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Sensitized photoxygenation of piroxicam in neat solvents and solvent mixtures

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Abstract

Detection of $O_2(^1\Delta_g)$ phosphorescence emission, $\lambda_{max}=1270$ nm, following laser excitation and steady state methods were employed to determine the total rate constant, k_T, for the reaction between the non-steroidal anti-inflammatory drug piroxicam (PRX) and singlet oxygen in several solvents. Values of k_T ranged from $0.048\pm0.003\times10^6$ M^{-1} s^{-1} in chloroform to $71.2\pm2.2\times10^6$ M^{-1} s^{-1} in *N*,*N*-dimethylformamide. The chemical reaction rate constant, k_R, was determined by using thermal decomposition of 1,4-dimethylnaphthalene endoperoxide as the singlet oxygen source. In acetonitrile, the k_R value is equal to $5.0\pm0.4\times10^6$ M^{-1} s^{-1}, very close to the k_T value. This result indicates that, in this solvent, the chemical reaction corresponds to the main reaction path. Dependence of total rate constant on the solvent parameters π^* and β can be explained in terms of a reaction mechanism that involves the formation of a perepoxide intermediate. Rearrangement of the perepoxide to dioxetane followed by ring cleavage and transacylation accounts for the formation of *N*-methylsaccharine and *N*-(2-pyridyl)oxamic acid, the main reaction products. Data obtained in dioxane–water (pH 4) mixtures with neutral enolic and zwitterionic tautomers of piroxicam in equilibrium show that the zwitterionic tautomer reacts with singlet oxygen faster than the enolic tautomer.

Keywords: Singlet oxygen; Piroxicam; Photosensitization; Perepoxide; Solvent effect; LSER

1. Introduction

Piroxicam (PRX) is a non-steroidal anti-inflammatory drug (NSAID) that causes adverse light-induced biological effects [1–8]. Its phototoxicity has been ascribed to singlet molecular oxygen ($O_2(^1\Delta_g)$) production by 2-methyl-4-oxo-2H-1,2-benzothiazine-1,1-dioxide, a metabolite that could be synthesized preferentially and that accumulates in human skin [8]. In addition, piroxicam photoproducts could be involved in its photosensitizing reactions [9], mainly those arising from reactions of piroxicam with singlet molecular oxygen [10]. Miranda et al. [10] have found that the same products, *N*-(2-pyridyl)oxamic acid and *N*-methylsaccharine, are formed both by direct irradiation of PRX in oxygen-saturated solutions or by photosensitized oxidation of the drug with tetraphenylporphine. This distribution is explained in terms of a plausible mechanism that involves a dioxetane intermediate. PRX is photostable in anaerobic conditions [10–12] and generates $O_2(^1\Delta_g)$ with very low quantum yields [8], then, phototoxic effects due to the products of the reaction between piroxicam and singlet oxygen would be relevant only for systems undergoing oxidative stress. Several questions must be answered to account for this possibility: how reactive is PRX towards $O_2(^1\Delta_g)$? How different are the rates of chemical and physical quenching of singlet oxygen by PRX? What is the dependence of reaction rate between PRX and $O_2(^1\Delta_g)$ on the tautomeric form of PRX that predominates in a given medium?

In this paper we report the kinetic results obtained from the sensitized photo-oxidation of PRX in several solvents and solvent mixtures. The main objective of this work was to explain the media effect on PRX reactivity towards singlet oxygen.

2. Experimental

Piroxicam (Sigma), rubrene, 5,10,15,20-tetraphenyl-21H,23H-porphine (TPP), methylene blue (MB), 1,4-dimethylnaphthalene (DMN) and 1,3-diphenylisoben-

zofurane (DPBF) (Aldrich) were used without further purification. 1,4-Dimethylnaphthalene endoperoxide was synthesized according to a previously described method [13,14]. Rose Bengal (Fluka) was recrystallized from ethanol prior to use. All solvents (Merck) were of spectroscopic or HPLC quality.

Time-resolved experiments were carried out by measuring the phosphorescence of $O_2(^1\Delta_g)$ at 1270 nm. TPP and MB were irradiated by the 500-ps light pulse of a PTI model PL-202 dye laser (414 or 655 nm, ~200 μJ per pulse). A PTI model PL-2300 nitrogen laser was employed to pump the dye laser. When Rose Bengal was used as the sensitizer, samples were excited with the second harmonic (532 nm, ~15 mJ per pulse) of the 6-ns light pulse of a Quantel Brilliant Q-Switched Nd:YAG laser. The singlet oxygen emission was detected by using liquid nitrogen cooled North Coast model EO-817P germanium photodiode detector equipped with a built-in preamplifier. Further experimental details have been reported elsewhere [15,16]. Total rate constants were also determined by steady state competitive experiments measuring the rubrene autoxidation rate [16]. In these experiments irradiation was performed with a visible Par lamp, 150 W, using a Shott cut-off filter at 450 nm.

The chemical reaction rate constant in acetonitrile was determined by using thermal decomposition of 1,4-dimethylnaphthalene endoperoxide as the singlet oxygen source. The reaction rate was obtained by measuring spectrophotometrically the PRX consumption. The rate of singlet oxygen production in the same experimental conditions was measured using DPBF as actinometer [18].

Fluorescence experiments were carried out in a Spex Fluorolog Tau 2 spectrofluorimeter with a sample holder thermostated at 22 °C. UV–visible absorption experiments were performed on a thermostated Unicam UV-4 spectrophotometer. A Fisons Plattform II mass spectrometer interfaced with a Hewlett-Packard 1050 liquid chromatograph was used to perform the HPLC–MS experiments.

Equation coefficients and statistical parameters of LSER correlations were obtained by multilinear correlation analysis with STATVIEW 5.0 (SAS Institute). Results agreed with the *t*-statistic of descriptors.

3. Results and discussion

3.1. Reaction of PRX with singlet oxygen in neat solvents

Total (physical and chemical quenching) rate constants, k_T, for the reaction of $O_2(^1\Delta_g)$ with PRX in several solvents were obtained from experimentally measured first-order decays of $O_2(^1\Delta_g)$ in the absence (τ_o) and in the presence of PRX (τ) according to Eq. (1). In the same solvents, triplet decay of sensitizer, TPP, was not affected by the addition of higher concentrations of PRX than those used to quench excited oxygen.

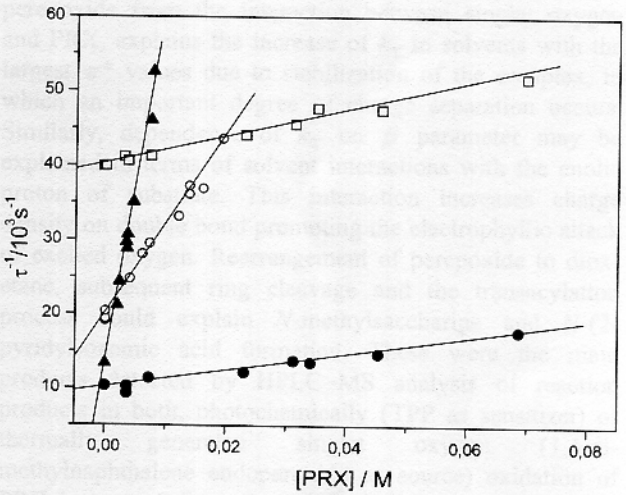


Fig. 1. Stern–Volmer plots for singlet oxygen deactivation by piroxicam. ▲, Acetonitrile; □, dioxane; ○, acetone; ●, methylene chloride.

$$\tau^{-1} = \tau_o^{-1} + k_T\,[PRX] \quad (1)$$

Linear plots were obtained for all the solvents employed (Fig. 1). Values of k_T calculated from the slope of these plots are given in Table 1. Quenching was more efficient in polar solvents and decreased considerably in less-polar solvents such as chloroform or dioxane. Because of the low solubility of PRX in benzene, the rate constants in this solvent were obtained from competitive steady-state inhibition of the rubrene autoxidation rate [17] by addition of PRX. In other solvents, such as acetonitrile or dioxane, the k_T values determined in steady-state experiments were similar to those obtained from $O_2(^1\Delta_g)$ luminescence decays (Table 1). Besides, in these steady-state experiments, quenching of the sensitizer excited states by PRX

Table 1
Values of k_T for the reaction between PRX and $O_2(^1\Delta_g)$ in different solvents

	Solvent	k_T (10^6 M^{-1} s^{-1})
1	Chloroform	0.048±0.003[a]
2	Methylene chloride	0.115±0.005[a]
3	Dioxane	0.166±0.009[a]
		0.189±0.010[b]
4	Benzene	0.709±0.035[b]
5	Acetone	1.19±0.07[a]
6	Tributylphosphate	3.80±0.13[a]
7	Acetonitrile	4.81±0.26[a]
		6.58±0.34[b]
8	*N*,*N*-Dimethylformamide	71.2±2.15[a]

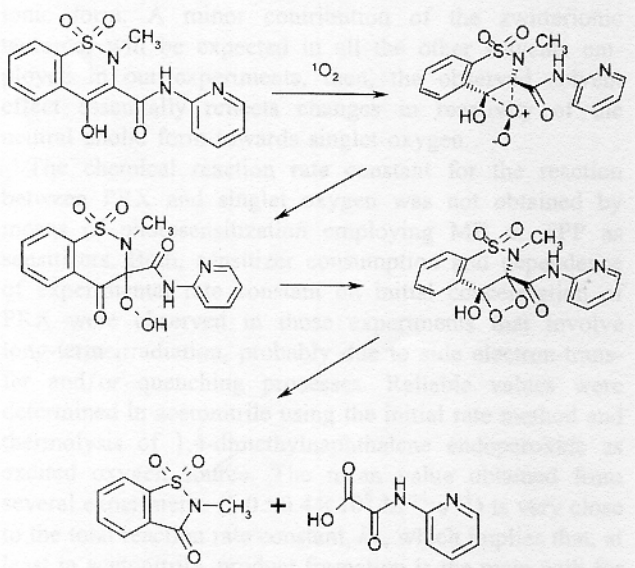
[a] Sensitizer: TPP or MB, time-resolved method.
[b] Sensitizer: rubrene, steady-state method.

can be disregarded since k_T values were independent of rubrene and PRX initial concentrations (up to 40 mM).

The total quenching rate constants increased by more than three orders of magnitude when the solvent changed from chloroform to *N,N*-dimethylformamide. However, the k_T increase cannot be associated only with changes in the macroscopic parameter dielectric constant. For instance, in chloroform ($\epsilon = 4.81$) or methylene chloride ($\epsilon = 8.93$), k_T values are smaller than those determined in dioxane ($\epsilon = 2.21$) or benzene ($\epsilon = 2.27$). This may indicate the occurrence of specific solute–solvent interactions. Thus, in order to have an insight into the solvent effect on singlet oxygen interaction with PRX we analyzed the quenching rate constant dependence on microscopic solvent characteristics using the semiempirical-solvatochromic equations of Taft, Kamlet and co-workers [19,20]. Data correlation obtained from a limited set of solvents due to the low PRX solubility, led to Eq. (2) which gives the dependence of k_T on solvent parameters

$$\text{Log } k_T = 2.703 + 3.415\pi^* + 2.516\beta \quad (2)$$

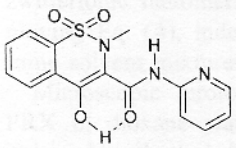
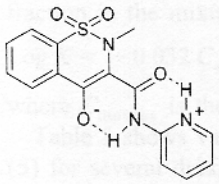
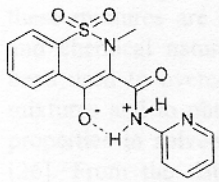
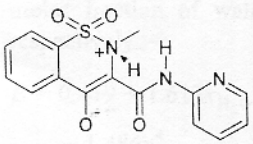
The dependence of k_T on both parameters, dipolarity-polarizability, π^*, and solvent ability as hydrogen bond acceptor, β, can be understood in terms of the formation of an exciplex intermediate with charge separation. A mechanism involving a dioxetane intermediate has been proposed to explain product distribution in the reaction between singlet oxygen and PRX [10]. However, dioxetane formation via 2+2 cycloaddition is not compatible with the large increase of k_T in polar organic solvents and its dependence on π^* and β parameters. Our data supports a reaction mechanism (Scheme 1) in which a perepoxide intermediate forms prior to dioxetane. The formation of a perepoxide from the interaction between singlet oxygen and PRX, explains the increase of k_T in solvents with the largest π^* values due to stabilization of the complex, in which an important degree of charge separation occurs. Similarly, dependence of k_T on β parameter may be explained in terms of solvent interactions with the enolic proton of substrate. This interaction increases charge density on double bond promoting the electrophyllic attack of excited oxygen. Rearrangement of perepoxide to dioxetane, subsequent ring cleavage and the transacylation process could explain *N*-methylsaccharine and *N*-(2-pyridyl)oxamic acid formation. These were the main products detected by HPLC–MS analysis of reaction products in both, photochemically (TPP as sensitizer) or thermally generated singlet oxygen (1,4-dimethylnaphthalene endoperoxide as source) oxidation of PRX in acetonitrile and methylene chloride. This mechanism is compatible with proposition of Nascimento et al. [21], who observed direct and sensitized light emission during the peroxidative metabolism of the nonsteroidal anti-inflammatory tenoxicam, both by horseradish peroxidase and by polymorphonuclear leukocytes. Chemiluminescense detected in these processes involves electronically excited metabolites of oxicam produced by thermal cleavage of a dioxetane intermediate.



Scheme 1.

Furthermore, piroxicam behavior in solution should be considered to support the mechanism depicted in Scheme 1. It may be possible that several tautomeric forms of piroxicam (see Table 2) are present in different solvents [22], which would affect the interpretation of the solvent effects. Experimental results indicate that the neutral enolic form predominates largely in non-polar and moderately-polar organic solvents. In addition, the formally zwitterionic tautomer corresponding to structure C in Table 2 is the most probable tautomer in equilibrium with the enol in highly-polar organic solvents as predicted by the properties obtained from simplest molecular orbital calculations. For these calculations, geometries were fully optimized at the SCF-level with the PM3 method of Gaussian. Absorption spectra were obtained with ZINDO/S method followed by configuration interaction calculations, which include only single excitations. Theoretical data obtained for enolic tautomer (structure A in Table 2) are in fair agreement with both the data of Bicca et al. [23] and with the experimental spectrum ($\lambda_{max.} = 325$ nm in several solvents). Moreover, data in Table 2 indicate that the zwitterionic tautomer B contribution to the equilibrium could be disregarded because no absorption above 400 nm was observed in polar and/or non-polar solvents. Furthermore, the low intensity of the absorption bands predicted for tautomer D are not compatible with experimental behavior. On the other hand, Geckle et al. [24] have estimated that about 10% zwitterionic tautomer is present in dimethylformamide at room temperature. Assuming a rate constant in the order of 10^8 M^{-1} s^{-1} for singlet oxygen quenching by the zwitterionic tautomer in dimethylformamide, not

Table 2
Formation heat and spectral parameters of several tautomers of piroxicam

Structure	ΔH_f (kcal mol^{-1})	λ_{Calc} (nm)	λ_{Exp}(dioxane) (nm)	Oscilator strength, f
 **A**	−69.06	309.0	326	0.518
 **B**	−50.73	449.2 366.0	366	0.131 0.363
 **C**	−47.07	339.2	366	0.490
 **D**	−34.08	368.1 337.9	366	0.078 0.025

more than 4% of the total quenching will be caused by the ionic form. A minor contribution of the zwitterionic tautomer will be expected in all the other solvents employed in our experiments, then, the observed solvent effect essentially reflects changes in reactivity of the neutral enolic form towards singlet oxygen.

The chemical reaction rate constant for the reaction between PRX and singlet oxygen was not obtained by means of photosensitization employing MB or TPP as sensitizers. Both, sensitizer consumption and dependence of experimental rate constant on initial concentration of PRX were observed in those experiments that involve long-term irradiation, probably due to side electron-transfer and/or quenching processes. Reliable values were determined in acetonitrile using the initial rate method and thermolysis of 1,4-dimethylnaphthalene endoperoxide as excited oxygen source. The mean value obtained from several experiments ($5.0\pm0.4\times10^6$ M^{-1} s^{-1}) is very close to the total reaction rate constant, k_T, which implies that, at least in acetonitrile, product formation is the main path for the reaction between singlet oxygen and PRX.

3.2. Reaction of piroxicam with singlet oxygen in solvent mixtures

Excited-state intramolecular proton transfer of PRX has been studied in dioxane–water mixtures [25]. To quantify reactions rate constant of both PRX tautomers, enolic and zwitterionic, for the reaction of this drug with singlet oxygen, we employ a similar approach measuring the total deactivation rate constant, k_T, in several dioxane–water (pH 4) mixtures as a function of composition. In these experiments, MB and TPP were used as sensitizers.

The contribution of enolic and zwitterionic tautomers to the total reaction rate constant, can be evaluated according to Eq. (3)

$$k_T = f_N k_N + f_Z k_z \quad (3)$$

where f_N and f_z are the fraction of enolic and zwitterionic forms present at equilibrium and k_N and k_z are the reaction rate constants for enol and zwitterion, respectively. Then, k_Z can be calculated according to Eq. (4) from k_T and k_N values determined separately.

$$k_z = (k_T - k_N)\frac{K+1}{K} + k_N \quad (4)$$

where K is the equilibrium constant between the enolic and zwitterionic tautomers. Additionally, to calculate k_z employing Eq. (4), independent determinations of K in the same solvent mixtures are necessary.

Microscopic protonation–deprotonation equilibria of PRX in dioxane–water mixtures have been studied by Takács-Novák et al. [22]. They found that the equilibrium constant for the enol zwitterion depends on the dioxane fraction in the mixture according to Eq. (5)

$$\text{Log}\, K = -0.032\, C_{\text{dioxane}} + 1.57 \quad (5)$$

where C_{dioxane} is the wt.% of dioxane.

Table 3 shows values of $(K+1)/K$ calculated from Eq. (5) for several dioxane–water mixtures.

Values of k_N in dioxane–water mixtures could be predicted using Eq. (2) if the solvent parameter values in these mixtures are known. Probes of different structures and chemical nature, that yield convergent results have been used to overcome preferential solvation in aqueous mixtures and to obtain approximate values of the solvent properties in solvent mixtures valid for LSER treatments [26]. From the data of Marcus [26], we found that in dioxane–water mixtures values of β and π^* depend on the molar fraction of water according to Eqs. (6) and (7), respectively

$$\beta = 0.419 + 1.613x_{H_2O} - 4.095x^2_{H_2O} + 4.019x^3_{H_2O} - 1.486x^4_{H_2O} \quad (6)$$

$R = 0.999$; S.D. $= 0.0026$; $n = 11$; $P < 0.0001$

$$\pi^* = 0.387 + 2.299x_{H_2O} - 9.988x^2_{H_2O} + 22.284x^3_{H_2O} - 25.778x^4_{H_2O} + 12.018x^5_{H_2O} \quad (7)$$

$R = 0.996$; S.D. $= 0.0182$; $n = 11$; $P < 0.0001$

Table 3 provides values of k_N, calculated by using Eqs. (2), (6) and (7) and k_z values obtained from Eq. (4).

The results listed in Table 3 show that k_N values are almost constant between $x(H_2O) = 0.34$ and 0.84. This independence of k_N value on water composition can be due to the following reasons: (i) between $x(H_2O) = 0.2$ and 0.6 solvent microscopic parameters, π^* and β, are almost constant and quite close; (ii) for $x(H_2O) > 0.6$ the increase in π^* is compensated by the decrease in β. The larger k_N at $x(H_2O) > 0.9$ can be explained by both larger π^* value for this solvent mixture and/or preferential solvation at this water composition. Table 3 also shows that k_z values are about a factor of 100 larger than k_N values. Enhanced reactivity of the zwitterionic tautomer can be explained by the increase of charge density on reactive site, the carbon–carbon double bond neighbor to the keto group, which facilitates the electrophylic attack of singlet oxygen. This result could be taken into account to explain in vivo PRX photosensitizing effects. Piroxicam exists in several prototropic species with the anionic one dominating at neutral biological pH [25]. Then, under in vivo like conditions, the enolic tautomer should be solubilized preferentially in non-polar microenvironments whereas zwitterionic and/or anionic tautomers should be located in more polar microdomains. Also, it is expected that anionic PRX reacts with $O_2(^1\Delta_g)$ faster than zwitterionic PRX due to the formal negative charge in the vicinity of the reactive center. This concept is supported by our experimental data. Values of k_T in dioxane–water mixtures at pH 9.2 were equal to 2.7 ± 0.11 and $2.8 \pm 0.12 \times 10^8$ M^{-1} s^{-1} at molar fractions of water of 0.34 and 0.67, respectively. Values of k_T obtained in these solvent mixtures are one order of magnitude larger than k_T values in mixtures dioxane–water at the same $x(H_2O)$ but at acidic pH, suggesting that anionic PRX is more reactive towards singlet oxygen than enolic and zwitterionic species. In view of these results, it is reasonable to assume that adverse photobiological effects of PRX mediated by its photo-oxidation products, could be very dependent on the PRX microlocation, with reactions of neutral enolic form predominating in hydrophobic microdomains and processes involving the more polar zwitterionic and anionic tautomers dominating in hydrophilic microenvironments.

In conclusion, PRX is a moderate quencher of singlet oxygen, and is a more effective quencher in polar solvents. A reaction mechanism involving a perepoxide intermediate that evolves to dioxetane from which products arise seems to be the main reaction path. The zwitterionic tautomer that

Table 3
Values of k_N, k_z and k_T for the reaction between piroxicam and singlet oxygen in dioxane–water mixtures

x (water)	k_N (10^6 M^{-1} s^{-1})	k_z (10^8 M^{-1} s^{-1})	k_T (10^7 M^{-1} s^{-1})	$(K+1)/K$
0.34	1.76	2.62	1.35	22.2
0.54	1.22	1.93	1.84	11.2
0.67	0.88	2.53	4.35	5.92
0.76	0.91	1.37	4.16	3.36
0.78	0.99	1.67	5.75	2.93
0.84	1.67	1.18	6.18	1.93
0.91	6.58	1.51	11.80	1.30

predominates in polar medium reacts about 100 faster than neutral enolic form. This may be relevant to account for the observed in vivo photobiological effects of PRX, when they are due to photo-oxidation products.

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References

[1] B. Fjellner, Photosensitivity induced by piroxicam, Acta Derm. Venerol. 63 (1983) 557–558.
[2] B.L. Diffey, T.J. Daymond, H. Fairgreaves, Phototoxic reactions of piroxicam, naproxen and tiaprofenic acid, Br. J. Rheumatol. 22 (1983) 239–242.
[3] R.S. Stern, Phototoxic reactions of piroxicam and other nonsteroidal antiinflamatori agents, New Engl. J. Med. 309 (1983) 186–187.
[4] K.J. McKerrow, D.E. Greig, Piroxicam-induced photosensitive dermatitis, J. Am. Acad. Dermatol. 15 (1986) 1237–1241.
[5] I.E. Kochevar, W.L. Morison, J.L. Lamm, D.J. McAuliffe, A. Western, A.F. Hood, Possible mechanism of piroxicam-induced photosensitivity, Arch. Dermatol. 122 (1986) 1283–1287.
[6] G. Serrano, J. Bonillo, A. Aliaga, E. Gargallo, C. Pelufo, Piroxicam-induced photosensitivity, J. Am. Acad. Dermatol. 11 (1984) 113–120.
[7] G. Serrano, J. Bonillo, A. Aliaga, J. Cuadra, C. Pujol, P. Cervero, M.A. Miranda, Piroxicam-induced photosensitivity and contact sensitivity of thiosalicylic acid, J. Am. Acad. Dermatol. 23 (1990) 479.
[8] A. Western, J.R. Van Camp, R. Bensasson, E.J. Land, I.E. Kochevar, Involvement of singlet oxygen in the phototoxicity mechanism for a metabolite of piroxicam, Photochem. Photobiol. 46 (1987) 469–475.
[9] B. Ljunggren, The piroxicam enigma, Photodermatology 6 (1989) 151–154.
[10] M.A. Miranda, F. Vargas, G. Serrano, Photodegradation of piroxicam under aerobic conditions. The photochemical keys of the piroxicam enigma?, J. Photochem. Photobiol. B: Biol. 8 (1991) 199–202.
[11] A. Figueiredo, C.A. Fontes-Ribeiro, S. Goncalo, M.M. Caldeira, A. Polares-Baptista, F. Texeira, Piroxicam-induced photosensitivity, Contact Dermatitis 17 (1987) 73–79.
[12] J.L. Cirne de Castro, E. Vale, M. Martins, Mechanism of photosensitive reactions induced by piroxicam, J. Am. Acad. Dermatol. 20 (1989) 706–707.
[13] N.J. Turro, M.F. Chow, J. Rigaudy, Mechanism of thermolysis of endoperoxides of aromatic compounds. Activation parameters, magnetic field and magnetic isotope effects, J. Am. Chem. Soc. 103 (1981) 7218–7224.
[14] K. Jesse, R. Markert, F.J. Comes, R. Schmidt, H.D. Brauer, Picosecond photochemistry: the mechanism of photocycloreversion of aromatic endoperoxides, Chem. Phys. Lett. 166 (1990) 95–100.
[15] A.L. Zanocco, G. Günther, E. Lemp, J.R. de la Fuente, N. Pizarro, Kinetics and mechanism of the photosensitized oxidation of furosemide, Photochem. Photobiol. 68 (1998) 487–493.
[16] A.L. Zanocco, G. Günther, E. Lemp, J.R. de la Fuente, N. Pizarro, Solvent effects on the sensitized photooxygenation of lidocaine, J. Photochem. Photobiol. 140 (2001) 109–115.
[17] D.J. Carlsson, G.D. Mendenhall, T. Suprunchuk, D.M. Wiles, Singlet oxygen ($^1\Delta_g$) quenching in the liquid phase by metal(II) quelates, J. Am. Chem. Soc. 94 (1972) 8960–8962.
[18] G. Günther, Reacciones de fotooxidación sensibilizada de xenobioticos de interés farmacológico, Ph.D. Thesis, University of Chile, 2000.
[19] C. Reichardt, Solvents and Solvents Effects in Organic Chemistry, VCH, Weinheim, 1990.
[20] M.J. Kamlet, J.L.M. Abboud, M.H. Abraham, R.W. Taft, Linear solvation energy relationships. 23. A comprehensive collection of the solvatochromic parameters π^*, α and β, and some methods for simplifying the generalized solvatochromic reactions, J. Org. Chem. 48 (1983) 2877–2887.
[21] A.L. Nascimento, J.A. Escobar, G. Cilento, The peroxidative metabolism of tenoxicam produces excited species, Photochem. Photobiol. 57 (1993) 362–366.
[22] K. Takács-Novák, J. Kökösi, B. Dodanyi, B. Noszál, R.S. Tsai, G. Lisa, P.A. Carrupt, B. Testa, Microscopic protonation/deprotonation equilibria of the anti-inflammatory agent piroxicam, Helv. Chim. Acta 78 (1995) 553–562.
[23] R. Bicca, J.D. Da Motta, Theoretical studies on non-steroidal antiiflammatory drugs, benoxaprofen, chlorpromazine and piroxicam, Int. J. Quantum Chem. 22 (1995) 123–136.
[24] J.M. Gekle, D.M. Rescek, E.B. Whipple, Zwitterionic piroxicam in polar solution, Magn. Reson. Chem. 27 (1989) 150–154.
[25] S.M. Andrade, S.M.B. Costa, Hydrogen bonding effects in the photophysics of a drug, piroxicam, in homogeneous media and dioxane–water mixtures, Phys. Chem. Chem. Phys. 1 (1999) 4213–4218.
[26] Y. Marcus, The use of chemical probes for the characterization of solvent mixtures. Part 2. Aqueous mixtures, J. Chem. Soc. Perkin Trans. 2 (1994) 1751–1758.