On the photophysical and photochemical behavior of fenbufen: a study in homogeneous media and micellar environments

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The photochemistry of fenbufen, γ-oxo-[1,1′-biphenyl]-4-butanoic acid (FB), has been investigated in homogeneous solutions and in micellar environments. The photochemical and photophysical properties of the drug are mediated by its lowest excited triplet states. The presence of a low-lying π,π* triplet is responsible for the inefficient Norrish Type I photocleavage, whereas an upper n,π* triplet promotes a large intersystem crossing yield. FB interacts with both anionic and cationic micelles. The different binding mode of the drug with the microheterogeneous environments can significantly change its photoreactivity. These changes are rationalized on the basis of "micellar cage effects".

Drug photochemistry has been the object of a considerable amount of attention, especially in the last decade. Such interest has been promoted by the fact that even though many of these compounds are excellent therapeutical agents, they are also known to often cause phototoxic and/or photoallergic phenomena.[1–3] Drug photodegradation reactions, leading to transient species and stable photoproducts, as well as drug photoinduced energy transfer processes, giving rise to singlet oxygen photosensitization, are mainly responsible for such undesirable side effects. Due to the awareness among the scientific community of the higher levels of solar UV radiation reaching the earth, interest in the present topic has recently increased markedly. Several reviews concerning the mechanisms of both drug photodegradation and photosensitization were recently published.[4–6]

From this picture, it emerges that shedding light on the photophysical properties and photochemical behavior of these therapeutic agents is a prerequisite for an understanding of the molecular basis of both their photostability and photosensitized biological effects. Moreover, beyond the concern for both drug photostability and phototoxicity, from a strict chemical point of view this subject offer opportunities to gain more insight into fundamental aspects related to mechanisms of photoinitiated processes in homogeneous solution, organized systems and constrained media. Actually, for an appropriate correlation between the phototoxicity and the photochemical properties of a given drug to be obtained, an adequate study should investigate the photochemistry of the photosensitizer both in homogeneous solution and in the presence of either a biological target or simpler model systems. The physical properties of the photosensitizer (*i.e.*, hydrophobicity, geometry, size, shape and charge) often lead to the formation of supramolecular aggregates with biomolecules. These aggregates can display different photochemical properties compared to those of the "free photosensitizer". The nature of the lowest excited states, the efficiency of the photophysical and photochemical pathways, the redox potentials, the fate of the reaction intermediates and the opening of new photoreactive channels are some of the properties of the photosensitizer that can be modulated by its compartmentalization in a biological microenvironment.[7,8] Among the several biological mimetic systems, micelles represent one of the most exploited; photochemical investigations in such an environment are always an active area of research.

Fenbufen (FB), γ-oxo-[1,1′-biphenyl]-4-butanoic acid, is believed to act as a prodrug for active anti-inflammatory metabolites such as 4-biphenylacetic acid and its hydroxylated derivatives, which are inhibitors of the cyclooxygenase enzymes.[9] Although the incidence of erythema photoinduced by FB is low, studies have shown that this prodrug stimulates the production of interleukin-1 in keratinocytes exposed to UV radiation.[10] Moreover, it has been shown that FB is capable of inducing both photohemolysis of red blood cells and the photoperoxidation of unilamellar phosphatidylcholine liposomes upon UVA irradiation.[11]

FB
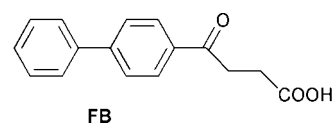
This paper reports on the spectroscopic and photochemical behavior of FB in homogeneous solution and in micellar environment by coupling both steady-state and time-resolved techniques to theoretical calculations.

Experimental

Reagents

Fenbufen and benzophenone were purchased from Sigma Chemical Company. The latter was recrystallized twice from ethanol. Sodium dodecyl sulfate (SDS) and cetyltrimethylammonium chloride (CTAC) were purchased from Fluka. Water was purified through a Millipore Milli-Q system. The phosphate buffer (10^{-2} M, pH 7.4) was prepared from reagent grade products. All the organic solvents were of the highest purity available and used without further purification.

Steady-state experiments

Irradiations were performed using monochromatic light obtained from a Series 200 He–Cd 325 nm laser (Liconix, St.

Clara, CA, USA). The incident photon flux was *ca.* 5×10^{15} quanta s^{-1}. The experimental irradiation procedures and the light intensity measurements have been described previously.[12,13]

The photodegradation quantum yields of FB, both in the absence and in the presence of micelles, were determined through high performance liquid chromatography (HPLC) analysis, from the disappearance of the starting compound up to 25% conversion. Due to the low quantum yield and the necessity of analyzing at a low conversion percentage, the values of Φ obtained in aqueous and in micellar solutions were affected by a fairly large error ($\pm 20\%$). HPLC analysis was performed on a Hewlett Packard 1100 chromatograph equipped with an on-line photodiode array detector (DAD). The chromatograms were recorded by monitoring at 230, 260 and 290 nm. The quantitative separation of the photoproducts was achieved on a LiChroCart RP-18 column (5 µM packing, 4×250 mm; HP) by eluting with a linear gradient of CH_3CN in 0.01 M phosphate buffer (pH 7) from 0 to 75% in 15 min, and a flow rate of 1 mL min^{-1}.

The absorption and fluorescence spectra were recorded with a Beckman DU 650 spectrophotometer and a Spex Fluorolog-2 (model F-111) spectrofluorimeter, respectively. Fluorescence quantum yields were obtained using quinine sulphate in 1 N H_2SO_4 ($\Phi_{flu} = 0.54$) as the standard. The absorbance values of the samples at the excitation wavelength were lower than 0.1 for a 1 cm pathlength. The fluorescence lifetime in degassed solution was determined by means of a time-correlated single photon counting system (IBH Consultants Ltd.). A nitrogen-filled lamp was used for excitation at 337 nm. The phosphorescence emission spectrum was measured at 77 K in an ethanol glass using a Perkin-Elmer LS 50. The triplet energy was determined from the onset of the phosphorescence spectrum.

Nanosecond laser flash photolysis and time-resolved detection of singlet molecular oxygen

The laser flash photolysis system employed in these studies has been previously described.[14,15] FB solutions ranging in concentration from 0.3 to 1.0 mM were excited with a Molectron UV-24 nitrogen laser (337 nm). All transient spectra were recorded employing a flow system with a 7×7 mm^2 Suprasil quartz cell with a 2 mL capacity and were purged with N_2 or O_2 for 30–45 min before as well as during data acquisition. A Surelite Nd : YAG laser (355 nm, 8 ns pulse) was used to excite 3.5 mM solutions of benzophenone (BP) in acetonitrile with or without FB for the determination of the triplet molar absorption coefficients ($\Delta\varepsilon_T$) by T–T energy transfer and intersystem crossing yields (Φ_{ISC}) by comparative techniques. The literature values for benzophenone in acetonitrile are ($\Delta\varepsilon_T = 6500$ mol L^{-1} cm^{-1} at 520 nm and $\Phi_{ISC} = 1.0$.[16] At the FB concentrations employed, more than 99% of the BP triplets were quenched. Nevertheless, kinetic corrections for incomplete quenching of the benzophenone triplet (using an experimentally determined value for the rate of quenching of the benzophenone triplet by FB of 4×10^9 M^{-1} s^{-1}) were performed in order to accurately determine the $\Delta\varepsilon_T$ for FB.

Time-resolved detection of the singlet molecular oxygen O_2 ($^1\Delta_g$) phosphorescence at 1270 nm was achieved by exciting at 308 nm with a Lumonics EX-530 laser. An EG&G Judson J16 8SP ROM5 (5 mm) germanium photodiode was mounted on a modified BNC connector. A 3 mm silicon filter (Oriel Corporation, Model 54081) was positioned between the sample cell and the detector to remove any shorter wavelength IR or visible interference. The signal was amplified using a Stanford Research Systems preamplifier equipped with short and long bandpass filters, and captured by a Tektronix 2440 digitizer. Data acquisition and processing were done with software developed in the LabVIEW 3.1.1 environment from National

Instruments. The determination of the O_2 ($^1\Delta_g$) quantum yield (Φ_Δ) was carried out using optically matched oxygen-saturated solutions of FB and phenalenone ($\Phi_\Delta = 0.98$)[17] by comparing the linear slopes of plots for the dependence of the signal intensity (extrapolated to zero time) against the laser energy.

Results and discussion

Absorption and emission

Fig. 1 shows the absorption and the fluorescence spectra of FB recorded in buffer solution at pH 7.4. The absorption spectrum is characterized by a main band centered at 284 nm ($\varepsilon = 20\,800$ M^{-1} cm^{-1}) and a weak tail extending beyond 350 nm. A shift towards shorter wavelengths was observed as the solvent polarity decreased. In particular, the main band shifted to 280 nm in both acetonitrile and isopropanol. The fluorescence spectrum in buffer solution exhibits a band centered at 400 nm. The fluorescence quantum yield (Φ_{flu}) of FB under these experimental conditions is *ca.* 0.001. A red shift of *ca.* 10 nm of the fluorescence maximum accompanied by a 4-fold reduction of Φ_{flu} was observed in acetonitrile whereas in isopropanol solution FB was basically non-fluorescent. The values of Φ_{flu} obtained are much lower than those reported for biphenyl in similar solvents ($\Phi_{flu} \approx 0.2$), accounted for by a low-lying and scarcely emitting n,π* singlet state (*vide infra*). This is not surprising given the molecular structure of FB, which can be viewed either as a substituted biphenyl or as a substituted phenyl alkyl ketone.

Fig. 2 shows the phosphorescence emission spectrum of FB in an ethanol glass at 77 K. A triplet energy of *ca.* 60 kcal mol^{-1} can be estimated from the onset of the band. This value is quite low if compared to that reported for biphenyl itself (*ca.* 70 kcal mol^{-1})[18] but in good agreement with that reported for acylbiphenyl derivatives (*ca.* 65 kcal mol^{-1}).[19] Literature data concerning the nature of the lowest triplet states of *para*-substituted phenyl ketones suggest a predominantly ^{3}π,π* symmetry for such electronic states.[19] Similarly to what
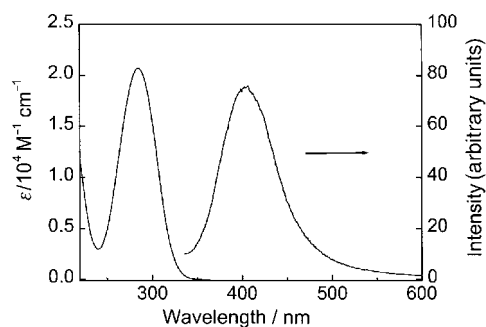

Fig. 1 Absorption and fluorescence ($\lambda_{exc} = 310$ nm) spectra of FB in phosphate buffer (1×10^{-2} M, pH 7.4).

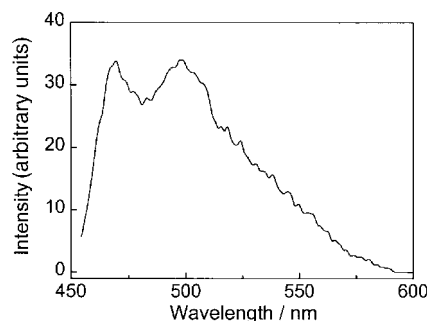**Fig. 2** Phosphorescence emission spectrum ($\lambda_{exc} = 320$ nm) of FB in an ethanol glass at 77 K.

has been observed in these cases, the presence of two close-lying triplets of n,π* and π,π* symmetry can be suggested for FB. This proposal is confirmed by both theoretical calculations and photochemical experiments (*vide infra*).

We performed semi-empirical quantum mechanical calculations (ZINDO/S) starting from a ground state optimized geometry (MM + force field)[20] and obtained the results reported in Table 1. In general, one can describe FB as a biphenyl perturbed by a carbonyl substituent that plays a substantial role in the photophysics of the whole molecule by providing low-lying states of an n,π* nature. The first two triplets, calculated to be at 580 and 471 nm respectively, are of a different nature, the first being a π,π* and the second an n,π* state. Although the calculated energy gap between these states is large (0.5 eV), there is the possibility of a proximity effect due to the fairly large vibronic couplings brought about by the C=O stretching mode (typically up to 1500 cm^{-1} in carbonyl compounds).[21]

The first calculated singlet (414.8 nm) corresponds in orbital nature to the second triplet $T_2(n,π*)$ and has null oscillator strength: it can be activated through vibronic coupling and corresponds, in energy, to the fluorescent state. The first sizeable singlet (responsible for the main absorption band) is calculated to lie at 298 nm as S_3 (π,π*) and corresponds to the intense $^1A \rightarrow {}^1L_a$ of the parent biphenyl, which hides the less intense $^1A \rightarrow {}^1L_b$ transition (calculated to be 304.6 nm). Taking into account the calculated dipole moments of the states involved (Table 1), one can explain the shifts observed in the absorption and emission bands with the decrease of the solvent polarity.

Laser flash photolysis

The transient absorption spectra obtained after 337 nm laser excitation of FB in N_2-saturated acetonitrile solution and recorded at various delay times with respect to the laser excitation are shown in Fig. 3. The spectra are characterized by a broad absorption band extending from 320 nm to beyond 500 nm, with a maximum centered at 420 nm. This transient species decays mono-exponentially with a rate constant of 6.7×10^4 s^{-1} (inset Fig. 3) and no relevant new bands form concurrently with its decay. Moreover, this transient can be quenched by oxygen ($k_q = 8.2 \times 10^8$ M^{-1} s^{-1}) and its formation can be sensitized by benzophenone ($k_q = 4 \times 10^9$ M^{-1} s^{-1}, see Experimental). These results are basically the same as those obtained by Navaratnam and Jones in the same solvent.[22] Thus, this band can be safely attributed to the T–T absorption of FB. This triplet absorption maximum for FB is red-shifted compared to the T–T absorption spectra reported for biphenyl,[23] probably due to the additional conjugation induced by the presence of the carbonyl substituent.

As mentioned previously, the presence of this carbonyl substituent has profound effects on the photophysical properties
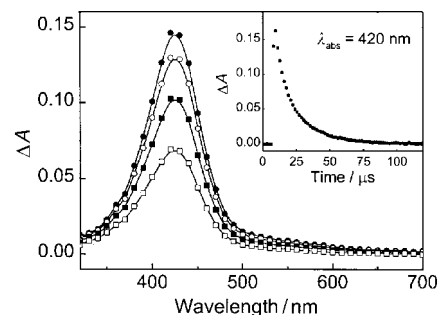
Fig. 3 Transient absorption spectra observed in a 1×10^{-3} M N_2-saturated solution of FB in acetonitrile (●) 0.40, (○) 1.28, (■) 3.20 and (□) 6.80 µs after excitation by a 2.0 mJ laser pulse at 337 nm. The inset shows the decay trace monitored at 420 nm.
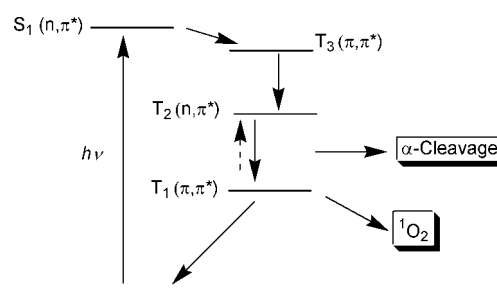
of FB. In particular, it promotes intersystem crossing (ISC) and the photogeneration of singlet oxygen, O_2 ($^1\Delta_g$). We obtained values of *ca.* 0.85 and 0.45 for Φ_{ISC} and Φ_Δ, respectively, in acetonitrile. The value of Φ_Δ is somewhat lower than that reported by Navaratnam and Jones.[22] The discrepancy could be due to both the different excitation wavelengths and temperatures. The Φ_{ISC} value is clearly higher than that reported for biphenyl ($\Phi_{ISC} \approx 0.6$) and can be accounted for by an assisted ISC mediated by the upper n,π* triplet state (Scheme 1). Fast internal conversion to a lower triplet state of π,π* symmetry can explain the relatively large value for Φ_Δ and the inefficient α-cleavage undergone by this ketone (*vide infra*). In addition, the value obtained for the efficiency of O_2 ($^1\Delta_g$) formation ($S_\Delta = 0.53$) is of the order of magnitude reported for biphenyl itself whose lowest triplet state has a π,π* nature ($S_\Delta = 0.42$).[24] Several groups have tried to explain the intermediate S_Δ values ($0.35 < S_\Delta < 0.85$) for naphthalene, biphenyl, and other aromatic systems. They have proposed that the equilibrium between the singlet and triplet states of the charge-transfer complex formed between the photosensitizer and oxygen could be slower than its decay.[24,25] Although it would be tempting to suggest that for FB the value of S_Δ reflects the fact that there is a considerable mixing between triplets of n,π* and π,π* symmetry, this certainly does not explain the trend observed for other aromatic systems for which mixing between states does not occur (*i.e.* biphenyl). Literature data report weak vibronic mixing between two triplet states whenever they are as close as the n,π* and π,π* triplets, even for biphenyl ketones.[26] The wave function Ψ_{T_1} of the lowest triplet can be represented by a linear combination of two unperturbed wave functions Ψ, with the mixing coefficients *a* and *b* dependent on the energy gap between the two pure states and on the availability and efficacy of mixing vibrations of proper symmetry:[26]

$$\Psi_{T_1} = a\Psi(π,π*) + b\Psi(n,π*)$$

Taking into account the calculated energy gap between the two states and a reliable vibronic coupling induced by the C=O stretching mode, we can infer that a mixing of $b/a = 0.37$ can be expected.

Table 1 Calculated energies, dipole moments, oscillator strengths and nature of the low-lying states of FB

| State	Energy/ kcal mol^{-1}	Dipole/D	Osc. strength	Nature
S_0	0	6.93		
T_1	49.3	7.49		π,π*
T_2	60.6	2.79		n,π*
T_3	67.0	7.04		π,π*
S_1	68.9	2.60	0.0	n,π*
T_4	77.0	7.93		π,π*
T_5	79.2	8.65		π,π*
T_6	81.5	7.70		π,π*
T_7	84.4	7.08		π,π*
T_8	84.8	4.85		n,π*
S_2	93.9	4.85	0.0008	n,π*
S_3	95.5	12.73	1.074	π,π*

Scheme 1

Our interpretation is confirmed by laser flash photolysis experiments in buffer solutions at pH 7.4. The transient spectrum recorded in 10^{-2} M phosphate buffer saline (PBS) undergoes a 25 nm red shift ($\lambda_{max} = 450$ nm) and decays mono-exponentially with a rate constant of 1.4×10^5 s^{-1} (data not shown). The large red shift in the T–T absorption spectra is in agreement with the π,π^* nature of the lowest triplet state. In addition, the rate constant for the quenching of the FB triplet by oxygen in PBS is 5×10^8 M^{-1} s^{-1}, which is similar, within experimental error, to that obtained in acetonitrile. Due to the low solubility of benzophenone in water, we were not able to determine Φ_{ISC} by energy transfer, and ground state depletion methods are difficult to perform with an N$_2$ laser, yielding unreliable data. We determined a Φ_Δ value for FB in D$_2$O of 0.74. Assuming the same Φ_{ISC} as in acetonitrile would result in an S_Δ value of ca. 0.8, which is closer to the value expected for aromatic systems having lowest triplet states of π,π^* symmetry. In a more polar environment, mixing between the n,π^* and π,π^* triplet states decreases (see Table 1) and the lowest triplet state of FB has a predominantly π,π^* nature.

Photoreactivity in aqueous solution

Fig. 4 shows the changes in the absorption spectra observed in a nitrogen-saturated solution of FB at pH 7.4 at different irradiation times. Previous work pointed out that these results were consistent with the formation of a main stable photoproduct, 4-biphenylcarboxyaldehyde.[11] An α-cleavage process (Norrish Type I fragmentation) accounts for the formation of this stable photoproduct. It is well known that the nature and multiplicity of the excited state involved in the photodegradation are two of the parameters affecting the quantum efficiency of the α-cleavage process. The rate constants for α-cleavage are much faster for n,π^* excited states than for π,π^* excited states.[27] In the former case, the half-vacant non-bonding orbital on oxygen can overlap with the α-bond undergoing homolysis.[28,29] Furthermore, a low activation energy is expected for a triplet state mediated α-cleavage process compared to the singlet state mediated process.[30] As a consequence, the most efficient Norrish Type I reactions are expected to occur from triplet states with n,π^* character. In our case, we obtained a photodegradation quantum yield for FB (Φ_{-FB}) in buffer solution of 4×10^{-4}. This low value is in agreement with the results concerning the nature of the lowest excited triplet state of FB (vide supra), which is a scarcely reactive π,π^* triplet state. For similar molecules, the observed reactivity has been attributed to mixing of the lowest triplet with the upper n,π^* triplet state[31] and, as proposed by Wagner and Leavitt,[32] from an equilibrium concentration of the upper n,π^* triplet (Scheme 1). In our case, the Boltzmann distribution calculated at 298 K (ca. 2% population of T$_2$) is consistent with the low value of the photodegradation quantum yield obtained. Given the n,π^* nature of the lowest
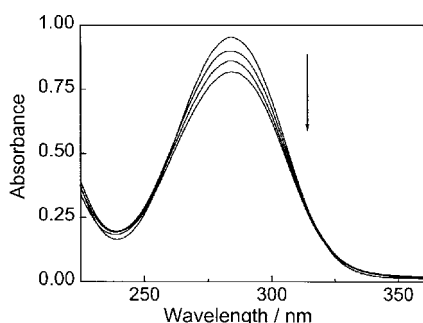
singlet state and the low photodegradation quantum yield, it is also valid to propose a singlet-mediated Norrish Type I photocleavage. In this context, we measured an upper limit of 0.2 ns for the singlet lifetime of FB in aqueous solution, which rules out any relevant quenching by oxygen on the singlet state. Since the photodegradation quantum yield of FB dropped threefold in oxygen-saturated solutions, it is reasonable to propose a photodecomposition mediated by a long-lived triplet state.

Photoprocesses in micellar environments

The ground state absorption and emission spectra for FB in 0.1 M SDS were identical to those recorded in aqueous solution, suggesting the absence of specific interactions between the surfactant and both the ground and excited states of FB. Nevertheless, the association between FB and SDS was demonstrated by fluorescence quenching experiments performed by using iodide, a quencher that is confined mainly in the aqueous environment. The quenching results, performed at two different concentrations of SDS, are reported in Fig. 5 and analyzed by using eqn. (1):[33]
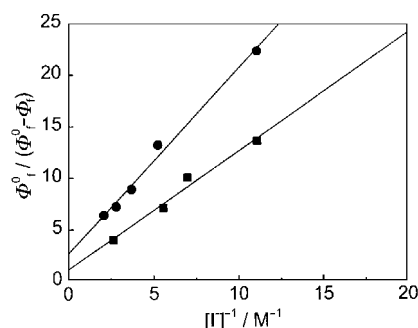
$$\Phi_f^0/(\Phi_f^0 - \Phi_f) = [(a\Phi_{fm}^0/b\Phi_{fw}^0) + 1][1 + 1/k_q\tau_{fw}^0[Q]] \quad (1)$$

where Φ_f^0 is the total fluorescence quantum yield in the absence of quencher, Φ_{fw}^0 and Φ_{fm}^0 are the fluorescence quantum yields in water and in the micelles, respectively, a and b are the fractions of the concentration of the drug in the micelle and water, respectively, and the product $k_q\tau_{fw}^0$ is the Stern–Volmer quenching constant. From the ratio a/b derived from the intercept of the two plots in Fig. 5, an association constant k_{assoc} between FB and the SDS micelles of $(2.5 \pm 0.5) \times 10^3$ M^{-1} was obtained by using eqn. (2):[33]

$$K_{assoc} = k_+/k_- = a/b[M]^{-1} \quad (2)$$

The low value for K_{assoc} is not surprising since FB is present in its anionic form at neutral pH (pK_a = 4.5). Deep incorporation of the drug in the micelle core is not favored from a thermodynamic point of view due to the coulombic repulsion caused by the negatively charged micelle and the chromophore. Besides, localization of FB in a deep region of the micellar environment would have led to changes both in the absorption and emission spectra according to the polarity effect on the excited states of the drug (vide supra and infra). The combination of both quenching and spectroscopic data suggest an incorporation mode of FB involving localization of the biphenyl chromophore near the Stern layer with the carboxyl head mainly exposed to an aqueous environment. Interactions of this kind have already been reported for other negatively charged alkyl–aryl chromophores.[33]

When the irradiation of FB was performed in the presence of SDS micelles, a 1.5-fold reduction in the photodegradation quantum yield compared to that reported in aqueous solution was observed. While a strong effect on the energy gap of the
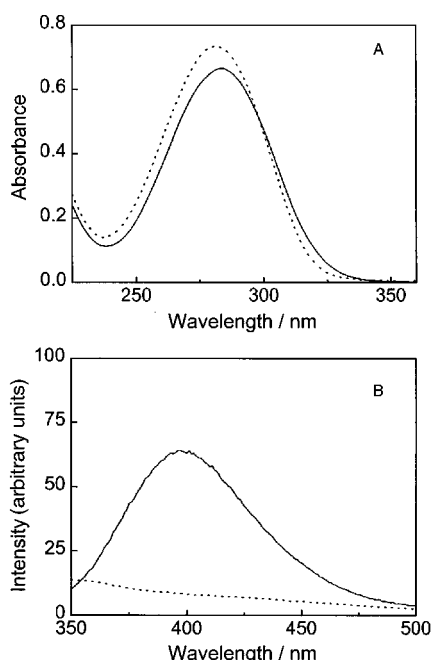

Fig. 4 Spectral changes of FB in phosphate buffer solutions (1×10^{-2} M, pH 7.4) observed under anaerobic conditions after 0, 10, 30 and 60 min irradiation with 325 nm light excitation.

Fig. 5 Plots of the data according to eqn. (1) for the quenching of the fluorescence of FB by I$^-$ in (■) 0.05 and (●) 0.1 M SDS.

low lying triplets seems unlikely on the basis of the rather weak incorporation of the drug in the anionic micelles, a possible reason for the lower photoreactivity of FB in SDS micelles could be related to the "cage effect". In fact, it is known that localization of the drug in the micellar environment promotes radical pair recombination before their diffusion.[34–36] Once produced, the radical pair originated by C–C homolysis can either recombine or evolve towards the formation of photoproducts. In the micellar environment, the former process plays a dominant role and, as a consequence, intersystem crossing in the radical cage is competitive with rotation of the caged radical pair and diffusion into the solvent. The small reduction in the photodegradation quantum yield of FB in SDS is in agreement with both the spectroscopic data and the association constant, thus corroborating the hypothesis that the chromophore localizes near the micellar surface.

Fig. 6(A) shows the absorption spectrum of FB in the presence of 0.1 M CTAC and a similar spectrum in aqueous solution. The observed blue shift in the maximum is in agreement with what is observed in solvents of lower polarity than water (*vide supra*) and indicates deep incorporation of the drug in the cationic micelle. From the dependence of the absorbance changes on the concentration of CTAC an association constant $K_{assoc} = (3.5 \pm 0.5) \times 10^4$ M^{-1} was obtained by applying eqn. (3):[37]

$$1/(A - A_w^0) = 1/(A_m^0 - A_w^0)(1 + 1/K_{assoc}[M])$$ (3)

where A_w^0 is the absorbance in the absence of CTAC, A is the absorbance in the presence of CTAC and A_m^0 is the limiting absorbance upon complete incorporation of FB in the micellar phase. The value of K_{assoc} is higher than that found for SDS and can be reasonably attributed to the additional electrostatic interaction involving the opposite charges of the chromophore and the external surface of the micelle. These interactions can play a key role in decreasing the rate constant for the exit of FB from the micellar cage. Since the association of solutes with micelles is diffusion controlled, the differences in equilibrium constants are related to differences in the exit rate constants.[38] Although no relevant differences in the absorption spectrum of FB were observed when the concentration of CT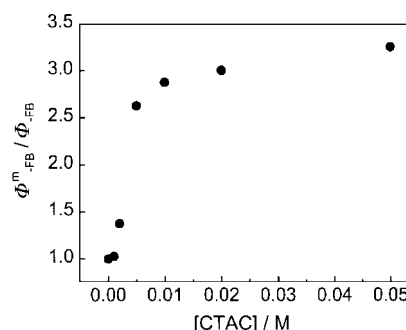AC was kept below the CMC, we cannot rule out the formation of pre-micellar aggregates between molecules of the drug and surfactant.

The fluorescence emission of FB was also affected by CTAC if its concentration was kept above the CMC. Fig. 6(B) shows the relevant quenching of the fluorescence emission upon incorporation in the micelles. The decrease in the fluorescence intensity can be attributed to the effect of the hydrophobic microenvironment on the relative energies of the excited states involved. Consequently, other non-radiative deactivation channels (*i.e.* ISC) might take place with higher efficiencies. This hypothesis is based on our findings of an n,π* symmetry for the lowest excited singlet state of FB, which is reasonably stabilized by its incorporation in the low-polarity environment of the micelle. This can lead to a strong spin–orbit coupling with the close-lying $^3\pi,\pi^*$, promoting an increase in the ISC efficiency. This behavior is similar to that observed in solvents less polar than water (*i.e.* acetonitrile and isopropanol, *vide supra*) and for the FB–β-cyclodextrin inclusion complex.[39]

Irradiation of FB in the presence of CTAC micelles leads to a completely different behavior than that observed either in aqueous solutions or in SDS micelles. The dependence of the ratio between the photodegradation quantum yield of FB in the presence, Φ^m_{-FB}, and in the absence, Φ_{-FB}, of CTAC as a function of the surfactant concentration (Fig. 7) indicates a marked increase of the photodegradation, which reaches a limiting value *ca.* 3 times higher than that reported in the absence of surfactant. The main photoproduct under these conditions was the same as that observed in aqueous solution, 4-biphenylcarboxyaldehyde. From Fig. 7 it can be seen that the increase in Φ_{-FB} occurs around the CMC, suggesting that such an enhancement reflects a photoprocess occurring in the micellar environment, as reported in the literature.[40,41] The increase of the photodegradation efficiency in the presence of CTAC can be rationalized on the basis of the dual role played by the micellar environment, acting both as a solvent and a reactant.

The spectroscopic results obtained in the CTAC micelles and the high value of the association constant suggest deep incorporation of FB into these structures. Besides the decrease in the polarity induced by the incorporation of FB into the micelles, we cannot exclude an additional contribution from this microenvironment in promoting a slight increase in the rotational barrier between the two phenyl rings. Although planarization for biphenyl is expected to be very fast even in the micelles, we believe that the electrostatic interaction between FB and the CTAC micellar surface could make this barrier moderately high. Such effects on the rotational barrier have been reported before for biphenyl-like chromophores; specifically, a reduction in the rotational motion has been observed in the case of positively charged molecules bearing a biphenyl-like chromophore incorporated in anionic micelles.[42] The combination of these two effects could lead to the same

Fig. 6 Absorption (A) and emission (B) spectra of FB in phosphate buffer (1×10^{-2} M, pH 7.4) (——) in the absence and (– – –) in the presence of 0.1 M CTAC.

Fig. 7 Dependence of the ratio between the quantum yield of photodegradation of FB in the presence, Φ^m_{-FB}, and in the absence, Φ_{-FB}, of CTAC as a function of the surfactant concentration.

phenomena as proposed by Yang and coworkers;[31,43] an increase of the vibronic mixing between the π,π^* and n,π^* states caused by the smaller energy gap accompanied by the higher efficiency of the α-cleavage photoprocess. Since this process in the CTAC micelles is expected to take place in a deeper region than in the SDS micelles, the formation of the photoproduct could also be favored by the fact that the benzoyl radical intermediate may abstract hydrogen from the surfactant, leading to the formation of the stable photoproduct. Similar processes have been reported in the literature, where an increase in the yield of aldehyde derivatives formed through α-cleavage processes in the presence of suitable hydrogen donors has been observed.[44] Nonetheless, the increase in the photodegradation quantum yield of FB in CTAC micelles ($\Phi^m_{FB} \approx 1.2 \times 10^{-3}$) still compares well with those reported for aryl alkyl ketones characterized by lowest $^3\pi,\pi^*$ states that are weakly mixed with upper $^3n,\pi^*$ states.[44]

In conclusion, this study has demonstrated that the photoreactivity of FB is mediated by its lowest excited triplet state of π,π^* nature and having an upper n,π^* triplet as precursor. The presence of the carbonyl substitutent promotes an assisted intersystem crossing, resulting in high yields for triplet population compared to biphenyl itself. The mixing between the two low-lying triplets is modulated by solvent polarity and plays a key role in the efficiency of singlet oxygen photogeneration. In light of the high quantum yield of this transient species, a Type II photosensitization mechanism seems to be the most relevant pathway to explain the phototoxic effect of FB.

The formation of carbon-centered radicals results from an inefficient cleavage process (Norrish Type I). The photodegradation of the drug can be altered by its incorporation into micellar structures and depends on the nature of the surfactant. These findings suggest that localization of the drug in specific regions of a biosubstrate with particular hydrophobicity and charge can lead to an increase in the photodegradation efficiency and, as a consequence, to an increase in the formation of free radicals.

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