Multifaceted Photoreactivity of 6-Fluoro-7-aminoquinolones from the Lowest Excited States in Aqueous Media: A Study by Nanosecond and Picosecond Spectroscopic Techniques

Sandra Monti,*[a] Salvatore Sortino,[b] Elisa Fasani,[c] and Angelo Albini[c]

Abstract: Nanosecond and picosecond absorption and emission spectroscopic techniques were applied to the investigation of the reactivity from the lowest excited states of some 6-fluoro-7-piperazino-4-quinolone-3-carboxylic acids (FQs) in aqueous media at neutral pH, in the absence and presence of different sodium salts. Following the detection of various transients, we proposed a mechanism for the cleavage of the carbon−fluorine bond that proceeded through different reaction pathways, dependent on the molecular structure and the characteristics of the medium. The drug lomefloxacin (LOM), a 6,8-difluoroquinolone derivative, underwent heterolytic cleavage of the C8−F bond from the excited singlet state. With the 6-monofluoroquinolone norfloxacin (NOR) and the corresponding 1,8-naphthyridinone enoxacin (ENX), the lowest singlet state was not significantly reactive and an important deactivation channel was intersystem crossing (ISC) to the triplet manifold. The lowest triplet state underwent cleavage of the C6−F bond through a solvent mediated process possibly via a cyclohexadienyl anionic adduct. In the presence of sulfite or phosphate buffer a novel defluorination mechanism, induced by electron transfer from the inorganic anions to the FQ triplet state, was observed. The correlation between the transients observed and the final photoproducts in the different media was elucidated.

Keywords: drug research · fluorine · laser chemistry · photochemistry · quinolones

Introduction

Fluoroquinolones (FQs) are widely used antibacterial agents that develop their pharmacological activity through the inhibition of bacterial gyrase, an enzyme involved in DNA replication and repair.[1] Interest in the photochemistry of these compounds arises from their phototoxic and photo-carcinogenic activity, a serious drawback for their use in therapy (ref. [2] and references therein). From a chemical point of view, the interest is motivated by the recent evidence that the photoprocess for many of these drugs is heterolytic photodefluorination,[2, 3] an unexpected process with fluoroaromatics due to the strength of the C−F bond (dissociation energy ca. 120 kcal mol^{-1}). The sparse precedents are limited to polymethoxyfluorobenzenes,[4] 4-fluorophenol[5] and 4-fluoroaniline.[6] From a photobiological point of view the photodefluorination process is suggested to play a determining role in the photosensitization reactions with biological substrates.[7] Thus, a mechanistic investigation of the photo-induced C−F bond cleavage in fluoroquinolone derivatives is required to rationalize this unusual photoprocess as well as to substantiate hypotheses on the molecular basis of photo-toxicity.

Virtually all of the FQs currently used in therapy are 1-alkyl-6-fluoro-7-dialkylamino (most often piperazino)-4-oxoquinoline-3-carboxylic acids (see general formula FQ). A few studies have recently addressed the characterization of stable and transient species in the photodegradation of some of these derivatives[2, 3, 8–12] but the detailed photophysical and photochemical behavior of FQs is not yet known. The steady state photolysis of some such drugs (see above) has recently been investigated in neutral aqueous medium.[2, 8] In most cases defluorination was shown to be the only process that occurred and its efficiency increased in the order of decreas-

[a] Dr. S. Monti
Istituto di Fotochimica e Radiazioni d'Alta Energia CNR
Area della Ricerca, Via P. Gobetti 101
40129 Bologna (Italy)
Fax: (+39) 051639-9844
E-mail: monti@frae.bo.cnr.it

[b] Dr. S. Sortino
Dipartimento di Scienze Chimiche
Università di Catania, Viale A. Doria 8
95125 Catania (Italy)
Fax: (+39) 09558-0138
E-mail: ssortino@dipchi.unict.it

[c] Prof. E. Fasani, Prof. A. Albini
Dipartimento di Chimica Organica
Università di Pavia, Via Taramelli 10
27100 Pavia (Italy)
Fax: (+39) 0382507323
E-mail: albini@chifis.unipv.it
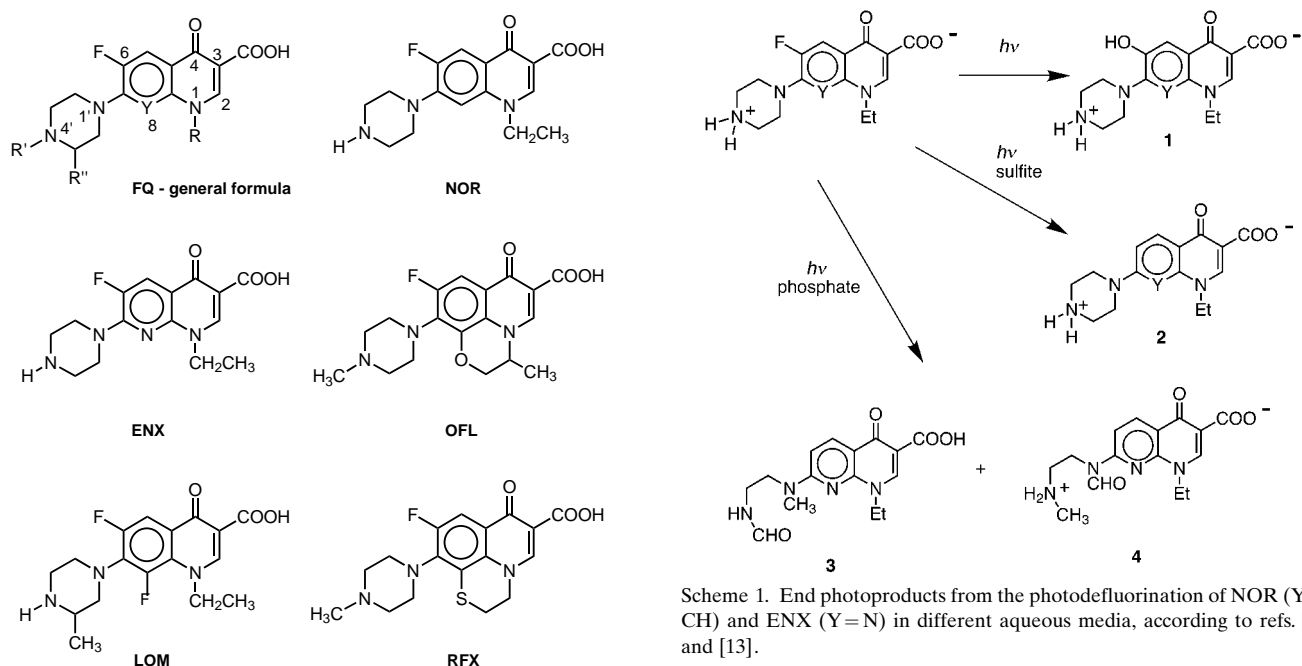
Scheme 1. End photoproducts from the photodefluorination of NOR (Y = CH) and ENX (Y = N) in different aqueous media, according to refs. [8] and [13].

ing mesomeric electron-donating effect of the group at position 8.[2] Accordingly, loss of fluoride did not occur in rufloxacin (RFX, Y = C−SR),[11, 12] and accounted for only 35 – 50 % of the photodegradation of ofloxacin (OFL, Y = C−OR).[2] On the contrary, in lomefloxacin (LOM, Y = C−F), norfloxacin (NOR, Y = C−H) and the related 8-aza naphthyridinone derivative enoxacin (ENX, Y = N) the only photoreaction was defluorination, as ascertained by the quantitative yield of drug degradation with release of fluoride.[2]

In addition to the dependence on the molecular structure, the FQ photodecomposition exhibited a dependence on the characteristics of the medium. In neutral sulfite or phosphate buffer the quantum yields were lower than in neat water.[2, 8] The difference was a major one in the case of NOR and ENX, where a correlation with the phosphate buffer concentration was observed.[2] Although in every case defluorination remained the only process that occurred, the structure of the photoproducts changed according to the medium. Irradiation of NOR and ENX in neat water (or in the presence of 5×10^{-4} M sodium hydrogen carbonate at pH 7.2) led to the corresponding phenols (Scheme 1, product **1**) through a formal nucleophilic substitution of the fluorine in position 6 with an OH group. On the contrary, in neutral sulfite buffer no phenols were formed and only reductive defluorination was observed (Scheme 1, product **2**).[8] With the 6,8-difluoroder-ivative LOM, release of fluoride selectively occurred from position 8. In water the only photoproduct resulted from cyclization onto the N-ethyl chain (Scheme 2, product **5**) while in sulfite buffer this product was accompanied by the 8-hydro derivative (Scheme 2, product **7**).[8] In neutral phosphate buffer further changes were observed. In the case of NOR the defluorinated photoproducts were not isolated, but with ENX and LOM new products formed by both reductive defluorination of the heteroaryl ring and oxidative fragmen-

tation of the piperazine moiety were obtained (Scheme 1, products **3** and **4**; Scheme 2, products **8** and **9**).[13]

As it appears from the above, the product distribution varies greatly with structure and conditions. Different de-fluorination mechanisms were proposed for the rationalization of such a medium dependent behavior. In water, formation of an aryl cation by photoheterolysis of the C−F bond was hypothesized.[2, 8] By contrast in both sulfite and phosphate buffer a reductive step was suggested to precede fragmentation.[8, 13]

Some pieces of evidence supporting part of this proposal have been previously reported.[2, 8, 13] It appeared appropriate to carry out a systematic examination of the excited state deactivation pathways of NOR, ENX, and LOM in aqueous media at neutral pH by nanosecond and picosecond time resolved spectroscopy and fluorescence measurements as well as examination of the effect of different sodium salts. It will be shown that a new insight has been gained on the role of the excited singlet and triplet states in the photodefluorination reaction in the different media. Therefore we can propose a rationale for the unusual photochemistry of these hetero-cycles and discuss the correlation between transients and final photoproducts, and we also incorporate in a unitary mecha-nism the effect of sulfite and phosphate ions.

Results

Absorption and emission: In 1×10^{-2} M phosphate buffer at pH 7.4 the absorption spectra of NOR, ENX, LOM, and OFL were characterized by an intense absorption band which peaked in the region 260 – 280 nm, a weaker band at 320 – 340 nm and a long tail extending up to 380 nm. The intensity and position of other bands present below 250 nm were more dependent on the specific molecular structure. Aqueous
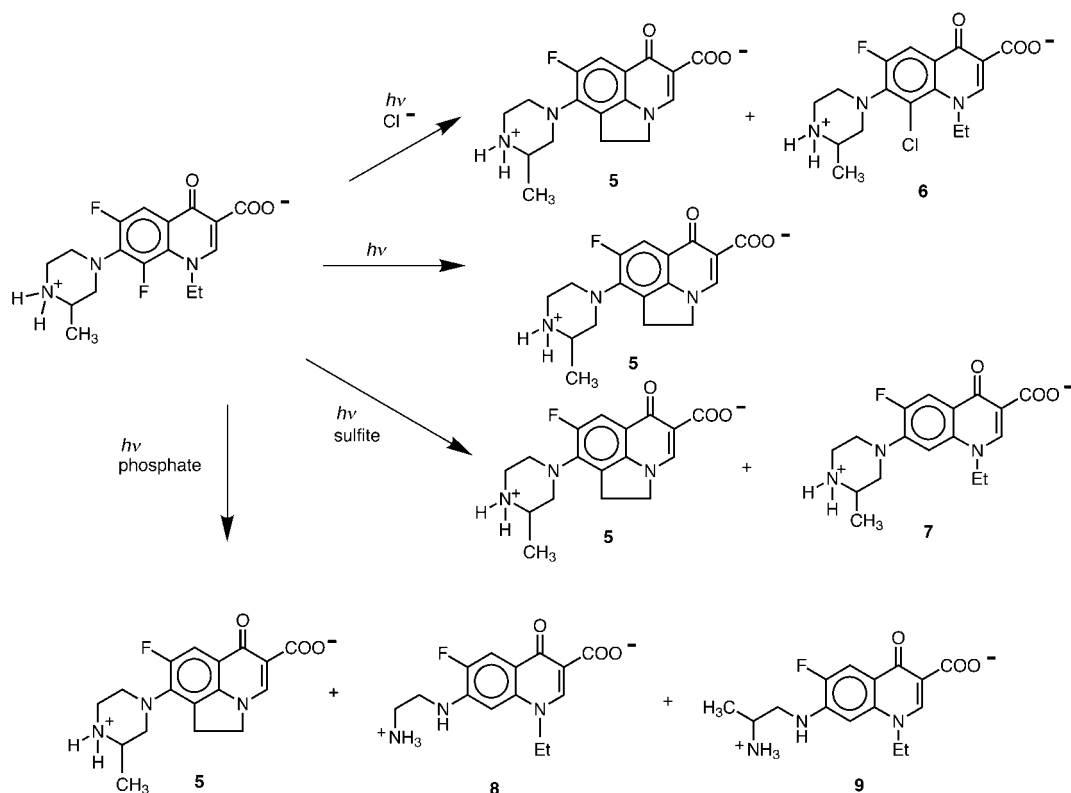
Scheme 2. End photoproducts from the photodefluorination of LOM in different aqueous media, according to refs. [8] and [13].

solutions where the pH had been adjusted by the addition of a little NaHCO₃ showed little difference. FQ molecules possess two proton binding sites, that is, the carboxy group and the piperazinyl ring, and are characterized by two main protolytic equilibria with pK_a at approximately 5.5–6.5 and approximately 8.5, respectively.[14] Thus at neutral pH the predominant structure is the zwitterion.

In air equilibrated solutions the fluorescence quantum yield was close to 0.1 for all the examined molecules, except that for ENX, where it was considerably lower (see Table 1).

Fluorescence lifetimes were extracted from the experimental time profiles taken at λ^f_{max} and are listed in Table 1. The lifetime of NOR is 1.5 ns and is in agreement with previous determinations,[2, 15] that of LOM is 1 ns and that of ENX is

considerably shorter. Data for OFL and rufloxacin (RFX)[12] are reported for comparison.

The fluorescence intensities (but not the shape of the spectra) of NOR, ENX, and LOM were considerably reduced in the presence of phosphate buffer at pH 7.4 (by 50–60% at 0.1M concentration). A lower reduction ($\leq 20\%$) was observed with all the studied derivatives at pH 7.4 with other salts, such as sodium sulfite or sodium chloride up to 0.1M, as well as sodium hydrogen carbonate up to 10^{-3}M (higher concentrations were not checked because of the significant pH change introduced). A Stern–Volmer treatment of the observed variations in the fluorescence intensity versus the total phosphate concentration afforded the quenching constants (K^f_{SV}). These were 16 and 15 M⁻¹ for NOR and ENX,

Table 1. Properties of photoexcited FQ drugs in aqueous solution at pH 7.4. Phosphate buffer 0.01M was used except when indicated.

	λ^f_{max}/nm	Φ_f	τ_f/ns	λ^{T-T}_{max}/nm	$\varepsilon^{T-T}_{max}\Phi_T$/M⁻¹cm⁻¹[d]	τ_T/µs	τ_X/µs[c]	$10^9 \times k_{O_2}$/M⁻¹s⁻¹
NOR	410	0.11	1.5[a]	620[c]	3400[c]	1.3[c]	3.6	2.7[c]
ENX	380	0.01	<0.5	520[c]	3500[e]	0.85[c]	≈2	2.4[c]
								(2.8)[g]
LOM	415	0.08	1.0[b]	500[c]	900[c]	≈0.1[c]	>0.2	
OFL	460	0.1	4.0	620	2300	1.8		1.7
RFX	470	0.07	4.8	640	2800[h]	10[f,h]		1.7
								(1.6)[h]

[a] τ_f is the same in pure water and 0.97 ns in 0.1M phosphate buffer, pH 7.4. [b] τ_f is 1.5 ns in pure water and 0.91 ns in 0.1M phosphate buffer, pH 7.4. [c] 1 × 10⁻³ M sodium hydrogen carbonate, pH 7.4. [d] values determined by comparison with $\varepsilon^{T-T}_{max}\Phi_T = 6500$ M⁻¹ cm⁻¹ for BP in acetonitrile (see Experimental Section). [e] From ref. [9]. [f] Limiting value at infinite substrate dilution. [g] Estimated on the basis of the photodegradation quantum yields in air equilibrated solutions from ref. [2], using the flash photolytically determined triplet lifetime. [h] From ref. [12]. The photoreaction is decarboxylation.

$10 \, M^{-1}$ for LOM and $< 1 \, M^{-1}$ for OFL and RFX (Figure 1). The buffer concentration had no significant influence on the emission lifetimes, except for NOR where a decrease from 1.5 ns at 0.01 M to 0.97 ns at 0.1 M phosphate was observed (see Table 1). It appears that dynamic quenching of the FQ excited
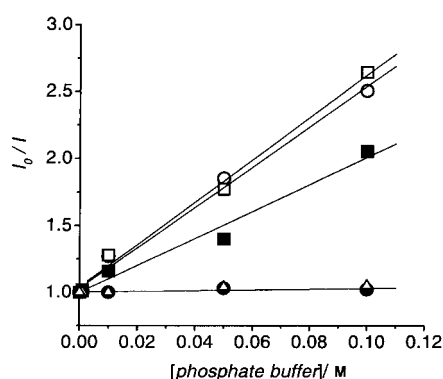

Figure 1. Stern–Volmer plots of the fluorescence intensity quenching by phosphate buffer at pH 7.4. Slope K_{SV}^f: NOR (□) $16 \, M^{-1}$, ENX (○) $15 \, M^{-1}$, LOM (■) $10 \, M^{-1}$, OFL (●) and RFX (△), $\leq 1 \, M^{-1}$.

singlet (i.e., quenching upon diffusive encounter of the excited state with the salt anion affecting the lifetime) is not the main process. The small lifetime decrease actually observed with NOR can account for only approximately 30% of the total K_{SV}^f. Thus, it is likely that a static effect (influencing the emission intensity but not the lifetime) caused by an interaction of the phosphate anions with NOR, ENX, and LOM in the ground state is involved.

Nanosecond laser flash-photolysis

Triplet spectra and quantum yields: Deoxygenated FQ solutions with a concentration of 0.4 to 1.5×10^{-4} M in aqueous media were used. In order to ensure that the zwitterion was the prevailing species, the pH was adjusted to 7.4 by the addition of 1×10^{-3} M NaHCO$_3$. Laser photolysis experiments performed in neat water showed that such a low carbonate concentration did not significantly affect the observed spectra or kinetics (see below).

The absorption changes produced by laser excitation (≤ 4 mJ pulse^{-1}) in Ar saturated solutions of NOR, ENX, and OFL were characterized by a large visible band, as shown by the differential absorptions in Figure 2, where the relative intensities are compared. Excitation of LOM led to a weaker transient absorption (Figure 2).

These transients were quenched by oxygen with rate constants k_{O_2} in the range $2 - 3 \times 10^9$ M^{-1}s^{-1}, and were assigned to the FQ triplet states on the basis of the reasonable lifetime (in the μs range, see below), effective oxygen quenching, and quenching by various additives (see below). The spectrum of ENX (λ_{max} 520 nm) was quite similar to that previously assigned to the triplet state in phosphate buffer.[9] For NOR and OFL the spectral features of the transients (λ_{max} 620 nm), their intensity and the oxygen quenching rate constants were similar to those observed with RFX (λ_{max} 640 nm)[12] and with OFL[18] and were likewise attributed to the triplet state. The
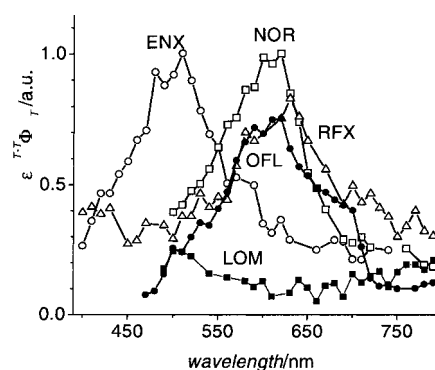

Figure 2. Triplet–triplet absorption spectra of FQs in aqueous media pH 7.4: NOR (□), ENX (○), LOM (■) in 1×10^{-3} M sodium hydrogen carbonate. OFL (●) and RFX (△, see ref. [13]) are in 1×10^{-2} M phosphate. Spectral profiles were taken 0.2 μs after the pulse for NOR, ENX, OFL, and RFX and 20 ns after the pulse for LOM.

weak absorption observed with LOM (λ_{max} ca. 500 nm) was tentatively assigned again to the population of the lowest triplet state. This was supported by the blue shift of λ_{max}, similar to that observed with ENX, the other molecule with an electron-withdrawing group in position 8.

The values obtained for the product $\varepsilon_{max}^{T-T} \Phi_T$ (Table 1) of ENX, NOR, OFL, and RFX were significantly higher than that of LOM. For the separate evaluation of the triplet quantum yields, the values of the molar absorption coefficients at λ_{max}^{T-T} are required. Previous determinations for ENX ($\varepsilon_{max}^{T-T} = 4100$ M^{-1}cm^{-1})[9] and RFX ($\varepsilon_{max}^{T-T} = 3900$ M^{-1}cm^{-1})[12] were obtained at high laser pulse energies by the singlet depletion method.[16] This may lead to an error if other phenomenon contribute at high pulse energy. For example, photoionization may occur and the solvated electron may contribute to the absorption. In order to clarify this, the transient signals at short time delay at 720 nm (λ_{max} of the solvated electron) were compared in Ar and N$_2$O saturated solutions as a function of the pulse energy. N$_2$O is known to be an efficient electron scavenger ($k_q = 9.1 \times 10^9$ M^{-1}s^{-1})[17] and in a few nanoseconds converts the solvated electrons according to Equation (1).[16]

$$N_2O + e^-_{aq} + H_2O \rightarrow N_2 + OH^\cdot + OH^- \tag{1}$$

For NOR, and ENX the end-of-pulse transient absorption at 720 nm was identical within the experimental uncertainty under both conditions for excitation energies up to 5 mJ pulse^{-1} (as in Figure 2). However, at higher laser energies the solvated electron absorption contributed significantly to the detected signal, as shown by the progressive increase of ΔA_{720} observed in Ar saturated solution up to $25 - 35$ mJ pulse^{-1}, whereas in the presence of N$_2$O, after total scavenging of the solvated electrons, the signal saturated at approximately $15 - 20$ mJ.

Thus evidence for both formation of a monophotonic transient and photoionization (apparently with a biphotonic contribution under the present conditions) was obtained at high pulse energy. Similar results were also obtained with RFX and OFL. For OFL monophotonic photoionization was recently reported by Navaratnam and Claridge.[18] These authors assigned a value of 11000 M^{-1}cm^{-1} to the molar

absorption coefficient of the triplet at 620 nm by the energy transfer method and this led to $\Phi_T = 0.33$.[18]

The $\varepsilon_{max}^{T-T} \Phi_T$ values in Table 1 were obtained at low laser energy (as described in the Experimental Section) and can be used to obtain information on Φ_T on the basis of the following additional evidence. We have previously studied RFX as an oxygen sensitizer and found that $\Phi_\Delta = 0.32$ at 75% triplet quenched,[12] and this implies that with this FQ, $\Phi_T \geq 0.4$ and $\varepsilon_{max}^{T-T} \approx 7000 \, M^{-1} cm^{-1}$ or lower. On this basis we can use the experimental $\varepsilon_{max}^{T-T} \Phi_T$ value (Table 1) for the evaluation of the lower limit for Φ_T, assuming, as it appears reasonable, that ε_{max}^{T-T} remains similar along the series. This gives $\Phi_T \geq 0.5$ for both ENX and NOR, ≥ 0.3 for OFL and $\approx 0.1-0.2$ for LOM.

Triplet lifetime: The decay of the FQ triplets was in the microsecond or submicrosecond range. In the case of NOR and ENX, the decay profile was not uniform, as apparent in the red tail of the spectra, where a slight rise followed by a slower decay occurred (see Figure 3 for the case of NOR). A
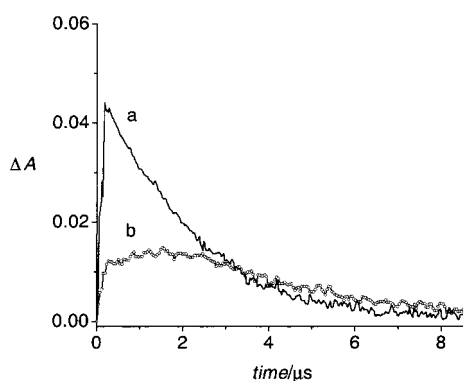

Figure 4. Absorption changes observed upon excitation of NOR $2.7 \times 10^{-4} M$ in neat water. $\lambda_{exc} = 355$ nm, ≈ 4 mJ pulse^{-1}, optical path 1 cm: (●) 0.250 μs; (○) 1.3 μs; (△) 4 μs and (■) 15 μs after pulse. Inset: time profiles at a) 520 nm and b) 720 nm.

Figure 3. Absorption time profiles observed upon excitation of NOR $2.7 \times 10^{-4} M$ at pH 7.4 in aqueous solution containing $10^{-3} M$ sodium hydrogen carbonate, a) 620 nm, b) 720 nm. $\lambda_{exc} = 355$ nm, ≈ 4 mJ pulse^{-1}, optical path 1 cm.

biexponential analysis applied fairly well. The time constants derived (reported in Table 1) were attributed to the triplet state (τ_T 1.3 μs for NOR and 0.85 μs for ENX) and to a further transient X formed from the triplet. Transient X was more red-shifted ($\lambda_{max}(X_{NOR})$ ca. 720 nm, $\tau_{X_{NOR}} = 3.6$ μs, and $\lambda_{max}(X_{ENX}) > 650$ nm, $\tau_{X_{ENX}} \approx 2$ μs). These features were somewhat dependent on the conditions. For NOR in unbuffered aqueous solution (pH ≈ 6.5) or when the pH was adjusted to 7.4 by the addition of NaOH (rather than by $10^{-3} M$ NaHCO$_3$ as in the previous case) biexponential kinetic analysis gave time constants of 1.4 μs and 4.1 μs for the triplet and X_{NOR} states, respectively (Figure 4).

The absorption for LOM in the red edge of the spectrum also decayed slower than in the blue side ($\tau_{510} \approx 100$ ns and $\tau_{750} > 200$ ns). However the unfavorable signal-to-noise ratios prevented a reliable biexponential treatment in this case.

Effect of phosphate buffer: Phosphate buffer at a concentration of $\geq 0.01 M$ significantly affected the observed transients. As is clearly observed for NOR (Figure 5), the effect was twofold. First, there was a decrease in the initial triplet
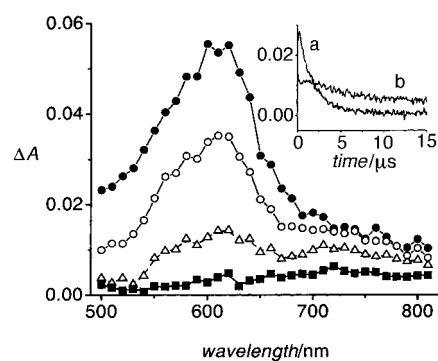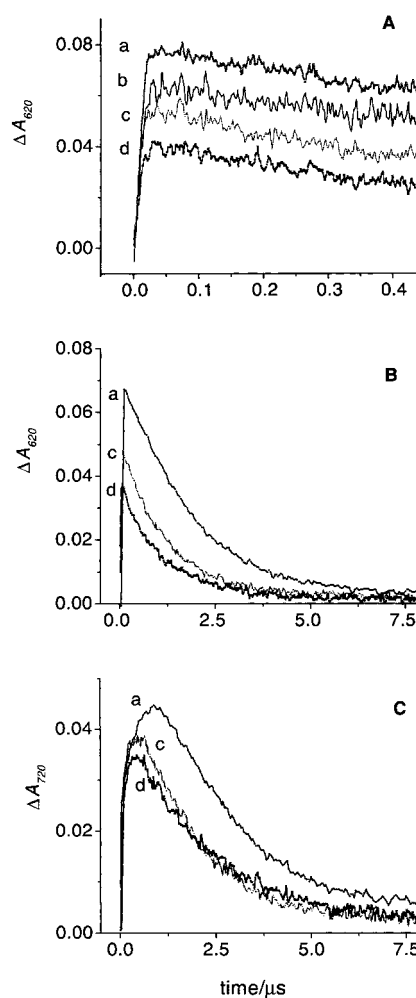

Figure 5. Dependence of the laser induced absorption changes in NOR ($A_{355} = 0.24$) on the concentration of phosphate buffer, pH 7.4: A) and B) 620 nm, C) 720 nm, a) 0.01 M, b) 0.0 M (neat H$_2$O, pH ≈ 6.5), c) 0.05 M, d) 0.1 M.

yield, as shown by the decreasing of the end-of-pulse intensity upon increasing the phosphate concentrations (Figure 5A).[19] A Stern–Volmer treatment of these quantities gave $K_{SV}^T = 13.5 \pm 3.5 \, M^{-1}$, in good agreement with the K_{SV}^f values derived from the quenching of the singlet emission intensity (see above). Second, there was a dynamic quenching and the

decrease of the 620 nm absorption band with elapsing time was accompanied by the concomitant formation of a new absorption around 700 nm, assigned to a further transient indicated as Y_{NOR} (Figure 5B and C and Figure 6). The two species appeared to have largely superimposed spectra. Thus in the whole range explored the time profiles of the
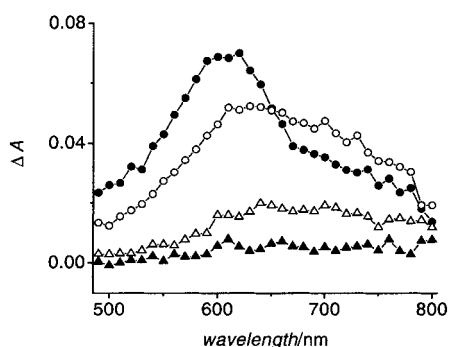

Figure 6. Absorption changes observed upon excitation of NOR 2.7×10^{-4} M in phosphate buffer 10^{-2} M. $\lambda_{exc} = 355$ nm, ≈ 4 mJ pulse^{-1}, optical path 1 cm: (●) 0.15 µs ; (○) 1 µs; (△), 4 µs ; (▲) 8 µs after pulse.

absorption changes were well described by a biexponential function. Kinetic analysis at 620 and 720 nm in 0.01 M phosphate buffer led to time constants of 0.64 µs and 2.0 µs, assigned respectively to the triplet state and to the transient Y_{NOR}. Extension of the biexponential analysis to 5×10^{-2} M and 1×10^{-1} M phosphate buffer, gave two bimolecular rate constants for the interaction with phosphate, $k_q^T = 8 \times 10^7$ M^{-1} s^{-1} and $k_q^Y = 6.5 \times 10^6$ M^{-1} s^{-1} for the triplet and for transient Y_{NOR}, respectively.

The effect was even more apparent with triplet ENX, where a quenching constant $k_q^T = 9.8 \times 10^8$ M^{-1} s^{-1} was obtained. This caused τ_T to be shortened from 0.85 µs in the absence of phosphate to 90 ns in 0.01 M phosphate buffer, at pH 7.4. An analogous transient Y_{ENX} was formed, in this case with absorption maximum at 670 nm.[9]

On the other hand, phosphate buffer up to 0.1 M had little effect on the LOM triplet lifetime ($\tau_T \approx 100$ ns), thus an upper limit of 1×10^7 M^{-1} s^{-1} was estimated for the triplet quenching constant.

Effect of sulfite buffer: *S*ulfite buffer had an effect similar to that of phosphate. The decay of triplet NOR was affected by the sulfite concentration according to a rate constant of $k_q^T = 2.6 \times 10^8$ M^{-1} s^{-1} and correspondingly a second kinetic component was observed at 700–750 nm, although there was no rising phase. The approximate spectra of the component Y_{NOR} in sulfite could be obtained by subtraction of the residual contribution of the triplet state from the transient absorption taken 200 ns after the pulse.[20] As it appears from Figure 7A, the spectrum of this transient was fairly consistent with that of the species Y_{NOR} obtained in phosphate.

In the case of ENX, quenching of the triplet by sulfite occurred with rate constant $K_q^T = 4.8 \times 10^8$ M^{-1} s^{-1} and growth of the intermediate at 670 nm was observed, as previously reported.[8] Again, a second transient absorption was detected quite similar to that observed in phosphate (Y_{ENX}, see Figure 7B).
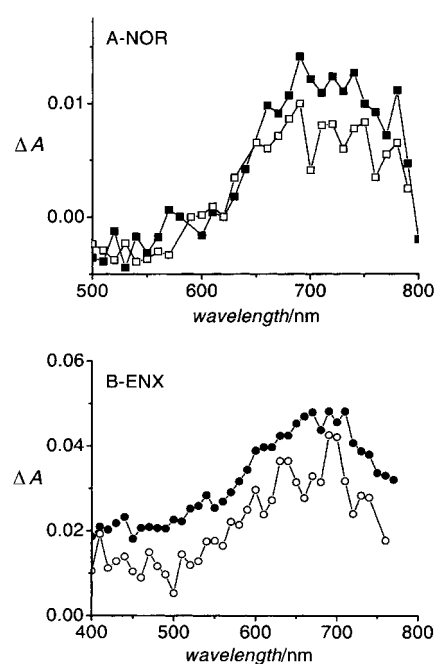

Figure 7. Absorption changes observed (B) in 6.5×10^{-5} M ENX, (●) in phosphate buffer 0.01 M, pH 7.4, 450 ns after pulse, (○) in sulfite buffer 0.01 M, pH 7.1, 430 ns after pulse. Laser energy ca. 2 mJ pulse^{-1}; A) 200 ns after pulse, after subtraction of the triplet contribution (see text), (■) in NOR 2.7×10^{-4} M in 0.01 M phosphate buffer pH 7.4; (□) in NOR 3.5×10^{-4} M in 0.005 M sulfite buffer, pH 7.1. Laser energy ca. 4 mJ pulse^{-1}.

The decay of the transients Y was sensitive to the buffer concentration. In Table 2 the rate constants for the quenching of the FQ triplets and of the transients Y, determined by biexponential analysis in the presence of phosphate and sulfite, are summarized.

Picosecond laser flash photolysis

Laser induced absorption with picosecond resolution was performed to compare the excited singlet behavior of LOM and ENX. These molecules, both with well characterized final photoproducts, were chosen because of their opposite properties, that is, the low Φ_T (see above) and suspected singlet photoreactivity[2] of LOM and higher Φ_T and predominant triplet photoreactivity of ENX.[9]

Aerated solutions of LOM 1.2×10^{-4} M in neat water were excited at 355 nm by laser pulses of 35 ps. Figure 8 shows the spectral changes observed 55 ps and 1.7 ns after time zero (see Experimental Section). A broad, symmetric, positive band peaking at 700 nm and a negative signal at 470 nm were detected. Since ground state LOM has no absorption in the 450–500 nm region, the negative signal could not be attributed to ground state depletion. In the inset the signal decay at 700 nm and recovery at 470 nm are represented. The profiles are exponential and the time constant, ≈ 1 ns at both wavelengths, is equal to the fluorescence lifetime. The negative signal at 470 nm was therefore attributed to fluorescence, reaching the detector along with transmitted light, and the 700 nm band was assigned to $S_1 – S_n$ absorption. In view of the discussion on the photochemical paths (see below), it is

Table 2. Reduction potential of ground state (E_{red}/V) and triplet energy (E^T/eV) of FQs; quenching rate constants (k_q/M^{-1}s^{-1}) of FQ intermediates, triplet and Y, by buffer salts.

	E_{red}/V vs Ag/AgCl (E^T/eV)	k_q^T/M^{-1}s^{-1} triplet state		k_q^Y/M^{-1}s^{-1} Transient Y	
		HPO$_4^{2-}$/H$_2$PO$_4^-$	SO$_3^{2-}$/HSO$_3^-$ ref. [8]	HPO$_4^{2-}$/H$_2$PO$_4^-$	SO$_3^{2-}$/HSO$_3^-$ ref. [8]
NOR	ca. −1.39 (2.9)	8.0×10^7 (1.3×10^8)[a]	2.6×10^8	6.5×10^6	7.5×10^7
ENX	−1.31 (2.8)	9.8×10^8 (2×10^8)[a]	4.8×10^8	$< 10^6$	1.2×10^8
LOM	−1.34	$< 10^7$	$> 10^8$		
OFL	ca. −1.4		1.0×10^8		

[a] Derived from the Stern–Volmer treatment of the photodegradation quantum yields,[2] using the steady state K_{SV} values from the photoreaction ($K_{SV} = 194$ M^{-1} for NOR and 179 M^{-1} for ENX)[2, 13] and the triplet lifetimes from flash photolysis.
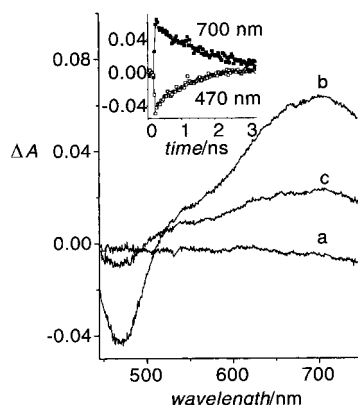

Figure 8. Absorption changes observed upon excitation of LOM 1.2×10^{-4} M in neat water. $\lambda_{exc} = 355$ nm, 35 ps, ≈ 4 mJ pulse^{-1}, optical path 1 cm: a) zero time (see Experimental Section); b) 55 ps; c) 1.7 ns. Inset: time profiles at 700 and 470 nm.

important to notice that addition of NaCl 0.1M did not produce any spectral or kinetic modification.

Laser excitation of ENX solutions, 1.4×10^{-4} M in water, induced absorption changes in the region 450–750 nm at 100 ps delay. After 200 ps a band with a maximum at ≈ 520 nm was recognized (Figure 9). In the explored delay time interval (up to 2.7 ns) the difference spectrum did not change

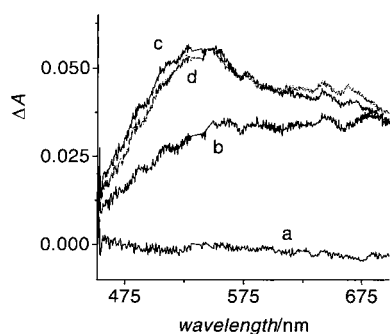Figure 9. Absorption changes observed upon excitation of ENX 1.4×10^{-4} M in neat water. $\lambda_{exc} = 355$ nm, 35 ps, ≈ 4 mJ pulse^{-1}, optical path 1 cm: a) zero time (see Experimental Section), b) 100 ps, c) 200 ps, d) 2.7 ns.

significantly. The 520 nm maximum was assigned to the $T_1 - T_n$ absorption on the basis of the triplet spectrum obtained by nanosecond laser flash photolysis. Comparison with the spectrum reported in Figure 2 indicates some discrepancy above 600 nm.
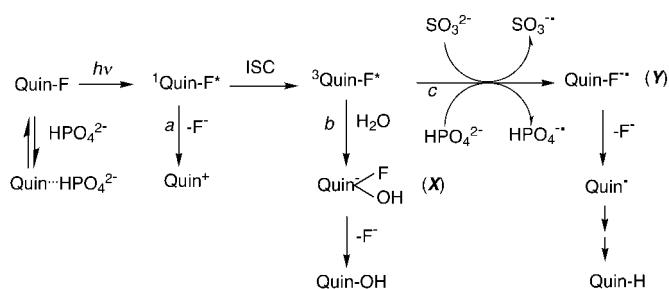
A contribution from a transient other than the triplet as well as the occurrence of some distortion of the triplet absorption profile are both possible and the very different conditions used in the picosecond experiment preclude unambiguous assignment.

Discussion

The above results can be used to examine the mechanism of the photoreaction of fluoroquinolones in the light of the steady state studies previously published. In order to facilitate the discussion, the photoproducts in the case of NOR, ENX, and LOM are indicated in Scheme 1 and 2 and the photodegradation quantum yields in the same media are reported in Table 3. OFL is much less reactive (Φ_{-FQ} ca. 0.001). Defluorination is the only significant reaction for the above molecules (see the structure and the yield of the photoproducts and the high percentages of fluoride released in Table 3). The photochemistry of FQs is discussed below in terms of reactions from the singlet and the triplet state, and the latter case involves further intermediates X and Y under some conditions (see Scheme 3).

Table 3. Photodegradation of FQ in neutral aqueous media.

	H$_2$O Ref. [2]	0.02 M sulfite Ref. [8]	0.1 M phosphate Ref. [2]	%F$^-$	Products (approx. yield, %)
LOM	0.55 Ar			100	5 (70)
	0.49 air			100	
		0.28		100	5 (45), 7 (15)
			0.4 Ar	95	5 (25), 8+9 (20)
			0.3 air	80	
ENX	0.13 Ar			99	1 (75)
	0.08 air			100	
		0.027		100	2 (55)
			0.01 Ar	100	3 + 4 (60)
			0.02 air	73	
NOR	0.06 Ar			100	1 (50)
	0.01 air			98	
		0.004		100	2 (40)
			0.0042 Ar	89	
			0.007 air	49	
OFL	0.0012 Ar			80	
	0.0016 air			87	
		< 0.0001		< 5	
			0.0015 Ar	35	
			0.0025 air	50	

Scheme 3. Proposed mechanisms for excited state reactivity of the examined fluoroquinolones.

Singlet state reaction: The high photodegradation quantum yield of LOM in water ($\Phi_{-FQ} > 0.5$), not significantly affected by oxygen and compared with a $\Phi_T < 0.2$, requires that the reaction mainly proceeds from the excited singlet state. As mentioned above, the process which occurs is defluorination. This would be expected to generate an aryl cation (Scheme 3, path a). However, no transient was revealed in the picosecond experiments except the singlet (see Figure 8), and there is no direct evidence for such a cation. The chemistry observed is carbene-like, with intramolecular insertion into the C—H bond of the neighboring N-ethyl group.[21a,b] This fits with the suggested cationic intermediate, since the parent phenyl cation has been shown to attack an alkyl chain at the *ortho* position.[21c] Further support for the cationic nature of the intermediate is given by the observed substitution of a chloro for a fluoro atom in chloride containing solutions. Thus, although intramolecular insertion is faster than water addition, a charged nucleophile is incorporated. The lifetime of the cation is estimated to be of the order of 10 ns, since trapping by Cl⁻ at low concentrations (1×10^{-2} M) is effective,[21b] and thus the failure in detection is due to either an unfavorable wavelength window or to low absorption coefficients rather than to the short lifetime.[22] It should also be pointed out that independently of the formation mechanism of this intermediate, the chemistry observed is reminiscent of a triplet and not a singlet phenylium cation. The triplet cation has a $\pi^5\sigma^1$, not a $\pi^6\sigma^0$ structure and a chemoselective reactivity.[23]

The reactivity of the singlet state appears to be related to the intramolecular charge transfer character of this state (from the amino group in 7 to the quinolone moiety). This character is indicated by the large Stokes shift of the fluorescence (ca. 6000 cm⁻¹).[23] In the case of LOM, this character is increased by the presence of a second electron with-drawing fluorine, and this makes heterolytic cleavage of the C—F bond fast enough to compete with ISC and emission. This reaction is typical of quinolones with a fluoro atom in position 8, since exactly the same pattern of reaction (selective loss of the fluoro in 8 followed by intramolecular attack onto the N-alkyl chain in water or, in the presence of chloride, formation of the 8-chloro derivative) has been observed in the case of fleroxacin (a 7-piperazinyl-5,6,8-trifluoroquinolone).[10, 21d] As previously reported,[2] regioselectivity in the heterolysis is in accord with a mesomeric formula with the charge on the 7-amino group and the pyridone aromatic ring, possible for the cation in position 8, but not for that in position 6.

The fast heterolytic cleavage from the excited singlet state in the case of LOM explains the relative insensitivity to the

presence of oxygen and to the environment, characteristic of the photoreaction of this molecule (compare with the much larger effects in the case of NOR and ENX, see Table 3),[2] although a minor contribution of the triplet state to the photoreaction is likely (see below).

Triplet state reaction: In the case of NOR and ENX oxygen strongly reduces the photodegradation quantum yield (see Table 3), and indicates a long-lived excited state as the main precursor. Sensitization by benzophenone was observed with ENX.[9] Indeed, a large triplet absorption is revealed by nanosecond flash photolysis with the above molecules as well as with the almost unreactive OFL. ISC is thus an efficient deactivation process from the excited singlet state for all of the considered FQs, except for the case of LOM where direct reaction predominates. Formation of the triplet can be followed by picosecond spectroscopy (see Figure 9). Effective oxygen quenching (rate constants in the range $2-3 \times 10^9$ M⁻¹s⁻¹) is consistent with the effect of air equilibration on the photodegradation quantum yields (see Table 1).

Thus, the reaction of NOR and ENX predominantly involves the triplet state. Both these molecules give the corresponding phenol as the only photoproduct in neutral aqueous solution, through a formal nucleophilic substitution of the fluorine in position 6 with an OH group (see Scheme 1). Two mechanisms can be envisaged: i) heterolytic cleavage of the triplet state to give an aryl cation, analogously to the case of the singlet seen above, and ii) reaction of the excited state with water forming a σ bond in a cyclohexadienyl (Meisen-heimer) anion, which subsequently releases fluoride (S_N2Ar^* mechanism). Mechanism ii) was shown to apply in several aromatic photosubstitutions via the triplet states,[27] and an increase in quantum yields of defluorination with increasing pH was observed. The strong pH dependence of reaction quantum yield in FQs, due to the above mentioned ionic equilibria of these zwitterions, precludes the possibility of a similar investigation in this case. However, the lack of any chloro-substituted derivative formation when NOR and ENX are photolysed in the presence of sodium chloride (up to 0.1 M)[8] suggests that no aryl cation is present in this case, contrary to the case of LOM and fleroxacin.

This was confirmed by flash photolysis which showed that with these two drugs the decay of the triplet is coupled with the formation of a further transient **X** absorbing around 700 nm (Figure 3). This transient is too long-lived to be identified as the aryl cation (see also above), but could be the π anion formed by addition of solvent (OH⁻ or H_2O) to the triplet state according to mechanism ii), see Scheme 3, path b. The exact characterization of **X** is difficult, but the visible absorption of the transient is in agreement with the spectral properties of such anions.[25] Furthermore, the more efficient defluorination of ENX (activated by aza substitution in 8) in comparison with NOR and the inefficient defluorination in the electron-donating substituted OFL and RFX support a nucleophilic attack in the reaction.

Effect of inorganic anions: A remarkable result of our previous steady-state studies was the complex effect of inorganic ions, that leads to large changes in both the

degradation quantum yield and product distribution.[2, 8, 13] A general salt effect with a moderate acceleration of the photoreactions has been documented,[2] but the most important effects evidenced in those works were specific and their rationalization was not straightforward since various steps of the photoprocess appeared to have been affected.

First, phosphate has a significant effect on the fluorescence quantum yield of NOR, ENX, and LOM, but not on OFL and RFX (Figure 1 and Table 1). The nature of the phenomenon is mainly static and can be rationalized by an electrostatic association ($K_a \approx 10\text{--}15\,\text{M}^{-1}$) of the phosphate dianion with the positively charged $>N(4')H_2^+$ moiety of these zwitterionic molecules with two hydrogen bonds (Scheme 4). Accordingly,

$$FQ\ +\ HPO_4^{2-}$$
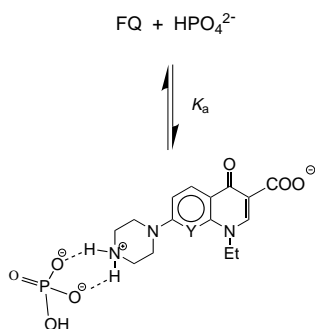
Scheme 4. Hypothesized structure of ground state associates of fluoroquinolones with phosphate dianions (Y = CH, N, CF).

the effect is small with OFL and RFX,[9] since in these cases the N(4') nitrogen is methylated and not capable of such strong interaction. Ground state association with phosphate precludes fluorescence, either by increasing the internal charge transfer interaction in the singlet excited state and channeling the energy into a radiationless path or by promoting the deprotonation of the nitrogen atom in the excited state through the action of the closely located phosphate dianion, which acts as a proton acceptor. A lower basicity of the piperazinyl N(4') in the excited state is indeed expected on the basis of the pH dependence of the FQ absorption spectra.[9, 18] The specific electrostatic effect of the phosphate anion, contrasting with the much smaller effects displayed by the sulfite anion, appears to be related to a more favorable geometry of the complex formed in the first case.

Anions have no significant effect on the singlet state chemistry, apart for the observed chloride trapping of the singlet derived carbocation in the case of LOM. However, sulfite and phosphate have a major effect both on the triplet-derived products and on the triplet evolution, as evidenced by product studies and flash photolysis, respectively.

In the case of sulfite, the quenching mechanism operative clearly involves electron transfer from the sulfite anions to the triplet state (path c in Scheme 3). This is supported by i) the marked exothermicity of the process (redox potentials $E(SO_3^{-\cdot}/SO_3^{2-}) = 0.63\,V$ and $E(SO_3^{-\cdot}/HSO_3^-) = 0.84\,V$ vs NHE,[26] E_{red} values for FQ in Table 2), justifying the observed high values of k_q^T ($>1 \times 10^8\,\text{M}^{-1}\text{s}^{-1}$), ii) the fact that the photoreaction under these conditions is reductive defluorination and iii) the analogy with the cathodic process, which leads to the same defluorinated products from ENX, NOR, and

LOM (again selectively from position 8 in the last case).[27a] From NOR and ENX quenching of the triplet leads to transients (**Y**) absorbing in the red (ca. 720 nm and 670 nm, respectively) which, on the basis of the above considerations, are identified as the corresponding radical anions. The absorbing properties of these species are in reasonable agreement with those of the radical anions of the related compounds flumequine[28] and OFL,[18] both extended up to 650 nm. The few literature precedents available suggest that cleavage of the strong C−F bond is not an efficient process in aromatic radical anions,[27b,c] and consistently the measured quantum yields are low (a few percent or lower, see Table 3). The overall process which leads to the final even-electrons products must involve further intermediates. In fact, in the case of ENX, flash photolysis[8] evidenced at least one further long-lived transient (λ_{max} 650 nm) formed upon decay of transient $\boldsymbol{Y}_{ENX}$.

In the case of LOM, the intersystem crossing quantum yield is low, and, although sulfite quenching of the triplet state was evidenced,[8] the resulting transient was too weak for unambiguous characterization. With LOM the quantum yield of decomposition is only marginally affected by the presence of sulfite and the main path of the photoreaction remains the same as in neat water, with reductive defluorination only as a minor path.

The effect of phosphate is in many respects similar to that of sulfite. Indeed, the same transients **Y** are observed from both NOR and ENX (Figure 7, Scheme 3) and, again, the reaction quantum yield is strongly diminished and reductive defluorination occurs though accompanied in this case by degradation of the piperazine side-chain. This supports electron transfer to the FQ triplet from phosphate (probably from the more reducing dianion HPO_4^{2-}, see below). Phosphate is certainly a poorer reducing agent than sulfite. However, there are indications that it could act as a reducing agent with excited states.[27d]

In the case of FQs, the reduction potential is about $-1.3/-1.35\,V$ vs Ag/AgCl[30a] and the triplet can be located close to $2.7\text{--}2.9\,eV$ on the basis of the onset of the low temperature phosphorescence spectra.[9, 15] The potential of the couple $HPO_4^{-\cdot}/HPO_4^{2-}$ is estimated to be significantly lower than $1.9\,V$ vs NHE.[29] These data support that reduction of triplet FQs by phosphate is close to thermoneutral. Quenching of the triplet is accompanied by quenching of the photodecomposition, and the flash photolysis determined rate constants (k_q^T) can be compared with the values (reported in parentheses in Table 2) derived from the Stern–Volmer treatment of the decomposition quantum yields versus the phosphate buffer concentration ($K_{SV} = k_q \times \tau$), by assuming the FQ triplet as the reacting state.

Given the different approach involved, the two values are acceptably close for NOR ($K_{SV} = 194\,\text{M}^{-1}$ vs $112\,\text{M}^{-1}$), while in the case of ENX the steady state derived value is significantly lower than expected from the triplet quenching ($K_{SV} = 179\,\text{M}^{-1}$ vs $760\,\text{M}^{-1}$). In other words, the overall photochemical degradation is less diminished when the concentration of the phosphate buffer increases than would be expected from the shortening of the triplet lifetime. Indeed, although the photoreaction in neat water is depressed by phosphate, quenching is accompanied by induction of a different

reaction, which is one order of magnitude more efficient with ENX than with NOR.[27]

A further mechanistic complication may be due to the role of the singlet excited state in the chemical reaction at high phosphate buffer concentrations. A significant fraction of molecules associated to the phosphate anions may on one hand lower the reaction quantum yield by lowering the efficiency of triplet formation (as it is the case with NOR), but on the other side introduce a new contribution to the reductive decomposition. In the latter case the slope of the (Φ^0/Φ versus [phosphate] plot would be decreased, as it appears to be the case with the more easily reducible ENX.[13] In the case of LOM, the low effect of phosphate on the photoreaction quantum yield even at a high concentration (>0.1M) is consistent with a singlet photoreactivity of prevailing unimolecular nature. This is in accord with the fact that reductive ring defluorination and side-chain oxidation are only minor processes with this molecule, for which the C−F bond heterolysis from the singlet remains the main path also in the presence of the above anions.[13]

As for the final photoproducts in the presence of phosphate, we have suggested elsewhere[13] that the oxidized phosphate radical abstracts hydrogen from the piperazine side chain initiating the degradation of this moiety and that fluoride loss and hydrogen abstraction both occur in cage before separation and diffusion of the radical ions. In this respect, the observed FQ anion (Figure 7) would represent the escaped fraction. If an absorption coefficient at λ_{max} of 2500 $M^{-1}cm^{-1}$ applies (as that of the OFL anion[18]), the quantum yield of this species, estimated from the intensity of the absorption at 670 and 700 nm with ENX and NOR, respectively, is of the same order of magnitude as that of the triplet (≥ 0.5). Thus the low values of Φ_{-FQ} ($\approx 10^{-2}$, Table 3) in these derivatives are attributed to back electron transfer after reduction to the radical anions.

There are some further complications. The decay of the FQ radical anion (transient **Y**) is faster at higher sulfite and phosphate concentrations, and this suggests that bimolecular quenching of the radical anion by the inorganic anions plays some role (Table 2). This is reasonable in the case of sulfite, where further reduction of $FQ^{-\cdot}$ to the dianion FQ^{2-} seems viable,[8] but not in the case of phosphate. Care should be exerted in the rationalization, since the observed effect might be because of a nonappropriate exponential description of $FQ^{-\cdot}$ decay at different phosphate concentrations.[34]

Electron transfer is probably a general phenomenon with triplet FQs, since a favorable thermodynamic balance applies to other anions, for example, to sodium hydrogen carbonate (potential of the couple $CO_3^{-\cdot}/CO_3^{2-} = 1.5$ V vs NHE).[32] However, we could not investigate this point since sodium hydrogen carbonate could be used only at a low concentration ($<10^{-3}$M, where the FQ triplet lifetimes are practically the same as in water), without affecting the pH of the solution.

Conclusion

The present investigation reveals that *three* mechanisms are involved in the photodegradation of FQs and correlates the observed behavior with the molecular structure and the medium characteristics. i) Significant heterolysis appears to occur in the singlet state and for a precise structure, that is, when electron-withdrawing substituents increase the internal charge-transfer character of the excited state and a strongly stabilized cation (in position 8) is formed. In this case, the reaction is quite efficient ($\Phi_{-FQ} = 0.55$ for LOM), while in the other case (F in position 6 only) the cleavage of the strong C−F bond does not compete with ISC. ii) A reaction also occurs in the triplet state, but in this case an addition–elimination mechanism via a cyclohexadienyl anion is involved. The importance of this process is proportional to the electrophilicity of the molecule (ENX > NOR ≫ OFL;RFX) and leads to Φ_{-FQ} varying from 0.13 to ≤ 0.001 in the series. iii) With (weakly) electron-donating anions a third type of photochemisty is observed that, besides association in the ground state, involves electron transfer to the triplet state and formation of the drug radical anion and of an inorganic radical. This is the least efficient of the three processes (Φ_{-FQ} in the order of 0.01), but formation of odd-electron species by interaction with inorganic anions is noteworthy. Such photoinduced electron transfer with inorganic ions may be a general phenomenon, though it has little precedent. This and other aspects of this work suggests that the photochemistry of organic molecules in aqueous and in saline solution may reveal new features and warrants further studies.

Finally, the debate of the origin of the phototoxic effects of drugs is open. Most often, oxygen sensitization is assumed to be the main mechanism, while the role of photoreactions of drug molecules has been scarcely documented as yet. The present investigation shows that fluoroquinolones react with largely variable quantum yield (0.001 to 0.55) by different mechanisms. Hopefully, the present results may contribute to the rationalization of the phototoxic effects of these drugs and be useful in further photobiological studies, for which these drugs are particularly suited substrates in view of their absorption in the UV-A.

Experimental Section

Materials: Norfloxacin and enoxacin were purchased from Sigma Chemicals (Milan) and used as received. Lomefloxacin hydrochloride was obtained from the same supplier and the free base was prepared according to the procedure described.[2] Water was purified by passage through a Millipore MilliQ system. $Na_2HPO_4 \cdot 2H_2O$, $NaH_2PO_4 \cdot H_2O$, Na_2SO_3, $Na_2S_2O_5$ and $NaHCO_3$ for the preparation of buffers at pH 7–7.4 were pro analysis products from Merck. The pH of the solutions was measured through a glass electrode.

Spectroscopic measurements: UV/Vis absorption spectra were taken by means of a Perkin–Elmer Lambda 5 spectrophotometer. Emission spectra were obtained by means of a Spex Fluorolog-2 F-111 spectrofluorimeter. The fluorescence quantum yields were obtained using quinine sulfate in H_2SO_4 (1N) as standard. The absorbance of the samples at $\lambda_{exc} = 340$ nm was ≤ 0.1 for a 1 cm pathlength.

Time resolved emission: The fluorescence lifetime in aerated solutions 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. Fluorescence decays, well described by a monoexponential function, were extracted from the observed emission signals by deconvolution of the instrumental response function. The software package was provided by IBH Consultants LTD.

Nanosecond laser flash photolysis: The setup for the nanosecond absorption measurements was described previously.[35] The minimum response time of the detection system was approximately 2 ns. The laser beam (Nd/YAG at $\lambda = 355$ nm, either a JK-Lasers system, pulse width 20 ns FWHM, or a Continuum Surelite II-10 system, pulse width 7 ns FWHM) was focused on a 3 mm high and 10 mm wide rectangular area of the cell and the first 2 mm were analyzed at a right angle geometry. The incident pulse energies used were ≤ 4 mJ pulse^{-1}. The bandwidth used in the spectrokinetic measurements was 2 nm (1 mm slit width). The spectra were reconstructed point by point from time profiles taken each 10 nm. The sample absorbance was 0.2–0.5 at 355 nm over 1 cm. Oxygen was removed by vigorously bubbling the solutions with a constant flux of argon, previously passed through a water trap to prevent evaporation of the sample. The same procedure was used to prepare either oxygen or N_2O saturated solutions. The solution (in a flow cell of 1 cm pathlength) was renewed after each laser shot. The temperature was 295 ± 2 K. The detector system was perturbed at $\lambda < 475$ nm by the intense emission of FQ, generated by the laser excitation. For this reason transient spectra in this wavelength region were not significant at short delays.

The product of the triplet absorption coefficient times the quantum yield of triplet formation, $\varepsilon_{max}^{T-T} \Phi_T$, was obtained by comparison of the absorbance of triplet FQ at λ_{max} of the T–T spectra with that found with an optically matched solution of benzophenone (BP) in acetonitrile, for which a unitary Φ_T and an absorption coefficient at 520 nm of 6500 M^{-1} cm^{-1} were assumed.[36] The FQ and BP ground state concentrations were chosen as to give $A_{355} = 0.02$–0.1 across the 2 mm width of the analysis beam. The slope of linear plots at different incident pulse energies below 2 mJ pulse^{-1} were compared. The uncertainty is $\pm 10\%$.

Acquisition and processing of absorption signals were performed by a home made program using Asyst 3.1 (Software Technologies, Inc.). Nonlinear fitting procedures by the least square method and χ^2 and distribution of residuals were used to judge the goodness of the fit.

Picosecond laser flash photolysis: Transient absorption spectra in the subnanosecond time region were obtained by a pump–probe technique, already described in detail.[37] Pump pulses of 35 ps (FWHM) at 355 nm were generated by an actively-passively mode-locked Nd-YAG laser source (Continuum PY62–10). The probe was a 35 ps pulse with a continuum spectral distribution from 400 nm to the near infrared, split in two beams to interrogate both excited and unexcited portions of the sample. The pump and probe beams were positioned to pass colinearly through the sample and to overlap over 1 cm pathlength. Sample and reference beams emerging from the cell were focused into two optical fibers coupled to a spectrograph (Spectrapro 275, Acton Research Corp., 0.275 cm focal length) and to an optical multichannel analyzer, with a double diode array detector placed on the focal plane (Princeton Instruments). The spectral resolution was 0.58 nm. An integrated software package (P-OSMA), provided by Spectroscopy Instruments GmbH, controlled the experiment. Time resolution was obtained by variation of the pathlength of the excitation beam by means of a mechanical, computer-controlled, delay line on which dichroic mirrors were mounted. In this way, the time interval between excitation and probing was adjusted. The instrumental response function was checked by detecting the S_1–S_n absorption of Rhodamine 6G in methanol.[37] The duration of the laser pulse limited the time resolution of the system. Consecutive absorption spectra were taken at several time delays and allowed the reconstruction of the decay profiles. The maximum delay achieved was ca. 3 ns. Zero time was assumed to be that corresponding to the onset of the build-up of the fluorescence signal of LOM at 470 nm, contemporarily detected. Aerated solutions (typically, 1000 mL by volume, 0.8–1.5 absorbance over 1 cm at 355 nm) were circulated in a flow quartz cell by a peristaltic pump to minimize the photodegradation. The laser, run at 10 Hz, delivered typically 2–4 mJ pulse^{-1}.

Acknowledgement

[1] J. M. Domagdala, L. D. Hann, C. L. Heifetz, M. P. Hutt, T. F. Mich, J. P. Sanchez, M. Solomon, *J. Med. Chem.* **1986**, *29*, 394–404.

[2] E. Fasani, A. Profumo, A. Albini, *Photochem. Photobiol.* **1998**, *68*, 666–674.

[3] L. J. Martinez, G. Li, C. F. Chignell, *Photochem. Photobiol.* **1997**, *65*, 599–602.

[4] G. Zhang, P. Wan, *J. Chem. Soc. Chem. Commun.* **1994**, 19–20.

[5] A. P. Durand, R. G. Brown, D. Worral, F. Wilkinson, *J. Chem. Soc. Perkin Trans. 2* **1998**, 365–370.

[6] M. Fagnoni, M. Mella, A. Albini, *Org. Lett.* **1999**, *1*, 1299–1301.

[7] a) S. Sortino, G. Condorelli, G. De Guidi, S. Giuffrida, *Photochem. Photobiol.* **1998**, *68*, 652–659; b) L. J. Martinez, R. H. Sik, C. F Chignell, *Photochem. Photobiol.* **1998**, *67*, 399–403.

[8] E. Fasani, F. F. Barberis Negra, M. Mella, S. Monti, A. Albini, *J. Org. Chem.* **1999**, *64*, 5388–5395.

[9] S. Sortino, G. De Guidi, S. Giuffrida, S. Monti, A. Velardita, *Photochem. Photobiol.* **1998**, *67*, 167–173.

[10] T. Morimura, Y. Nobuhara, H. Matsukura, *Chem. Pharm. Bull.* **1997**, *45*, 373–377.

[11] G. Condorelli, G. De Guidi, S. Giuffrida, S. Sortino, R. Chillemi, S. Sciuto, *Photochem. Photobiol.* **1999**, *70*, 280–286.

[12] S. Sortino, G. Marconi, S. Giuffrida, G. De Guidi, S. Monti, *Photochem. Photobiol.* **1999**, *70*, 731–736.

[13] E. Fasani, M. Mella, S. Monti, A. Albini, *Eur. J. Org. Chem.* **2001**, 391–397.

[14] K. Takacs-Novak, B. Noszal, I. Hermecz, B. Kereszturi, B. Podanyi, G. Szasz, *J. Pharm. Sci.* **1990**, *29*, 1023–1028.

[15] P. Bilski, L. J. Martinez, E. B. Koker, C. F. Chignell, *Photochem. Photobiol.* **1996**, *64*, 496–500.

[16] R. V. Bensasson, E. J. Land, T. G. Truscott, *Flash photolysis and Pulse Radiolysis*, Pergamon Press, Oxford, **1983**, Chapter 1.

[17] G. V. Buxton, C. L. Greenstock, W. P. Helman, A. B. Ross, *J. Phys. Chem. Ref. Data* **1988**, *17*, 513–886.

[18] S. Navaratnam, J. Claridge, *Photochem. Photobiol.* **2000**, *72*, 283–290.

[19] In neat water the signal of the triplet was approximately 20% lower than in 0.01M phosphate buffer at pH 7.4, and this is likely to be due to the lower zwitterion concentration.

[20] This was performed by assuming that the triplet spectral profile was that registered in the presence of sodium hydrogen carbonate 1×10^{-3} M and, for the sake of simplicity, that only the triplet absorbed at 620 nm in both buffers.

[21] a) E. Fasani, M. Mella, D. Caccia, S. Tassi, M. Fagnoni, A. Albini, *Chem. Commun.* **1997**, 1329–1330; b) E. Fasani, M. Rampi, A. Albini, *J. Chem. Soc. Perkin Trans. 2* **1999**, 1901–1907; c) K. Hori, T. Sonoda, M. Harada, S. Yamazaki-Nishida, *Tetrahedron* **2000**, *56*, 1429–1436; d) T. Morimura, K. Kohno, Y. Nobuhara, H. Matsuhura, *Chem. Pharm. Bull.* **1997**, *45*, 1828–1832.

[22] Phenylium cations do not absorb in the wavelength range explored, see a) S. Steenken, M. Askokkuna, P. Maruthamuthu, R. A. McClelland, *J. Am. Chem. Soc.* **1998**, *120*, 11925–11930; b) L. S. Romsted, J. Zhang, L. Zhang, *J. Am. Chem. Soc.* **1998**, *120*, 10046–10051.

[23] a) S. Gasper, C. Devadoss, G. Schuster, *J. Am. Chem. Soc.* **1995**, *117*, 5206–5211; b) H.-R. Park, H.-C. Lee, T. H. Kim, J.-K. Lee, K. Yang, K.-M. Bark, *Photochem. Photobiol.* **2000**, *71*, 281–293; c) B. Guizzardi, M. Mella, M. Fagnoni, A. Albini, *Tetrahedron* **2000**, *56*, 9383–9390.

[24] a) J. Cornelisse, in *CRC Handbook of Organic Photochemistry and Photobiology* (Eds.: W. H. Horspool, P. S. Song), CRC Press, Boca Raton, **1995**, pp. 250–265; b) A. M. J. Van Eijk, A. M. Huizer, C. A. G. O. Varma, J. Marquet, *J. Am. Chem. Soc.* **1988**, *111*, 88–95.

[25] a) C. F. Bernasconi, *J. Org. Chem.* **1970**, *35*, 1214–1216; b) K. Yokoyama, R. Nakagaki, J. Nakamura, K. Mutai, S. Nakafumo, *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2472–2480; c) J. A. Zoltewicz, T. M. Oestreid, *J. Org. Chem.* **1991**, *56*, 2805–2809.

[26] At the pH used the two sulfite ions are practically equimolar. The redox potentials mentioned were determined by kinetic methods, see R. E. Huie, P. Neta, *J. Phys. Chem.* **1984**, *88*, 5665–5669.

[27] a) A. Profumo, E. Fasani, A. Albini, *Heterocycle*s **1999**, *50*, 1499 – 1502; b) V. A. Afanas'ev, O. N. Efimov, G. N. Nesmerenko, O. M. Nivovarov, B. G. Rodachev, M. L. Kidechel', *Izv. Akad. Nauk SSSR* **1988**, 806 – 809; c) K. J. Houser, D. E. Bartak, M. D. Hawley, *J. Am. Chem. Soc.* **1973**, *95*, 6033 – 6040; d) V. A. Kuzmin, A. K. Chibisov, *Dokl. Akad. Nauk SSSR* **1973**, *212*, 1146 – 1148.

[28] M. Bazin, F. Bosca, M. L. Marin, M. A. Miranda, L. K. Patterson, R. Santus, *Photochem. Photobiol.* **2000**, *72*, 451 – 457.

[29] This estimate is in agreement with the weaker oxidative power of $HPO_4^{-\bullet}$ with respect to $H_2PO_4{}^{\bullet}$ (the potential of the couple $H_2PO_4{}^{\bullet}$/ $H_2PO_4^-$ is close to 2.4 V vs NHE).[30] This is inferred by taking into account that $HPO_4^{-\bullet}$ is able to oxidize bromide to bromine with a rate constant of $6.5 \times 10^6 \, \text{M}^{-1}\text{s}^{-1}$,[31] a low rate consistent with a potential lower by several tenths of Volt than that of the Br/Br^- couple, that is, 1.92 V vs NHE,[33] on the basis of the Rehm – Weller (or Marcus) equation for electron transfer rates.[32]

[30] D. Stanbury, *Adv. Inorg. Chem.* **1989**, *33*, 69 – 138.

[31] P. Maruthamuthu, P. J. Neta, *J. Phys. Chem.* **1978**, *82*, 710 – 713.

[32] D. Rehm, A. Weller, *Isr. J. Chem.* **1970**, *8*, 259 – 271.

[33] Notably the yield of the OH-substituted photoproduct, formed from ENX by the triplet mediated nonreductive pathway, is suppressed by phosphate to the same extent as the triplet itself.[13]

[34] Under conditions with a large extent of triplet quenching and, consequently, high radical concentrations, the acceleration of the FQ radical anion decay could be indicative of back electron transfer to the oxidized inorganic anion. This is supported by the fact that quenching constants are higher with the less stabilized NOR radical anion than with ENX. Moreover the low values of the degradation quantum yields and the nature of the photoproducts (where *both* reactions, ring defluorination *and* piperazine degradation, occurred concomitantly), are consistent with a predominant in-cage photochemistry, and further supports back electron transfer as the most likely channel for the out-of-cage radical decay.[13]

[35] S. Monti, N. Camaioni, P. Bortolus, *Photochem. Photobiol*. **1991**, *54*, 577 – 584.

[36] R. V. Bensasson, J. C. Gramain, *J. Chem. Soc. Faraday Trans. 1* **1980**, *76*, 1801 – 1810.

[37] P. Bortolus, F. Elisei, G. Favaro, S. Monti, F. Ortica, *J. Chem. Soc. Faraday Trans.* **1996**, *92*, 1841 – 1851.