pH Effect on the efficiency of the photodeactivation pathways of naphazoline: a combined steady state and time resolved study†

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The effect of pH on the spectroscopic behavior of naphazoline [NP, 2-(1-naphthylmethyl)imidazoline] has been examined in aqueous solution by combining steady state and time resolved spectroscopic experiments. The photodeactivation pathways are modulated by the pH changes and can be rationalized on the basis of the chemical structure of the compound. The naphthalene-localized singlet formed upon light excitation is partially deactivated *via* a reversible energy transfer process involving the imidazoline moiety when NP is present in its cationic form. This process leads to an imidazoline-assisted intersystem crossing being responsible for the population of the lowest naphthalene-localized triplet state.

In the case of the neutral form, the imidazoline ring behaves as an efficient electron donor, which is able to quench the excited naphthalene *via* a photoinduced electron transfer. Such a process is responsible for the decrease in the naphthalene-localized triplet generation as well as for the reduction of drug photodegradation.

The topic of drug photochemistry has always received great attention but recently this interest has markedly intensified due to the increase in the UV portion of the sun's spectrum reaching the earth. Problems related to drug photostability are of relevance not only in the context of the involvement of UV–visible exposure after topical application on the skin or the eyes, but also in their production and storage. Knowledge concerning both drug photochemistry and photophysics helps in the design of more photostable drugs as well as in the development of photoprotective systems against potential adverse phototoxic reactions triggered by drugs. Much of the work reported in the last few years on the mechanisms of drug photodegradation and drug photosensitization appears in some recent reviews.[1–3] However, not many papers have focused attention on the dominant role played by pH on the photochemical behavior of drugs.

Many drugs belonging to different pharmacological classes are characterized by one or more protonation sites and, as a consequence of the relative prototropic equilibria, different forms can be present at different pH values. Due to this, markedly different photobehavior can be observed for the drugs depending upon the prototropic form present. In some cases this behavior can be characterized by unusual photophysical and photochemical deactivation pathways for the chromophore. The antiinflammatory drug ketoprofen is one of the most recent and interesting cases in which the dominant role played by pH on drug photochemistry has been demonstrated.[4,5] The typical benzophenone photochemistry observed for the acid form of the drug is not observed at the physiological pH in which the basic form is present. An efficient photodecomposition triggered by a fast deactivation of the excited state, accompanied by the absence of singlet oxygen generation, was observed at neutral pH.

Due to the presence of two different protonation sites, many fluoroquinolone antibacterials can be present in their cationic, zwitterionic and anionic forms.[6] In the case of enoxacin[7] different photophysical and photochemical behavior was observed for the three different species and in particular the strong pH dependence of the photodegradation quantum yield reached its maximum value around the physiological pH. Similar behavior of the photophysical properties was found for norfloxacin,[8] another fluoroquinolone antibacterial. The zwitterionic form, resulting from the dissociation of the carboxyl group and the protonation of the piperazinyl ring, was proposed to be the species responsible for toxic reactions because of its capability to generate singlet oxygen. The influence of pH on the photochemistry of the nonfluorinated parent member of fluoroquinolones, nalidixic acid, was also investigated.[9–11] Its triplet state was characterized at pH 9.2[9] and photodecarboxylation was found to occur in alkaline solution.[10] A significant pH dependence for the production of singlet oxygen was also observed.[11]

Finally, it has to be considered that the different prototropic species of a drug can be characterized by differing abilities to interact with biological targets. The consequence of different drug–biomolecule binding modes can be important in determining the efficiency of the photosensitizing activity of the drug.

Naphazoline (NP), 2-(1-naphthylmethyl)imidazoline is a drug belonging to the vasoregulator class, present in the market as eye drops. Recent studies concerning its photochemistry[12] and phototoxicity[13] showed that hydrated electrons, nitrogen-centered radicals and singlet oxygen are produced upon laser excitation. Hydrated electrons and nitrogen-centered radicals have been reported to be responsible for DNA photocleavage under anaerobic conditions whereas OH radicals have been identified as triggering species for DNA damage in an aerobic environment.[13] Because the nitrogen of the imidazoline moiety can be protonated, naphazoline may be present in two different forms depending on pH. The aims of the present study are to gain more insight into the overall deactivation pathways of NP upon excitation at different pH values, in order to contribute to the general picture of NP photobehavior.

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Experimental

Naphazoline hydrochloride (molecular weight 246.7) was purchased from Sigma Chemical Company. 2-Methyl-2-imidazoline and naphthalene were purchased from Aldrich. Water was purified through a Millipore Milli-Q system and the pH of solutions was measured through a glass electrode. For the experiments at different pH, we used 10^{-2} M phosphate buffer (pH 6.6–8.6), 10^{-2} M carbonate buffer (pH 9–11) and NaOH for higher pH.

The steady state absorption spectra were obtained using a Cary 1E spectrophotometer. Luminescence spectra were recorded using a Perkin Elmer LS 50 instrument. Naphthalene in cyclohexane was employed as a standard for the fluorescence quantum yield determination.[14]

All the transient spectra and kinetics were recorded by employing a flow system with a 7 × 7 mm^2 Suprasil quartz cell with a 2 mL capacity, and were purged in a storage tank with N_2 for 30 min before as well as during the acquisition. A similar laser flash photolysis system has been previously described.[15,16] Briefly, the samples were excited with a Lumonics EX-530 laser with Xe–HCl–Ne mixtures, generating pulses at 308 nm of ~6 ns and ⩽60 mJ $pulse^{-1}$. The signals from the monochromator–photomultiplier system were initially captured by a Tektronix 2440 digitizer and transferred to a PowerMacintosh computer that controlled the experiment with software developed in the LabView 3.1.1 environment from National Instruments.

Fluorescence time resolved studies were carried out using the fourth harmonic (*i.e.* 266 nm) pulse from a Continuum PY-61 Nd : YAG laser (35 ps, 4 mJ $pulse^{-1}$) as the excitation source. A Hamamatsu C-4334 streak camera was used for detection and acquisition of the data.

Results and discussion

The absorption spectra of the acidic and basic forms of NP are shown in Fig. 1. The observation of two isosbestic points at 256 and 280 nm confirms the presence of two different forms of NP. The relative prototropic equilibrium (Scheme 1) is characterized by a pK_a of *ca.* 10[12] reflecting the high basicity exhibited by the imidazoline moiety. Recognizing that the nitrogens in the imidazoline ring are equivalent, the double bond in the protonated structure can be considered as delocalized across the carbon between the two nitrogens. The difference in the molar absorptivity observed for the neutral and the cationic species can be explained on the basis of the structures shown in Scheme 1. Indeed, a conjugation links the two parts of the molecule in the case of one of the two tautomers of the basic form of NP. The relatively slight effect on the absorption spectrum indicates that the dominant structure of the deprotonated form of NP is the central one in Scheme 1.

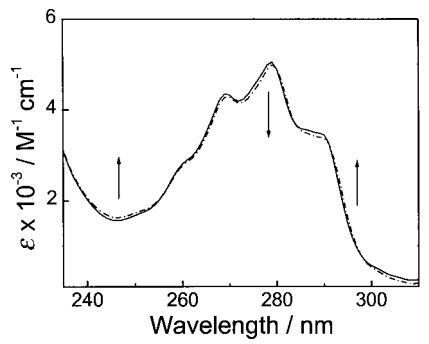
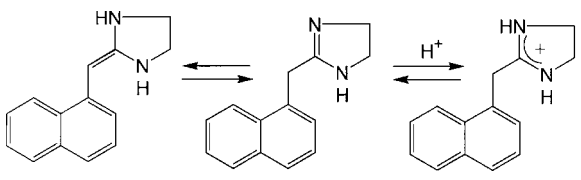


Fig. 1 Absorption spectra of the cationic (——) and neutral (– · – ·) forms of NP.



Scheme 1

In contrast, the emission properties of NP were greatly affected by pH changes. A strong decrease in the fluorescence intensity was observed upon going from the cationic to the neutral form of NP (Fig. 2). By monitoring the fluorescence intensity at 325 nm as a function of pH (inset, Fig. 2), a sigmoidal curve with an inflection point around pH 10 is obtained. These results are in accordance with the pK_a value obtained from absorption measurements.[12] The reduction in the fluorescence intensity with increasing pH was also accompanied by a reduction of the fluorescence lifetime. Monoexponential decays of 8 ns for pH values lower than 8 and of 0.35 ns for pH values higher than 12 were obtained. These two lifetimes were attributed to the cationic and to the neutral forms of NP, respectively. At intermediate pH values the decay was biexponential and characterized by the same lifetimes. The prototropic equilibrium leads to an increase and a decrease of the relative weights of the shorter and the longer components with changing pH (Fig. 3).

On the basis of its chemical structure, NP can be considered as a bichromophoric molecule formed by a sub-unit 1 (the naphthalene moiety), a spacer (the methylene) and a sub-unit 2 (the imidazoline ring), as pictorially represented in Scheme 2.‡ Given that the naphthalene is the chromophore dominating the absorption, the marked difference in the fluorescence

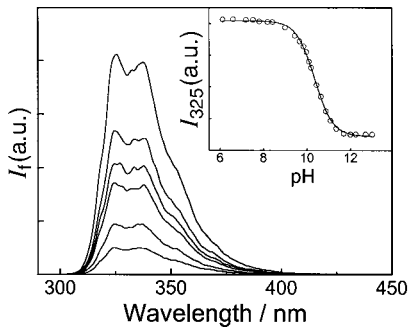


Fig. 2 Dependence of NP fluorescence spectra on pH; λ_{exc} = 290 nm. Inset: pH dependence of the fluorescence intensity at 325 nm.

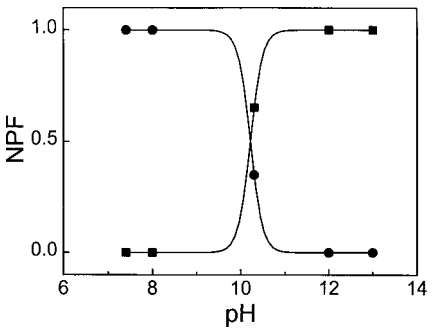
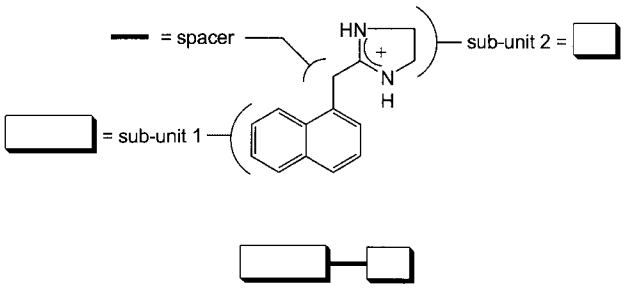


Fig. 3 pH Dependence of the normalized pre-exponential factors (NPF) related to the (●) slow and (■) fast components of the NP fluorescence decay.

‡ Even though the conjugated form reported in Scheme 1 would account for the active spacer, the slight effect observed in the absorption spectra upon deprotonation suggests that such a structure does not play a dominant role compared with its tautomeric form.



Scheme 2

properties observed for the neutral and cationic forms of NP suggest that intramolecular interactions between the two chromophores could be involved. In light of this, we considered it useful to perform some fluorescence experiments in the presence of naphthalene and 2-methyl-2-imidazoline as model compounds for sub-units 1 and 2, respectively.

We observed that naphthalene fluorescence was quenched by both the acidic and the basic form of imidazoline, as shown in the Stern–Volmer plots reported in Fig. 4. Taking into account that the fluorescence lifetime of naphthalene in aqueous solution is 30 ns, bimolecular quenching constants of 1.5×10^8 and 2.3×10^9 M^{-1} s^{-1} were obtained at pH 7 and 12, respectively. These findings suggest that either when naphazoline is present in its cationic or neutral form, the imidazoline moiety can act as an intramolecular quencher of the naphthalene-localized excited singlet. Confirmation that a quenching process is operative even when the protonated form of NP is present is provided by a comparison of both the fluorescence quantum yield and fluorescence lifetime of NP with the values reported for naphthalene. The obtained values of $\Phi_f = 0.027$ and $\tau_f = 8$ ns for the cationic form of NP are in fact *ca.* three times smaller than those observed for naphthalene in the same solvent.[14,17] Photoinduced energy transfer (ET) and electron transfer (et) processes involving the two chromophores of the molecule can be proposed as being responsible for the observed behavior. Of these two processes, the former is more likely to occur in the protonated form of NP. A consideration of the energetics of such a process and the value related to the singlet oxygen quantum yield support our hypothesis.

From the absorption spectra of the protonated form of 2-methyl-2-imidazoline it is possible to place the energy of this compound at around 393 kJ mol^{-1}. This value was confirmed by semiempirical calculations performed by means of the Zerner intermediate neglect of differential overlap/spectroscopic (ZINDO/S) method (Hyperchem software package). The energies calculated for the lowest singlet and triplet excited states of 2-methyl-2-imidazoline were in fact 410 and 285 kJ mol^{-1}, respectively. These values could be slightly overestimated given that the solvent effect has not been taken into account. Insomuch as the energy of the lowest excited singlet state in aqueous solution for naphthalene is *ca.* 385 kJ mol^{-1},[18] the electronic states of the donor and the acceptor are approximately isoenergetic. These values for the energy of the donor and the acceptor account for the not very efficient energy transfer observed in the case of the protonated form of NP. On the basis of these results we believe that the excitation energy resides on both chromophores.

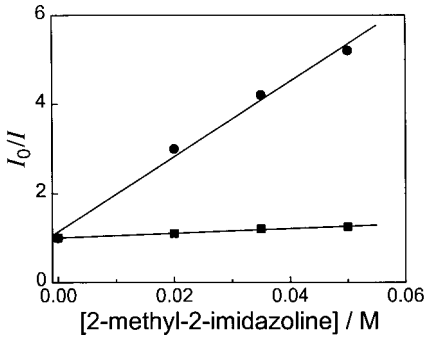


Fig. 4 Stern–Volmer plots of the quenching of naphthalene fluorescence by 2-methyl-2-imidazoline at (■) pH 7 and (●) pH 12.

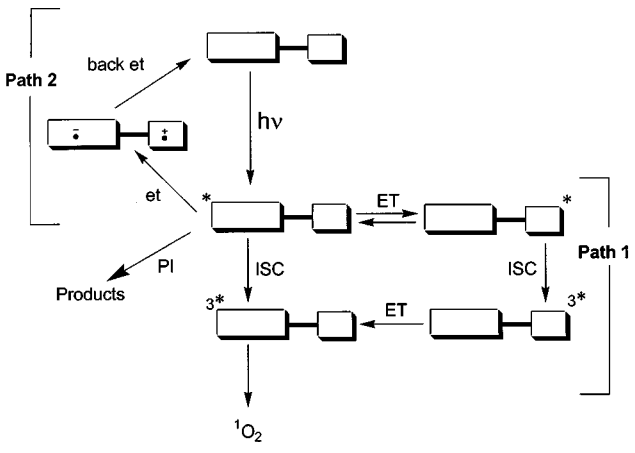
The singlet oxygen quantum yield found for the cationic form of NP was 0.19.[12] Such a value is actually comparable to that of other substituted naphthalenes,[19] accounting for the singlet–singlet energy transfer mechanism proposed. We believe that once formed, the imidazoline-localized singlet undergoes fast intersystem crossing (ISC) followed by an efficient triplet–triplet energy transfer involving the imidazoline-localized triplet and the naphthalene chromophore. This process is responsible for the assisted formation of the naphthalene-localized triplet (see path 1 Scheme 3). Indeed, as mentioned above, the energy of the imidazoline triplet state is estimated to be greater ($E_T \approx 285$ kJ mol^{-1}) than that of the naphthalene triplet ($E_T \approx 251$ kJ mol^{-1}). The suggested mechanism is not uncommon. In the case of nabumetone, a non-steroidal anti-inflammatory drug, the butanone side-chain was proposed to act as an intramolecular quencher of the naphthalene singlet as well as a photosensitizer of the naphthalene triplet.[19] On the other hand, an electron transfer process involving the excited naphthalene unit and the protonated imidazoline unit would have led to a reduction in the ISC quantum yield with a consequent decrease of the singlet oxygen photogeneration.

As reported earlier, our results account for the higher quenching efficiency of the naphthalene singlet by the basic form of the imidazoline compared with the acidic one. In the case of the neutral form of NP, a photoinduced electron transfer involving the excited naphthalene and the imidazoline ring is reasonably proposed as the mechanism responsible for the observed behavior. Some considerations of the electrochemistry of the system provide a first confirmation of the photoinduced electron transfer process. Indeed, by taking into account that the naphthalene and imidazoline potentials are $E^{\circ}_{NA/NA^{\cdot -}} \approx -2.3$ eV and $E^{\circ}_{IM^{\cdot +}/IM} < 1$ eV, respectively,[20] and that the lowest excited naphthalene-localized singlet lies around 3.9 eV[18], the thermodynamic balance

$$E_{NP}^{*} + E^{\circ}_{NA/NA^{\cdot -}} - E^{\circ}_{IM^{\cdot +}/IM} > 0.6 \text{ eV}$$

accounts for the energetically favorable quenching of the excited naphthalene-localized singlet *via* an electron transfer mechanism.

As a consequence of observed influence of the pH on the modulation of the photophysical properties of NP, an effect on the photochemistry of the drug is expected as well. A detailed study of the transients involved in the NP photochemistry in neutral aqueous solution has been recently carried out.[12] This work pointed out that a mixture of mono-



Scheme 3

and biphotonic photoionization (PI) processes leading to the formation of hydrated electrons are involved in NP photodegradation. The radical cation formed after electron photoejection from the naphthalene ring was efficiently quenched from the imidazoline moiety through a fast intramolecular electron transfer. This process generated a long-lived resonance-stabilized nitrogen centered radical with λ_{max} around 330 nm. The NP triplet state formation (λ_{max} 410 nm) was also observed and singlet oxygen was efficiently formed as already mentioned. These results allowed us to define NP as a potential Type I and Type II photosensitizer.

Fig. 5 shows the transient spectra of NP recorded in N_2 saturated solution at pH 7 and 12 and taken 0.1 μs after the laser pulse. It can be seen that the magnitude of the signal from the neutral form of NP is much smaller than that of the cationic form, both in the UV and in the visible bands. Nevertheless, the decays of the 330 nm transient, of the NP triplet and of the hydrated electrons were not affected by the pH change. By taking into account that both the cationic and the neutral form of ground state NP are characterized by the same extinction coefficient at the excitation wavelength used (308 nm), and that the molar absorptivity of the transients involved in NP are not expected to change with pH, we can rule out that the lowering of intensity observed is due to trivial factors. These findings suggest that the increase in pH does not change the nature of the photochemical processes but only reduces their efficiency.

By following the maximum absorbance change (ΔA) at the three main wavelengths as a function of pH (Fig. 6), we observed sigmoidal behavior with inflection points around pH 10. The observed behavior is in good agreement with the fluorescence results previously discussed. As a consequence, the reduction of the photodegradation efficiency can be interpreted as due to the efficient photoinduced electron transfer process between the naphthalene and the imidazoline. Such a process is dominant when 100% of the neutral form is present (see path 2 in Scheme 3).

The lower intensity of the band relative to the NP triplet observed for the neutral form of naphazoline deserves further consideration. As shown in Fig. 6(B) the amount of the naphthalene-localized triplet decreased as the pH increased. This result fits well with the proposed electron transfer mechanism. Actually, if an energy transfer, like that suggested for the cationic form of NP, was responsible for the quenching observed at high pH values an increase in the assisted ISC quantum yield would be expected.

The observation of the same transient intermediates for the neutral form of NP compared to those observed for the protonated form, indicates that the proposed electron transfer quenching of the naphthalene-localized singlet does not generate any other new detectable transient species. As a consequence it is reasonable to propose that a back electron transfer leads to the ground state of NP (path 2 in Scheme 3).

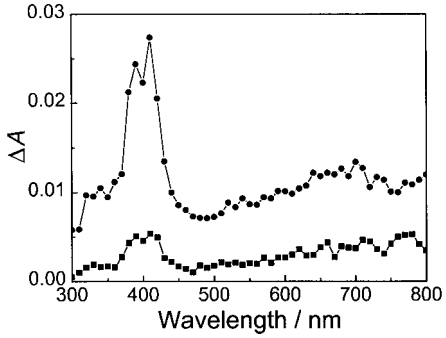


Fig. 5 Transient absorption spectra observed in a 4×10^{-4} M NP N_2 saturated solution upon 308 nm laser excitation and taken 0.1 μs after the pulse at (●) pH 7 and (■) pH 12.

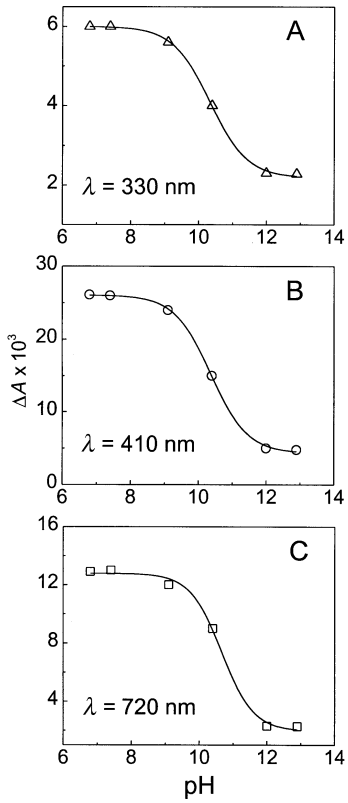


Fig. 6 pH Dependence of ΔA observed in a 4×10^{-4} M NP N_2 saturated solution upon 308 nm laser excitation and taken (A) 30 μs and (B, C) 0.1 μs after the pulse.

In view of these results, the neutral form of NP is not only the most photostable species but, in light of the smaller ISC quantum yield, is not expected to sensitize singlet oxygen formation efficiently. The overall processes in accordance with the results obtained are reported in Scheme 3, in which path 1 and path 2 are dominant for the cationic and neutral forms of NP, respectively.

In conclusion, NP provides an interesting example of a drug characterized by photoinduced intramolecular energy and electron transfer processes. We have found that the naphthalene chromophore dominates the absorption leading to its fluorescent singlet state. When NP is present in its protonated form, the imidazoline moiety can act as a singlet energy acceptor and, given that the two excited states are almost isoenergetic, the excitation energy can be considered as localized on either chromophore. This fact is responsible for the imidazoline-assisted ISC leading to the population of the lowest naphthalene-localized triplet state.

The quenching efficiency of the naphthalene-localized singlet state increases upon NP deprotonation. Under these conditions, the imidazoline ring acts as an efficient quencher *via* a photoinduced electron transfer mechanism. This process does not trigger any new photochemical reaction but leads only to a significant increase in NP photostability. The photoproduction of hydrated electrons generated after naphthalene photoionization and of the consequent nitrogen-centered radicals was, in fact, remarkably reduced.

Similarly, the production of the naphthalene-localized triplet state was also markedly inhibited in the case of the neutral form of NP. The electron transfer process competes indeed with the ISC in the deactivation of the naphthalene-localized triplet. Therefore, protonation and deprotonation of the imidazoline ring play a key role in the efficiency of the photophysical and photochemical deactivation pathways of the drug, modulating its potential properties as a Type I and Type II photosensitizer. The neutral form of NP is characterized not only by a higher photostability but also by a lower

efficiency as a singlet oxygen generator. The present investigation suggests that the ideal approach to improving drug photostability and in reducing drug phototoxicity could lie in the design of molecules characterized by chromophoric units able to originate "*safe photochemistry and photophysics*". Knowledge about the mechanisms of the drug photodeactivation pathways is the first step towards this goal.

Acknowledgements

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References

1 G. Guidi, S. Giuffrida, P. Miano and G. Condorelli, in *Modern Topics in Photochemistry and Photobiology*, ed. F. Vargas, Research Signpost, Trivandrum, India, 1997, pp. 65–102.
2 F. Boscà and M. A. Miranda, *J. Photochem. Photobiol. B: Biol.*, 1998, **43**, 1.
3 Symposium-in-print on drug: photochemistry and phototoxicity: *Photochem. Photobiol.*, 1998, **68**, 633–697.
4 S. Monti, S. Sortino, G. De Guidi and G. Marconi, *J. Chem. Soc., Faraday Trans.*, 1997, **93**, 2269.
5 L. J. Martinez and J. C. Scaiano, *J. Am. Chem. Soc.*, 1997, **119**, 11066.
6 K. T. Kovak, B. Noszal, I. Hermecz, G. Kereszturi, B. Podanyi and G. Szasz, *J. Pharm. Sci.*, 1990, **79**, 1023.
7 S. Sortino, G. De Guidi, S. Giuffrida, S. Monti and A. Velardita, *Photochem. Photobiol.*, 1998, **67**, 167.
8 P. Bilski, L. J. Martinez, E. B. Korer and F. Chignell, *Photochem. Photobiol.*, 1996, **64**, 496.
9 G. Vermeesch, J. C. Ronfard–Haret, M. Bazin, V. Carillet, P. Morliere and R. Santus, *Photochem. Photobiol.*, 1991, **54**, 661.
10 N. Detzer and N. Huber, *Tetrahedron*, 1975, **31**, 1937.
11 P. Dayhaw-Barker and T. G. Truscott, *Photochem. Photobiol.*, 1988, **47**, 765.
12 S. Sortino and J. C. Scaiano, *Photochem. Photobiol.*, 1999, **70**, 590.
13 S. Sortino, S. Giuffrida and J. C. Scaiano, *Chem. Res. Toxicol.*, 1999, **12**, 971.
14 D. F. Eaton, in *Handbook of Organic Photochemistry*, ed. J. C. Scaiano, CRC Press, Boca Raton, Florida, 1989, vol. 1, pp. 231–240.
15 J. C. Scaiano, *J. Am. Chem. Soc.*, 1980, **102**, 7747.
16 J. C. Scaiano, M. Tanner and D. Weir, *J. Am. Chem. Soc.*, 1985, **107**, 4396.
17 V. Wintgens, in *Handbook of Organic Photochemistry*, ed. J. C. Scaiano, CRC Press, Boca Raton, Florida, 1989, vol. 1, pp 405–418.
18 N. J. Turro, *Modern Molecular Photochemistry*, Benjamin/Cummings Publishing Co., Menlo Park, CA, 1978.
19 L. J. Martinez and J. C. Scaiano, *Photochem. Photobiol.*, 1998, **68**, 646.
20 G. J. Kavarnos, *Fundamentals of Photoinduced Electron Transfer*, VCH Publishers, Inc., New York, 1993.

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