Photochemistry of Diflunisal in Nonionic (Brij-35) Micelles: Influence of the Microenvironment on Photoionization, Electron Trapping, and Persistent Radical Effect

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The photochemistry of diflunisal (DF) in nonionic micelles of polyoxyethylene lauryl ether (Brij-35) has been investigated by combining steady-state and time-resolved spectroscopic techniques. Photophysical and photochemical measurements account for an efficient aggregation of the drug with the micellar system. Shortening of the singlet and lengthening of the triplet lifetimes of DF is observed upon incorporation into micelles, respectively. The former effect is responsible for changes in the relative weight of the mono- and biphotonic pathways of photoionization. The quenching constant related to the trapping by the ground state of DF of the hydrated electrons photoejected is markedly reduced in the presence of Brij-35. The exit dynamic of DF from the micellar cage is the almost exclusive rate-controlling step for the electron-trapping process. Both nature of the photoionization and efficiency of the electron trapping provide basic information about the localization of DF in the micellar cage. The "persistent radical effect" responsible for the cross-combination reaction between phenoxy and σ aryl radicals in aqueous solution is suppressed in the presence of micelle. Mobility and different reactivity in the micellar environment of the two main radical species generated upon DF photolysis are proposed as a rationale for this observation. The relation of the overall results to the phototoxic effects displayed by the drug is also commented upon briefly.

Introduction

Photochemical investigation in organized assemblies mimicking biological systems is an extremely active area of research in the wide arena of supramolecular photochemistry.[1−4] Among micelles, monolayers, vesicles, and cyclodextrins, the first have represented one of the most exploited as simple model systems for cell membranes[5−7] since they do not possess a bilayer structure and have in many cases an appropriate spherical form. In this field, much of the interest of the photochemists has been focused in gaining more insight on both structural and dynamic features of biological membranes, on the entry/exit dynamic of stable and transient species as well as to the development of membrane-mediated photoprocesses for practical purposes. Studies performed in simple membrane-mimicking systems like micelles are particularly important in the case of phototoxic compounds. In this regard, much considerable attention has been recently devoted to topics concerning drug photochemistry.[8−10] Although investigations addressing the elucidation of the photochemistry of these molecules in homogeneous media are the first step to understand the molecular basis of the phototoxic effects, photoreactivity in homogeneous media and phototoxicity are often not directly correlated each other. This lack of correlation is due to the fact that the physical properties of the drug (i.e., hydrophobicity, geometry, size, shape, and charge) lead often the formation of host−guest supramolecular aggregates with biomolecules. These aggregates can display different photobehavior when compared to that of the "free molecule". As already reported for a large variety of systems,[1−7] the nature of the lowest excited states, the efficiency of the photophysical and photochemical pathways, the fate of the reaction intermediates, and the opening of new photoreactive channels are some of the parameters of the guest molecule that can be remarkably affected by its compartmentalization in particular sites of a biological microenvironment. As a consequence, a sequential approach consisting in exploring the photobehavior of phototoxic compounds in biological mimicking environments of increasing complexity represents an adequate strategy for a more appropriate correlation between phototoxicity and photochemical properties.

The picture emerging from these considerations suggests clearly that beyond the concern for both drug photostability and phototoxicity, from a strict physicochemical point of view, this subject offers opportunities to get more insight on fundamental aspects related to mechanisms of photoinitiated reactions in homogeneous, organized, and constrained media.

Diflunisal (DF), 2′,4′-difluoro-4-hydroxy-[1,1′-biphenyl]-3-carboxylic acid, is one of the most common nonsteroidal antiinflammatory drugs used in the market and it is known to induce phototoxic effects both in vitro[11,12] and in vivo[13] upon UVA irradiation. A previous study concerning its steady-state photochemistry in neutral aqueous solution[11] has shown that under UVA excitation DF undergoes photodefluorination, a not common reaction in fluoroaromatic derivatives due to the strength of the C−F bond (dissociation energy ca. 125 kcal/mol). The main photoproduct isolated in the absence of oxygen was the 2′-(2‴,4‴-difluoro-3″-carboxy-[1″,1‴-biphenyl]-4″-oxy)-4′-fluoro-4-hydroxy-[1,1′-biphenyl]-3 carboxylic acid (PhP) (see Scheme 1). The high toxic activity displayed by PhP itself toward cell membranes led to the labeling of this compound as the main species responsible for the photoinduced damage.[11] A recent detailed report concerning the transient photochemistry of DF in neutral aqueous solution has shed light on the molecular

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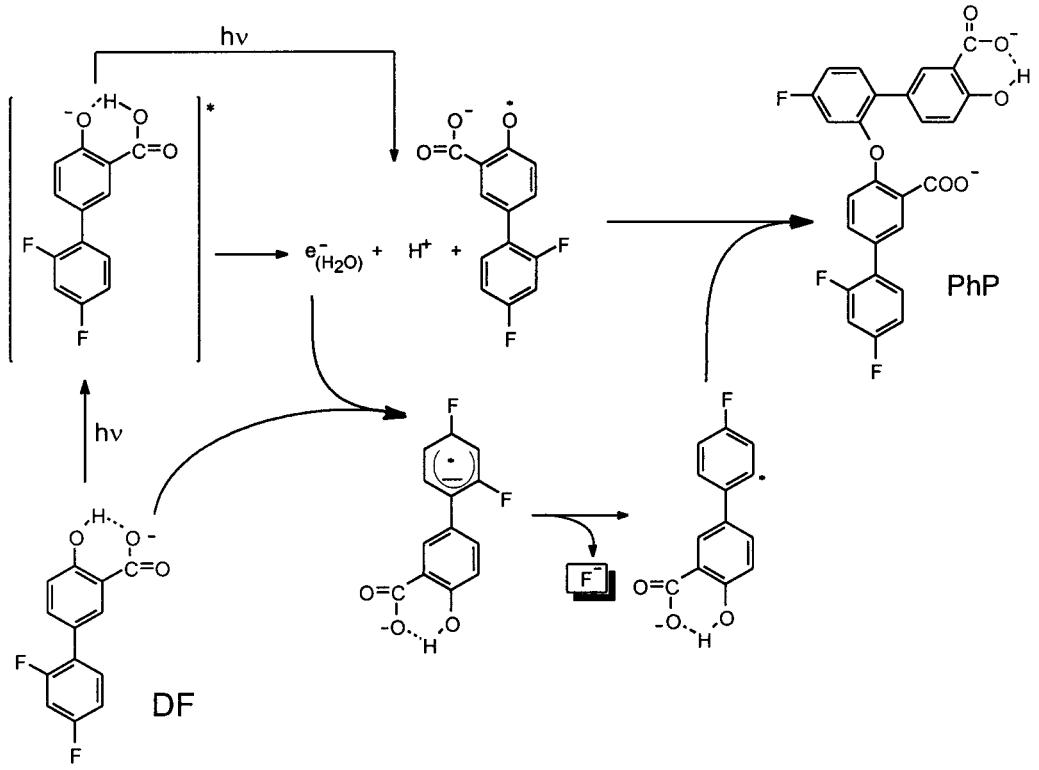
SCHEME 1



mechanism of drug photodegradation.[14] For the sake of clarity, the overall results are recalled in Scheme 1. Such investigation demonstrated that the main process involved in the DF photochemistry is a photoionization occurring from the singlet state through a mixture of mono- and biphotonic pathways. The main intermediate originating from electron photoejection has been identified as a phenoxy radical absorbing at 350 and 540 nm. It has also been ascertained that the defluorination process does not occur from an excited state but takes place via a radical anion intermediate formed after an efficient trapping by the ground state of DF of the electrons photoejected. A σ aryl radical is originated after that loss of fluoride has occurred. The second-order decay observed for the phenoxy radical was attributed to a cross-combination reaction with the σ radical. Such pathway was proposed as responsible for the formation of PhP as the main stable photoproduct. From a strict mechanistic point of view, this behavior provided an example a reaction controlled by the persistent radical effect. Indeed, in systems involving a radical (i.e., σ radical) more reactive than another one (i.e., phenoxy radical), the almost exclusive formation of the cross-combination product is quite common and can be explained as a result of the so-called "Fisher−Ingold persistent radical effect".[15,16]

In this work we report a combined steady-state and time-resolved investigation on the photochemistry of DF in nonionic micelles of polyoxyethylene lauryl ether (Brij-35), presenting a comparison with the study already performed in the absence of them.[14] The present study is aimed at gaining insight into the influence of the microenvironment on both the nature and efficiency of the overall drug photoprocesses. Such a goal is of interest not only for fundamental aspects concerning the general picture of the photochemistry in organized media but, in light of the reasons stated above, also for its relevance in providing significant basis for a more appropriate correlation between photochemical and phototoxic properties.

Experimental Section

Diflunisal (molecular weight 250.2) and Brij-35 (critical micellar concentration (cmc) $= 6 \times 10^{-5}$, aggregation number (N) $= 40$) were purchased from Sigma Chemical Co. (St. Louis, MO) and used as received. Phosphate buffer, 10^{-2} M, pH 7.4, was prepared from reagent grade products. The pH of solutions were measured with a glass electrode. Acetonitrile, from Carlo Erba, was used as received.

Steady-state absorption and emission spectra were recorded using a Beckman 650 DU spectrophotometer and a Spex Fluorolog 2 (Mod. F111) spectrofluorimeter, respectively. High-performance liquid chromatography (HPLC) was performed on a Hewlett-Packard 1100 chromatograph equipped with on-line photodiode array detector (DAD). The UV traces were monitored at 250 and 310 nm. The UV spectra (DAD) were recorded between 200 and 400 nm. The quantitative separation of PhP was achieved on an LiChroCart RP-18 column (5 μm packing, 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 retention times for DF and PhP were 11.2 and 10.5 min, respectively.

Fluorescence time-resolved studies were carried out by using the fourth harmonic (i.e., 266 nm) pulse from a Continuum PY-61 Nd:YAG laser (35 ps, 4 mJ/pulse) as excitation source. A Hamamatsu C-4334 streak camera was used for detection and acquisition of the data.

Nanosecond Laser Flash Photolysis. All the transient spectra and kinetics were recorded by employing a flow system with a 7×7 mm^2 Suprasil quartz cell with a 2 mL capacity, and were purged in a storage tank with N_2 for 30 min before as well as during the acquisition. A similar laser flash photolysis system has been previously described.[17,18] Briefly, the samples were excited with a Lumonics EX-530 laser with a Xe/HCl/Ne mixtures generating pulses at $\lambda_{exc} = 308$ nm of ~6 ns and ≤60 mJ/pulse. The signals from the monochromator/photomultiplier

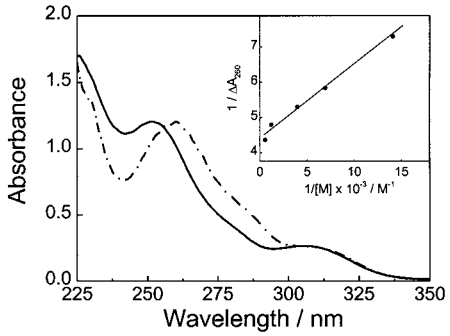


Figure 1. Absorption spectra of DF 1×10^{-4} M in 10^{-2} M phosphate buffer pH 7.4 (—) in the absence and (- · - · -) in the presence of 0.2 M Brij-35. Cell path: 1 cm. Inset: linear fitting of the absorbance differences, at 260 nm, on micelle concentration according to eq 1.

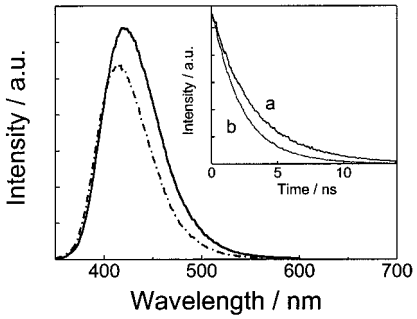


Figure 2. Fluorescence spectra of DF 1×10^{-4} M in 10^{-2} M phosphate buffer pH 7.4 (—) in the absence and (- · - · -) in the presence of 0.2 M Brij-35. $\lambda_{exc} = 306$ nm. The inset shows the decay traces (a) in the absence and (b) in the presence of 0.2 M Brij-35 observed in the region 400−440 nm.

system were initially captured by a Tektronix 2440 digitizer and transferred to a PowerMacintosh computer that controlled the experiment with software developed in the LabView 3.1.1 environment from National Instruments.

Steady-State Photolysis. Aliquots of DF (5×10^{-4} M) in the presence of Brij-35 (0.2 M) were irradiated under nitrogen flux in a Rayonet photochemical reactor equipped with a variable number of "black light" phosphor lamps with an emission in the 310−390 nm range with a maximum at 350 nm. The fluency at the irradiation position was about 800 μW/cm^2. The incident photon flux on the 3 mL solution in quartz cuvettes was ca. 5×10^{15} quanta s^{-1}. The experimental procedures for the light intensity measurement have been previously described.[19]

Results and Discussion

Absorption and Emission. The absorption spectrum of DF in phosphate buffer solution pH 7.4 was only slightly affected by addition of Brij-35 if its concentration was kept below the critical micellar value, indicating the lack of specific interactions between the ground state of the drug and the surfactant monomers. On the contrary, significant spectral changes were noticed when the surfactant was used in higher concentration. This suggests the aggregation of Brij-35 to form micelles and the incorporation of DF into these structures. Figure 1 shows the absorption spectra of the drug in the presence of 0.2 M Brij-35 and, for comparison, in its absence. In order to obtain the association constant for the host−guest system, an analysis of the dependence of the absorbance changes on the nonionic micelles' concentration was performed according to eq 1:[20]

$$1/(A - A^0_w) = 1/(A^0_m - A^0_w)(1 + 1/K_{ass}[M]) \qquad (1)$$

where A^0_w and A are the absorbance values in the absence and in the presence of surfactant respectively, A^0_m is the limiting absorbance upon complete incorporation of DF in the micellar phase and [M] = ([Brij-35] − cmc)/N is the Brij-35 micelles concentration. (The absorbance values in the presence of Brij-35 were taken by using a reference solution containing the same concentration of surfactant.) From the linear plot reported in the inset of Figure 1, an association constant of $K_{ass} = k_{+1}/k_{-1} = 2.3 \times 10^4$ M^{-1} ± 3000 according to the following micellization equilibrium was obtained.

$$DF + M \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} DF{-}M \qquad (2)$$

Such a value is in good agreement with literature data concerning the biphenyl itself,[21] taken as model compound, and suggests that the association of the drug with the surfactant aggregates is particularly favored. Spectrophotometric titrations performed both in the absence and in the presence of 0.2 M Brij-35 (DF incorporation > 95%) provided useful information about the localization of the drug in the micellar microenvironment. The values of p$K_{a1} = 3.1$ and p$K_{a2} = 10.6$ related to the carboxyl and hydroxyl moieties, respectively, were in fact basically unchanged in the presence of micelles. On the basis of this result it is reasonable to infer that such groups are not localized in a deep region of the micelle but reside near the micellar surface and thus are mainly exposed to water molecules. This hypothesis is in agreement with literature data reporting the effect of neutral micelles on the prototropic equilibria of incorporated guest molecules.[22,23] According to the approach proposed by Fernandez and Fromherz,[22] a localization of the carboxyl and hydroxyl groups in a region characterized by a dielectric constant lower than water would have led to changes in the pK_a values as result of a destabilization of the charged DF anions in the low polarity medium of micelle. Furthermore, the hypothesis of an aqueous environment surrounding the hydroxyl and carboxyl moieties is corroborated well by the laser flash photolysis experiments performed in the presence of Brij-35 (*vide infra*). On the contrary, based on these considerations and by taking into account the remarkable absorption spectral changes observed in the 260 nm band, it is reasonable to propose a localization mode in which only the phenyl rings are protruding into the micellar interior.

Both fluorescence spectrum and lifetime of DF were also affected by Brij-35 only if its concentration was kept above the cmc. As reported in Figure 2, addition of neutral surfactant led to a slight decrease of the fluorescence intensity accompanied by a blue shift (ca. 6 nm) of the emission maximum. Besides, a shortening of the fluorescence lifetime was observed under the same conditions if compared to that observed in the absence of micelle (inset Figure 2). The good correlation between the ratios of fluorescence quantum yields ($\Phi_{fw}/\Phi_{fm} = 1.25$) and fluorescence lifetimes ($\tau_{fw}/\tau_{fm} = 1.40$) in the absence and in the presence of micelles, respectively, rules out the involvement of optical effects due to the micellar phase. In fact, such effects are quite common and have been often reported as responsible for incongruity between these two photophysical parameters.[20]

As can be noticed from Figures 1 and 2, the large Stokes shift observed between the absorption and emission maxima accounts for a large difference between the geometry of the ground and the excited state. The recent study concerning the photochemical properties of DF in aqueous medium[14] pointed out that such a shift cannot be rationalized simply on the basis of the planarity change of the two phenyl rings upon light

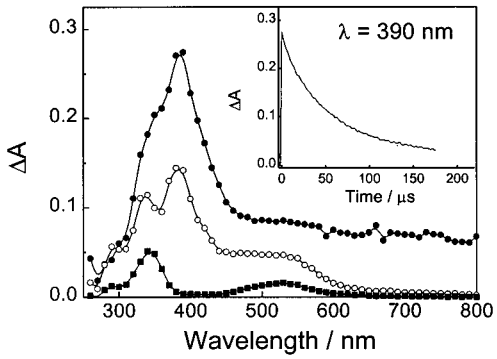


Figure 3. Transient absorption spectra observed in a DF 4×10^{-4} M N_2-saturated solution in phosphate buffer 10^{-2} M pH 7.4 in the presence of 0.2 M Brij-35, upon 308 nm laser excitation: (●) 0.1 μs, (○) 25 μs, (■) 160 μs after the pulse. The inset shows the decay trace recorded at 390 nm.

excitation (twisted in the ground and coplanar in the excited state).[24] An additional contribution was attributed to a photoinduced intramolecular proton transfer. Indeed, the proton involved in the intramolecular hydrogen bond from the hydroxyl to the carboxyl moieties in the ground state can shift toward the carboxylic oxygen in the excited state (Scheme 1). This behavior is typical of salicylic acid derivatives.[25,26] In light of these facts and by considering that the photophysical parameters of chromophores containing hydroxyl substituents have been reported to be extremely sensitive to the environment,[27−29] it would be conceivable to suggest that an effect of the micellar medium on the prototropic shift, on the rotational barrier of the phenyl rings or on both, could play a role in the slight blue shift of the fluorescence spectra observed in the presence of Brij-35. Nevertheless, we believe that neither the former nor the latter are factors responsible for the behavior noticed. Actually, as proposed on the basis of the absorption results (vide supra) and according to the laser flash photolysis experiments in the presence of Brij-35 micelles (*vide infra*), both the hydrophilic carboxyl and hydroxyl groups are believed to be mainly exposed to a water environment. As a consequence, an effect of the surfactant on the photoinduced proton transfer seems highly unlikely. Likewise, the hypothesis consistent with an increase in the rotation barrier of the two phenyls rings due to steric constrains in the micellar cage was also discarded on the basis of experiments performed in a binary mixture water/acetonitrile 50:50 (v:v). In such a solvent both fluorescence spectrum and lifetime of DF were very similar to those observed in the presence of 0.2 M Brij-35 (see Figure 2 and related inset). As a consequence, the effect of the nonionic micelles on the emission properties of DF can be ascribed simply to a polarity effect of the microenvironment on the lowest excited singlet state of the guest molecule. Although this hypothesis could seem apparently in contrast with the lack of polarity effect on the fluorescence of the biphenyl itself,[30] it is consistent with the chemical structure of DF. Indeed, it needs to be considered that due to the presence of the two fluorine atoms the molecule should bear a significant fraction of positive charge at least on one of the two phenyl rings. This would confer a certain polar nature to both ground and excited state. Thus, the blue shift observed might be consistent with a destabilization of the energy of the polar singlet state due to the localization of the drug into regions of lower local microscopic polarity.

Transient and Steady-State Photochemistry. Figure 3 shows the transient spectra recorded in nitrogen-saturated solution containing DF in the presence of nonionic micelles upon 308 nm laser excitation at three different delay times respect to the laser pulse. The spectrum taken 0.1 μs after the pulse shows a shoulder around 350 nm, a maximum around 380 nm and a long absorption extending beyond 800 nm. Moreover, it should be noticed that once the decay of the 380 nm band and the broad absorption in the visible region are complete, two clear bands centered at 350 and 540 nm appear. These spectroscopic features are basically the same as those observed in the absence of micelle[14] and are indicative of no generation of new transient intermediates in the micellar environment during the initial light excitation, besides those observed in homogeneous aqueous medium. Hence, we can safely assign the broad absorption extending beyond 800 nm to the hydrated electrons formed after DF photoionization, the peak around 380 nm to the triplet state of DF, and the two bands at 350 and 540 nm, observed on longer time scale, to the phenoxy radical generated after electron photoejection. At first glance, the lack of significant spectral changes of the T−T absorption if compared to that of an optically matched aqueous solution of DF could be consistent with a low sensitivity of the DF triplet state for the micellar environment. Nevertheless the triplet decay (inset Figure 3) was profoundly influenced by the presence of micelles. The related rate constant decreased in fact from $k_{Tw} = 1.6 \times 10^5$ s^{-1}, observed in water solution,[14] to $k_{Tm} = 2.6 \times 10^4$ s^{-1}, providing a strong indication for a confinement of such an excited state mainly in the interior of the micellar cage. The lengthening of the triplet decay is quite common with triplets upon incorporation in the micellar system and is generally related to the protection exerted by the micellar cage against external quenching impurities and to a perturbation of the intersystem crossing process to the ground state.[7] However, inhibition of triplet state−ground state interactions due to incorporation of only one molecule per micelle can also be responsible for the behavior observed.[7] The above hypotheses are consistent with the lack of any relevant effect on the triplet lifetime observed in organic solvents (i.e., acetonitrile and methanol) and thus provide a clear indication that the lengthening of the triplet decay time is not correlated to changes of the local micropolarity.

Time-resolved luminescence experiments performed in neutral aqueous solution pointed out that the DF triplet state is able to sensitize singlet oxygen O_2 ($^1\Delta_g$) formation through energy transfer to O_2.[31] Inasmuch as this harmful species was confirmed to play a significant role in the photosensitizing effect of DF against cell membranes,[11,12] the behavior of the DF triplet in the Brij-35 micelles deserves some brief comments. The high affinity of the drug triplet for the investigated membrane-mimicking system confers an important kinetic significance increasing the potential of DF as regard type II-mediated photosensitization processes. Actually, by taking into account both the remarkable lengthening of the triplet lifetime and the higher solubility of oxygen in the micellar phase,[7] the fraction of DF triplet quenched by oxygen can be significantly larger than that in aqueous environment. Since such effects might be also present in native biological systems, an increase in the efficiency of $O_2(^1\Delta_g)$ photogeneration cannot be ruled out. What is suggested accords well with recent studies on other nonsteroidal antiinflammatory drugs that have shown how micellar aggregates can strongly influence the ability of the drugs in photosensitizing the singlet oxygen generation.[32]

Photoionization Process. To gain insight into the influence of the micellar microenvironment, both on the efficiency and on the nature of the photoionization process of DF, we performed a laser power effect on the absorbance of the hydrated electrons, monitored at 720 nm ($\epsilon = 19\,000$ M^{-1} cm^{-1}),[33] in the presence and, for comparison, in the absence of micelles.

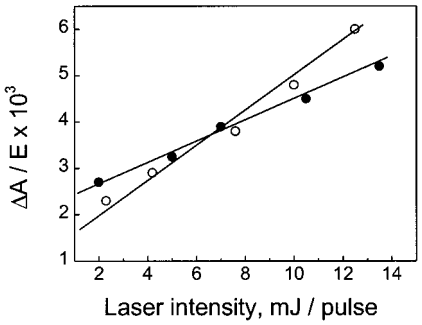


Figure 4. Laser intensity dependence of the ratio between the absorbance changes, at 720 nm 0.1 μs after pulse, and the pulse energy according to eq 3, observed in a DF 4×10^{-4} N_2-saturated solution in phosphate buffer 10^{-2} M pH 7.4 upon 308 nm laser excitation (○) in the absence and (●) in the presence of 0.2 M Brij-35.

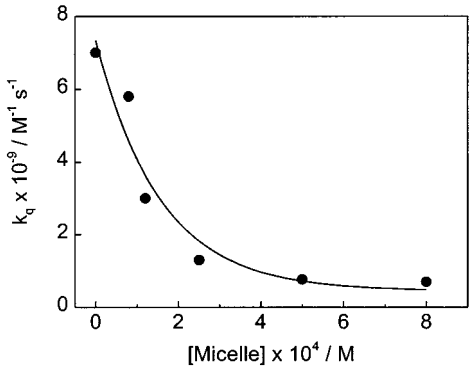


Figure 5. Quenching constants related to the trapping of the hydrated electrons by DF ground state as a function of the Brij-35 micelle concentration in phosphate buffer 10^{-2} M pH 7.4.

Also, in this environment photoionization occurs through a mixture of mono- and biphotonic mechanisms, similarly to what already observed in aqueous medium.[14] Nevertheless, changes in the relative efficiency of these pathways were noticed. As shown in Figure 4, the experimental data fit eq 3well:

$$\Delta A/E = a + bE \tag{3}$$

where E is the laser intensity, a is a coefficient depending on the quantum yield of the one-photon process, and b is a factor depending on the extinction coefficients and yields of the intermediate steps of the consecutive two-photon process.[34] From the slopes and the intercepts of the linear plots, it is straightforward to realize that the accommodation of DF in the micellar cage leads to an increase in the monophotonic and a decrease in the biphotonic photoionization efficiency, respectively. This behavior is in good agreement with the emission properties observed in micelles. Given that neutral micelles cannot affect per se the efficiency of the electron photoejection due to the lack of charged surface,[7] the changes in the relative weights of the two-photon and one-photon components can be easily understood on the basis of the microenvironment effect on the singlet lifetime of DF. The shortening of the time constant of the intermediate state S_1 should in fact decrease the probability of the absorption of a second photon during the laser pulse (ca. 6 ns) leading to a concomitant increase in the one-photon pathway efficiency. This behavior is in accordance with the opposite trend observed for photoionization processes in the case of molecules whose singlet lifetimes increase upon formation of supramolecular adducts.[35,36] The results reported in Figure 4 corroborate the hypothesis proposed in the previous section concerning the localization mode of DF in the micelle. Indeed, the quantum yields related to the one-photon photoionization pathways involving phenol moieties are expected to be particularly sensitive to the surrounding environment and the structure of the solvent around the OH groups is known to play a dominant role. In particular, as extensively reported in the literature, the formation of H-bonded supramolecular structures in which the OH binds two water molecules by acting as both H-donor and H-acceptor is believed to be the key step in determining the efficiency of the monophotonic mechanism.[27,28,37,38] In this connection, elegant studies performed by Monti and co-workers in supramolecular systems containing phenol derivatives[35] have pointed out a remarkable reduction in the efficiency of the one-photon photoionization pathway when the hydroxyl group resides essentially in an nonaqueous region of the supramolecular assembly. Since in our case the weight of the one-photon process was even higher than that observed in the absence of micelle, it is reasonable to believe that the incorporation of DF in the these systems does not provoke any relevant hindrance to the formation of the H-bonded supramolecular structures between water molecules and the hydroxyl moiety. This finding is in good agreement with the unaffected value of the pK_a observed in the presence of Brij-35 (vide supra) and provides a further evidence that the confinement mode of the drug inside the micellar system can be consistent with the hydroxyl and carboxyl groups of the molecule exposed mainly to an aqueous environment and the biphenyl chromophore protruding toward the micellar interior.

These results concerning the nature of the photoionization pathways can be of relevance for the understanding of the electron photoejection processes in biological environment. Indeed, similar compartmentalization mode of the drug in biological pockets can lead to a decrease in the biphotonic photoionization efficiency and an increase in the monophotonic one. This latter is dominant in typical environmental conditions due to the low light intensity level of irradiation.

Electron Trapping Process. As reported in our recent investigation,[14] the trapping of the hydrated electrons by ground-state molecule of DF in aqueous solution was very efficient ($k_{qw} = (7.0 \pm 0.3) \times 10^9$ M^{-1} s^{-1}). Such a process was demonstrated to trigger the drug defluorination via a radical anion intermediate leading to loss of fluoride and to the formation of a σ aryl radical (see Scheme 1). The quenching of the electron photoejected was investigated in the presence of different Brij-35 micelle concentrations. Figure 5 shows that the k_{qw} is remarkably decreased with increasing micelle concentration up to a limiting value of $k_{qm} = 6.8 \times 10^8 \pm 0.4$ M^{-1} s^{-1} corresponding to the reaction between e_{aq}^- and totally micellized DF. (Under our experimental conditions, the percentage of photodegradation is negligible so that the DF concentration is basically unaffected by the electron photoejection.) It is noteworthy that the ratios k_{qw}/k_{qm} at a given concentration of micelle are in fairly good agreement with the ratio a/b, where a and b are the fractions of DF in the micelle and water respectively, derived by substituting the value of K_{ass} previously determined in eq 4:[20]

$$K_{ass} = k_{+1}/k_{-1} = a/b\ [\mathrm{M}]^{-1} \tag{4}$$

This finding suggests that DF associated with micelle does not react efficiently with hydrated electrons and that the trapping process takes places mainly in the bulk solution. Moreover, this result provides another confirmation about the association mode proposed earlier. Similar behaviors have been observed in the case of chromophores deeply incorporated in nonionic micelles.[7] The lowering of the quenching constant observed in the micellar

system reflects the fact that the exit dynamic of DF from the micelles cage can be the almost exclusive rate-controlling step for the electron-trapping process. Since in the absence of charge repulsion with the micellar surface, as in our case, k_{+1} are reported to be of the order of 2.5×10^{10} M^{-1} s^{-1} [20,39] an upper limit of ca. 1.2×10^6 s^{-1} for the exit rate (k_{-1}) of DF can be calculated by means eq 4.

On the basis of the values of k_{qm} and by considering that the decay of the hydrated electrons in the absence of DF, k_0 (estimated from the intercepts value of the quenching plots related to Figure 5) was ca. 3×10^5 s^{-1}, it can be readily seen that in the whole range of therapeutic concentrations of DF ($[0.2-2] \times 10^{-3}$ M)[40] the hydrated electron no longer reacts quantitatively, according to eq 5.

$$k_{obs} = k_0 + k_{qm}[DF] \quad (5)$$

This observation stimulates some comments. A potential compartmentalization of DF in specific sites of a native biological system may provoke a slowing down of the drug mobility with consequent decrease in the efficiency of the electron capture by DF ground-state molecules. As a consequence, although a lower degree of the drug photodecomposition is expected to occur in these conditions, competitive processes in the scavenging of the electron photoejected involving a direct participation of specific biological sites might play not negligible roles in the photosensitization process. This hypothesis is not unprecedented. Indeed, recent studies of drugs undergoing photoionization upon light excitation have confirmed that protein residues provoke a remarkable shortening of the lifetime of the hydrated electron photoejected.[41,42] Among the several protein sites for the scavenging process, the carbonyl groups of the peptide chains have been proposed as the more likely candidates.[41,42]

Cross- and Self-Combination Reactions. As outlined in the Introduction section, the recent study performed in aqueous medium pointed out that the formation of PhP as the main stable photoproduct in the DF photolysis was attributed to a cross-combination reaction involving phenoxy and σ radicals (see Scheme 1). Given the known different self-reactivity of these two radicals, the almost exclusive generation of PhP was explained as a result of the "Fisher−Ingold persistent radical effect".[15,16] Indeed, according to this theory, when a radical (i.e., σ radical) is less persistent than another one (i.e., phenoxy radical) and its self-termination reaction takes place, the concentration of the more persistent species will increase in time to high levels and steer the system toward cross termination. Basically, the self-termination of the less persistent radical (fast process) will be suppressed by the slow self-termination of the more persistent radical.

When DF was irradiated in the presence of 0.2 M Brij-35 (DF incorporation > 95%) under nitrogen-saturated solution, the situation changed drastically. The chromatographic analysis of the irradiated mixture, performed below 20% conversion of the starting compound, revealed that no formation of PhP at all occurs in these experimental conditions. Besides, a kinetic analysis performed at 540 nm showed a significant lengthening of the phenoxy radical lifetime if compared to that observed in the absence of micelle (Figure 6).

These findings can be explained by considering the different reactivity toward the micellar environment of the two radicals involved in the cross reaction to give PhP. A simplified description of the probable processes occurring in the micellar system and consistent with the present results is proposed in the Scheme 2 and discussed below.

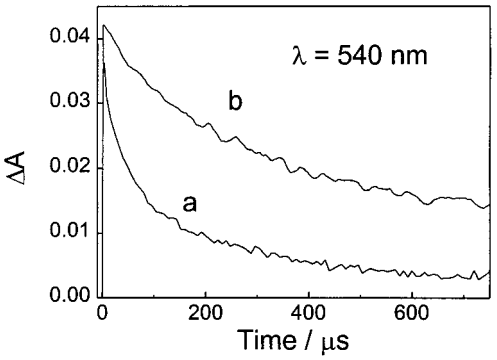
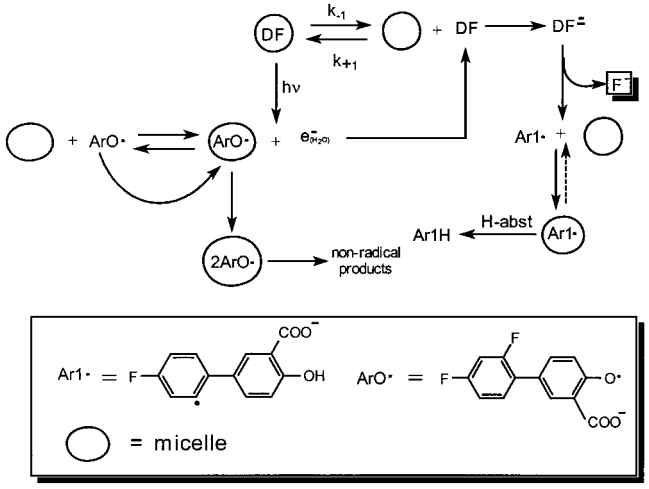


Figure 6. Kinetic traces for the decay of the phenoxy radical at 540 nm recorded in a DF 4×10^{-4} M N_2-saturated solution in phosphate buffer 10^{-2} M pH 7.4, (a) in the absence and (b) in the presence of 0.2 M Brij-35 upon 308 nm laser excitation.

SCHEME 2



The electron-trapping processes by DF ground state were demonstrated to be the rate-determining step for defluorination, via radical anion intermediate, and consequent formation of the σ radical.[14] Though the electron capture takes place in the bulk solution, it is difficult to ascertain if the σ aryl radical is generated either in the aqueous environment or after a reentry of the radical anion intermediate into the micelle. Taking into account the more polar nature of this transient species when compared with DF itself, the former hypothesis appears the more likely. In contrast, once formed, the σ radical will be preferentially located in the micellar phase. Indeed, considering the chemical structure of this radical, it is reasonable to propose that such a species is characterized by either similar or even higher affinity for the micelle than that of DF. As a consequence, given the high hydrogen abstraction (H-abs) reactivity of the σ radicals,[43,44] it is quite reasonable to propose that in our case such a radical might decay in the micellar interior probably via a H-abs intramicellar reaction. This hypothesis is supported well by kinetic considerations. Indeed, it has to be considered that the limiting values for H-abs reactions involving similar σ radicals are $k_H \approx 10^6-10^7$ M^{-1} s^{-1}.[43,44] Moreover, as has been demonstrated for typical supramolecular systems, the confinement of radicals in restricted spaces of organized assemblies can lead to relevant increase in the efficiency of the H-abs process[45,46] (due to the high local concentration of abstractable hydrogen) when compared to that observed for single monomers. Thus, one can readily see that under our experimental conditions the H-abs intramicellar reaction can be highly competitive (k_H[Brij-35] $\approx 10^5-10^6$ s^{-1}) with the exit dynamic of the σ

radical from the micellar cage ($k_{-1} \approx 10^5-10^6$ s^{-1}). Unfortunately, due to the known optical transparency of the σ radical in the monitored wavelength range,[43,44] it was not possible to gain any direct evidence for the kinetic behavior of this transient species. Nevertheless, the high reactivity of the σ radical with the micellar interior was reflected in the significant lengthening of the decay related to the phenoxy radical observed in the micellar solution and monitored at 540 nm (Figure 6). (The first part of the decay in the micelle is influenced by the decay of the triplet that, under these conditions, is longer lived than in water solution.)

A hypothesis concerning this decay could be consistent with a self-combination reaction of the phenoxy radical giving rise to nonradical products. In this regard, once free of its partner, ArO$^\bullet$ has two main pathways to decay depending on its preference either for the aqueous or the micellar phase. In the former case, the concentration of the phenoxy radicals in the aqueous phase will be relatively high so that radical−radical reaction in that environment will be the dominant decay mechanism. On the contrary, in the latter case, the radical concentration in the aqueous phase will be low. In this scenario the decay will occur through a mechanism in which ArO$^\bullet$ visits a certain number of micelles by repeated exit and entry processes until it finds one which contains another radical to react with, leading to a final product.[47,48] At this point, it is worthy to notice that the lengthening of the decay related to the phenoxy radical could reflect both cases. Actually, in accordance with the Fisher−Ingold persistent radical theory[15,16] a lengthening of the decay of the less reactive phenoxy radical is expected even if the self-combination reaction would take place in the aqueous phase. This is due to the inhibition of the cross-combination reaction caused by the sequestering of the more reactive σ radical in the micellar cage. Although the lack of micellar effect on the absorption maxima of the phenoxy radical might be consistent with a localization of such a radical mainly in the aqueous phase, we believe that the hypothesis of a termination reaction occurring in the micelles seems the more likely. Indeed, a substantial decrease of the partition coefficient, between micellar and aqueous phases, of the phenoxy radical with respect to that of the parent compound is unreasonable. Rather, on the basis of a slightly more hydrophobic nature of the phenoxy radical, its association constant with the micelle should be even higher than that of DF. Moreover, it has also to be considered that under our experimental conditions the radical concentration ($\approx$10 μM, based on $\epsilon_{340} = 3800$ M^{-1} cm^{-1})[14] is much lower than the micelle concentration ($\approx$5 mM). Finally, literature data report no relevant effects of the micellar environment on the absorption maxima of similar radical species.[47,49] All these reasons drive us to suggest that the phenoxy radical self-recombination reaction could occur mainly within the micelle cage.

The hypothesis proposed rules out, of course, any attack of the ArO$^\bullet$ radical to the micellar material (i.e., H-abs). Despite the low reactivity of the phenoxy radical toward H-abs, such processes have been observed in similar biological-mimicking systems in the case of highly hydrophobic phenoxy radicals.[50] However, this is not the case. Again, kinetic considerations confirm well our hypothesis. By taking into account that bimolecular rate constants for H-abs by phenoxy radical are $k_H \ll 10^6$ s^{-1}, the intramicellar abstraction reaction is so slow that ArO$^\bullet$ radical would need to "wait" in the micelle a time $\gg$5 μs (k_H[Brij-35] $\ll 2 \times 10^5$ s^{-1}). Due to the fact that, as proposed above, the mobility of the phenoxy radical can be comparable to that of DF ($k_{-1} \approx 1 \times 10^6$ s^{-1}), the residence time of such a radical in the micelle can be estimated as ca. 1 μs. As a consequence, one can readily see that an H-abs process by ArO$^\bullet$ radical from the micellar interior is highly unlikely.

We believe that the present scenario might be directly applicable to biological systems due to their microheterogeneity and the presence of abstractable hydrogen atoms. Therefore, the picture that emerges from these experiments on the fate of the two main radicals generated upon DF irradiation may provide helpful contributes in the understanding of the photosensitizing effects displayed by the drug in anaerobic conditions.[11,12] It stands to reason that probably the formation of the σ radical could play a major role in determining the level of the photoinduced damage. Actually, although produced in the bulk solution, this radical can be efficiently sequestered within hydrophobic pockets of a biological environment similarly to what proposed for micelles. The confinement time into these regions might be long enough to make a harmful process such as H-abs from biological sites highly likely. On the basis of the total lack of the cross-combination product PhP observed in the micellar system, it is hard to believe that such a compound is formed in appreciable amounts when DF is irradiated in the presence of biological systems. Thus, even though previous photosensitization experiments performed exclusively in the presence of PhP showed a strong efficiency of this compound in provoking the breakage of red blood cells,[11] it is not unreasonable to suggest that a direct participation of this compound in either toxic or phototoxic effects observed upon direct irradiation of DF could play only a minor role. In contrast, we cannot rule out a potential involvement of new photoproducts (i.e., self-combination product of the phenoxy radical) formed in the presence of micelles, in determining the extent of the photoinduced damage on biological target.

Conclusions

The present investigation has pointed out how both nature and efficiency of the photophysical and photochemical pathways of DF are markedly influenced by the presence of nonionic micelles. Photoionization and electron-trapping studies have represented a valid tool to gain basic information about the incorporation of DF in the micellar cage. The related results account for an association mode consistent with the hydroxyl and carboxyl groups of the molecule exposed mainly to an aqueous environment and the biphenyl chromophore protruding toward the micellar interior. The persistent radical effect controlling the formation of the cross-combination reaction in aqueous solution is suppressed in the presence of micelles probably due to an intramicellar reactivity of the σ aryl radical. In conclusion, localization of DF and its transient species photogenerated upon light excitation in the micellar microenvironment, their mobility between aqueous and micellar phase, and their different reactivity in the micellar aggregates are the main factors responsible for the profound changes in the overall photobehavior of DF. Beyond contributing to the general picture of the photoprocesses in organized assemblies, this study provides an example of how photochemical investigations in a biological mimicking system like micelles may represent an adequate strategy for a better understanding of the molecular basis of drug photoreactivity in highly complex biological media.

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