visible spectrophotometer Beckman DU-65. All measurements were made in a 1 cm stoppered quartz cell at room temperature. To determine the decadic molar absorption coefficient, ε (M^{-1} cm^{-1}), concentrations in the range 10^{-6}–10^{-4} M were used. To study the pH dependence of the absorption spectrum, solutions of the dyes with an absorbance of 1.5 at the absorption maximum were acidified with sulfuric or hydrochloric acid.

Fluorescence spectra and fluorescence quantum yields were determined using a spectrofluorimeter PTI QM-1 Quanta Master. These spectra were performed either under air, N$_2$ or

O$_2$ saturation, bubbling the gas in the solution for 15 min. Quantum yields of fluorescence were obtained as described elsewhere[13–15,35] by using quinine sulphate in 1 N sulphuric acid as a reference ($\Phi_f(r) = 0.546$).[16]

Time resolved phosphorescence detection (TRPD) was used for singlet molecular oxygen [O$_2$($^1\Delta_g$)] measurements. For excitation, a Q-Switched Nd:YAG laser (7 ns FWHM) operating at 355 nm was used as a source (8 mm diameter in the cell). The near-IR luminescence of O$_2$($^1\Delta_g$) was observed at 90° geometry through a 5 mm thick anti-reflective coated silicon metal filter with wavelength pass >1.1 μm and an interference filter at 1.27 μm by means of a preamplifier (low-impedance) and a Ge photodiode (Applied Detector Corporation, time resolution 1 μs). The measurements were performed in neutral solutions saturated with O$_2$. Measurements were performed averaging the signals generated by 512 laser shots for a better signal to noise ratio. A simple exponential analysis of the emission decay was performed with the exclusion of the initial part of the signal. Phenalenone, with $\Phi_{\Delta Ph} = 0.95$, was used as a reference.[36]

Photoacoustic measurements were performed using a set-up described previously.[37] A Q-Switched Nd:YAG laser (7 ns FWHM) operating at 355 nm was used as an excitation source (1 mm diameter in the cell). A home-made ceramic piezoelectric (4 × 4 mm) transducer with an appropriate amplifier was used to detect the acoustic signals. The resolution time of the experiments was $\tau_R = 1$ μs. Measurements were performed by averaging the acoustic signals generated by 64 laser shots for a better signal to noise ratio. The absorbance of the solutions was checked before and after each set of laser shots. For all the studied dyes neither photobleaching nor photoproducts were observed. 2-HBP was used as a CR.[38] Sample and reference solution concentrations were matched within 2% to absorbance values between 0.1 and 0.2 at the laser excitation wavelength. Signals were also mathematically processed by convolution methods, combined with an appropriate kinetic model, using Sound Analysis software, version 1.50D (Quantum Northwest Inc., Spokane, WA). Experiments were performed in N$_2$ or O$_2$ saturated solutions by bubbling for 30 min with a solvent-saturated gas previous to the measurements.

4. Conclusions

The absorption and fluorescence parameters of the five dyes studied depend strongly on both the solvent and the gas present. Fluorescence quantum yields in N$_2$ are 40% higher than those obtained in O$_2$ and acidification of the solutions causes a dramatic decrease of the fluorescence yield.

Dyes **1–3** in both solvents and dye **5** in acetonitrile have O$_2$($^1\Delta_g$) production quantum yields between 0.1 and 0.25 in saturated O$_2$ solutions, while dye **4** in both solvents and dye **5** in ethanol show no O$_2$($^1\Delta_g$) production. Oxygen totally quenched the triplet state in both solvents. On the other hand, with the exception of dye **5** in ethanol, two types of

deactivation processes take place in the absence of O$_2$: fast processes taking place in less than a few nanoseconds, delivering heat to the medium, and a slower triplet state deactivation, in a time of the order of the time resolution of our experiment. By using convolution methods, the lifetimes of the triplet states of the five acridinedione dyes are estimated to have lifetimes less than several tens of ns. Dye **5** in ethanol has the same behavior as the reference. This behavior is rationalized by considering the absence of the NH moiety in its structure. Thus the excited state of compound **5** in ethanol solution shows a rapid ethanol-assisted keto–enol equilibrium. This process is similar to that undergone by the electronic excited state of CR, such as 2-HBP.[18] The octahydro-γ-pyridyl-acridine structure of compound **5** and the decahydro-γ-pyridyl-acridine structure of dyes **1–4** account for this different behavior observed.

Furthermore, the presence of basic moieties in the dye structure (secondary amine –NH– and/or the pyridine moiety) and the protic character of ethanol can explain why the quantum efficiency of triplet formation in this solvent is lower than that in acetonitrile. This also explains the dramatic decrease in fluorescence quantum yield when the proton concentration is increased, as observed in experiments at low pH values.

Acknowledgements

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