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Visible light-mediated photodegradation of imidazoline drugs in the presence of Riboflavin: Possible undesired effects on imidazoline-based eye drops

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ABSTRACT

The imidazoline-based ophthalmic drugs oxymetazoline and xylometazoline are widely used as ocular decongestants in pharmaceutical preparations. In this paper, the degradation of these drugs and the model compound 2-methyl-2-imidazoline, was studied in the presence of the vitamin B_2 (Riboflavin) and visible light.

The photogenerated Riboflavin electronically excited triplet state interacts with oxymetazoline and xylometazoline and as a result different free radicals and reactive oxygen species are produced. These species interact with the drugs in further steps, producing their degradation.

Oxymetazoline is more easily photo-degradable than xylometazoline towards reactive oxygen species. Particularly, oxymetazoline reacts four orders of magnitude faster than xylometazoline with singlet oxygen. This fact is due to the presence of an OH-group in the benzene ring of oxymetazoline, increasing the oxidability of the drug. The degradation of xylometazoline by reactive oxygen species becomes more important as its concentration increases. This finding should warn against long-time treatments with xylometazoline. An eventual local accumulation of the drug may cause adverse effects in the ocular organ in the presence of Riboflavin. In parallel, the present results advise for a moderate precaution in relation to light exposure after topical application of the imidazoline derivatives oxymetazoline and xylometazoline.

1. Introduction

Aside from the skin, the most susceptible organ to UV–vis radiation induced damage is the eye [1]. Drugs which have been developed for long-term medication and/or drugs which are administered in a high accumulative dosage are more likely to cause photosensitivity responses *in vivo* [2]. In this context, ophthalmic drugs, very especially those of topical administration, constitute a family of particular risk and deserve interest.

The diversity of structures of these potentially-photosensitive drugs indicates that different mechanisms are likely to be responsible for initiating photoreactions in the eye. Upon adequate light irradiation, these drugs may either generate eventually toxic products; simply degrade, lowering their specific therapeutic effects, or generate their electronic excited states and start an unpredictable cascade of reactive and physical processes. Since most ophthalmic drugs are transparent to day-light, photodamage could occur through the photosensitising action of molecules which are usually co-administered, used in diagnostic tests and/or tissue molecules belonging to the biological environment [1,3,4].

Riboflavin (Rf), one of the B_2 vitaminic complex components, is a natural constituent of the eye [5]. By exposure to UV–vis light Rf produces free radicals and reactive oxygen species (ROS) [6]. Rf can also directly oxidize a suitable substrate by electron abstraction in the absence of molecular oxygen [7].

On the other hand, in the last decade, a new therapy (corneal collagen crosslinking) based on topical application of Rf and UV light has been developed. This relatively non-invasive procedure has proven to be a first treatment to halt keratoconus and secondary corneal ectasia progression. In this treatment Rf acts as photosensitiser releasing ROS and free radicals that cause hydrogen bond or cross-link formation between the amino acids on the collagen chains [8–10].

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In this context, the presence of Rf (natural or topically applied) or any other suitable molecule on the ocular organ that can act as light absorbing agent, could start unpredictable series of photo-processes, many of them driven through the action of the ROS.

ROS are involved in an extensive variety of health-related effects [11–17]. In particular, sensitised photopromoted injuries on eye constituents and the capability of natural biomolecules acting as defences against ROS damage, were and still are being investigated with remarkable interest [18–20]. Although a considerable number of papers report on the clinical and therapeutic properties of drugs in general, the eventual ROS-mediated photodegradation of the family of medicaments selected for this work and their photosensitising effects as potential generators of chemically aggressive species have not been explored.

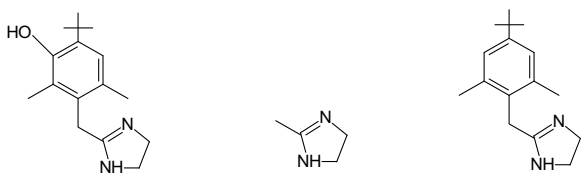
Substrates chosen in the present study are oxymetazoline (OZ) and xylometazoline (XZ) drugs and 2-methyl-2-imidazoline (IZ) like model compound (Scheme 1). From here on, these substrates will be denoted as OD. The primary action mechanism of imidazoline derivates OZ and XZ is vasoconstriction, accomplished by direct stimulation of α-receptors on blood vessels [21]. OZ is usually found as a decongestant in various pharmaceutical preparations used in the treatment of eye-irritation and nasal-congestion derived from cold, rhinosinusitis and/or allergic symptoms. XZ acts locally as a nasal decongestant. It is also indicated for the symptomatic relief of redness of the conjunctiva in ocular surface mild irritation, such as those due to smoke, dust, stale air and allergies [22–24].

In the work under study we investigate the OZ and XZ degradation in the presence of Rf and visible light, in order to obtain information about the interactions taking place between these ophthalmic drugs and biologically relevant oxidative species. The substrate 2-methyl-2-imidazoline (IZ) was used as model compound with the purpose of investigating the reactive target of ophthalmic drugs. Further, the ability of OZ and XZ to act by themselves as potential $O_2(^1\Delta_g)$ photosensitisers for ulterior oxidative damage was studied.

2. Experimental

2.1. Materials

Oxymetazoline (OZ), 3-(4,5-dihydro-1H-imidazol-2-ylmethyl)-2,4-dimethyl-6-*tert*-butyl-phenol, xylometazoline (XZ), 2-[(2,6-dimethyl-4-*tert*-butyl-phenyl)methyl]-4,5-dihydro-1H-imidazole, 2-methyl-2-imidazoline (IZ), Riboflavin (Rf), Perinaphthenone (PN), sodium azide (NaN_3), D-Mannitol, benzoic acid sodium salt (NaBz), catalase from bovine liver (CAT), superoxide dismutase (SOD) from bovine erythrocytes and D_2O (99.9%) were purchased from Sigma Chem. Co. Furfuryl alcohol (FFA) was from Riedel de Haën.



Oxymetazoline (OZ) | 2-methyl-2-imidazoline (IZ) | Xylometazoline (XZ)

Scheme 1. Chemical structures of oxymetazoline, xylometazoline and 2-methyl-2-imidazoline.

The solvents employed were methanol (MeOH) and acetonitrile (MeCN) HPLC quality, both purchased from Sintorgan.

2.2. Methods

For the stationary photolysis experiments, a 150 W quartz halogen lamp was employed as excitation source. Proper cut-off filters, at 420 nm for experiments with Rf and at 320 nm for experiments with PN, were used in order to remove the radiation below those wavelengths and to ensure that the light was only absorbed by the photosensitisers. The irradiation device has been described elsewhere [25,26].

Rf-sensitised photooxygenation rates of OD were determined from the initial slopes of the plots of oxygen consumption *vs.* irradiation time, employing a specific oxygen electrode (Orion 97-08). In order to investigate the eventual involvement of ROS, experiments of oxygen uptake in the absence and in the presence of different additives with ROS-scavenging capacity were made. The following compounds were employed as ROS scavengers: 5.0 mM NaN_3 for singlet oxygen, $O_2(^1\Delta_g)$; 1 mg/100 mL SOD for superoxide anion radical, $O_2^{\bullet-}$; 1 mg/100 mL CAT for hydrogen peroxide, H_2O_2; 5.0 mM benzoic acid sodium salt (NaBz) or 5.0 mM D-Mannitol for hydroxyl radical, $HO^{\bullet}$.

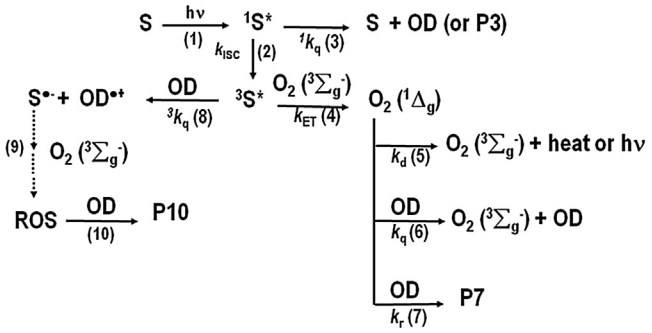
Values of the reactive rate constant for O2(1g)-deactivation by OD, k_r, were determined by a comparative method [27], employing FFA as a photooxidisable reference and PN as photosensitiser. FFA is a current reference for $O_2(^1\Delta_g)$ reactions, with a reported pH-independent rate constant k_{rRef} of $1.2 \times 10^8\ M^{-1}\ s^{-1}$ in water [28]. The k_r values were determined through the expression $k_r/k_{rRef} = slope_{OD}/slope_{Ref}$, where $slope_{OD}$ and $slope_{Ref}$ are the respective slopes of the first-order plot for oxygen uptake *vs.* the irradiation time for OD and FFA, assuming that only the reaction of OD with $O_2(^1\Delta_g)$ leads to OD oxygen consumption. In all cases, conversions lower than 10% were employed in order to avoid possible interference from photoproducts.

The laser-kinetic spectrophotometer for time resolved O_2 $(^1\Delta_g)$ phosphorescence detection (TRPD) has been previously described [26,29]. Briefly, it consisted on a Nd:YAG laser (Spectron) as the excitation source. The output at 355 nm was employed to excite the photosensitiser PN. The emitted radiation (mainly at 1270 nm) was detected at right angles using an amplified Judson J16/8Sp germanium detector, after passing through appropriate filters. $O_2(^1\Delta_g)$ lifetimes were evaluated in the absence (τ_0) and in the presence (τ) of OD, and the ratio $1/\tau$ was plotted as a function of the quencher concentration, according to a Stern-Volmer treatment: $1/\tau = 1/\tau_0 + k_t\,[OD]$, where k_t ($= k_q + k_r$) is the overall rate constant for O_2 $(^1\Delta_g)$ quenching by the OD. The determinations of k_t were made in D_2O due to the enlargement of the $O_2(^1\Delta_g)$ lifetime in this solvent [28].

The determinations of k_r and k_t values were carried out using PN as auxiliary photosensitiser, in order to quantify the exclusive participation of the species $O_2(^1\Delta_g)$. This dye is an extremely efficient $O_2(^1\Delta_g)$-photosensitiser for aqueous media [30].

The ability of OD to generate $O_2(^1\Delta_g)$ upon direct UV-irradiation (evaluated through the quantum yield Φ_Δ) was determined in MeCN, employing Naphazoline as reference compound. The 266-nm output of the already mentioned Spectron Nd:Yag laser was used as excitation source [26]. The initial intensities of the emission at 1270 nm were measured for optically matched solutions of the OD and Naphazoline. A Φ_Δ value for $O_2(^1\Delta_g)$ production by the reference compound of 0.2 was employed in the calculations [31].

For the stationary Rf fluorescence experiments, a Spex Fluoromax spectrofluorometer was employed. Rf-fluorescence lifetimes were measured using a time-correlated single photon counting technique on an Edinburgh FL-9000CD instrument. The



Scheme 2. Possible reaction pathways in the sensitised photoirradiation of oxymetazoline, xylometazoline and 2-methyl-2-imidazoline. S represents the photosensitiser (Riboflavin or Perinaphthenone) and OD the ophthalmic drugs.

excitation and emission wavelengths were 445 and 515 nm, respectively. In order to determine the values of the quenching rate constant ^1k_q, a Stern-Volmer treatment of the data was applied ($1/^1\tau = 1/^1\tau_0 + {}^1k_q$ [OD]). The lifetimes $^1\tau_0$ and $^1\tau$ correspond to the fluorescence of Rf in the absence and presence of the OD, respectively. The experiments were carried out at 25 ± 1 °C. In all cases quartz cells of 1.0 cm path-length were employed.

Argon-saturated solutions of 0.01 mM Rf were photolysed using a laser flash photolysis apparatus [32]. The decay of the electronically excited triplet state of Rf was measured at low Rf concentration (typically 0.01 mM) and at laser energy low enough to avoid undesired effects such as self-quenching and triplet-triplet annihilation, respectively. The values of the quenching rate constant of triplet Rf by the OD, ^3k_q, in MeOH and aqueous solutions were obtained from Stern-Volmer treatments. The transient absorption spectra of 0.01 mM Rf in the absence and in the presence of OD were determined in Argon-saturated aqueous solutions.

3. Results

3.1. ROS-mediated photodegradation of OD

Results were interpreted and discussed on the bases of the Scheme 2. Briefly, this scheme can be described as follows: in the presence of UV–vis light, a dye or pigment (sensitizer, S) can absorb this radiation and gives rise to its electronically excited singlet state ($^1S^*$) and, through intersystem crossing, to its electronically excite triplet state ($^3S^*$) (processes (1) and (2), respectively). $^3S^*$ can be deactivated by molecular oxygen ($O_2(^3\Sigma_g^-)$), generating the reactive oxygen species, $O_2(^1\Delta_g)$ (process (4)), and/or by an electron donor substrate, yielding the semi-reduced form of the sensitizer $S^{\bullet-}$ (process (8)). This species, in an aerated environment, is able to generate different ROS (processes denoted by (9)) that in further steps can react with the OD (process (10)). On the other hand, $O_2(^1\Delta_g)$ can decay either by collision with surrounding solvent molecules (process (5)) and by physical and/or chemical interaction with the OD (processes (6) and (7), respectively).

3.2. Stationary photolysis

The irradiation of aerated aqueous solutions of 1.0 mM OZ or IZ with visible light in the presence of 0.02 mM Rf produced changes in the OD absorption spectrum (Fig. 1, main) and also in the absorption bands of Rf, the latter only after prolonged irradiation. In parallel, oxygen consumption by OZ and IZ was observed (Fig. 1, inset I) but not in the dark or in the absence of these OD within the irradiation times employed in these experiments. These results indicate that OZ, IZ and Rf suffer chemical transformations under

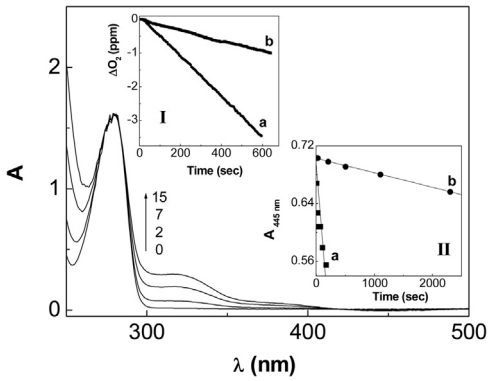


Fig. 1. Spectral evolution of *ca.* 1.0 mM oxymetazoline + 0.02 mM Riboflavin *vs.* 0.02 mM Riboflavin, upon visible-light photoirradiation (λ > 420 nm) in aqueous solution. Numbers on the spectra represent the irradiation times, in minutes. Inset I: Oxygen consumption in aqueous solution upon 0.02 mM Riboflavin sensitised photoirradiation (λ > 420 nm) of a: 1.0 mM oxymetazoline; b: 1.0 mM 2-methyl-2-imidazoline. Inset II: Rates of *ca.* 0.02 mM Rf consumption a: in the absence and b: in the presence of 5.0 mM OZ, in deoxygenated aqueous solution.

irradiation with visible light, in the presence of the vitamin Rf. Under similar conditions, for 1.0 mM XZ, no spectral modifications were observed. In addition, oxygen consumption could not be detected. Nevertheless, when solutions containing 5.0 mM XZ and 0.02 mM Rf were photolysed, oxygen uptake was observed. These facts demonstrate that the chemical degradation of XZ, in the presence of Rf as daylight absorbing agent, depends on the XZ concentration.

In deoxygenated solutions, the presence of 5.0 mM OZ produced a decrease in the rate of 0.02 mM Rf consumption, as estimated by monitoring the absorbance decrease of the absorption band of Rf at 445 nm (Fig. 1, inset II). Similar results with 5.0 mM XZ were obtained. Since photodegradation of Rf is known to proceed from its electronically excited states [33,34], the mentioned delay in the degradation rate of Rf constitutes an indication of the deactivation of the pigment excited states by the OD.

The preceding results strongly suggest that when exposed to environment light, Rf interacts with the OD and releases ROS into the surrounding medium. In order to obtain further information about these processes, several experiments were performed as outlined below.

3.3. Interaction of the OD with the electronically excited states of Rf

In air-equilibrated MeOH solution, Rf shows a fluorescence emission band. In the presence of ≥ 20.0 mM OD, the intensity of this band decreased. In parallel, the fluorescence decay of Rf was evaluated in the absence and in the presence of the OD by means of time-resolved methods (see Experimental section). Results show that the fluorescence lifetime of Rf diminishes in the presence of OD. This fact demonstrates interaction between the electronically excited singlet state of Rf and the OD (process (3), Scheme 2). The values of ^1k_q were graphically determined (Fig. 2, Table 1).

Table 1 shows that OZ and IZ deactivate the electronically excited singlet state of Rf with rate constants ^1k_q of $\approx 10^9\ M^{-1}s^{-1}$, which are close to the diffusion control in MeOH. In the case of OZ, the value of ^1k_q falls within the range obtained by Cardoso et al. [35] for a variety of phenolic compounds.

In order to evaluate the interaction between the electronically excited triplet state of Rf and OD, the decay rate of this state was monitored from the absorbance at 670 nm. In this way, it was observed that the lifetime of the excited triplet state of Rf appreciably decreased in the presence of the OD (process (8), Scheme 2) evidencing interaction among both species. As before, a

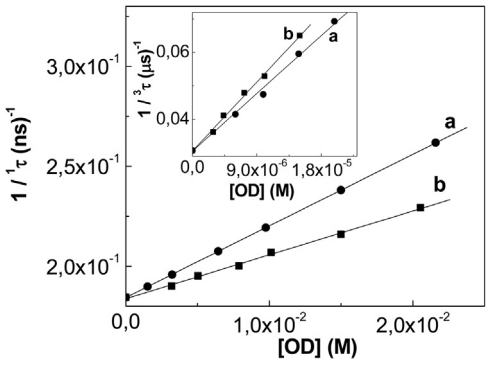


Fig. 2. Stern-Volmer plots for the time-resolved quenching of electronically excited singlet state of Riboflavin in MeOH. Inset: Stern-Volmer plots for the quenching of electronically excited triplet state of Riboflavin in Argon-saturated aqueous solution by a: oxymetazoline; b: 2-methyl-2-imidazoline.

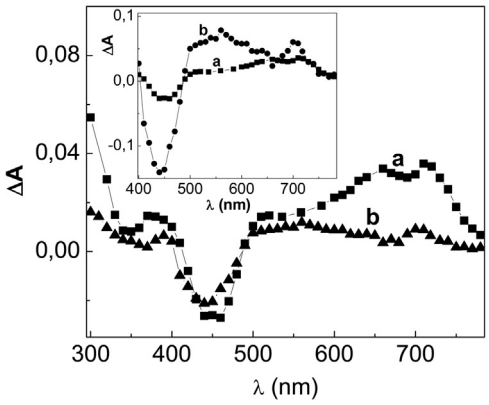


Fig. 3. Transient absorption spectra of a: 0.02 mM Riboflavin and b: 0.02 mM Riboflavin + 0.1 mM oxymetazoline taken at 1 and 20 μs after the laser pulse, respectively, in Argon-saturated aqueous solutions. Inset: Transient absorption spectra of a: 0.02 mM Riboflavin (1 μs) and b: 0.02 mM Riboflavin + 0.1 mM oxymetazoline (20 μs), normalized at 670 nm, in Argon-saturated aqueous solution.

Stern-Volmer treatment (Fig. 2, inset) yielded the bimolecular rate constants ^3k_q. Table 1 shows the values of these constants determined in MeOH and in aqueous solutions. As can be seen, these constants increase with the solvent polarity. This fact supports the ionic character of the species involved in process (8).

For the purpose of obtaining more information about the interaction between the electronically excited triplet state of Rf and OD, transient absorption spectra of Rf in aqueous solution, were determined in the absence and in the presence of the OD. Transient absorption spectrum of Rf, obtained 1 μs after the laser pulse is similar to the reported [36] one for the species ^{3}Rf*. Under identical conditions but in the presence of the OD the spectra show an important decrease in the region 500–750 nm (see Fig. 3 for OZ, as typical example). If the transient absorption spectrum of Rf (1 μs) is compared to the spectrum of Rf in the presence of 0.1 mM OZ (*ca.* 65% of triplet state of Rf quenched by OZ), taken at 20 μs after the laser pulse and normalized at 670 nm (Fig. 3, inset), a new absorption band centred at 570 nm is observed. Similar results were found for the spectrum of Rf in the presence of 0.1 mM XZ or 0.1 mM IZ. These results are in good agreement with previous reported [36] for the interaction of ^{3}Rf* with other substrates and supports the electrons transfer from the OD to ^{3}Rf*.

3.4. Participation of ROS in the Rf-mediated photodegradation of the OD

It is well known that [37,38], in the presence of $O_2(^3\Sigma_g^-)$, the excited triplet state of Rf can generate different ROS (processes denoted as (9) in Scheme 2) and begin a cascade of photoprocesses.

To obtain information on the nature of the ROS involved in the Rf-mediated OD photodegradation, experiments of oxygen uptake in the absence and presence of different additives with ROS-scavenging capacity were made. The scavengers SOD, CAT, NaN_3 and NaBz were employed in order to elucidate the eventual participation of $O_2^{\bullet-}$, H_2O_2, $O_2(^1\Delta_g)$ and $HO^\bullet$ respectively [6,39–46]. The results with NaBz were confirmed using D-Mannitol as additional scavenger of $HO^\bullet$. Similar concentrations of D-Mannitol, have been previously used as an efficient trapper in $HO^\bullet$-mediated photooxidations [36,42,47].

The rate of oxygen uptake by OZ was reduced in comparative irradiations of mixtures of 1.0 mM OZ and 0.02 mM Rf in the presence of all the ROSscavengers investigated (Table 1).

Table 1
Relative rates of oxygen photo-consumption by oxymetazoline (OZ) xilometazoline (XZ) and 2-methyl-2-imidazoline (IZ) sensitised by Perinaphthenone ($v_{-O2(rel)}$ PN) and Riboflavin ($v_{-O2(rel)}$ Rf); rate constants for the overall (k_t) and reactive (k_r) quenching of singlet oxygen; rate constants for the quenching of electronically excited singlet state of Riboflavin (^1k_q); rate constants for the quenching of electronically excited triplet state of Riboflavin (^3k_q); slope of oxygen uptake sensitised by Riboflavin in the absence ($slope_0$) and in the presence of different scavengers (NaN_3, sodium azide; SOD, superoxide dismutase; CAT, catalase and NaBz, benzoic acid sodium salt). $O_2(^1\Delta_g)$ quantum yields (Φ_Δ) determined in acetonitrile.

	Oxymetazoline (OZ)	Xilometazoline (XZ)	2-methyl-2-imidazoline (IZ)
$v_{-O2(rel)}$ Rf	1	NR	0.26
$v_{-O2(rel)}$ PN	1	NR	< 0.05
k_t x 10^{-8} ($M^{-1}s^{-1}$)	4.2 ± 0.4	0.006 ± 0.001	2.2 ± 0.2
k_r x 10^{-8} ($M^{-1}s^{-1}$)	1.6 ± 0.2	< 0.001 [(a)]	< 0.08
^1k_q x 10^{-8} ($M^{-1}s^{-1}$) MeOH	36 ± 4	1.0 ± 0.1	22 ± 2
^3k_q x 10^{-8} ($M^{-1}s^{-1}$) MeOH	11 ± 1	< 0.001	2.8 ± 0.3
^3k_q x 10^{-8} ($M^{-1}s^{-1}$) H_2O	20 ± 2	18 ± 2	22 ± 2
slope $_{NaN3}$/$slope_0$	0.05	NR	0.16
slope $_{SOD}$/$slope_0$	0.50	NR	1.0
slope $_{CAT}$/$slope_0$	0.92	NR	0.75
slope $_{Bz}$/$slope_0$	0.76	NR	1.0
Φ_Δ	0.08 ± 0.01	0.11 ± 0.01	ND

(a) Estimated value; NR: not reaction at 1×10^{-3} M; ND: not determined

As mentioned above, when the system 1.0 mM XZ/0.02 mM Rf was photolysed, oxygen consumption was not observed (Table 1). However, the addition of SOD, CAT or NaBz to the system 5.0 mM XZ/0.02 mM Rf produced a decrease in the rates of oxygen consumption as compared to the experiment in the absence of these scavengers.

For the experiment 1.0 mM IZ/0.02 mM Rf only the inhibitors CAT and NaN_3 were able to reduce the rate of oxygen consumption (Table 1).

In order to confirm the OD reactivity against H_2O_2, the thermal reaction between 5.0 mM XZ and 20.0 mM H_2O_2 was tested. An increase in the XZ absorbance at 264 nm upon addition of the peroxide indicates the occurrence of chemical transformations in the system XZ/H_2O_2. Similar results were found in the cases of 1.0 mM OZ or 1.0 mM IZ and 20.0 mM H_2O_2.

As additional information, and in order to eliminate possible interference of the scavengers in the oxygen uptake, control experiments with SOD, CAT and NaBz were performed. These compounds did not produce any noticeable oxygen consumption when exposed to visible-light in the presence of 0.02 mM Rf. Besides, they did not interact with the electronically excited states of Rf under the described conditions.

In order to evaluate the participation of the species $O_2(^1\Delta_g)$ and to achieve as much information as possible about pure potential interactions OD-$O_2(^1\Delta_g)$, the photosensitiser PN was used. This dye is frequently employed in $O_2(^1\Delta_g)$-reactions because it generates $O_2(^1\Delta_g)$ with good yield [30]. Both overall and reactive interactions between $O_2(^1\Delta_g)$ and the OD were quantified through the rate constants k_t and k_r, respectively, as described in the experimental section.

k_r values were evaluated through oxygen uptake experiments (Fig. 4, Table 1). In the case of XZ, it was not possible to detect any oxygen consumption, even at concentrations as high as 5.0 mM. Considering the detection limit of the electrode used in the measurements of oxygen consumption, an upper-limit of 1×10^5 $M^{-1}\,s^{-1}$ for the rate constant k_r was quoted.

Fig. 4, inset shows the Stern-Volmer graphs from which the k_t values were determined (Table 1).

3.5. Determination of O_2 ($^1\Delta_g$) quantum yields (Φ_Δ)

In order to investigate the ability of OD to act by themselves as potential photosensitisers, the quantum yields of $O_2(^1\Delta_g)$ production (Φ_Δ) were determined. Due to the optical features of OD (absorption bands below 300 nm, see Fig. 1 not irradiated spectrum), Φ_Δ values were determined upon direct irradiation at 266 nm, as mentioned in Section 2.2.

OZ and XZ produce $O_2(^1\Delta_g)$ when they are exposed to UV-irradiation. The signals for $O_2(^1\Delta_g)$ generation were determined as described in the experimental section. The Φ_Δ values are shown in Table 1.

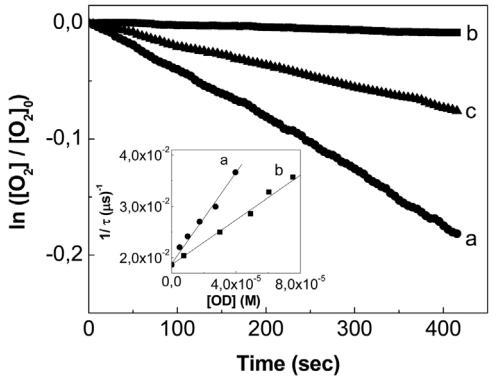


Fig. 4. First-order plots of oxygen consumption upon irradiation (λ > 320 nm) in aqueous solution in the presence of Perinaphthenone (A_{366} = 0.6) plus a: 1.0 mM oxymetazoline; b: 1.0 mM 2-methyl-2-imidazoline; c: 1.0 mM furfuryl alcohol. Inset: Stern Volmer plots for the quenching of O2 ($_1\Delta$g) by a: oxymetazoline; b: 2-methyl-2-imidazoline, in D_2O solution with Perinaphthenone (A_{355} = 0.2).

4. Discussion

Table 1 also shows the relative slopes of the oxygen uptake plots for irradiated solutions in the absence of added scavengers, in the presence of Rf ($v_{-O2(rel)}$ Rf, Table 1) or PN ($v_{-O2(rel)}$ PN, Table 1). The results obtained for Rf point to different processes for oxygen consumption, if compared to the same results but in the presence of PN, where an exclusively $O_2(^1\Delta_g)$-mediated mechanism operates. In the case of Rf, an additional source of oxygen consumption must be related to processes which involve other reactive species.

Oxygen uptake results, spectroscopic evidence in the Rf-sensitised photoprocesses and auxiliary specific ROS experiments strongly support the effective participation of electronically excited states of the pigment and different ROS generated from these excited states. In the case of XZ, the results depend on the range of concentrations employed. With 1.0 mM XZ, the Rf-sensitised photodegradation does not occur within the irradiation times employed in our experiments; however, with 5.0 mM XZ the oxygen uptake was detected.

Although the deactivation of the electronically excited singlet state of Rf was observed in MeOH at relatively high OD concentration, this process (process (3)) is not significant under the experimental conditions of the present work when aqueous solutions were employed (*ca* 1.0–5.0 mM for OD). That is, in this concentration range, OD would not be degraded by interaction with excited singlet state of Rf.

Regarding the interaction between ^{3}Rf* and OD, the increase of ^3k_q with increasing solvent polarity and the appearance of a band centred at 570 nm in the transient absorption spectra of Rf in the presence of OD, corresponding to the semireduced form of Rf, constitute clear evidences of the participation of radical ionic species in the mechanism of deactivation of ^{3}Rf* by OD.

Electron transfer reactions, with the concomitant production of the Rf radical anion and the radical cation of the substrates, have been reported for a great variety of compounds (phenol, imidazole, amine and indole derivatives) [32,36,48–53]. Additionally, Huvaere et al. [49] investigated the deactivation of ^{3}Rf* by imidazole derivatives, and through primary kinetic isotope effects they proposed that the interaction between ^{3}Rf* and imidazole derivatives occurs *via* electron transfer mechanism. In a previous paper we studied the intermediates formed after the reaction of the sulfate radicals with several imidazoline derivatives (naphazoline, tetrahydrozoline, oxymetazoline, xylometazoline and 2-methyl-2-imidazoline) through flash-photolysis and compared the experimental absorption spectra to those obtained from theoretical calculations. We confirmed the one-electron oxidation of the imidazoline derivatives and the formation of N-centred radicals for XZ and IZ and phenoxyl radicals for OZ [48].

Due to the ionic character of the species involved in the deactivation of ^{3}Rf* by OD, the electron transfer mechanism would be favoured by the medium polarity. A larger stabilization of the radical ions with solvent polarity is expected to facilitate the electron transfer from de OD to ^{3}Rf*.

The larger enhancement of XZ reactivity toward ^{3}Rf* in water with respect to MeOH, compared to OZ and IZ, could be due to the different nature of the radical ionic intermediates formed, as

reported in a previous paper for the oxidation of these drugs by inorganic radicals [48]. The stabilization of these radical ions could be dependent on the solvent polarity, thus affecting the probability of deactivation of $^3Rf^*$.

Results demonstrate that Rf, when excited to its triplet state by UV exposure, in the presence of OD, releases free radicals and triggers a cascade of processes producing the oxidative species $O_2(^1\Delta_g)$, $O_2^{\bullet-}$, H_2O_2 and/or $HO^\bullet$. The experiments in the presence of additives with ROS-scavenging capacity show the participation of different ROS in the Rf-sensitised photooxidation of the OD. The decrease in the rate of oxygen consumption of the system OZ-Rf in the presence of SOD, CAT and NaBz, accounts for the respective presence of $O_2^{\bullet-}$, H_2O_2 and $HO^\bullet$. This fact suggests that these oxidative species are effectively generated and potentially react with OZ. In the case of IZ, the lack of any effect on the oxygen uptake in the presence of SOD would rule out the direct participation of $O_2^{\bullet-}$ as oxidative agent. However, the observed decrease in the rate of oxygen consumption in the presence of CAT strongly suggests that H_2O_2 is involved in the mechanism. Hence, the species $O_2^{\bullet-}$should also be generated because the formation of H_2O_2 occurs *via* $O_2^{\bullet-}$ [25,29,54]. The absence of any inhibitory effect of NaBz on the oxygen consumption rules out the participation of $HO^\bullet$ in the oxidative process. For 5.0 mM XZ, the observed decrease in the rate of oxygen consumption in the presence of SOD, CAT and NaBz strongly suggests that the species $O_2^{\bullet-}$, H_2O_2 and $HO^\bullet$, respectively, are formed during the oxidative process and may react with 5.0 mM XZ. The result obtained from the thermal reaction between XZ and H_2O_2 supports the viability of H_2O_2-mediated oxidation of 5.0 mM XZ.

With regard to the participation of $O_2(^1\Delta_g)$ in the Rf-sensitised photoreaction, for 1 mM OZ or 1 mM IZ the decrease in the oxygen uptake in the presence of NaN_3 indicates that the species $O_2(^1\Delta_g)$ is involved in the mechanism of photooxidation. In the case of 5.0 mM XZ, the lack of any effect exerted by NaN_3 banishes a significant participation of $O_2(^1\Delta_g)$-mediated mechanism.

The interaction of OD with $O_2(^1\Delta_g)$ was quantified by TRPD and oxygen uptake experiments using PN as photosensitiser. k_t and k_r values of OZ, compared to XZ, clearly demonstrate that the presence of an OH-group on the benzene ring increases the reactivity of the OD. The quenching of $O_2(^1\Delta_g)$ by phenols in polar and non-polar solvents has been extensively reviewed [55]. The hydroxyl substituent increases the electron donor ability of the aromatic compound towards the electrophilic species $O_2(^1\Delta_g)$, with regard to the non-substituted compound. This effect has been explained on the basis of a mechanism involving an intermediate complex possessing charge-transfer character. The deactivation of the encounter complex will merely lead to physical quenching (process (6)), whereas a complete electron transfer will produce chemical oxidation of the substrate [55] (process (7)).

On the other hand, the model compound IZ, which constitutes the basic structure of OZ and XZ, is photodegraded to a lesser extent than OZ but more than XZ. A kinetic balance structure-reactivity of the OD indicates that the photodegradative behaviour is not merely the additive contributions of each component moiety of the molecule, but depends on the reactivity of the molecule as a whole.

Upon direct UV-light irradiation OZ and XZ generate the oxidative species $O_2(^1\Delta_g)$ with similar efficiency, but they can be considered fairly good photodynamic photosensitisers. In the case of XZ, although the self-promoted oxidation *via* O_2 $(^1\Delta_g)$ could be ruled out, the sensitised oxidation of other surrounding molecules can occur. This pro-oxidant behaviour that possess the OD, should advise for moderate precaution towards light exposure during storage and managing and, especially, after the topical application of these drugs.

5. Conclusions

Upon UV-exposure OZ and XZ generate the oxidative species $O_2(^1\Delta_g)$ with similar efficiency and they can be considered fairly good photodynamic photosensitisers. These results should advise for moderate precaution towards light exposure during storage and managing and, especially, after the topical application of the ophthalmic drugs OZ and XZ.

Rf is a natural constituent of the eye and under visible light-irradiation can trigger a cascade of processes producing highly reactive species. The substrates OZ and XZ suffer degradation in the presence of Rf and visible light, due to the generation of different free radicals and reactive oxygen species. Thus, the simultaneous presence of Rf and OZ or XZ in the ocular organ could reduce the therapeutic effect of the ophthalmic drugs and endanger their clinical effectiveness. The decomposition of XZ occurs with very low efficiency and depends on the concentration range employed. This result should warn patients treated with XZ due to high concentrations of this drug may cause adverse effects in the eye in the presence of Rf.

Our results are also of critical relevance in ophthalmological treatments such as corneal collagen crosslinking. Given that OD is present during the treatment, the therapeutic effect of the drugs could be reduced, their clinical effectiveness endangered, and undesired reaction products could be accumulated.

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