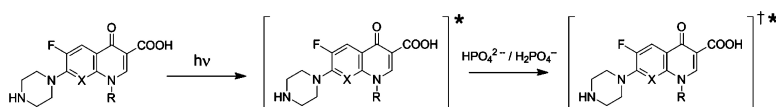Communication

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Formation of Secondary Triplet Species after Excitation of Fluoroquinolones in the Presence of Relatively Strong Bases

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The photophysical and photochemical properties of fluoroquinolone antibiotics (FQs) have been extensively studied in recent years in order to elucidate the mechanism which leads to photosensitivity associated with this family of compounds.[1] These studies have revealed some unexpected features, such as the photocleavage of the strong C−F bond, which has been associated with phototoxic behavior.[2] This process is thought to occur via nucleophilic substitution by a hydroxyl group in the triplet excited state of monofluorinated FQs in neat water or via the production of the corresponding anion radical in the presence of reducing buffers such as phosphate and sulfite.[2,3] Our studies with structurally related FQs enoxacin (ENX), ciprofloxacin (CIP), and norfloxacin (NFX) (see Chart 1 for structures) show an additional remarkable process associated with their triplet excited states. We have found that bicarbonate and phosphate buffer enhance the transformation of the primary triplet state into a secondary triplet state, whose photophysical properties have been characterized by energy transfer experiments.

Laser flash photolysis (LFP) of aqueous solutions of ENX, CIP, and NFX at a pH of 7.4 leads to the population of the excited triplet state (^{3}A*, see absorption maxima in Table 1), followed by the formation of a second transient species (B) with a lifetime less than 2 μs which absorbs in the longer wavelength region (Figure 1A). The nature and mechanism of formation of B are not completely clear. It has been suggested, in the presence of phosphate buffer, that B is the anion radical formed by electron transfer from the phosphate dianion to ^{3}A*.[3,4] However, no further attempt to confirm the nature of B and characterize it has been reported. We, on the other hand, found that the presence of phosphate buffer in the medium affects the transient absorption spectra observed after LFP of CIP, as shown in Figure 1. A similar trend was observed in the case of ENX and NFX as well. The intensity and rate of formation of B increased with phosphate concentration (see Figure 2A), to the extent that only B was seen at buffer concentrations higher than 0.1 M. The absorption profile of B resulting from LFP of ENX, CIP, and NFX was analyzed at different phosphate buffer concentrations (up to 20 mM), and the plot of the rate of buildup of the transient absorption at the correspondent λ_{max} resulted in straight lines (Figure 2B) from whose slopes the quenching rate values were derived (Table 1).

Replacing the phosphate buffer by bicarbonate, we observed similar effects on aqueous solutions of CIP. The rate of quenching of ^{3}A* by both phosphate and bicarbonate increased with pH ((2.3 to 5.6) × 10^8 M^{-1} s^{-1} and (0.5 to 6.0) × 10^8 M^{-1} s^{-1} for phosphate and bicarbonate quenching, respectively, from pH 5.8 to pH 9.0). Addition of sodium hydroxide (up to 0.1 mM) had a
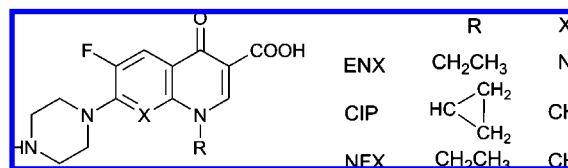
Chart 1. Structure of Fluoroquinolones Studied

Table 1. Photophysical Properties of the Triplet Excited States of ENX, CIP, and NFX

	ENX	CIP	NFX
^{3}A λ_{max} (nm)	520	610	620
^{3}B λ_{max} (nm)	670	700	700
ϵ (^{3}A) (M^{-1}cm^{-1})	6900[a]	7200[b]	7900[a]
ϵ (^{3}B) (M^{-1}cm^{-1})[b]	5800	9600	8900
10^9 × k_{ET} (^{3}A+NAP) (M^{-1} s^{-1})	2.3[a]	1.9[c]	2.2[a]
10^9 × k_{ET} (^{3}B+NAP) (M^{-1} s^{-1})[c]	0.6	1.4	1.2
10^8 × k_{ET} (^{3}A+Phosphate) (M^{-1} s^{-1})[d]	9.9	2.3	0.8
10^8 × k_{ET} (^{3}A+Bicarbonate) (M^{-1} s^{-1})[d]		1.5	

[a] Data from ref 6. [b] Molar absorption coefficients ϵ were obtained at the corresponding maximum wavelength by the energy transfer method, using NAP as standard (ϵ_{430} = 9500 M^{-1} cm^{-1} for ^{3}NAP).[7] [c] Quenching rate constants were obtained using 0.0−1.0 mM concentrations of NAP at appropriate conditions for the observation of ^{3}A (no buffer) and ^{3}B (0.2 M phosphate buffer). [d] Rates for the formation of ^{3}B were obtained using 0.0−20 mM phosphate or bicarbonate concentrations at pH 7.4.

similar effect in methanol solutions, but no transients were observed in aqueous solutions above pH 9. Moreover, quenching of ^{3}A* was not accelerated in the presence of acetate or oxalate anions up to 40 mM at pH 7.4.

In the case of CIP, B was quenched by oxygen with a rate of 2.6 × 10^9 M^{-1} s^{-1}. This near-diffusion rate is common in energy transfer reactions[5] and therefore suggests that B is a triplet excited state (^{3}B*). This assignment is in contradiction with the suggestion that electron transfer occurs between the excited triplet state and phosphate buffer, where one would expect to observe the anion radical of the FQ as in the case of the sulfite reaction. We have shown that, at the end of the sulfite reaction, the absorption due to CIP anion radical is observed at 600 nm (which was confirmed by pulse radiolysis, data not shown). Hence, it is clear that transient B is not the CIP anion radical. The assignment of B as the triplet was confirmed by sensitization experiments using quenchers such as naproxen (NAP), a naphthalene derivative with relatively low triplet energy (259 kJ mol^{-1}).[8] LFP of ENX, CIP, and NFX was performed in the presence of 0.2 M phosphate buffer (pH 7.4), so that triplet ^{3}A* is completely converted to ^{3}B* within the pulse and increasing concentrations of NAP (0 to 2 mM so that it does not compete with phosphate for triplet ^{3}A*). The decay of the T−T absorption originating from ^{3}B* was measured for all compounds

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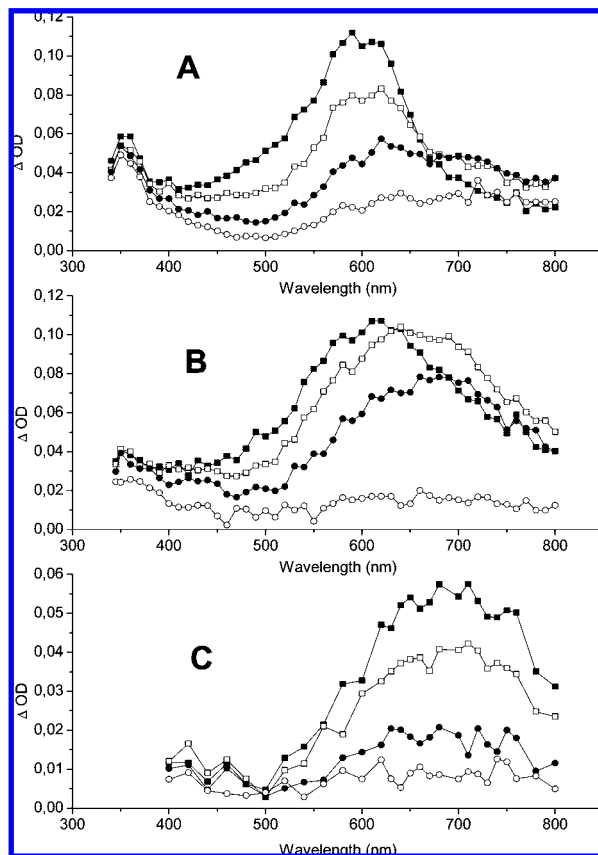‡ Manchester Metropolitan University.
§ University of Salford.

Figure 1. Transient absorption spectra observed after laser flash excitation of a 0.5 mM CIP aqueous solution in nitrogen containing no buffer (A), 0.01 M (B), and 0.5 M (C) phosphate buffer. The spectra are taken at 300 ns (—■—), 800 ns (B, C —□—), 1.4 μs (A —□—), 2.2 μs (B, C —●—), 3.5 μs (A —●—), and 8.5 μs (—○—). Laser energies were 10 mJ.
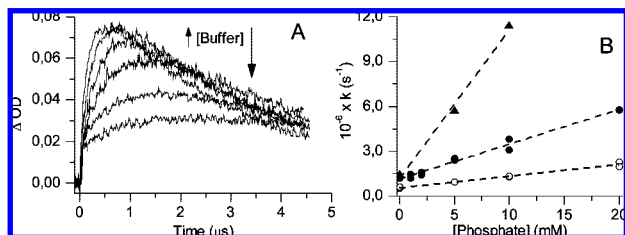

Figure 2. (Left) Transient absorption profiles observed at 700 nm after laser flash photolysis of a 0.4 mM CIP aqueous solution containing phosphate buffer ranging in concentration from 0.0 to 0.02 M. (Right) Plot showing the rate of buildup of the transient species B (enoxacin (▲), ciprofloxacin (●), and norfloxacin (○)) at its respective maximum wavelength vs concentration of the phosphate buffer.

and was found to be similar in value to the growth of the absorption due to the T–T absorption of NAP at its maximum wavelength (λ = 430 nm),[8] as shown in Figure 3A for NFX. When the rates of decay of ^{3}B* were plotted against NAP concentrations, straight
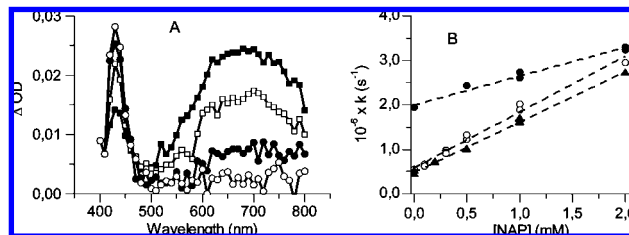

Figure 3. (Left) Transient absorption spectra observed at 70 ns (—■—), 210 ns (—□—), 1.0 μs (—●—), and 2.2 μs (—○—) after laser flash photolysis of a 0.2 mM norfloxacin solution in water containing 0.2 M phosphate buffer (pH 7.4) and 1.0 mM naproxen. The averaged laser energy employed was 9 mJ. (Right) Plot showing the rate of decay of the transient species B (enoxacin (▲), ciprofloxacin (●), and norfloxacin (○)) at its respective maximum wavelength vs concentration of NAP.

lines were obtained (see Figure 3B), and the quenching rate values were obtained from the slopes (see Table 1).

Sensitization of ^{3}NAP allowed us to calculate the molar absorption coefficient of ^{3}B*, and the different values obtained are summarized in Table 1.

When quenchers with a higher triplet energy such as 4-biphenylcarboxylic acid (E_T = 265 kJ mol^{-1})[9] were used, triplet ^{3}B* of any of the FQs studied was not quenched, as opposed to triplet ^{3}A*, indicating that the energy level of ^{3}B* is lower than that of ^{3}A*.

In conclusion, we have shown that the conversion of ^{3}A* of the FQs studied to a secondary triplet ^{3}B* with a lower energy is accelerated in the presence of bicarbonate or phosphate in aqueous solutions and by NaOH in methanol solutions. Since this is not observed when weaker bases such as acetate or oxalate are present, we suggest that it is a base-catalyzed deprotonation process in the triplet excited state of the FQs. This appears to be an unusual process, especially because no triplet is formed by direct excitation of the anionic ground state. This may have some implications in mechanisms of photoreactivity based essentially on triplet energy levels.

References

(1) Albini, A.; Monti, S. *Chem. Soc. Rev.* **2003**, *32*, 238–250.
(2) Fasani, E.; Negra, F. F. B.; Mella, M.; Monti, S.; Albini, A. J. O. *Chem.* **1999**, *64*, 5388–5395.
(3) Monti, S.; Sortino, S.; Fasani, E.; Albini, A. *Chem.—Eur. J.* **2001**, *7*, 2185–2196.
(4) Mella, M.; Fasani, E.; Albini, A. *Helv. Chim. Acta* **2001**, *84*, 2508–2519.
(5) Bensasson, R. V.; Land, E. J.; Truscott, T. G. *Flash Photolysis and Pulse Radiolysis*; Pergamon Press: Oxford, 1983.
(6) Lhiaubet-Vallet, V.; Cuquerella, M. C.; Castell, J. V.; Bosca, F.; Miranda, M. A. *J. Phys. Chem. B* **2007**, *111*, 7409–7414.
(7) Bosca, F.; Cosa, G.; Miranda, M. A.; Scaiano, J. C. *Photochem. Photobiol. Sci.* **2002**, *1*, 704.
(8) Martinez, L. J.; Scaiano, J. C. *Photochem. Photobiol.* **1998**, *68*, 646.
(9) Schulman, E. M.; Parker, R. T. *J. Phys. Chem.* **1977**, *81*, 1932.

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