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Letter

Drastic photochemical stabilization of lomefloxacin through selective and efficient self-incorporation of its cationic form in anionic sodium dodecyl sulfate (SDS) micelles†

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It is shown that a 30-fold photochemical stabilization of the highly photoreactive and photocarcinogenic fluoroquinolone lomefloxacin can be achieved through a selective and efficient sequestering of its cationic form by a suitable micellar system at neutral pH.

Lomefloxacin (LM) is a drug belonging to the class of fluoroquinolone (FQ) antibacterials. Recently, these compounds have been the object of increasing interest due to their photobiological and photochemical relevance. Indeed, despite FQs being excellent therapeutic agents, the great majority are very active in inducing toxic and carcinogenic effects upon UVA excitation.[1–6] From a strict photochemical point of view, the interest around these molecules has been stimulated by the fact that many of them undergo photodefluorination, a very uncommon reaction in fluoroaromatics given the strength of the C–F bond (dissociation energy *ca.* 125 kcal mol^{-1}). In this overall scenario, LM is not only much more phototoxic, photomutagenic and photocarginogenic than other FQs,[3,5,6] but also one of the most photodegradable.[7–9] Albini and co-workers have indeed demonstrated that photodefluorination of LM occurs from position 8 with an unusually high quantum yield ($\Phi_{-LM} \approx 0.6$), leading to a mesomeric carbocation-carbene as the main intermediate in the photodecomposition.[7–9] This species has been invoked as the most likely candidate responsible for the LM photoinduced toxic effects.[3,7,10] The picture emerging from these considerations suggests that there is a critical need to find suitable systems to improve the drug photostability, which, as mentioned, seems to be directly related to the high photosensitizing activity displayed by LM. With this in mind we have explored the effect of sodium dodecyl sulfate (SDS) micelles on the photobehavior of LM in 10^{-2} M phosphate buffer at pH 7.2.

Like other FQs, LM can be present in three different forms depending on the pH (Scheme 1) and characterized by fairly different absorption and fluorescence spectra (Fig. 1).

The pK_a values related to the protonation of the carboxyl and piperazinyl groups, determined by both spectrophotometric and spectrofluorimetric titrations, were pK_{a1} = 5.9 and pK_{a2} = 8.2, respectively, and are in good agreement with previous literature data.[11] Given the isoionic pH of *ca.* 7.1, the zwitterionic form dominates at physiological pH. It is interesting to note that, similarly to what was reported

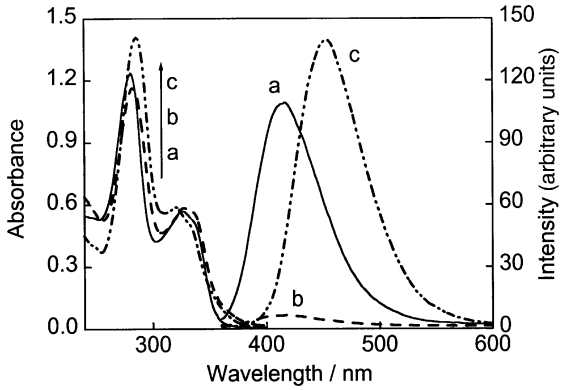
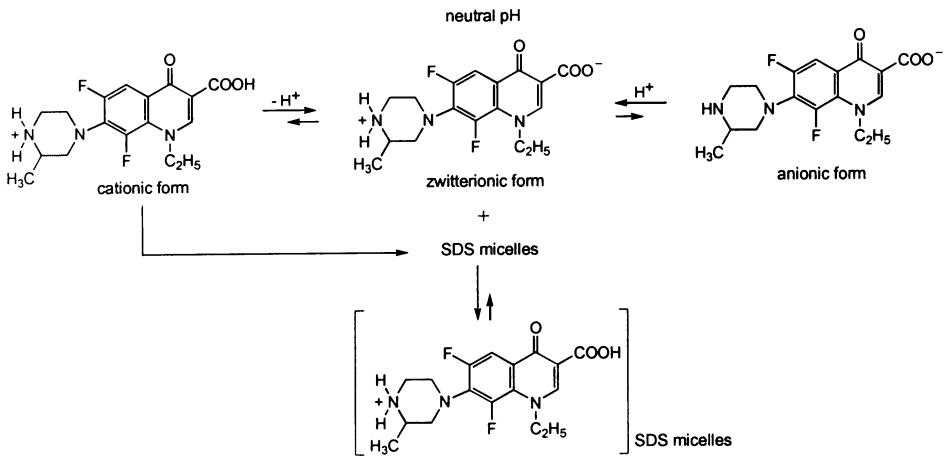


Fig. 1 Absorption (240–600 nm) and fluorescence (360–600 nm) spectra of LM at pH 7.2 (a), 12.0 (b) and 4.0 (c). λ_{exc} = 325 nm (isosbestic point).

† Dedicated to Professor Giuseppe Condorelli on the occasion of his 70th birthday.



Scheme 1

for other FQs,[12,13] this species is the most photolabile.[12] Recent picosecond and nanosecond time-resolved studies[14] have confirmed the hypothesis put forward in the last few years that the photodefluorination is mainly triggered by a short-lived singlet state[7,10] rather than, as observed for other FQs, by triplet states.[9,13,14] Such a singlet photoreactivity appears to be necessarily related to the intramolecular charge-transfer character of this state,[7,14] as indicated by the large Stokes shift of the fluorescence (see Fig. 1). The presence of the two electron-withdrawing fluorines plays a key role in the photoreactivity of LM, making the heterolytic cleavage of the C–F bond fast enough to compete with intersystem crossing (ISC).[7,14]

Fig. 2 shows the absorption and fluorescence spectra of LM in the presence of increasing amounts of SDS surfactant. As shown in the inset, the sharp changes in both the absorbance and the fluorescence intensity occur around the critical micellar concentration (CMC = 8×10^{-3} M), suggesting clearly that the behavior observed is directly related to the formation of micelles. Interestingly, as can be seen by comparison with Fig. 1, the absorption and emission spectra in the micellar solution show virtually the same characteristics as the cationic form of LM. This is a strong indication that SDS micelles interact almost exclusively with this prototropic species although it is not possible to rule out a weak interaction with the zwitterion. In brief, as shown in Scheme 1, SDS micelles make the cationic form of LM the most abundant at neutral pH. This proposal is corroborated by spectrophotometric and spectrofluorimetric titrations. The values of the apparent pK_a related to the carboxylic and piperazinyl moieties shift from 5.9 and 8.2 in aqueous solution to 7.9 and 9.5 in 0.1 M SDS, probably due to a change of the local hydronium concentration at the micellar interface and consistent with Hartley's model.[15] The deep incorporation of the cationic form in the micellar cage was further confirmed by quenching experiments carried out by using iodide (in the range 0–0.1 M), a quencher confined mainly in the aqueous environment. The results (data not shown) were analyzed by using eqn. (1) according to the method proposed by Quina and Toscano[16]

$$\Phi_f^0/(\Phi_f^0 - \Phi_f) = [(a\Phi_{fm}^0/b\Phi_{fw}^0) + 1][1 + 1/k_1\,\tau_{fw}^0[Q]] \quad (1)$$

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, the product $k_q\tau_{fw}^0$ is the Stern–Volmer quenching constant and Q is the quencher concentration. From the ratio a/b derived from the intercept of the linear plot (correlation coefficient 0.99), an association constant K_{ass} between LM and the SDS micelles of 5×10^4 M^{-1} was obtained by using eqn. (2):[16]

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

The high value of K_{ass} (related to an overall association constant in which the fraction in solution is defined by the pH of the solution) accounts for an extremely efficient incorporation process in the pseudo-phase. The structure of the cationic LM suggests that attractive electrostatic, hydrogen-bond and hydrophobic interactions may play key roles in making such an association particularly favorable. In this regard, a binding mode consisting of the positively charged piperazinyl group anchored to the negatively charged micellar surface and the neutral aromatic chromophore protruding towards the micellar interior can be reasonably proposed. This hypothesis is in good agreement with the remarkable pK_a changes discussed above.

The most important effect of the selective and efficient sequestering of the cationic form of LM by the micellar cage is reflected in the photoreactivity of the drug. Fig. 3 shows the dependence of Φ_{-LM} as a function of the SDS concentration.‡ It is obvious that the incorporated species is characterized by an extremely high photostability if compared with that of the free molecule. Indeed, Φ_{-LM} drops *ca.* 30-fold upon complete incorporation in the micelle, reaching the limiting value of 0.02. It is worthwhile noting that also in this case the sharp change in Φ_{-LM} occurs around the CMC, thus reflecting the formation of micellar aggregates. This remarkable inhibition of Φ_{-LM} cannot be simply ascribed on the basis of the shift provoked by the micelles on the prototropic equilibria. Indeed, the reactivity of the monocationic form of LM in aqueous solution is only 1.6 times lower than that of the zwitterionic form.[12] Therefore, the reasons for this drastic inhibition of the photodecomposition might lie in the peculiar role of the micellar microenvironment as reaction medium. Different effects can be invoked to explain the behavior observed.

As discussed earlier, the strong intramolecular charge-transfer character of the singlet state of LM makes the defluorination process fast enough to compete with ISC. In this respect, a rotation toward planarity of the piperazinyl group, which appears strongly out-of-plane in the optimized structure of the ground state, has been proposed to accompany the change in the dipole moment.[14] Given the low polarity of the micellar interior, a destabilization of this charge-transfer state by the SDS microenvironment could be inferred. This hypothesis accords well with the 10 nm blue shift observed in the maximum of the emission band of the totally incorporated LM when compared with that of the cationic form in aqueous solution. However, the slight reduction of Φ_{-LM} in a 50 : 50 buffer–acetonitrile mixture suggests that the polarity effect can

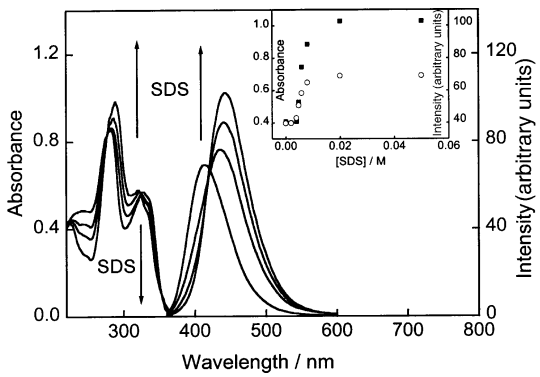


Fig. 2 Absorption and emission spectra of LM in 10^{-2} M phosphate buffer (pH 7.2) in the presence of increasing amounts of SDS in the range 0–0.05 M (some spectra are omitted for clarity). λ_{exc} = 325 nm (isosbestic point). The absorbance (at 300 nm, ○) and fluorescence (at 445 nm, ■) changes *vs.* SDS concentration are shown in the inset.

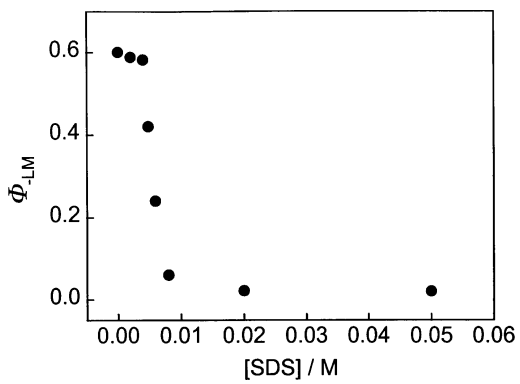


Fig. 3 Photodegradation quantum yield of LM in 10^{-2} M phosphate buffer at pH 7.2 as a function of SDS concentration.

not be the only factor responsible for the finding observed in micelles.

The high viscosity of the micellar medium and/or steric constraints in its interior may also be factors contributing to the decrease of the charge-transfer character by hindering the twisting of the piperazinyl substituent in the excited singlet state. Direct evidence for a decrease in the rotational motion upon micellar complexation is provided by fluorescence polarization measurements. In the absence of SDS, fluorescence from LM is only weakly polarized ($p = 0.008$), due to the tumbling motion of the chromophore in aqueous medium. In contrast, a degree of polarization varying in the range 0.05–0.06 over almost the entire excitation range is observed in the presence of 0.05 M SDS. Although the fluorescence quantum yield of the cationic form of LM in SDS was *ca.* 1.6 times higher than in water solution, such an increase does not follow the 30-fold decrease in Φ_{-LM}. This finding indicates that the inhibition effect by SDS micelles on LM photodecomposition can be only partly attributed to the increase of the radiative deactivation pathways.

Another possible parameter responsible for the drastic photostabilization observed could also be represented by the micelle as a reaction medium of confined space. In this regard, literature data report that charged micelles often lead to efficient separation of ionic couples and prevention of the back-neutralization reaction because of coulombic effects.[17] Nevertheless, it should be considered that in our system reasonable hydrogen-bond interactions involving the fluorine of LM with the aliphatic chain of the SDS may occur. In light of this and by taking into account the above discussed electrostatic binding involving the positively charged piperazinyl ring with the oppositely charged SDS surface, the occurrence of cage-recombination bimolecular processes between the two photogenerated partner ions cannot be ruled out. Therefore, the dramatic reduction of Φ_{-LM} could likely be mediated by the multifaceted role played by the micellar microenvironment as a solvent of low micropolarity, as a medium of high microviscosity and as a reaction environment of confined space. Nevertheless, it remains difficult to ascertain the relative weights of the individual factors.

Finally, it is worth noting that experiments performed in either neutral or cationic micelles have shown only a weak affinity of LM for the micellar environment with consequent small effects on the photodegradation efficiency. In conclusion, the present investigation demonstrates that the suitable choice of an appropriate micellar system leads to a dramatic enhancement in the photostability of the highly photoreactive LM. This effect is achieved through a highly selective and efficient self-incorporation of the cationic form of the drug in the micellar cage at physiological pH. Preliminary experiments undertaken in our laboratory have confirmed that the described effects are also observed for others FQs characterized by similar chemical structures but different photodefluorination mechanisms. The related results will be reported in a subsequent full paper. We believe that the present work provides basic information that will be useful in controlling FQ photoreactivity under physiological pH conditions. Thus, this investigation may prove a useful tool in the design of biocompatible drug-carrier devices able to improve the drug photostability and, as a consequence, reduce the high phototoxic effects induced by FQs. This topic is currently under investigation in our laboratory.

Experimental

The absorption and fluorescence spectra were recorded with a Beckman DU 650 spectrophotometer and a Spex Fluorolog-2 (model F-111) spectrofluorimeter, respectively. Monocromatic radiation of 325 nm, obtained from a He-Cd continuum laser, was used as irradiation source for the photodegradation experiments.

The photodegradation quantum yields of LM were determined through high performance liquid chromatography (HPLC) analysis, from the disappearance of the starting compound up to the 12% transformation. HPLC analysis was performed on a Hewlett Packard 1100 chromatograph equipped with an on-line photodiode array detector (DAD). A LiChroCart RP-18 column (5 μm packing, 4 × 250 mm; HP) was used. The irradiated mixture was eluted with a linear gradient of CH_3CN in buffer (triethylamine–phosphoric acid, pH 3) from 0 to 75% in 15 min, and a flow rate of 1 mL min^{-1}. Both retention time and integrated area for the non-irradiated LM in the presence of SDS were the same as in the absence of surfactant, suggesting that no complex existed during the elution.

Acknowledgements

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‡ The nature of the photoreaction did not change in the presence of SDS.

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