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Methyl anthranilate as generator and quencher of reactive oxygen species: A photochemical study

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ABSTRACT

Methyl anthranilate (MA) is a naturally occurring aromatizer and flavorant agent frequently employed in food industry and in personal care products. Due to the property of MA of being a UV-A radiation-absorber it is incorporated to topical sunscreen formulations. Within the framework of the wide variety of practical uses and applications, the photodegradation of MA becomes a topic of interest from health to an economic point of view. In this context, kinetics and mechanistic aspects of its photo-oxidation has been studied, in order to know the degradation pathways of MA under natural-like photoirradiation. The assays were performed in the absence and in the presence of riboflavin (Rf, vitamin B_2) acting the later as a native photosensitizer. The results showed that MA is capable to generate singlet molecular oxygen ($O_2(^1\Delta_g)$) upon direct UV-A irradiation, with a quantum yield value of 0.13 ± 0.02. The anthranilate also quenches the oxidative species with an overall rate constant value $k_t = 2.1 \pm 0.2 \times 10^7\ M^{-1}\ s^{-1}$ while the reactive interaction between MA and $O_2(^1\Delta_g)$ showed a rate constant value in the range of $5.9–6.8 \times 10^6\ M^{-1}\ s^{-1}$. The k_r/k_t ratio, lower than 0.35 indicates that only one third of the $O_2(^1\Delta_g)$-quenching events effectively lead to oxidation of the scavenger, being this an acceptable property for a sunscreen component. Regarding the Rf-photosensitized visible-light irradiation of MA–Rf mixtures, the anthranilate quenches the electronically excited singlet and triplet states of the vitamin. As a result the oxidative species $O_2^{\bullet-}$ and H_2O_2, are generated, being the later effectively deactivated by MA. At first, the described results would show MA as a candidate for UV-A and visible-light mediated degradation. Nevertheless, at the same time MA acts as a moderate physical scavenger of photogenerated oxidative species in a sort of self-protector against photoinduced degradation and protector of oxidizable surrounding compounds.

1. Introduction

Methyl anthranilate (MA, Scheme 1), a naturally occurring compound, is present in a variety of flowers and fruits [1–3] and is widely utilized in different fields of the industry [4,5]. It is mainly responsible for the fragrance of Concord grapes, contributing to the characteristic fruity aroma of wines [6]. It also present in several essential oils such as neroli and bergamot [7]. Because of its pleasant aroma, MA is used in a variety of personal care products such as body lotions, perfumes and makeups [8]. Besides, due to the property of MA of being a UV-A radiation-absorber, it is incorporated as a photoprotector to topical sunscreens formulations [9]. Finally, despite its pleasing scent for both humans and other mammals, MA and its derivatives are aversive to birds and have been used as a non-lethal bird repellent [10,11]. On this regard it is added to water surfaces in order to avoid avian depredation of fish at hatcheries, repel birds from water at airports and from rice fields [12].

Within the framework of the wide variety of practical uses and applications of MA in water, cosmetics, foodstuffs and specially in sunscreens, its natural fate constitutes a particularly interesting topic. MA, is non-hydrolizable, but it is susceptible to photoreaction when exposed to UV radiation [10]. Research works have been performed on the UV photoirradiation of MA in oxygen-free solutions, reporting a MA-trimer as main photoproduct [10]. However there are no studies about any direct or sensitized photoprocess leading aerobic MA degradation.

The direct and sensitized photodecomposition of some structurally-related MA compounds has been studied. Staudemayer and Roberts performed the photolysis of N-acetyl anthranilic acid [13]. The authors reported that, in oxygenated solution, the photodegradation took place from the excited singlet state via photo-Fries-like cleavage and rearrangement, to yield anthranilic acid, 3-aceto-2-aminobenzoic acid and 5-aceto-2-aminobenzoic acid as the major products. Otherwise in deoxygenated benzene solution, the photodecomposition occurred from excited triplet state giving benzoxazinone as the main product. Moreover Patel

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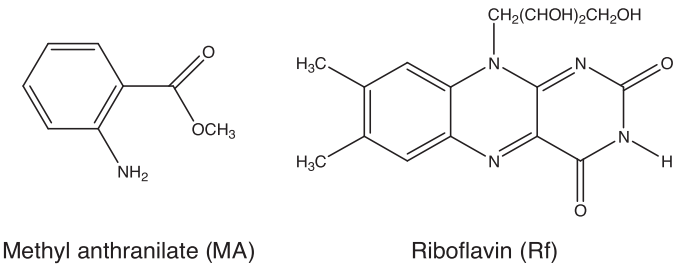
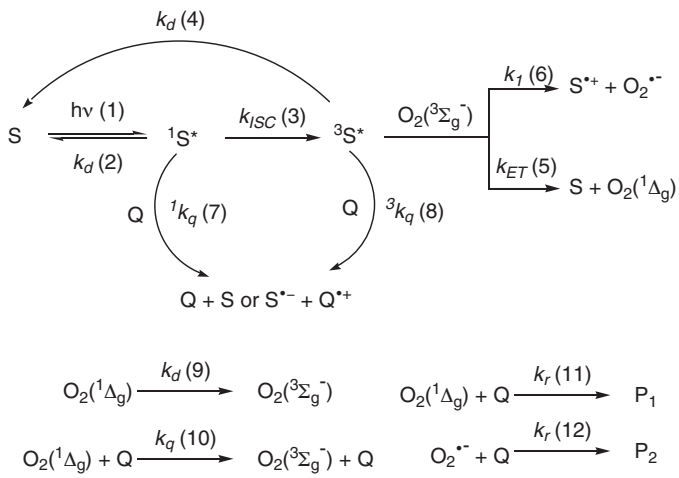


Scheme 1. Structural formulae of methyl anthranilate and riboflavin.



Scheme 2. Possible reaction sequence in a photosensitzed process.

has reported that the aminobenzoic acids undergo photosensitized reaction in the presence of methylene blue reacting with $O_2(^1\Delta_g)$ to give nitrobenzene as a photoproduct [14].

A partially daylight absorbing compound such as MA can be spontaneously degraded by photochemical processes initiated from its electronically excited state, resulting in the breakage of molecular bonds, the direct reaction with ground state oxygen in the medium, and the reaction with reactive oxygen species (ROS) generated by energy transfer or electron transfer from the excited substrate to oxygen. Also, the degradation of this compound in an aquatic natural environment can occur through its reaction with ROS if a photosensitizer is present in the medium. Riboflavin (Rf, Scheme 1), is a naturally-occurring pigment present as traces in water courses, lakes and seas, and all kind of foodstuffs [15,16]. It can play the role of a photosensitizer because upon visible light irradiation, generates $O_2(^1\Delta_g)$ and $O_2^{\bullet-}$ with quantum yields of 0.47 and 0.009, respectively [17,18].

According to our knowledge, no systematic kinetic or mechanistic studies have been carried out on the potential photodegradation process applied to MA under aerobic conditions this kind of study could help to foresee the natural degradation of this flavoring compound. The present research was undertaken in order to know the fate of MA under natural-like photoirradiation in the absence and in the presence of the ubiquitous Rf, acting the latter as a photosensitizer.

2. Materials and methods

2.1. Materials

Riboflavin (Rf), deuterium oxide 99.9% (D_2O), superoxide dismutase (SOD) from bovine erythrocytes, methyl anthranilate (MA), ascorbic acid (AA), sodium azide (NaN_3), were purchased from Sigma Chem. Co. Rose Bengal (RB), furfuryl acetate (FAc) and furfuryl alcohol (FFA) were from Aldrich. All chemicals were used as received. Water was triply distilled. Methanol and acetonitrile (MeCN), HPLC quality, were provided by Sintorgan.

2.2. Stationary photolysis

Stationary aerobic photolysis of aqueous solutions containing MA and Rf or RB was carried out in a special equipment developed in our laboratory. This device uses interchangeable blue or green LEDs (Light Emitting Diode) as photoirradiation source. Commercially available LEDs with emission maximum at 467 nm (26 nm FWHM) and 510 nm (40 nm FWHM) was employed respectively for Rf and RB photosensitized process.

Continuous aerobic photolysis of aqueous solutions of MA as sensitizer and different substrates was carried out in a home made photolyser with a 150 W quartz-halogen lamp. In all cases continuous irradiation was performed at $\lambda > 330$ nm (cut-off filter).

Ground state absorption spectra were obtained employing a Agilent HP 8452A diode array spectrophotometer.

2.3. $O_2(^1\Delta_g)$ experiments

The phosphorescence emission of singlet oxygen ($O_2(^1\Delta_g)$) was determined by time-resolved phosphorescence detection (TRPD) using a system previously reported [19]. Briefly, it consisted in a Nd:Yag laser (Spectron) as the excitation source. The emitted radiation ($O_2(^1\Delta_g)$ phosphorescence, 1270 nm) was detected at right angle using an amplified Judson J16/8Sp germanium detector, after having passed through two Wratten filters. The output of the detector was coupled to a digital oscilloscope and to a personal computer to carry out the signal processing. Usually, the average of 16 shots were needed so as to achieve a good signal to noise ratio, from which the decay curve was obtained. Air equilibrated solutions were employed in all cases.

D_2O and MeCN were employed as solvents in time resolved phosphorescence detection of $O_2(^1\Delta_g)$ in order to enlarge the lifetime of the emissive species within the detection limits of our equipment.

2.4. Quantum yield of singlet oxygen generation

The efficiency of $O_2(^1\Delta_g)$ production by MA in MeCN was determined by the comparative method already described [20], The initial intensities of the emission decay curves at 1270 nm (I_0) were determined for air-equilibrated solutions (Abs_{532} ca. 0.2) as a function of laser fluence (L_E). The output at 355 nm of the aforementioned Spectron Nd:Yag laser was employed as the excitation source. L_E was varied using neutral density filters. Φ_Δ values for MA was obtained by comparison of the slopes of the linear plots I_0 vs. L_E with that of a reference compound. The absorbance of all solutions was matched at 355 nm. Perinaphthenone was employed as a reference compound (PN) with a $\Phi_{\Delta PN} = 1$ for $O_2(^1\Delta_g)$ generation [21].

2.5. Determination of k_r and k_t

The reactive rate constant (k_r, see process (11), Scheme 2) for chemical reaction of $O_2(^1\Delta_g)$ with MA was determined using Eq. (1) for which the knowledge of the reactive rate constant for the photo-oxidation of a reference compound R is required [22]:

$$\frac{\text{slope}}{\text{slope}_R} = \frac{k_r}{k_{rR}} \quad (1)$$

Slope and slope_R are the respective slopes of the first-order plots of MA or O_2 and reference consumption under sensitized irradiation. MA was monitored by absorption measurement and FAc was

used as reference ($k_r = 5.5 \times 10^7\ M^{-1}\ s^{-1}$) [23]. Oxygen uptake in water was monitored with a 97–08 Orion electrode. Assuming that the reaction of $O_2(^1\Delta_g)$ with the quencher is the only means of oxygen consumption, the ratio of the first order slopes of oxygen uptake by the substrate and the reference compound, turns out k_r/k_{rR}. The reference compound used was FFA, which has a reported pH-independent k_r value of $1.2 \times 10^8\ M^{-1}\ s^{-1}$ [24].

The total quenching rate constant (k_t) for $O_2(^1\Delta_g)$ deactivation by MA, was determined by time resolved near-IR phosphorescence detection (TRPD). $O_2(^1\Delta_g)$ lifetimes were evaluated in the presence (τ) and in the absence (τ_0) of the quencher and the data were plotted as a function of quencher concentration, according to a simple Stern–Volmer treatment (Eq. (2)).

$$\tau^{-1} = \tau_0^{-1} + k_t[MA] \quad (2)$$

D_2O was employed as a solvent in the TRPD experiments in order to enlarge the $O_2(^1\Delta_g)$ phosphorescence lifetime within the temporal range attainable by our photodetector [24].

2.6. Fluorescence measurements

Fluorescence lifetimes were determined with a time-correlated single photon counting technique (SPC) on an Edinburgh FL-900CD instrument, equipped with a blue LED (PicoQuant PLS-8-2-208). Excitation and emission wavelengths for Rf were 450 and 540 nm, respectively. Measurements were performed at 25 ± 1 °C in air-equilibrated solutions.

In order to determine the rate constants for interactions between ^{1}Rf and MA (reaction (7)) a classical Stern–Volmer treatment of data was applied through the following equation:

$$\frac{^1\tau_0}{^1\tau} = 1 + {}^1k_q\,{}^1\tau_0[Q] \quad (3)$$

where $^1\tau$ and $^1\tau_0$ are the lifetimes for Rf fluorescence in presence and in absence of the quencher.

2.7. Laser flash photolysis experiments

Transient absorption spectra were determined in Argon-saturated 0.04 mM Rf aqueous solutions using a flash photolysis apparatus. The above described laser was employed to generate ^{3}Rf*. A 150 W Xenon lamp was used as source to the monitor beam. The detection system is comprised by a PTI monochromator and a red-extended photomultiplier (Hamamatsu R666). The signal, acquired and averaged by a digital oscilloscope (Hewlett-Packard 54504A), was transferred to a PC via a HPIB parallel interface, where it was analyzed and stored. ^{3}Rf* disappearance was monitored from the first-order decay of the absorbance at 670 nm, a zone where the interference from other possible species is negligible. To avoid self-quenching and triplet–triplet annihilation, the triplet decay was measured at low Rf concentration (typical 0.05 mM) and at low enough laser energy.

For determination of the rate constant for interaction of ^{3}Rf*–MA, the Stern–Volmer expression (Eq. (4)) was employed.

$$\frac{1}{^3\tau} = \left(\frac{1}{^3\tau_0}\right) + {}^3k_q[MA] \quad (4)$$

where $^3\tau$ and $^3\tau_0$ are the experimentally determined lifetimes of ^{3}Rf* in presence and absence of MA. Since both Rf and MA absorb at 355 nm, the quenching of ^{3}Rf* by MA was made so that the amount of absorbed light by MA was not greater than 10%.

3. Results and discussion

The following reaction scheme (Scheme 2) was employed for the evaluation and discussion of the experimental results.

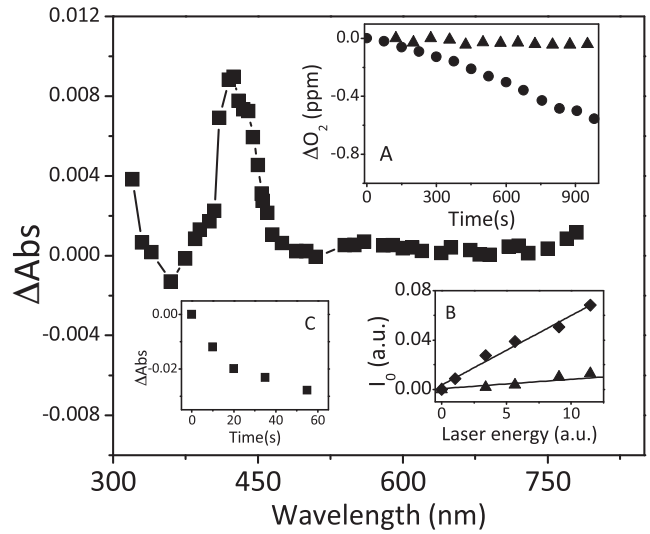


Fig. 1. Transient absorption spectra of MA (172 μM) in argon-saturated aqueous solution taken at 4 μs after the laser pulse. *Inset A*: oxygen uptake as a function of photoirradiation time by aqueous solutions of: (▲): 0.1 M mM MA + 0.5 mM ascorbic acid + 1 mM sodium azide (●): 0.1 M mM MA + 0.5 mM ascorbic acid. *Inset B*: laser fluence dependence on the amplitude of $O_2(^1\Delta_g)$ phosphorescence emission at zero time (I_0) in MeCN. (◆): PN ($Abs_{355\,nm} = 0.3$); (▲): AM ($Abs_{355\,nm} = 0.3$). *Inset C*: aerobic self-sensitized photooxidation of aqueous 0.01 mM of MA irradiated at 360 nm.

According to Scheme 2, the absorption of incident light promotes the sensitizer (S, in ground state) to electronically excited singlet and triplet states (^{1}S* and ^{3}S* respectively (processes (1–3)). Both states can either decay to ground state or can be quenched through processes (2)–(4) and/or (7)–(8). ^{3}S* may react with ground state oxygen ($O_2(^3\Sigma_g^-)$) forming the species superoxide radical anion ($O_2^{\bullet-}$) (process (6)) [18,25]. ^{1}S* or ^{3}S* can be physically quenched by Q, or through an electron transfer process could give rise to the respective semireduced ($S^{\bullet-}$) and semioxidized ($Q^{\bullet+}$) forms (process (7) or (8)). An energy transfer process from ^{3}S* to $O_2(^3\Sigma_g^-)$ dissolved in the medium, generates the species $O_2(^1\Delta_g)$ (process (5)). The excited oxygenated species can decay either by collision with surrounding solvent molecules (process (9)) or by interaction with Q through physical and/or chemical/reactive processes (steps (10) and (11) respectively).

3.1. Triplet excited MA transient absorption and $O_2(^1\Delta_g)$ generation by MA

The transient absorption spectrum of MA in aqueous solution is shown in Fig. 1. The difference spectrum obtained by laser flash photolysis show two absorption bands in the 350–700 nm range with a maximum at 425 nm and a less intense band centered at 560 nm. The spectrum exhibit a negative band (centered ca. 350 nm) caused by depletion of the ground state and all decays were monoexponential, including the recovery in the bleaching regions. All these observations strongly suggest that the observed absorption spectrum correspond to a single transient species, which is assigned to the triplet excited state of MA ($^3MA^*$). The determined lifetime of this state in aqueous solution is 280 μs.

The photoirradiation ($\lambda_{irr} > 330$ nm) of a solution containing 0.1 mM MA + 0.5 mM Ascorbic Acid (AA) gave rise to oxygen uptake (Fig. 1, Inset A). Oxygen consumption was practically suppressed when the same experiment was performed in the presence of a specific quencher of $O_2(^1\Delta_g)$, in this case a 1 mM NaN_3 was used. Under the experimental conditions the only light-absorber was MA. AA is an oxidizable biological target that reacts with $O_2(^1\Delta_g)$ with a reported rate constant $k_t = 1.42 \times 10^8\ M^{-1}\ s^{-1}$ (process (11) with

Table 1
Quantum yield of $O_2(^1\Delta_g)$ generation by MA in acetonitrile; rate constants ($M^{-1}\,s^{-1}$) for overall (k_t) and reactive (k_r) quenching of $O_2(^1\Delta_g)$ by MA; rate constants ($M^{-1}\,s^{-1}$) for the quenching of $^3Rf^*$ (^3k_q) and $^1Rf^*$ (^1k_q) by MA in water and methanol respectively.

Φ_Δ	$O_2(^1\Delta_g)$–MA		Rf*–MA	
	$k_t \times 10^7$	$k_r \times 10^6$	$^3k_q \times 10^9$	$^1k_q \times 10^9$
0.13 ± 0.02	2.1 ± 0.2	5.9 ± 0.6	3.0 ± 0.2	8.4 ± 0.3

AA instead of Q) [26]. The effect of NaN_3 strongly suggests that the reaction could be mediated by $O_2(^1\Delta_g)$, photosensitized by MA.

The ability of MA as a $O_2(^1\Delta_g)$–generator was quantified through TRPD as described in the experimental section. A quantum yield value $\Phi_\Delta = 0.13 \pm 0.01$ of $O_2(^1\Delta_g)$ generation by MA was determined in MeCN as a solvent. Data shown in Fig. 1, inset B and in Table 1.

The possible self-sensitized photooxidation of MA (reactions (5) + (11) with $^3MA^*$ instead of $^3S^*$) was also evaluated. An aqueous solution of 0.01 mM MA was aerobically irradiated at 360 nm. The degradation of MA can be quantified by monitoring the decrease of its absorption band centered at 327 nm, as shown in Fig. 1, inset C.

The preceding experiments confirm the production of $O_2(^1\Delta_g)$ upon photoirradiation of MA. The ROS can react with surrounding oxidizable targets or with the very MA. The final result will be regulated by both the relative concentrations and the respective reactive rate constants k_r of MA and the oxidizable targets.

3.2. Interaction $O_2(^1\Delta_g)$–MA. Determination of the rate constants k_t and k_r

As shown in Fig. 2, visible light irradiation of the mixture RB ($Abs_{549} = 1.2$) + 0.4 mM MA in water generates spectral changes attributable to chemical transformations in MA. RB produces $O_2(^1\Delta_g)$ with quantum yield of 0.75 [27] and it is one of the most widely employed generators of this oxidative species [28].

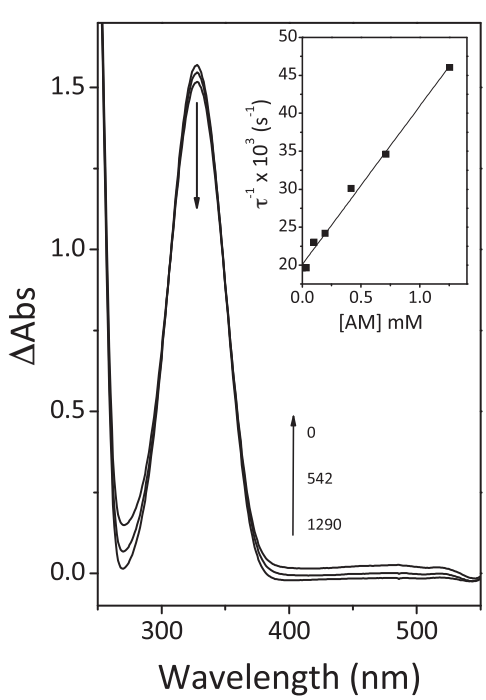


Fig. 2. Difference absorption spectra of aqueous RB ($Abs_{549} = 1.2$) + 0.4 mM MA vs RB ($Abs_{549} = 1.2$) upon irradiation at 510 nm under air-saturated conditions. Numbers on the spectra represent the irradiation time in seconds. Inset: Stern–Volmer plot for the quenching of $O_2(^1\Delta_g)$ phosphorescene by MA.

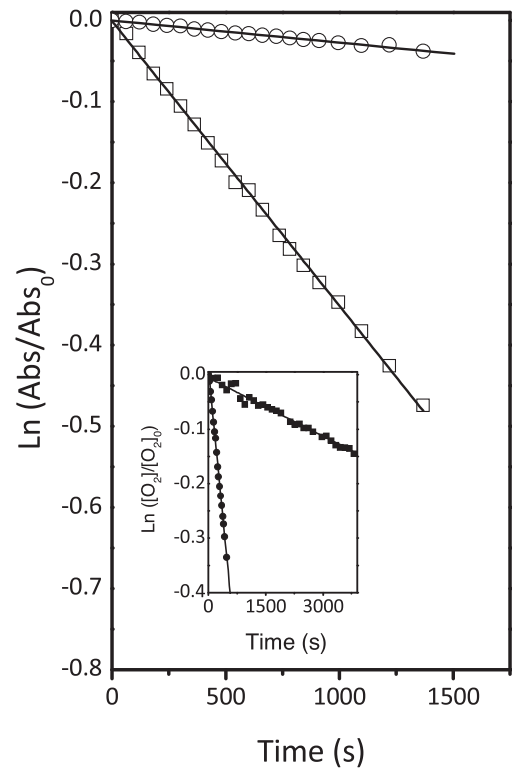


Fig. 3. First order plots of MA consumption (followed by UV–vis spectroscopy) of aqueous solutions of RB ($Abs_{549} = 1.2$) containing (○) AM (0.4 mM); (□) FAc (0.4 mM) upon visible-light irradiation. *Inset:* first-order plots for oxygen uptake upon visible light irradiation (cut-off 440 nm) in aqueous solutions containing: (●) RB ($Abs_{549} = 0.44$) + 0.45 mM FFA and (■) RB ($Abs_{549} = 0.44$) + 0.45 mM AM.

The spectral changes suggest the participation of $O_2(^1\Delta_g)$ in the degradation of MA (processes (10) and (11)). This possibility was evaluated through TRPD experiments.

MA quenches $O_2(^1\Delta_g)$ phosphorescence with an overall rate constant value $k_t = 2.1 \pm 0.2 \times 10^7\,M^{-1}\,s^{-1}$ (Table 1), employing RB as a sensitizer in D_2O as a solvent. The corresponding Stern–Volmer plot is shown in Fig. 2, inset. This experiment unambiguously demonstrates an interaction between $O_2(^1\Delta_g)$ and MA, which may be in nature merely physical (process (10)), purely reactive (process (11)) or a composition of both mechanisms operating simultaneously. The overall rate constant for the interaction MA–$O_2(^1\Delta_g)$, in the range of $10^7\,M^{-1}\,s^{-1}$ is close to that reported for aniline [29], $k_t = 1.06 \times 10^7\,M^{-1}\,s^{-1}$, a molecule which presumably represents the reactive moiety of the anthranilate.

The k_r value was determined by two different experiments: (a) by means of oxygen uptake measurements (Fig. 3, inset) and (b) by monitoring MA consumption from the decrease in the 327 nm absorption band, by means of UV–vis spectroscopy (Fig. 3, main). Rate constant values k_r of $5.9 \pm 0.6 \times 10^6\,M^{-1}\,s^{-1}$ and $6.8 \pm 0.7 \times 10^6\,M^{-1}\,s^{-1}$ respectively were obtained. The coincidence between both values is within the estimated experimental error. Each value was obtained from an independent experiment and referred to different independent reference literature values (see experimental). Table 1 reports the average value of the results obtained by both methods.

The quantum efficiency of a $O_2(^1\Delta_g)$-mediated oxidation can be evaluated through the expression $\Phi_r = k_r\,[Q]/(k_d + k_t\,[Q])$, where k_d is the rate constant of the $O_2(^1\Delta_g)$ deactivation by interaction with solvent molecules (process (9)). This expression cannot be applied when the substrate concentration is unknown as in the cases of complex biological or natural environments. In these cases a useful and simpler approach is the evaluation of the k_r/k_t ratio, i.e.

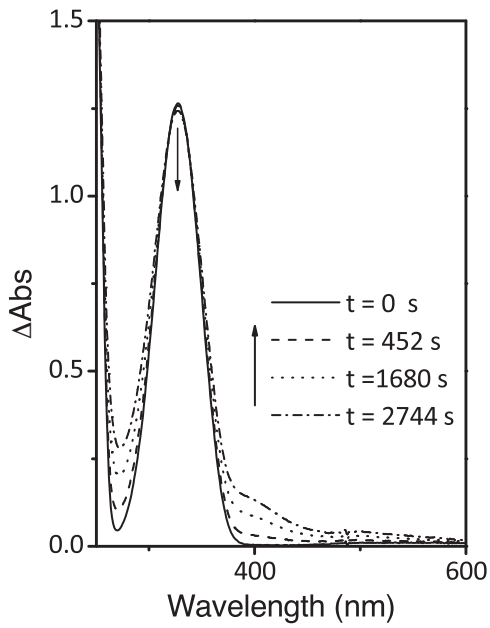


Fig. 4. Difference absorption spectra of aqueous solutions 0.033 mM Rf + 0.3 mM MA vs 0.033 mM Rf upon irradiation at 467 nm under air-saturated conditions. Numbers on the spectra represent irradiation time in seconds.

the fraction of overall quenching that effectively leads to chemical reaction. The moderately low value for the quotient $k_r/k_t \sim 0.3$ exhibited by MA could be a desirable quality when compared with salicylates and benzophenones which are among the oldest commercially available UV absorbers [30]. These species only exhibits reactive quenching in the alkaline pH range, and have been reported k_r/k_t values of 0.5 for 1′-hydroxy-2′-acetonaphthone [31], 0.01 for salicylic acid (SA) and 0.46 for methyl salicylate [32]. Although SA shows the lower and most favorable k_r/k_t ratio this only occurs in basic medium where the salicylate is the reactive species while in pH 7 water no interaction with $O_2(^1\Delta_g)$ could be detected. A low k_r/k_t value implies the elimination of $O_2(^1\Delta_g)$ without appreciable loss of the scavenger. This action, in a given environment, represents a protection for surrounding oxidizable molecules, a desirable property for sunscreens components.

3.3. Interaction Rf–MA

The difference spectrain Fig. 4, show that the photoirradiation of the mixture 0.03 mM Rf + 0.3 mM MA vs 0.03 mM Rf in water produces spectral changes, these are qualitatively similar to those shown in Fig. 3 for the case of RB-sensitization. In parallel, oxygen consumption was observed upon photoirradiation of an aqueous solution containing 0.04 mM Rf plus 0.6 mM MA. The experimental evidence strongly suggests the participation of Rf electronically excited states, the possible interaction of these states with MA and the possible involvement of ROS. In order to investigate these possibilities a systematic kinetic and mechanistic study was carried out.

The potential quenching of ^{1}Rf* by MA was studied in MeOH, a more suitable solvent due to the limited solubility of the anthranilate in water. Rf presents an intense fluorescence, centered at 543 nm, emission band. The reported fluorescence quantum yield (Φ_F) of Rf in MeOH is 0.39 [33]. MA quenches the fluorescence of ^{1}Rf* decreasing the stationary emission intensity, while the shape of the emission spectrum does not change. The fluorescence of ^{1}Rf* showed a single-exponential decay with a life-time ($^1\tau_0$) of 5.7 ± 0.1 ns, in agreement with published results [33]. The presence of MA in the range 5–30 mM produces a decrease in the lifetime

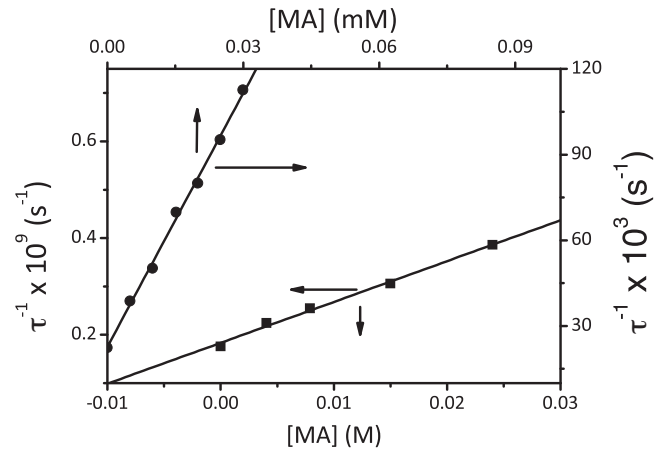


Fig. 5. (■) Stern–Volmer plot for the time-resolved quenching of the fluorescence of Riboflavin by MA in methanol. (●) Stern–Volmer plot for quenching of ^{3}Rf* by MA.

of ^{1}Rf*. From a simple Stern–Volmer treatment a rate constant $^1k_q = 8.4 \pm 0.3 \times 10^9\ M^{-1}\ s^{-1}$ was determined (Fig. 5 and Table 1).

Both, anaerobic and aerobic photodegradation rates of Rf, as monitored by the decrease in the Rf-absorption band at 445 nm, suffer an increase in the presence of 0.1 mM MA, strongly suggesting an interaction ^{3}Rf*–MA. The photodegradation of Rf is well known to occur from ^{3}Rf* in anaerobic conditions [25] and with the contribution of a $O_2(^1\Delta_g)$-mediated process in the presence of air [17]. The graphical representation of the anaerobic runs is shown in Fig. 4.

Results demonstrates an interaction between ^{3}Rf*–MA. Bimolecular quenching rate constant ^3k_q of $(3.0 \pm 0.2) \times 10^9\ M^{-1}\ s^{-1}$ (process (8) with Rf instead of S) was graphically obtained Fig. 5 for MA (Table 1). Regarding the interaction of Rf electronically excited states, ^{1}Rf* and ^{3}Rf,* with MA, a simple calculation through the Stern–Volmer equation, employing the ^1k_q value obtained indicates that a concentration of MA 0.5 mM, similar to those employed in the experiments of Rf-photosensitization, would produce a decrease in the lifetime of ^{1}Rf*, lower than 2%. Hence, under work conditions all effects derived from the interaction of the MA with electronically excited Rf could be exclusively ascribed to an interaction with ^{3}Rf*.

^{3}Rf* may generate ROS via processes 5 and 6 (with Rf instead of S), and the production of $O_2^{\bullet-}$ (process (6) with Rf instead of S) may be increased through processes (13)–(16). An electron transfer reaction generates the reduced species $Rf^{\bullet-}$ (reaction (8) which is rapidly protonated (step (14) [16,35]) and in a further step produces the ROS $O_2^{\bullet-}$ (reactions (15)–(17)).

$$Rf^{\bullet-} + H^+ \rightarrow RfH^{\bullet} \quad (13)$$

$$2RfH^{\bullet} \rightarrow RfH_2 + Rf \quad (14)$$

$$RfH_2 + O_2(^3\Sigma_g^-) \rightarrow RfH_2^{\bullet+} + O_2^{\bullet-} \quad (15)$$

$$RfH_2^{\bullet+} + O_2^{\bullet-} \rightarrow Rf + H_2O_2 \quad (16)$$

$$H_2O_2 + MA \rightarrow P(10) \quad (17)$$

In order to evaluate the eventual participation of $O_2^{\bullet-}$ in the reaction mechanism, the photolysis experiments was performed in the individual presence of 0.075 μM SOD. The enzyme is a specific $O_2^{\bullet-}$ quencher (reaction (18)) and it has been frequently employed, in similar concentrations, to confirm/discard the participation of the oxygenated species in a given reaction step [34,35]. Results,

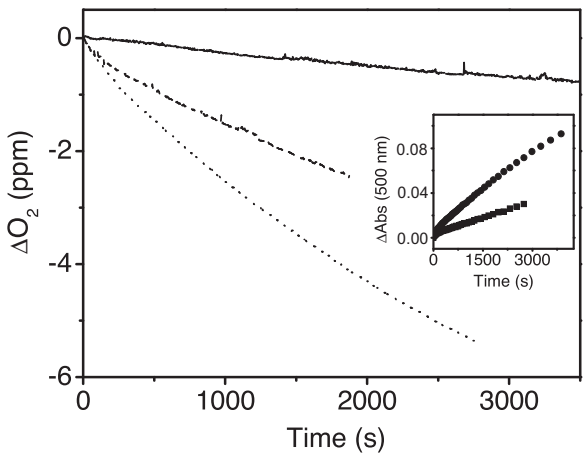


Fig. 6. Oxygen uptake upon visible-light irradiation (cut off 400 nm) of aqueous solutions containing: (——) Rf ($Abs_{445\,nm}$ = 0.4) (– –) Rf ($Abs_{445\,nm}$ = 0.4) + 0.6 mM MA; (• • • •) Rf ($Abs_{445\,nm}$ = 0.4) + 0.6 mM MA (0.6 mM) + 0.075 μM SOD. *Inset*: absorbance increase at 550 nm of aqueous solutions of Rf ($Abs_{445\,nm}$ = 0.4) + 0.6 mM MA (0.6 mM) (■) and Rf ($Abs_{445\,nm}$ = 0.4) + 0.6 mM MA + 0.075 SOD (●) upon photoirradiation at 467 nm.

shown in Fig. 6 main, indicate an increase in the rate of oxygen uptake in the presence of SOD.

$$2O_2^{\bullet-} + 2H^+ \xrightarrow{SOD} O_2(^3\Sigma_g^-) + H_2O_2 \quad (18)$$

The observed increase of oxygen consumption in the presence of SOD could be explained as follows, on the basis of scarcely effective reaction (12): process (18) (with SOD) yields one oxygen molecule from two $O_2^{\bullet-}$ radicals (generated from one oxygen molecule, process ((6) or (15)) and H_2O_2, and the latter oxidant, as well as the amount produced by process ((6) or (15)), can react with a MA (process (12)). Besides, the regeneration of oxygen through process (18) could favor the production of the reactive species $O_2(^1\Delta_g)$ (process (5)), which could react through process (11). This reaction sequence should favor the production of the reactive species $O_2(^1\Delta_g)$ (process (5)). In this way both competitive steps (5) and (8) would render $O_2(^1\Delta_g)$.

The photoirradiation of aerobic solutions of Rf ($Abs_{445\,nm}$ = 0.4) and MA (0.6 mM) results in a progressive oxidation of MA to a product whose accumulation could be followed at 500 nm, where Rf has non absorption. The presence of SOD, increased the rate of product formation, as shown in the inset of Fig. 5. This finding is in line with the interpretation on the effect of SOD above discussed.

4. Conclusions

Spectroscopic evidence, oxygen uptake results, time resolved $O_2(^1\Delta_g)$ phosphorescence assays and auxiliary specific ROS experiments strongly support the occurrence of effective quenching of the ROS $O_2(^1\Delta_g)$ and H_2O_2 by MA. Apparently the anilinic molecular moiety is responsible for $O_2(^1\Delta_g)$-mediated oxidative process.

MA generates $O_2(^1\Delta_g)$ in solution upon direct photoexcitation with a quantum yield of 0.13 ± 0.01, a non desirable property for a compound employed in sunscreens formulations. Nevertheless MA qualifies as an acceptable scavenger of $O_2(^1\Delta_g)$ provided that physically eliminates the oxidative species without a substantial self-degradation. Only one-third of overall $O_2(^1\Delta_g)$-quenching events effectively leads to chemical reaction.

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