Role of Excited State Intramolecular Charge Transfer in the Photophysical Properties of Norfloxacin and Its Derivatives

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The photophysical properties of 1-ethyl-6-fluoro-7-(1-piperazinyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (norfloxacin, NFX) and some of its derivatives have been studied to evaluate the role of the free carboxylic acid and the nonprotonated piperazinyl group in the behavior of the 1,4-dihydro-4-oxoquinoline ring. Steady state and time-resolved fluorescence measurements at different pHs provide clear evidence in favor of singlet excited-state deactivation of NFX and its N(4′)-methyl derivative pefloxacin (PFX) via intramolecular electron transfer from the N(4′) atom of the piperazinyl ring to the fluoroquinolone (FQ) main system. This is a very efficient, energy-wasting pathway, which becomes dramatically enhanced in basic media. Acetylation at N(4′) (as in ANFX) decreases the availability of the lone pair, making observable its fluorescence and the transient absorption spectrum of its triplet excited state even at high pH. It also reveals that the geometry of FQs changes from an almost sp^3 hybridization of the N(1′) of the piperazinyl substituent in the ground state to nearly sp^2 in the singlet excited state (rehybridization accompanied by intramolecular charge transfer, RICT); accordingly, the singlet energy of ANFX is significantly lower than that of NFX and PFX. The fluorescence measurements using acetonitrile as a polar nonprotic organic solvent further support deactivation of the singlet excited state of nonacetylated NFX derivatives via intramolecular electron transfer from the N(4′) atom.

Introduction

Elucidation of the factors affecting the photophysical properties of donor−acceptor compounds is important because excited-state intramolecular electron transfer is a basic and crucial step in a variety of photophysical, photochemical, and biochemical processes.[1−4] Photoinduced intramolecular electron transfer can take place in systems with acceptor and donor subunits formally linked by a simple bond. Several models have been studied to explain low-energy emission bands observed in some electron donor−acceptor compounds. They include twisted intramolecular charge transfer (TICT), wagged intramolecular charge transfer (WICT), and rehybridization by intramolecular charge transfer (RICT).[5] The important issues related to the photophysics of these compounds are the nature of the subunits, the electronic structure and conformation in the charge-transfer excited states and the influence of the medium polarity.[3−10]

In this context, most of the fluoroquinolones (FQs), which are increasingly being used as antibiotics to treat a broad range of bacterial infections,[11] can be viewed as electron donor−acceptor compounds. Dialkylamino groups are the electron-donating part and a 1,4-dihydro-4-oxoquinoline-3-carboxylic acid as the electron-accepting moiety. In fact, large Stokes shifts have been observed in the emission spectra of FQs in neutral aqueous media. This was initially attributed to a twisted geometry of their singlet excited states with respect to their ground states (TICT);[12] however, theorethical calculations (AM1 Cosmo model) on one FQ (ofloxacin) seem to favor rehybridization of an almost sp^3 N(1′) atom in the ground state to nearly sp^2 in the excited singlet (RICT).[13,14] Anyway, both hypotheses are in agreement with a charge-transfer excited state.

The interest in the photophysical and photochemical properties of these compounds initially arose from their potential to induce phototoxic and photocarcinogenic side effects.[15−22] Thus, a number of studies have addressed the characterization of transient species and photoproducts of some of these derivatives;[23] however, despite this effort, the photophysical and the photochemical behavior of FQs is not yet completely understood. It has been found that photodegradation of FQs in aqueous solution leads mainly to defluorination and cleavage of the amino substituent at C-7.[24−28] However, these processes strongly depend on the reaction medium, specially on the pH[25−29] and are affected by the presence of salts.[29−32] In this context, the photophysical and photochemical properties of 7-(1-piperazinyl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (norfloxacin, NFX) at different pHs have been previously studied.[13,14,30] In fact, it has been observed that NFX fluorescence data (spectrum, lifetime and quantum yield) are strongly dependent on the presence of water as well as on pH changes. It is worth mentioning that under basic conditions there is no significant emission. The NFX triplet excited state (^{3}NFX) quantum yield has been estimated at ca. 0.5 in aqueous solutions whereas the singlet oxygen quantum yield has been found to be lower than 0.1 in phosphate buffer and ca. 0.017 in methanol. Besides, photodegradation is more efficient in neutral media.

Most of the above effects have been associated with the interconversion between the zwitterionic form at neutral pH and the cationic or anionic forms in acidic or basic solutions, respectively. However, it is not clear why the different forms have diverging photophysical and photochemical properties. A better understanding of the behavior of this donor−acceptor model would be important to rationalize the FQ photobiological properties. Hence, in the present work, NFX has been chosen as the model FQ to perform a systematic study on the involvement of the carboxylic group and the N(4′) atom of the piperazinyl ring in the emission properties of this family of

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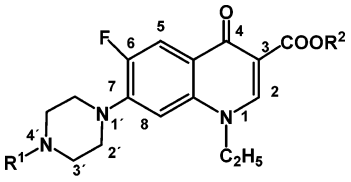
CHART 1: Structures of NFX and Its Derivatives



Compound	R^1	R^2
NFX	H	H
PFX	CH_3	H
EPFX	CH_3	CH_3
ANFX	CH_3CO	H
EANFX	CH_3CO	CH_3

drugs. For this purpose, the photophysical properties of NFX have been compared with those of some derivatives, such as pefloxacin (PFX, *N*-methylated NFX), its methyl ester (EPFX), *N*-acetyl norfloxacin (ANFX), and its methyl ester (EANFX) (Chart 1).

The obtained results provide experimental evidence for the role of several key processes in the emission from the singlet excited state of FQs: (a) formation of an intramolecular hydrogen bond between the nonionized carboxylic group and the adjacent ketone moiety, which clearly produces an increase in the fluorescence quantum yield as well as a decrease in the energy of its singlet excited state, (b) an intramolecular deactivation pathway by electron transfer from the N(4′) atom of the piperazinyl ring to the FQ singlet excited state and (c) the influence of changes in the geometry of N(4′) on the RICT effect of the NFX excited state with respect to its ground state.

Experimental Section

Materials. Norfloxacin (NFX), $CDCl_3$, and CD_3OD were obtained from Sigma Chemical Co. (St Louis, MO). Pefloxacin (PFX) was extracted from AZUBEN, produced by Ipsen Pharma laboratories (Barcelona, Spain). Sodium phosphate buffer was prepared from reagent-grade products using deionized water; the pH of the solutions was measured through a glass electrode and adjusted with NaOH to pH 7.4. Other chemicals were of reagent grade and used as received. 7-(4-Acetyl-1-piperazinyl)-1-ethyl-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (ANFX) was prepared from a solution of NFX (150 mg, 0.47 mmol) in Ac_2O (50 mL) that was refluxed for 7 h.[33] The solution was cooled to room temperature and concentrated. Afterward, the residue was dissolved in water, neutralized to pH ∼7.4, extracted with CH_2Cl_2 and concentrated to dryness. The synthesis of PFX methyl ester (EPFX) and ANFX methyl ester (EANFX) was achieved from solutions of PFX or ANFX (100 mg, 0.26 mmol) in CH_2Cl_2 (50 mL). After cooling these solutions in an ice bath, diazomethane (216 mg, 4 mmol) in diethyl ether (100 mL) was added dropwise, and the resulting mixtures were stirred at room temperature overnight. Then, they were concentrated under vacuum and submitted to purification by column chromatography on silica gel, eluting with CH_2Cl_2/MeOH (85/15 v/v).

Absorption and Emission Measurements. UV/vis-absorption measurements were performed on a Shimadzu UV-2101PC spectrometer. Fluorescence emission and excitation spectra were recorded on a Photon Technology International (PTI) LPS-220B fluorometer. All spectra, except for relative measurements of fluorescence quenching, are corrected for the instrument response. Lifetimes were measured with a lifetime spectrometer (TimeMaster fluorescence lifetime spectrometer TM-2/2003) from PTI by means of the stroboscopic technique, which is a variation of the boxcar technique. A hydrogen/nitrogen flashlamp (1.8 ns pulse width) was used as excitation source. The kinetic traces were fitted by monoexponential decay functions using a reconvolution procedure to separate from the lamp pulse profile. Measurements were done under aerated conditions at room temperature (25 °C) in cuvettes of 1 cm path length. The emission spectra of NFX, PFX, EPFX, ANFX and EANFX were obtained in the presence of acetonitrile and in neutral aqueous solutions (pH = 7.4), in the absence and in the presence of phosphate buffer (PB, 10 and 50 mM). Besides, the spectra were also recorded in basic and acidic media after addition of NaOH or HCl to the neutral, nonbuffered solution. The fluorescence lifetimes of NFX, PFX, EPFX, ANFX and EANFX were only obtained under neutral conditions (pH ca. 7.4, solutions were adjusted adding NaOH) and in acetonitrile. The procedure to determine fluorescence lifetimes and quantum yields was to adjust the absorbance of the solutions to the arbitrary value of 0.1 at the excitation wavelength of 340 nm. Quinine bisulfate in 1 N H_2SO_4 ($\phi_f = 0.546$) was used as standard. The singlet energies (E_{0-0}) of each FQ at the different pHs were estimated from the intersection of the normalized excitation and emission spectra.

Laser Flash Photolysis Measurements. A pulsed Nd:YAG SL404G-10 Spectron Laser Systems was used at the excitation wavelength of 355 nm. The single pulses were ∼10 ns duration and the energy was lower than 10 mJ/pulse. The detecting light source was a pulsed Lo255 Oriel xenon lamp. The laser flash photolysis system consisted of the pulsed laser, the Xe lamp, a 77200 Oriel monochromator, an Oriel photomultiplier tube (PMT) system made up of a 77348 side-on PMT tube, 70680 PMT housing and a 70705 PMT power supply. The oscilloscope was a TDS-640A Tektronix. The output signal from the oscilloscope was transferred to a personal computer.

All samples of NFX and (E)ANFX were in ACN or aqueous solutions at pH 7.4 and 12, and the absorbance was set at 0.26 at 355 nm. The basic medium was prepared after addition of NaOH to the neutral nonbuffered solution. Deaeration was achieved by bubbling nitrogen or N_2O.

Differential Normal Pulse Voltammetry. The reduction potentials of ANFX and NFX in 10 mM PB aqueous solutions were measured using the differential normal pulse voltammetry technique, with Au as working electrode and Ag/AgCl as the reference electrode. The samples were deaerated bubbling vigorously Ar for 15 min. The measurements were done under stirring, and the scan rate was 50 mV/s.

Results and Discussion

Absorption, emission and excitation measurements on norfloxacin (NFX) and its derivatives (PFX, EPFX, ANFX and EANFX) were performed systematically in neutral, acidic and basic aqueous media as the first step to determine the role of the carboxylic acid and the N(4′) of the piperazinyl group in the photophysical properties of the main ring. Thus, several singlet excited-state properties of these FQs were determined, such as their energies (E_{0-0}) wavelengths of the emission maxima or fluorescence quantum yields (ϕ_f) (see Table 1).

Absorption Spectra in Aqueous Solutions. The compounds containing a free carboxylic acid (NFX, PFX and ANFX) displayed superimposable absorption spectra with three maxima at 272, 322 and 336 nm and a long tail extending to 370 nm, both at pH 7.4 and at pH 11 (Figure 1A). When the pH was fixed at 3.5, NFX, PFX and ANFX showed again identical absorption spectra with a clear maximum at 278, diminished absorption at 310−330 nm and a long tail extending to 385 nm.

The absorption spectra of esters EANFX and EPFX were only recorded at pH 7.4 and 3.5, because these compounds were

TABLE 1: Photophysical Properties of Several Fluoroquinolones at Different pHs

	pH = 3.5				pH = 7.4				pH = 12.0			
compound	form[a]	E_{0-0}[b]	λ_{max}[c]	ϕ_f	form[a]	E_{0-0}[b]	λ_{max}[c]	ϕ_f	form[a]	E_{0-0}[b]	λ_{max}[c]	ϕ_f
NFX	A	74.5	442	0.21	B	79.2	410[d]	0.12[d]	C			0
PFX	A′	74.3	440	0.26	B′	79.6	408	0.07	C′			0
ANFX	D	73.9	442	0.04	E	76.2	428	0.13	E	76.0	428	0.12
EANFX	F	75.5	430	0.06	F	75.4	428	0.06				
EPFX	G	78.8	412	0.13	G	79.0	410	0.06				

[a] Predominating form at the indicated pH (structures in Figure 3). [b] In kcal/mol. [c] In nm. [d] In H_2O and D_2O.

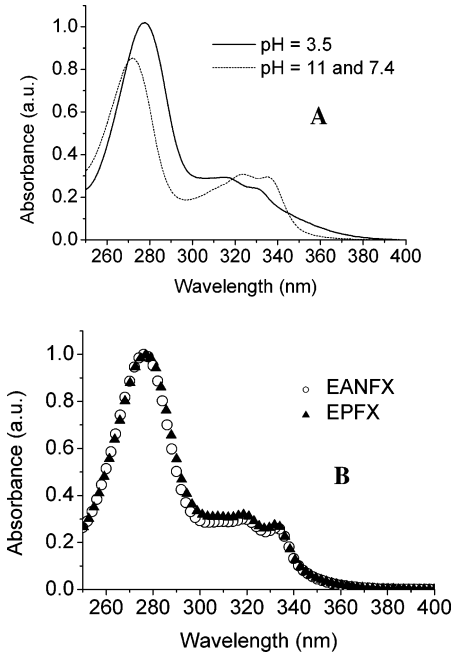


Figure 1. Absorption spectra of (A) NFX, PFX and ANFX at different pHs, and (B) EPFX and EANFX at pH 3.5 and 7.4.

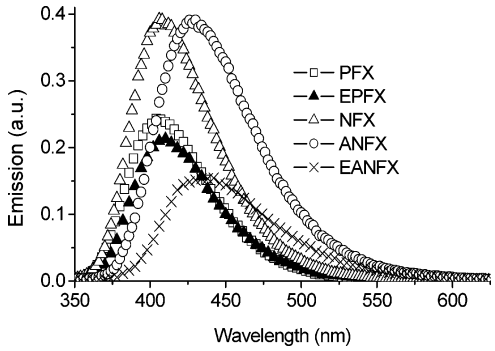


Figure 2. Emission spectra of NFX, PFX, ANFX and EPFX and EANFX at pH ca. 7.4.

hydrolyzed very fast at pH 11, to give ANFX and PFX, respectively. As shown in Figure 1B, the traces obtained for both esters were identical, and remained unaffected by the pH.

Emission Spectra in Aqueous Solutions. The emission spectra of NFX, PFX, and EPFX under neutral conditions (pH = 7.4) were very similar, with a band at λ_{max} ca. 410 nm and a long tail extending to 620 nm (Figure 2).

However, ANFX and its ester EANFX showed an emission band with a maximum at 428 nm, red-shifted (18 nm) with respect to NFX, PFX and EPFX. The fluorescence quantum yield of ANFX was found to be ca. 0.13, similar to that reported for NFX (ϕ_f = 0.12); in contrast, in the case of PFX, EPFX and EANFX the ϕ_f values were markedly lower. In addition, NFX, PFX and ANFX were also analyzed in aqueous solution at pH 12 and 3.5.

Thus, under basic conditions, PFX and NFX did not produce significant fluorescence (as previously described for NFX at pH > 11[30]) whereas the intensity of the ANFX emission band (not shown) was similar to that obtained at pH = 7.4 and appeared at the same wavelength.

In acidic media, NFX, PFX and ANFX all emitted at longer wavelength (λ_{max} ca. 440 nm). These data are summarized in Table 1. The excitation spectra were recorded for all compounds, at the three usual pHs to determine the singlet energies (E_{0-0}) of each FQ at the different pHs. From the intersection of the normalized excitation and emission spectra, the singlet energies (E_{0-0}) of the selected FQs were determined at the different pH values. They are also given in Table 1.

The above results cannot be clearly understood without considering which FQ forms (Figure 3) are present at the different pHs. Thus NFX is present as form A at pH 3.5, B at pH 7.4 and C at pH 12 on the basis of its two pk_a values (pk_{a1} ca. 6.22 and pk_{a2} ca. 8.51).[34] For PFX, with pk_{a1} ca. 6.02 and pk_{a2} ca. 7.80,[34] the major forms are also A′, B′, and C′ under the same conditions; however, at pH = 7.4 the amount of form C′ is significant. For ANFX only one pk_a (6.53)[34] is relevant in the studied pH range; hence form D overwhelmingly predominates at pH 3.5 and form E at pHs 7.4 and 12. In the case of the esters EANFX and EPFX forms F and G, respectively, are present not only at pH 3.5 but also at pH 7.4.

With this background, the photophysical properties of the FQs can be analyzed by considering separately their structural modifications.

Methylation of the Carboxylic Group. This modification produced unimportant changes in the properties of the singlet excited states (S_1). In fact, forms F and G have singlet energies and fluorescence spectra similar to those of forms E and B′, respectively (ANFX and PFX at pH 7.4). In contrast, when the free carboxylic group of fluoroquinolones ANFX, PFX and NFX is deprotonated (pH change from 3.5 to 7.4), the resulting carboxylate forms B, B′ and E exhibit photophysical properties dramatically different from those of the nondissociated acids A, A′ and D (Table 1). Thus, an intramolecular hydrogen bond between the carboxylic proton and the 4-oxo group of the FQ ring system must increase the intramolecular charge-transfer character not only in the ground state (long-wavelength absorption tail of NFX, PFX and ANFX at pH = 3.5, Figure 1A) but also in the singlet excited state, where the energies of A, A′ and D decrease with respect to B, B′ and E (see Table 1).

Acetylation at the N(4′) Atom. Acetyl substitution in the amino group produced important changes of the FQ emission properties, but not of the absorption spectra. Thus, ANFX displayed the same absorption and emission spectra at pH = 7.4 and 12, where form E largely predominates (Figure 1A and Table 1). In this context, the closely related forms C and C′ (NFX and PFX at pH 12) did not produce detectable fluorescence. This fact, which had been observed previously for NFX, was attributed to a decrease of the intrinsic radiative constant.[23] However, as it can be seen in Table 1, form E generates similar fluorescence at pH 12 as at pH 7.4. A possible rationalization

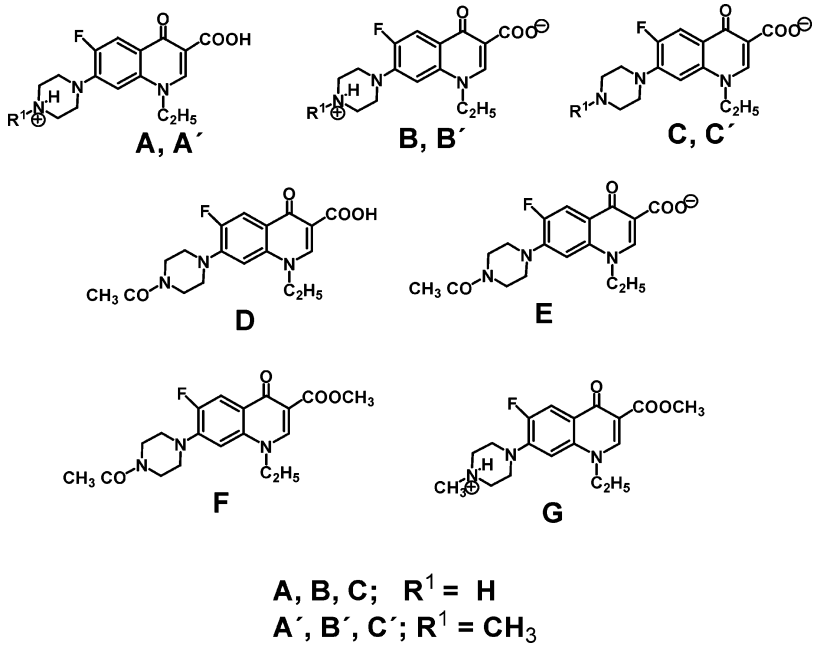


Figure 3. Structures of the fluoroquinolones in aqueous media.

of the lack of fluorescence of forms C and C′ at pH 12 is the occurrence of a deactivation process for their singlet excited states by intramolecular electron transfer (IET) from the N(4′) lone pair to the FQ system. This deactivation pathway is not possible for form E, whose N(4′) lone pair is blocked as a result of acetylation. In this context, several experiments were performed by laser flash photolysis looking for additional evidence in support of this deactivation process. Thus, the absorbance observed for the ANFX triplet excited state (at λ_{max} ca. 610 nm)[35] was the same at both pHs (7.4 and 12) whereas in the case of NFX the triplet signal (λ_{max} at ca. 620 nm)[23] was detected only at pH 7.4. Moreover, formation of the solvated electron (arising from the singlet excited state) was observed for ANFX under nitrogen at pH 12, whereas this band was also missing in the analogous experiments performed with NFX.

The large Stokes shift of the emission spectra of FQs in neutral aqueous media has been explained through TICT or RICT processes in the singlet excited state, associated with intramolecular charge transfer from N(1′) to the main ring.[12−14] In principle, the results obtained with NFX derivatives can be justified by either of the above processes. As an indication of the very low electron-donating effect of the N(1′) piperazinyl ring on the FQ main ring in the ground state, it was observed that the absorption spectra of forms E and B were very similar (Figure 1A). Likewise, the reduction potentials of ANFX and NFX were found to be nearly the same ($E_{red} = -1.56$ V for both compounds; see Figure 4). On the other hand, the large Stokes shift in the emission spectra of forms E and B is in accordance with the electron-donating effect of the N(1′) piperazinyl substituent on the FQ main ring in the singlet excited state. In this context, the fact that the energies of the singlet excited states of forms E and B were clearly different is indicative of the important role played by the whole piperazinyl ring in the intramolecular charge-transfer process from N(1′) to the main ring (Table 1 and Figure 3). Two hypotheses could justify this result:

(i) the lone pair of N(1′) would be partially blocked in B (the proton forming a bridge between both nitrogens of the piperazinyl ring); this is not possible for E, where protonation at N(4′) is inhibited by acetyl substitution. Actually, it is known that in the protonation of piperazines the two nitrogen atoms

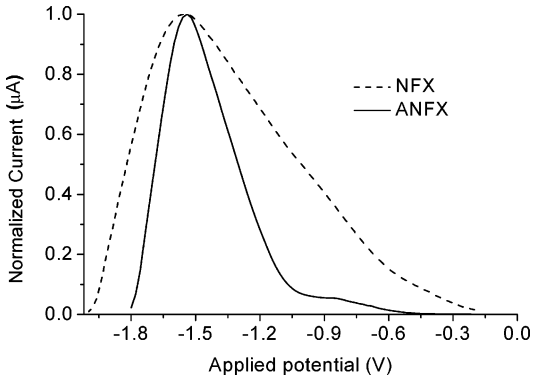


Figure 4. Measurement of the potential of ANFX and NFX in 10 mM PB aqueous solutions, using the differential normal pulse voltammetry technique.

do not behave independently, as both contribute to stabilize the first positive charge, giving rise to two different p*K* values (3.97 and 8.34).[36] However, the absence of any deuterium effect on the emission of NFX in D_2O (Table 1) seems to be in favor to the second hypothesis (see below).

(ii) The geometry of N(4′) in the piperazinyl ring changes from an sp^3 hybridization to a close sp^2 upon acetylation. This could facilitate a parallel rehybridization of the N(1′) of the piperazinyl substituent from an almost sp^3 hybridization in the ground state to nearly sp^2 in the singlet excited state. Theoretical calculations on ofloxacin support such rehybridization.[14]

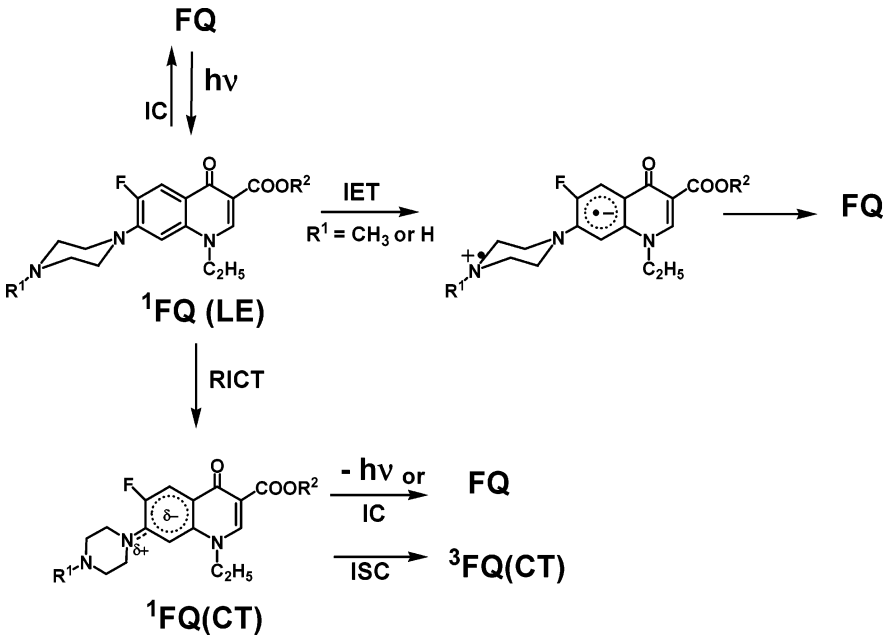
Methylation at the N(4′) Atom. Methyl substitution at the secondary amino group did not change the nature of the FQ singlet excited states (S_1). Thus, as shown in Figures 1, and 2 and Table 1, NFX and PFX at pH = 3.5 (forms A and A′) had identical absorption spectra and similar singlet excited-state energies. The same was true for B and B′ (pH = 7.4), whereas no significant fluorescence was observed for C and C′ (pH 12) due to deactivation via intramolecular electron transfer from the N(4′) lone pair to the FQ ring system in the singlet excited state. The fluorescence quantum yield differences between NFX and PFX at pH = 7.4 (0.12 and 0.07, respectively) are attributable to the fact that under these conditions more than 90% of NFX is in the form B ($pk_{a2} = 8.51$) whereas in the case

TABLE 2: Photophysical Properties of Several Fluoroquinolones in Acetonitrile and in Neutral Aqueous Solutions (pH ca. 7.4)

compound	λ_{max}, nm (ACN)	ϕ_f (ACN)	τ_f, ns (ACN)	k_r[a] (ACN)	k_{nr}[a] (ACN)	τ_f, ns (H_2O)	k_r[a] (H_2O)	k_{nr}[a] (H_2O)
NFX	440	<0.001	3.05[b]	3.3×10^5	3.2×10^8	1.5[c] (1[d])	8.0×10^7	5.9×10^8
PFX	438	<0.001				1.6 (1.1[d])	4.4×10^7	5.8×10^8
ANFX	445	0.018	4.1	4.4×10^6	2.4×10^8	0.9	1.4×10^8	9.6×10^8
EANFX	410	0.025	4.0	6.2×10^6	2.5×10^8	0.9	6.7×10^7	1.0×10^9
EPFX	400	<0.001				1.3	4.6×10^7	7.2×10^8

[a] The radiative (k_r) and nonradiative rate constans (k_{nr}) are in s^{-1}, and they were obtained by using the equations $k_r = \phi_f/\tau_f$ and $k_{nr} = 1/\tau_f - k_r$; the ϕ_f (H_2O) are in Table 1. [b] Taken from ref 14. [c] Measured in the present work; in good agreement with refs 13 and 37. [d] In the presence of 100 mM PB.

SCHEME 1: Photophysical Pathways of FQs Singlet Excited States



of PFX (pk_{a2} ca. 7.80) there is only ca. 60% of B′ and more than 30% of C′.

Effect of Phosphate Buffer. Fluorescence measurements were also performed in the presence of different amounts of PB buffer (from 10 to 100 mM) at pH ca. 7.4. In this context, previous studies had established that NFX singlet excited state is quenched by PB through static (mainly) and dynamic mechanisms, and that static quenching is inhibited when the piperazinyl ring is substituted at N(4′).[37] Results obtained with NFX and its derivatives (PFX, EPFX, ANFX and EANFX) in the presence of different amounts of PB buffer (from 10 to 100 mM) are in agreement with this picture. The emission band of NFX was reduced by ca. 60% at 100 mM PB. This reduction was only ca. 10% with PFX and EPFX under the same conditions. In the case of ANFX and its ester EANFX, the presence of PB did not change the intensity of the emission bands. The lack of dynamic quenching can be attributed to the singlet excited-state energies, which are lower for (E)ANFX (E_{0-0} ca. 75 kcal/mol) than for NFX and (E)PFX (ca. 79 kcal/mol).The fluorescence quenching rate constant by PB was found to be ca. 3×10^9 M^{-1} s^{-1} for NFX and PFX, as determined by fluorescence lifetimes for these FQs in neutral aqueous solutions with and without PB (100 mM) (Table 2). According to the Rehm and Weller equation and using a value of −1.56 V for the reduction potential of NFX and ANFX and the singlet energy levels described above and assuming that FQ-PB redox reaction is only thermodynamically permitted for the former, we can estimate an oxidation potential of the couple $HPO_4^{\bullet -}/HPO_4^{2-}$ in the range 1.87−1.7 V. This value is in agreement with previous studies, where it was postulated that this oxidation potential must be lower than 1.9 V.[26]

The present results agree with the hypothesized structure of the ground-state associates of FQs with phosphate dianions, which are responsible for static quenching[37] and help to understand the reasons for the different dynamic quenching of NFX derivatives.

Effect of Acetonitrile as Organic Solvent. When the FQ derivatives were studied in acetonitrile as an organic nonprotic solvent, it was observed that the emission quantum yields of all the compounds were lower than those determined in neutral aqueous solutions (see Tables 1 and 2). These results seem to be in accordance with the increase of the nonradiative rate constants of the FQs as previously proposed in studies on NFX.[23] Specifically, this was justified by an increase of the internal conversion (IC) rate. However, the fact that (E)ANFX fluorescence quantum yields in acetonitrile were only ca. 2 times lower than those determined for forms D and F in aqueous solutions suggest that, although IC could have increased, the main deactivation pathway of the singlet excited state of NFX is intramolecular electron transfer between N(4′) and the FQ main ring system (IET, see Scheme 1). In fact, all the nonacetylated FQs show a very weak fluorescence in acetonitrile. Further support to this process was obtained by laser flash photolysis experiments with NFX and EANFX, using acetonitrile as the solvent. Thus, although no transients were detected for NFX, in the case of EANFX a transient absorption spectrum was observed with λ_{max} ca. 590 nm (Figure 5). This species can be assigned to the EANFX triplet excited state by analogy to ^{3}NFX and on the basis of its efficient quenching by oxygen ($k_q = 2 \times 10^9$ M^{-1} s^{-1}) (Figure 5). Similar transient species and absorbances were observed when EANFX was studied in neutral aqueous solutions under N_2O. Thus, it seems that the NFX

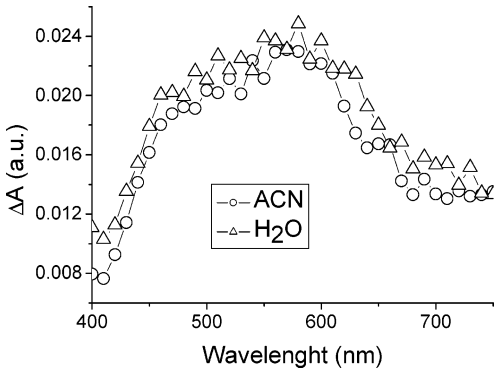


Figure 5. Transient absorption spectra of the EANFX triplet excited state obtained 100 ns after the laser pulse in deaerated ACN solutions (N_2) and in N_2O-purged neutral aqueous solutions.

derivatives intersystem crossing (ISC) is not significantly affected by the use of an organic nonprotic solvent as acetonitrile. On the other hand, it was observed that fluorescence lifetimes of ANFX and EANFX were similar to each other and had a value close to that described for NFX under similar conditions.[14] It seems that singlet excited states of NFX derivatives are longer lived in acetonitrile than in neutral aqueous solutions (Table 2).

All the above results seem to be in agreement with Scheme 1, whose main feature is the involvement of two singlet excited states, as previously described for systems with acceptor−donor subunits:[6−8]

(a) a nonfluorescent locally excited (LE) singlet, which would be quickly transformed by rehybridization (RICT) into a charge-transfer (CT) state unless other intramolecular reactions compete with this process (two of these reactions would be an intramolecular electron transfer (IET) from the N(4′) lone pair to the FQ system or an analogous intermolecular process with phosphate dianions as donors when they are associated with N(4′)) and

(b) the charge-transfer (CT) state (more stable and lower in energy than LE), whose deactivation would occur either by emission or by intersystem crossing (ISC) to the triplet excited state.

Conclusions

The above results show the influence of a diamine donor subunit on the photophysical properties of FQs, an unusual type of system with acceptor−donor groups. In fact, the lone pair of N(4′) acts as a switch. Clear evidence has been obtained supporting singlet excited-state deactivation of NFX and PFX via intramolecular electron transfer from the N(4′) atom of the piperazinyl ring to the FQ main system. This is a very efficient, energy-wasting pathway that becomes dramatically enhanced in basic media and in nonprotic organic solvents such as acetonitile. Acetylation at N(4′) decreases the availability of the lone pair, making fluorescence observable even at high pH. It also reveals that the geometries of FQs change by a RICT process; accordingly, the singlet energy of (E)ANFX is significantly lower than that of NFX and (E)PFX. Besides, further experimental evidence is provided confirming the previously proposed role of an intramolecular hydrogen bond between the nonionized carboxylic acid and the 4-oxo group, as well as the nature of static/dynamic fluorescence quenching by phosphate anions.

From the photochemical and photobiological point of view these results can help to explain the low singlet oxygen quantum yields observed when this type of compound is studied in organic solvents and the influence of the pH on the photodegradation of the FQs.

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