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Photodegradation of Prednisolone under UVB solar irradiation. Role of ROS-photogenerated in the degradation mechanism.

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Abstract

The use of biologically active substances with anti-inflammatory properties such as corticosteroids has increased considerably in the last decades. Particularly, the compound we are interested in, Prednisolone (Predn), is a glucocorticoid with high biological activity. This compound absorbs UV radiation and may participate in photochemical processes, which can result in its own decomposition. These processes could result in the formation of free radicals or reactive oxygen species (ROS).On these grounds, the kinetic and mechanistic aspects of the direct photodegradation of Predn have been studied in aqueous medium under different atmospheric conditions by stationary and time-resolved techniques. The mechanism involved in the photodegradation has been elucidated. Predn is capable of generating the excited triplet state ^{3}Predn* as a result of UVB light absorption. In the presence of oxygen, ^{3}Predn*allows the formation of ROS, of which the $O_2(^1\Delta_g)$ (Φ_Δ=0.014), H_2O_2 and the radical OH$^\bullet$ highlight. The latter is generated from spontaneous dismutation of $O_2^{\bullet-}$ and subsequent homolytic cleavage, photochemically promoted, of H_2O_2. Predn undergoes unimolecular photodegradation reactions in inert argon atmosphere.

In this study we found that in the presence of oxygen, the Predn photo consumption is improved. This implies that the attack by ROS involves a very important additional contribution to the photodegradation of Predn in aerobic conditions.

Keywords: Prednisolone, corticosteroids, photodegradation mechanism, reactive oxygen species.

1.　　Introduction

Any substrate possessing the ability to absorb photons can participate in a photochemical process; i.e. may be photoreactive, which can lead to its own degradation or degradation of substances associated to it/or present in the medium, among which compounds of biological origin such as proteins, sugars, lipids, or nucleic acids, are the most important. Moreover, a drug product can be exposed to natural or artificial light during production, storage and use. The great majorities of pharmaceutical substances absorb UV radiation and may participate in a photochemical process which can result in its own decomposition. These processes could cause the formation of free radicals or reactive oxygen species (ROS) [1].

On the other hand, the use of biologically active substances with anti-inflammatory properties has increased considerably in the last decades. In the present contribution, we are interested in the photochemical behavior of corticosteroids. These compounds are the main anti-inflammatory drugs currently used, they are synthetic derivatives of hydrocortisone, a steroid hormone produced in the adrenal gland [2]. They are highly used in many fields of modern medicine [3] because they possess the ability to inhibit immunological, inflammatory and allergic processes that occur in response to external harmful stimuli [4]. Particularly, the compound we are interested in, Prednisolone (Predn)is a glucocorticoid with high biological activity. It is part of the list of "Essential Medicines" created by the World Health Organization (WHO) [5], which nucleates all drugs that are considered important in health care of most of the population. Predn is the biologically active metabolite of prednisone, which is obtained by dehydrogenation of the

molecule of Cortisone in positions 1 and 2 of the cyclopentane-perhydro-phenantrene nucleus [6].

A very important and exhaustive compilation about photostability of drugs, has been done by H.H. Tønnesen [7]. Particularly, the photoreactivity of glucocorticosteroides have been investigates in solution as well in solid state and it has been demonstrated that these compounds must to be protected from light. Glucocorticosteroides cross conjugates such as prednisolone, prednisone betamethasone, triamacinolone and others result to be quite photoreactive. This fact may expect taking into account that the efficient photorearrangement of cyclohexadienones to bicycle hexanones is well known [8].

Another aspect that should be considered when studying the photochemical behavior of drugs is the new approach that has been given to the study of these compounds as environmental pollutants, specifically as water pollutants [9,10]because when these drugs are consumed by humans, they are not fully metabolized and are eliminated virtually unchanged through the urine or feces. Only some of these substances can be removed by waste-water treatment plants; consequently, the drugs reach water reservoirs [11,12], accumulate and cause ecological damage, as well as hormonal and immunological changes on aquatic populations[13]. These substances are part of the group of emergent contaminants and their impact on the environment is not fully understood.

Even when the toxicity of Predn and its photochemical derivatives on aquatic organisms have been investigated by other authors [14], the mechanism and kinetic aspects of the photodegradation process has not yet been elucidated.

Several studies related to photochemical behavior of Predn and structural related compounds under an inert atmosphere have been done. Beginning pioneering works related to the photochemistry of prednisone acetate and related substances [15], Williams

et al. decided to revisit the issue [16,17]. They propose to re-investigate the photochemistry of various steroids, finding differences in the photoproducts obtained in deoxygenated organic solvents and mixtures of acetic acid/water, and concluding that photoisomerizations of cross-conjugated cyclohexadienones take place via an n-π* excited triplet state and show a strong solvent dependence.

More recently, Ricci *et al.* [18] returned to the subject of photochemistry (in oxygen free atmosphere) of Pregna-1,4-dien-3,20-diones and related molecules in which fluorine or chlorine atoms were incorporated. They found different reaction products depending on the wavelength at which the compounds (dissolved in acetonitrile) were irradiated.

On the other hand, a study [19] of the photocatalytic transformation of dexamethasone using titanium dioxide as a photocatalyst have demonstrated that the photodegradation occurs through the formation of some hydroxyl derivatives. The main oxidation step occurs from the attack by one OH$^{\bullet}$ radical.

Despite this, very little is known about its photochemistry in aqueous medium under aerobic conditions. Here we present a study on the photochemistry of this emergent pollutant under advantageous conditions to achieve its natural degradation in the environment, i.e. solar irradiation of watercourses with dissolved oxygen. The focus was placed on the influence of ROS as intermediaries for the Predn degradation under such conditions.

The importance of studying the mechanism of photodegradation of Predn, which are of great pharmacological and environmental importance, is that the elucidation of pathways which are part of the mechanism, will allow access to important information, such as: a) the feasibly of these events occur under certain environmental condition, b) the

speed and efficiency of the process, c) the values of controllable variables to avoid the photodegradation, d) the possible photoproducts formed, to mentioned only a few.

The aims of this study were to identify the behavior of Predn under UVB sunlight irradiation and to determine the influence of oxygen on the degradation reactions of the drug. To accomplish these goals, the study of the photophysical and photochemical processes of the glucocorticoid Predn, under different experimental conditions, has been done by using different techniques in order to evaluate the participation of the different ROS and to propose a possible mechanism which accounts for the processes and species involved in the degradation of the compound.

2. Experimental

2.1. Materials

Prednisolone 99% (Predn), superoxide dismutase from bovine erythrocytes Biuret>95% (SOD) and catalase from bovine liver (CAT) Biuret> 95%. Sodium azide99% (NaN$_3$) and isopropanol 99.7% (isoOH) were purchased from Merck. Furfuryl acetate 99% (FFAc), Perinaphthenone 97% (PN) and Rose Bengal 95%were provided by Aldrich Chemical Company. Acetonitrile (MeCN), HPLC quality, were provided by Sintorgan.

All these compounds were used as received. The water used was triply distilled. All measurements were carried out with freshly prepared solutions at 298 K, except when other temperature is indicated. In order to increase the solubility of Predn, 1% MeCN was added. Where otherwise indicated, "aqueous solution" refers to water/MeCN 99/1% v/v mixture.

2.2. Methods

2.2.1. Stationary photolysis experiment

Stationary direct photolysis experiments were carried out by irradiation of aqueous solutions containing Predn, ([Predn]=4×10^{-5} M), which was irradiated with monochromatic light at the maximum of the absorption band (248 nm, see Figure 1) at room temperature. The equipment used consists on a Photon Technology International (PTI) unit provided with a high pass monochromator, and equipped with a 150 W Xenon lamp UV-visible absorption spectra which were recorded on a Shimadzu UV-2401PC spectrometer (Shimadzu Corporation, Tokyo, Japan).

Experiments in air-equilibrated aqueous solutions were carried out in a quartz spectrophotometric cell, open to the atmosphere and under constant magnetic stirring. In the experiments in which it was necessary to ensure a high concentration of oxygen, the reaction cell was continuously bubbled with this gas. Experiments under anaerobic conditions were made in a quartz cell provided with a hermetic seal and bubbled with argon for 15 min.

For the determination of the direct photodegradation quantum yields of Predn (Φ_R^{Predn}), measurements were performed according to the method described by Rahn *et al*. [20] using a KI/KIO$_3$ mixture in a borates buffer as actinometer.

To establish the possible involvement of different ROS in the photodegradation of Predn, direct photolysis experiments were carried out in the presence and in the absence of specific ROS scavengers.

In order to corroborate the ability of Pred to generate O$_2$($^1\Delta_g$), the photooxidation of FFAc in the presence of the corticoid was evaluated in oxygen and argon atmosphere following the decrease of the absorption band of FFAc at 215 nm. FFAc react with O$_2$($^1\Delta_g$) with a rate constant value of $5.5 \times 10^7 \, M^{-1} s^{-1}$ [21].

2.2.2. *Laser flash photolysis experiment*

Transient absorption measurements were performed using laser-flash photolysis equipment. A Spectron SL400 Nd:YAG laser generating 266 nm laser pulses (~18 ns pulse width) was used for sample excitation. The laser beam was defocused in order to cover all the path length (10 mm) of the analyzing beam from a 150 W Xenon lamp. The detection system comprises a PTI monochromator coupled to a red-extended Hamamatsu R666 PM tube. The signals initially captured by a HP54504 digitizing oscilloscope were averaged and then transferred to a computer for storage and analysis. The experiments were determined in argon-saturated MeCN solutions.

2.2.3. *Time-resolved singlet oxygen phosphorescence detection*

The time-resolved $O_2(^1\Delta_g)$ phosphorescence detection (TRPD) has been previously described [22]. Briefly, it consisted of a Nd:YAG laser (Spectron) as the excitation source. The output at 266 nm was employed to excite Predn and PN. The emitted radiation (1270 nm) was detected at right angles using an amplified Judson J16 ∕ 8Sp Germanium detector after passing through appropriate filters. The output of the detector was coupled to a digital oscilloscope and to a personal computer for processing the signal. Sixteen shots were averaged to get a good signal-to-noise ratio, from which the decay times were calculated [23].

The $O_2(^1\Delta_g)$ production by Predn (ϕ_Δ) was determined in MeCN by the comparative method already described [24]. The dependence of $O_2(^1\Delta_g)$ phosphorescence intensity at 1270 nm, extrapolated to zero-time (I_0), on the laser fluence (E_L) was measured for Predn and the reference solutions, changing the laser fluency by employing filters of neutral

density. ϕ_Δ value was calculated by comparison of the slopes of the linear plots of I_0 $vs.E_L$ for reference and sample.

It is known that the slope can be considered as proportionally constant, which accounts for electronic and geometric characteristics of the detection system and solvent properties. Hence, a reference is needed for calibration. The reference was PN in MeCN (ϕ_Δ=0.95) [25]. Measurements of sample and reference under identical experimental conditions, constant characteristics of irradiation wavelength and matched absorbances allow the evaluation of ϕ_Δ.

2.2.4. Semi-empirical molecular orbital calculations

Semi-empirical molecular calculations (AM1) were carried out using CS Chem3D Pro software from Cambridge Soft Corporation. AM1 was employed in structure geometry optimization and calculations for the solute dipole moment.

3. Results and Discussion

3.1.Photolytic kinetic and quantum yield of photodegradation

Although UVB radiation range comprises from 280 nm to 315 nm, the excitation of the sample was carried out in the maximum of the absorption band, in order to obtain a greater sensitivity in the observed changes.

The photodegradation of Predn was studied in aqueous solution by direct irradiation with monochromatic light at the maximum of the absorption band (248 nm) as a function of time, following the changes in the absorption spectra of the corticoid.

In order to ensure the absence of substrate aggregation effects in the solvent used in this study, the absorption spectra of Predn in the water/MeCN 99/1% v/v mixture were

determined at different substrate concentrations from 1.5×10^{-5} to 3.0×10^{-3} M. As shown in Figure 1-a (supplementary material) no spectral changes although the change in concentration is more than two orders of magnitude.

In addition, to confirm the absence of aggregation phenomena, absorption spectra were determined in different solvents, including MeCN and MeOH, for which the solubility of Predn is much higher than that corresponding to water. As can be seen in Figure 1-b (supplementary material) there is only a characteristic band shift for the variation of polarity of the medium, without hint of new bands or shoulders.

The solubility of the corticoid in the solvent mixture used is consistent with the moderate dipole moment calculated for Predn (5.2 D, see section 2.2.4.).

In order to evaluate the influence of the oxygen concentration in the process, equivalent experiments were made at different atmospheric condition: argon-saturated solution, oxygen-saturated solution and air-balanced solution. The irradiation at 248 nm produces changes in the Predn absorption spectra; the result obtained for oxygen and argon atmospheres are shown in Figure 1. In all cases, a decrease in the intensity of the absorption band with the increase of irradiation time was observed as a consequence of Predn photodegradation. Although the consumption of the corticoid is independent of the nature of the atmosphere, the spectral modifications in inert atmosphere conditions are different from those in the presence of oxygen. In oxygen atmosphere, a decrease of the band is observed without noticeable shifts in peak position as well as two clear isosbestic points, while in inert atmosphere, decreased absorption is observed with a progressive blue shift of the maximum. These results are a clear signal that the photoproducts formed in the absence of oxygen are different in nature from those obtained in the presence of this gas.

It must be noted; that the photoproducts of prednisolone, dexamethasone and prednisone have been already identified under sunlight irradiation in air saturated aqueous solution, through different techniques [14,26]. However, the mechanistic aspects have not been elucidated. In this context, we carry out several experiments with the aim to have evidence to propose a possible mechanism.

Based on the observed changes in the absorption spectra, the photodegradation quantum yield of Predn (Φ_R^{Predn}) was determined by actinometrical measurements using the equation below:

$$ln\left(\frac{10^A-1}{10^{A_0}-1}\right) = \pm\Phi_R I_0 \varepsilon l \times t \qquad \text{Equation 1}$$

where the + or - sign depends on whether we consider the formation of a product or disappearance of a reagent, respectively.

From the slope of the plot of the logarithmic terms *vs.* irradiation time in equation (1) and, knowing the actinometer quantum yield (Φ_R^{Act}) and its molar absorption coefficient (ε_{352}=26400 M^{-1}s^{-1}) [15] in the experimental conditions, it is possible to determine the value of the incident radiation intensity (I_0). Then, with the I_0 value and knowing Predn molar absorption coefficient (ε_{248}=18500 M^{-1}cm^{-1}, determined in the present work) in the same experimental condition that the actinometer, it is possible to calculate Φ_R^{Predn} from the slope of the plot of the logarithmic terms *vs.* irradiation time in equation (1). The respective values of Φ_R^{Predn} at different atmospheric condition are summarized in Table 1.

Considering that the substrate concentration is 3×10^{-5}mol/L, this represents the upper limit for the concentrations of all the reactive species formed (if its generation

efficiencies were unitary).Since the concentration of oxygen in air saturated water at 298 K

is 0.27 mmol/L [27], this value represents a large excess compared to the initial substrate

concentration, and for this reason no differences are observed when the concentration of

dissolved oxygen is increased. Then it reaches a saturation situation (plateau) which

justifies that the Φ_R^{Predn} determined in the atmospheres of air and oxygen are practically the

same. Experiments were performed in triplicate, always obtaining the same values.

On the other hand, considering that Predn photodegradation obeys a first-order

kinetic behavior and reordering equation 1, the apparent first-order rate constant (k_{app}) can

be determined from the semi-logarithmic plot of A_t/A_0 as a function of irradiation time

(equation (2)) for each experimental condition, based on the analysis of the decrease in the

absorbance at 248 nm.

$$ln\left(\frac{[A]_t}{[A]_0}\right) = -2.303\,\ell(\Phi_\lambda I_0)\varepsilon_\lambda \times t = -k_{app} \times t$$

Equation 2

The first-order plots are shown in Figure 2 and the k_{app} values are displayed in Table

1. Under complete or partial oxygenation conditions, it is observed that the

photodegradative k_{app} of Predn is higher than the one found in anaerobic conditions.

This provides evidence that molecular oxygen is involved, in some way in

photochemical reactions thus allowing inferring Predn can generate ROS as a result of the

UV-absorption. Self-sensitization capacity for the photochemical generation of ROS by

steroid compounds has been lately demonstrated in the case of the steroid estrogens, 17α-

ethynylestradiol[28].

Furthermore, it is very important to note that Predn also undergoes photochemical

reactions in the absence of oxygen. This behavior is probably due to typical unimolecular photochemical reactions, mainly lumiketone rearrangement following the UV light absorption[17,29].

However, other authors have done a very interesting research for two synthetic steroids with a great anabolic potency: Boldenone and Trenbolone [30]. They found a different behavior, observing that the rates of the photo consumption process are identical in the absence and in the presence of oxygen, in the reaction mixture. This fact implies that the ROS are not involved in the photodegradation mechanism of such steroids.

3.2.Interaction of Predn with photogenerated ROS

According to what was discussed above, molecular oxygen plays an important role in the process of direct photodegradation of Predn, and this process could be the result of the participation of photogenerated ROS, obtained by self-sensitization from the corticoid. In order to elucidate the nature of ROS involved in the process, photolysis experiments with monochromatic light irradiation in the presence and in the absence of additives with ROS-scavenging capacity were carried out in aqueous solution. For SOD, CAT and isoOH the wavelength irradiation was 248 nm, while for NaN_3 the experiment was carried out at 260 nm to avoid the absorption of this salt.

As shown in Figure 3, no change was observed in the rate of photo-consumption of Predn in the presence of 10 mM NaN_3 as compared to the respective photo-consumption in the absence of the salt. This salt is an exclusive physical quencher of $O_2(^1\Delta_g)$ with a rate constant of 3×10^8 M^{-1} s^{-1} in aqueous solution (*process 1*) and has been used previously, in similar concentration, to ascertain whether $O_2(^1\Delta_g)$ participates in a given reaction

mechanism [31,32]. Then, these results suggest that $O_2(^1\Delta_g)$ is not involved in Predn photodegradation under this work experimental conditions. Section 3.3 will show that the photoproduction of singlet oxygen is very low with a Φ_Δ of 0.014 ± 0.002.

To confirm that $O_2(^1\Delta_g)$ does not contribute to the consumption of Predn, a photolysis experiment sensitized by Rose Bengal (which has a quantum yield of $O_2(^1\Delta_g)$ formation of 0.7 in water [33]) was carried out. The purpose of this experiment was to generate $O_2(^1\Delta_g)$ at much higher concentration than that produced by energy transfer from the triplet excited state of Predn ($^3Predn^*$) to the ground state of oxygen. In this experiment (using cut-off filters to avoid direct excitation of the substrate) no changes in the concentration of Predn were observed even after prolonged irradiation. (See figure 2 in supplementary material). This result confirms clearly that there is no reaction of Predn with $O_2(^1\Delta_g)$.

$$O_2\left(^1\Delta_g\right) + N_3^- \;\rightarrow\; {}^3(N_3^-)^* + O_2\left(^3\Sigma_g\right) \qquad\qquad \textit{process 1}$$
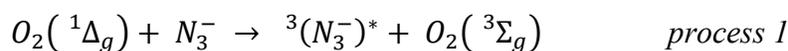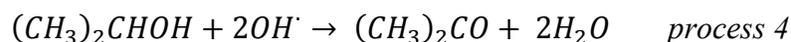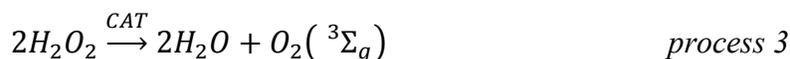
A similar experiment was performed in the presence of 1 mg/100 ml SOD. This enzyme, which catalyzes the dismutation of $O_2^{\bullet-}$ through *process 2,* has been utilized previously as a quencher of $O_2^{\bullet-}$ mediated photooxidation, in similar concentrations to those employed in this work [34-36].

$$2O_2^- + 2H^+ \xrightarrow{SOD} O_2\left(^3\Sigma_g\right) + H_2O_2 \qquad\qquad \textit{process 2}$$
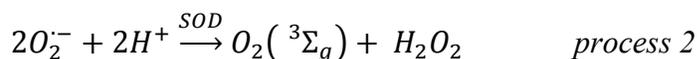
The increase in the rate of Predn photo-consumption induced by the presence of the enzyme, supports the idea of the involvement of the $O_2^{\bullet-}$ species. It is well known [37,38],

that in an $O_2^{\bullet-}$ mediated mechanism, SOD can either preclude or stimulate the oxidation of a given substrate, depending on the particular mechanistic role. That is, the latter effect will depend on whether ROS acts as a final oxidative species or as an intermediate one in the subsequent generation of other ROS. Due to the fact that eventually other ROS such as $OH^{\bullet}$ and H_2O_2 can be generated from $O_2^{\bullet-}$, equivalent experiments in the presence of specific scavengers for $OH^{\bullet}$ and H_2O_2 (0.2 M isoOH and 1 mg/100 ml CAT, respectively) have been performed. CAT and isoOH had been used previously for the detection of ROS [28,39-41]. The species H_2O_2 is decomposed by CAT according to *process 3* whereas $OH^{\bullet}$ reacts with isoOH by *process4*.

$$2H_2O_2 \xrightarrow{CAT} 2H_2O + O_2\left(^3\Sigma_g\right) \qquad\qquad process\ 3$$

$$(CH_3)_2CHOH + 2OH^{\cdot} \rightarrow (CH_3)_2CO + 2H_2O \qquad process\ 4$$

In Figure 3, a decrease in the Predn photo-consumption in the presence of both ROS-scavengers is observed. These results could indicate that, initially, the abovementioned species (H_2O_2 and $OH^{\bullet}$) are involved in the Predn photodegradation mechanism. The H_2O_2, generated from $O_2^{\bullet-}$ dismutation, undergoes homolytic cleavage giving $OH^{\bullet}$. The decomposition reaction of H_2O_2 photochemically catalyzed (*process 5*) has been extensively studied [42-44]. The irradiation conditions used in this work could facilitate the photogeneration of $OH^{\bullet}$ from H_2O_2.

$$H_2O_2 \xrightarrow{h\nu} 2OH^\cdot \qquad\qquad process\ 5$$

Similar results were found for 17α-ethynylestradiol upon addition of isoOH into solution. The degradation rate decreased. This indicates that the $OH^\cdot$, photogenerated from H_2O_2, is involved in the self-sensitization photodegradation of this steroid [28].

To reinforce the participation of the radical OH• in the photolysis of Predn, a similar experiment (to that carried out with isoOH) was done in the presence of mannitol, which is another typical OH• radical trap. The rate of consumption of Predn in the presence of mannitol decreases with respect to similar experience in the absence of this OH• scavenger.

Although photolysis experiments in the presence of NaN_3 do not present significant changes in Predn photodegradation followed by absorption, it is possible that $O_2\,(^1\Delta_g)$ could be generated in the medium, but does not react with the corticoid. To corroborate this, photooxidation experiments of a reference compound, furfuryl acetate (FFAc), were made in oxygen-saturated and argon-saturated aqueous solutions in the presence of Predn. FFAc selectively reacts with $O_2\,(^1\Delta_g)$ [45] through *process 6*.

$$FFAc + O_2\left(^1\Delta_g\right) \rightarrow Products \qquad\qquad process\ 6$$

The decrease in FFAc absorbance with the increase of irradiation time in oxygen-saturated condition (Figure 4), as well as the absence of changes in inert atmosphere (inset, Figure 4) allows us to conclude that $O_2\,(^1\Delta_g)$ is generated. However, this ROS does not interact with Predn, which justifies the consumption profile of the corticoid in the presence of NaN_3.

All the above evidence indicates that neither O_2 $(^1\Delta_g)$, nor $O_2^{\bullet-}$, nor H_2O_2 react with Predn, which allows us to assume that $OH^\bullet$ is the species responsible for the degradation of Predn. This agrees with the previously photodegradation reported for other related corticosteroid (dexamethasone) [19], in which the photoinduced transformation also was initiated only by $OH^\bullet$ radical attack.

3.3. Singlet oxygen generation by Predn

To corroborate the effects observed in the photolysis experiments with FFAc in the presence of Predn, the ability of the corticoid as a $O_2(^1\Delta_g)$ generator was quantified through TRPD as described in the experimental section. Quantum yield of $O_2(^1\Delta_g)$ generation by Predn (Φ_Δ) was determined in MeCN and by using PN as a reference. Results are shown in Figure5 and the value obtained: 0.014 ± 0.002 was determined based on equation 3:

$$\Phi_\Delta(Predn) = \frac{slope\ (Predn)}{slope\ (PN)} \times \Phi_\Delta(PN) \qquad \text{Equation 3}$$

Although Φ_Δ has a low value, Predn generates $O_2(^1\Delta_g)$ photochemically, as it was demonstrated by this experiment as well as by the FFAc photodegradation.

Other authors [46,47] have done a comprehensive investigation for other corticoids, flumethasone and fluocinoloneacetonide. They found that these substrates generate $O_2(^1\Delta_g)$ under UVB irradiation with a remarkable efficiency for fluocinoloneacetonide than for flumethasone.

However, $O_2(^1\Delta_g)$ is not involved in the photodegradation process of Predn as it was evidenced in the photolysis experiments with the ROS-scavenger NaN_3.

3.4.Transient species generation

Because the carbonyl photochemical reactions and the photogeneration of ROS can occur from ^{3}Predn*, laser flash photolysis measures were performed with the aim to detect the presence of this transient species.

The transient (difference) absorption spectrum of Predn in (deoxygenated) MeCN is shown in Figure 6.Thespectrum shows positive absorptions between 300-500 nm, where an intense band with a maximum at 330 nm and a shoulder near 350 nm is highlighted. Two minor bands centered near 400 nm and 480 nm can be clearly identified too.

The absorption kinetic profiles determined between 300 and 500 nm followed first-order kinetics with identical lifetimes (4 µs). From these results, it is possible to conclude that the absorption bands observed in the transient spectrum correspond to a unique transient species.

On the other hand, it is important to note that a possible intermolecular electron transfer is discarded due to the absence of new bands formed concomitantly to the disappearance of the triplet state of Predn (Figure 6). This is a good argument to infer the absence of intermolecular auto quenching processes. That is, the triplet state does not react with the substrate in the ground state neither with the solvent.

It should be noted that in the presence of oxygen, no significant transient absorption signals could be detected. This is probably due to the fact that with the dissolved oxygen in the solvent used the triplet state of Predn is completely deactivated (diffusional quenching), and that the products of this process (step 4, scheme 2) does not show significant absorption within the spectral and temporary range accessible to our equipment.

These observations, together with the triplet-triplet absorption spectrum previously reported for other steroid (17-β-hydroxyandrosta-4,6-dien-3-one (Δ6-testosterone))[48] which also has a dienone functionality, strongly suggest that the observed absorption spectrum corresponds to the triplet excited state of Predn (^{3}Predn*). To the best of our knowledge, the triplet-triplet absorption spectrum of Predn has not been reported yet in the literature. A possible reason for this is the apparently very low efficiency of the intersystem crossing process in these kinds of molecules.

3.5. Photodegradation mechanism proposed

Considering all the evidence presented, the mechanism shown in Scheme 2 is hereby proposed, which explains the photodegradation of Predn in aqueous solution.

The absorption of UVB light promotes Predn, in ground state, to electronically excited singlet and triplet states, ^{1}Predn* and ^{3}Predn*, respectively (reaction1 and 2).

It is assumed that the fluorescence quantum yield of Predn in aqueous solution is negligible, since its emission could not be detected.

In aerobic conditions (pathway I) ^{3}Predn* may react with ground state oxygen ($O_2(^3\Sigma_g)$) forming the species singlet oxygen ($O_2(^1\Delta_g)$) by energy transfer (reaction 3) or superoxide radical anion ($O_2^{\bullet-}$) by electron transfer (reaction4). $O_2^{\bullet-}$ could suffer spontaneous dismutation generating hydrogen peroxide (H_2O_2) by reaction 5. This species may experience homolytic photo-cleavage of the O-O bond and generate hydroxyl radical ($OH^{\bullet}$) by reaction 6. This highly reactive ROS is also involved in the photodegradation of Predn (reaction 7). It must be noted that H_2O_2, does not react with the corticoid (see Figure 3).

On the other hand, under inert atmosphere, the pathway II occurs, leading to the unimolecular photodegradation of Predn. This reaction occurs very likely from the ^{3}Predn* by intramolecular reorganization (reaction 8) [14]. It is important to note that in the presence of oxygen, both reactive pathways occur simultaneously.

In the literature, a photodegradation study of ibuprofen (IBP) under UV irradiation in aqueous media has been carried out [41], and there it was proved that the mechanism included direct photolysis and self-sensitization via ROS. However, in that case, the photo consumption of IBP is less effective in the presence of oxygen. This fact is indicating that the direct photolysis way is preponderant in the global mechanism.

Contrary to what was observed in the photolysis of IBP, in this study we found that in the presence of oxygen, the Predn photo consumption is enhanced (see Figure 2). This implies that the attack by ROS (via 1) involves a significant additional contribution to the photo degradation of the steroid in aerobic conditions. This fact is very important in order to take into account this emerging contaminant photodegradation in aqueous wastes by sunlight.

Conclusions

The main finding in this work is that the biologically active glucocorticoid Predn undergoes photo-induced degradation by irradiating with solar UVB light. This degradation includes two reactive ways: direct photolysis and self-sensitization via ROS.

The molecular oxygen has an important role in the Predn photodegradation mechanism in aqueous medium, making the formation of highly oxidizing ROS possible.

On the other hand, Predn is capable of generating the excited triplet state, ^{3}Predn* as a result of UVB light absorption. The spectrum of this transient species is reported here
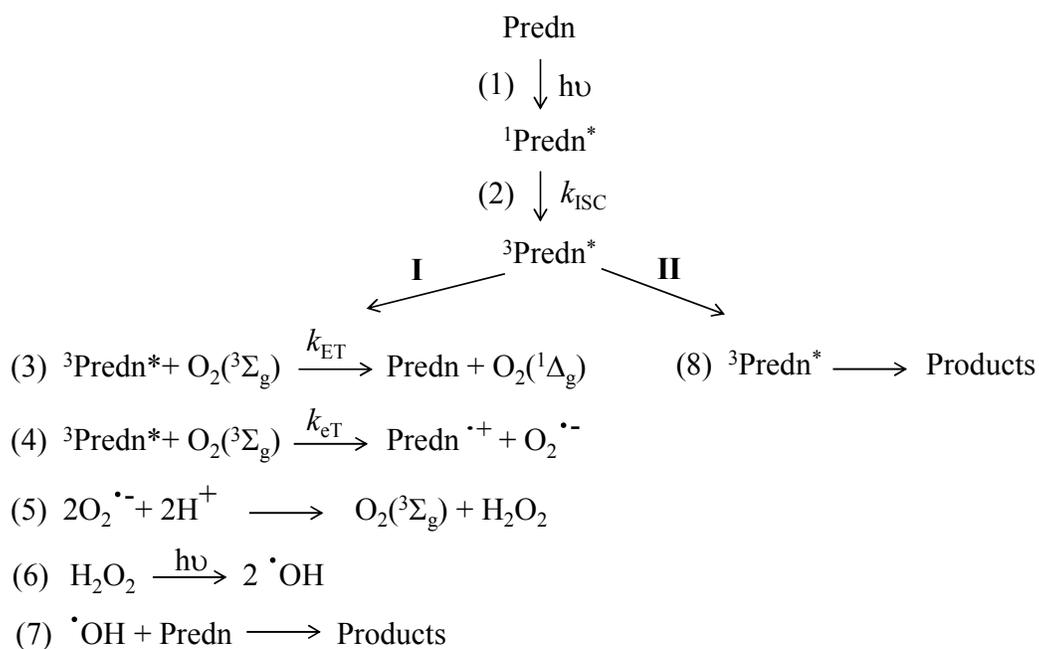
for the first time. ^{3}Predn* allows the formation of ROS, making $O_2(^1\Delta_g)$ ($\Phi_\Delta = 0.014$), H_2O_2 and the radical OH$^\bullet$ stand out.

Likewise, photodegradation of Predn in aqueous medium is controlled by the radical OH$^\bullet$, since the species $O_2(^1\Delta_g)$ and H_2O_2 do not interact chemically with the drug. Predn undergoes unimolecular photodegradation reactions in inert argon atmosphere.

In this study we found that in the presence of oxygen, the Predn photo consumption is improved. This implies that the attack by ROS involves a very important additional contribution to the photodegradation of Predn in aerobic conditions. These results are essential for the purpose of considering the photodegradation of this emerging contaminant in aqueous wastes by sunlight.

Figures, Schemes and captions
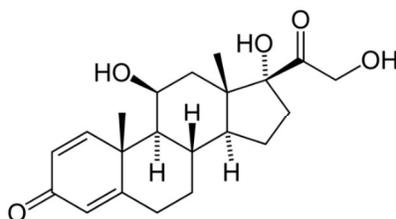
Scheme 1. Chemical structure of the corticoid Prednisolone (Predn)

Predn

$(1) \downarrow h\upsilon$

^{1}Predn*

$(2) \downarrow k_{ISC}$

^{3}Predn*

I   **II**

(3) ^{3}Predn$^* + O_2(^3\Sigma_g) \xrightarrow{k_{ET}}$ Predn $+ O_2(^1\Delta_g)$ (8) ^{3}Predn$^* \longrightarrow$ Products

(4) ^{3}Predn$^* + O_2(^3\Sigma_g) \xrightarrow{k_{eT}}$ Predn$^{\bullet +} + O_2^{\bullet -}$

(5) $2O_2^{\bullet -} + 2H^+ \longrightarrow O_2(^3\Sigma_g) + H_2O_2$

(6) $H_2O_2 \xrightarrow{h\upsilon} 2\ ^\bullet OH$

(7) $^\bullet OH + $ Predn $\longrightarrow$ Products

Scheme 2. Proposed pathway for photodegradation of Predn

Table 1. Quantum yield (Φ_R^{Predn}) and rate apparent constant (k_{app}) of photodegradation of

Predn, upon monochromatic irradiation at 248 nm, in different atmospheres condition.

|	argon	oxygen	air
Φ_R^{Predn}	(0.019 ± 0.002)	(0.034 ± 0.004)	(0.034 ± 0.005)
k_{app} (s^{-1})	$(0.7 \pm 0.2) \times 10^{-4}$	$(1.4 \pm 0.5) \times 10^{-4}$	$(1.4 \pm 0.4) \times 10^{-4}$
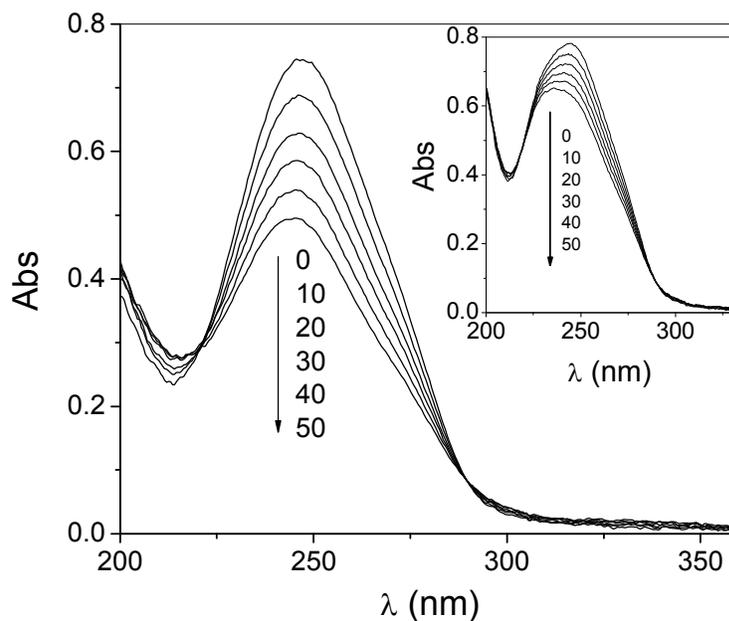
Figure 1. Spectral evolution of Predn (4×10^{-5} M) in aqueous solution (water/MeCN 99/1% v/v) irradiated at 248 nm, in oxygen-saturated condition. <u>Inset</u>: Spectral evolution of Predn (4×10^{-5} M) in aqueous solution (water/MeCN 99/1% v/v) irradiated at 248 nm, argon-saturated condition. Insert numbers indicate photoirradiation time in min.
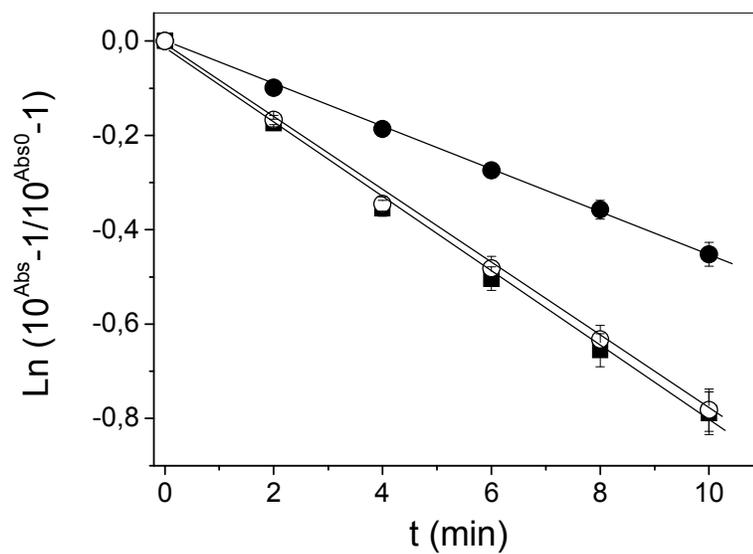
Figure 2. Predn photodegradation (4×10^{-5} M) at different atmosphere conditions: (●) argon saturated-solution; (■) air saturated-solution; (○) oxygen saturated-solution. Error bars indicate one standard deviation.
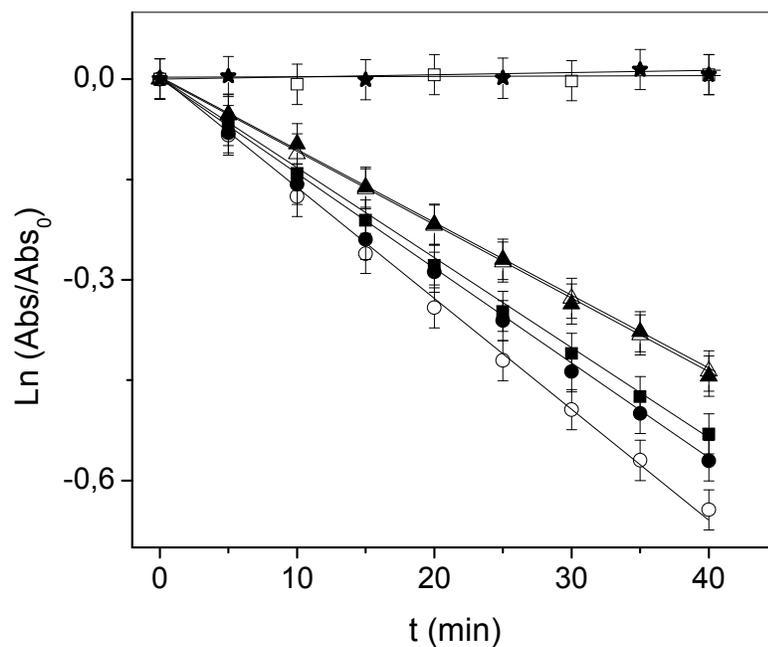
Figure 3. Effects of ROS-scavengers on Predn photoconsumption. (■) 4×10^{-5} M Predn; (□) 4×10^{-5} M Predn dark control; (★) 4×10^{-5} M Prend + 10 mM H_2O_2 dark control; (●) 10 mM NaN_3; (○) 1 mg% SOD; (▲) 1 mg% CAT; (△) 0.2 M IsoOH. Error bars indicate one standard deviation. All measurements were carried out at 298 K, except for CAT experience (310 K).
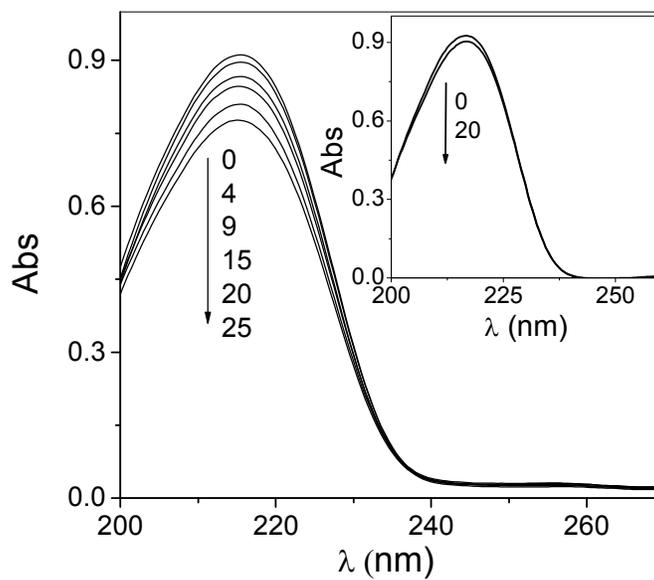
Figure 4. FFAc (1×10^{-4} M) photoconsumption in the presence of Predn (4×10^{-5} M) in aqueous solution (water/MeCN 99/1% v/v) upon 248 nm irradiation in air-saturated conditions. <u>Inset:</u> FFAc (1×10^{-4} M) photo consumption in the presence of Predn (4×10^{-5} M) in aqueous solution (water/MeCN 99/1% v/v) upon 248nm, irradiation in argon-saturated conditions. Numbers on the spectra represent the irradiation time in minutes.

As blank solution it was used Predn (4×10^{-5} M) in aqueous solution photolyzed at the same times as the FFAc (1×10^{-4} M) / Predn (4×10^{-5} M) sample.
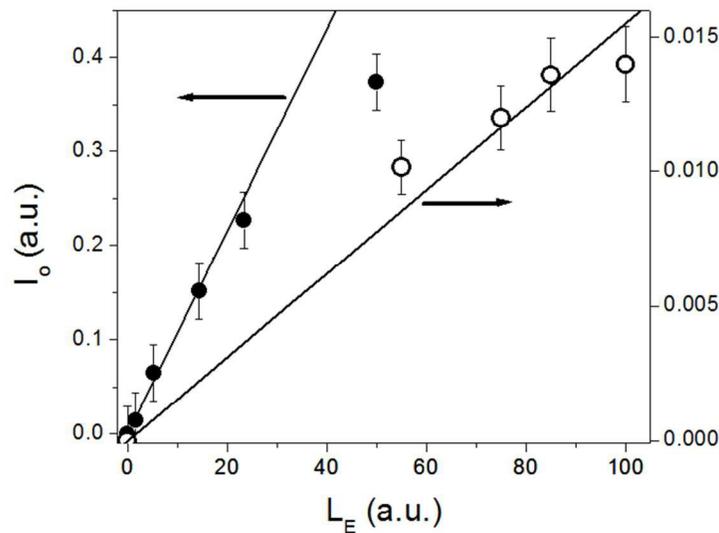
Figure 5. Laser fluence (E_0) dependence on the amplitude of $O_2(^1\Delta_g)$ phosphorescence emission at zero time (I_0) in MeCN. (●) PN; (○) Predn.
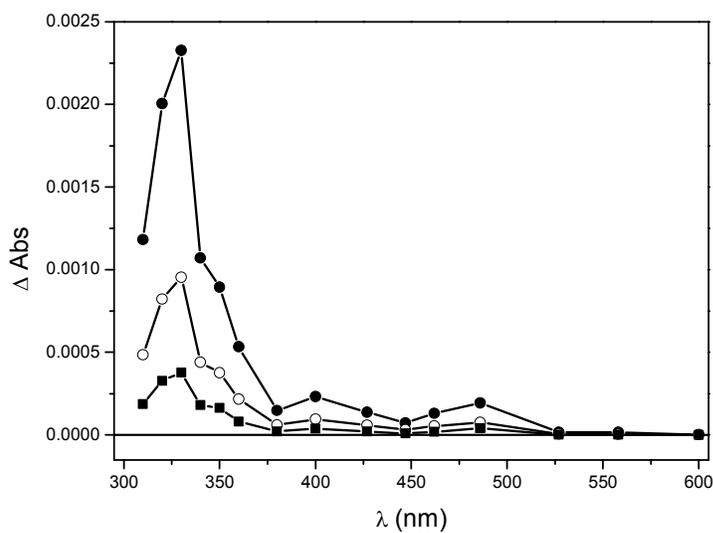
Figure 6. Transient absorption spectra of Predn in argon-saturated solution in MeCN, determined (●) 1µs, (○) 4 µs and (■) 8 µs after laser flash excitation at 266 nm.

References

[1] H.H. Tonnesen, Photoreactivity of Drugs. In Solar Radiation and Human Health, Espen Bjertness (Eds) (2008) 102-113.

[2] B.G. Katzung. Chapter 39, Adrenocorticosteroids & Adrenocortical Antagonists, in Basic & Clinical Pharmacology, McGraw-Hill, (Eds) (2007).

[3] A.C. Cato, E. Wade, Molecular mechanisms of anti-inflammatory action of glucocorticoid, Bioassays 18 (1996) 371-378.

[4] L.A Schwiebert, L.A Beck, C. Stellato, Glucocorticosteroids inhibition of cytokine production: Relevance to antiallergic actions, J. Allergy Clin. Immunol. 97 (1996) 143-152.

[5] 19th WHO Model List of Essential Medicines, W.H. Organization Editor (2015).

[6] M. Davis, R. Williams, J. Chakraborty, J. English, V. Marks, G. Ideo, Prednisone or Prednisolone for the Treatment of Chronic active Hepatitis? - A Comparision of Plasma Availability, J. Clin. Pharmac. 5 (1978) 501-505.

[7] Photostability of drugs and drugs formulations, Edited by H.H. Tønnesen, Second Edition, CRC Press, (2004) p. 23-25.

[8] H.J. Barton, J. Bojarski, A. Zurowska, Stereospecificity of the Photoinduced Conversion of Methylphenbarbital to Mephenytoin, Arch. Pharm. (Weinheim) 319 (1986) 457-461.

[9] T. Heberer, Occurrence, fate and removal of pharmaceutical residues in the aquatic environment: A review of recent research data, Toxicology Letters 131 (2002) 5-17.

[10] A.G. Trovó, S.A. Santos Melo, R. Fernandes Nogueira, Photodegradation of pharmaceuticals amixicillin, bezafibrate and paracetamol by the Photo-Fenton process, J. Photochem. Photobiol. A: Chem.198 (2008) 215-220.

[11] C. Desbrow, E.J. Routledge, G.C. Brighty, J.P. Sumpter, M. Waldock, Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening, Environ. Sci. Technol. 32(1998)1549-1558.

[12] E.M. Golet, A.C. Alder, A. Hartmann, T. Ternes, W. Giger, Trace Determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection, Anal.Chem.73 (2001) 3632-3638.

[13] B. Halling-Sorensen, S. Nors Nielsen, P.F. Lanzky, F. Ingerslev, H.C. Holten-Lutzhoft, S. E. Jorgensen, Occurrence, fate and effects of pharmaceutical substances in the environments, Chemosphere 36 (1998) 357-393.

[14] M. Della Greca, A. Fiorentino, M. Isidori, M. Lavorga, A. Previtera, M. Rubino, F. Temussi, Toxicity of prednisolone, dexamethasone and their derivatives on aquatic organisms, Chemosphere 54 (2004) 629-637.

[15] (a) D.H.R. Barton, W.C. Taylor, The photochemistry of prednisone acetate in neutral solution, J. Am. Chem. Soc. 80 (1958) 244-245. (b) D.H.R. Barton, W.C. Taylor, Photochemical transformations. Part IV. The photochemistry of prednisone acetate, J. Chem. Soc. 510(1958) 2500-2510. (c) H.E. Zimmerman, A New Approach to Mechanistic Organic Photochemistry, in Advances in Photochemistry, Volume 1, W. A. Noyes, G. S. Hammond and J. N. Pitts (Eds), John Wiley & Sons, Inc., Hoboken, NJ, USA.7 (1963) 183. (d) D.I. Schuster, Mechanisms of photochemical transformations of cross-conjugated cyclohexadienones, Acc. Chem. Res. 11 (1978) 65-73.

[16] J.R. Williams, R.H. Moore, R. Li, J. Blount, Structure and Photochemistry of Lumiprednisone and Lumipredisone Acetate, J Am. Chem. Soc. 17 (1979) 5019-5025.

[17] J.R. Williams, R.H. Moore, R. Li, C.M. Weeks, Photochemistry of 11α-and 11β-Hydroxyl Steroid 1,4-Dien-3-ones and 11α and 11β Hydroxy Steroidal Bicyclo [3.1.0] hex-3-en-2-ones in Neutral and Acidic Media, J. Org. Chem. 45 (1980) 2324-2331.

[18] A. Ricci, E. Fasani, M. Mella, A. Albini, General Patterns in the Photochemistry of Pregna-1,4-dien-3,20-diones, J. Org. Chem. 68 (2003) 4361-4366.

[19] P. Calza, E. Pelizzetti, M. Brussino, C. Baiocchi, Ion trap tandem mass spectrometry study of dexamthasone transformation products on light activates TiO_2 surface, J. Am. Soc. Mass Spectrom. 12 (2001) 1286-1295.

[20] R.O.Rahn, Use Of Potassium Iodide As a Chemical Actinometer, Photochem. Photobiol. 58 (1993) 874-880.

[21] J. Natera, W. Massad, N.A. García, The role of vitamin B6 as an antioxidant in the presence of vitamin B2- photogenerated reactive oxygen species. A kinetic and mechanistic study, Photochem. Photobiol. Sci. 11 (2012) 938-945.

[22] S. Criado, S. Bertolotti, N.A. García, Kinetic aspect of the Rose Bengal-sensitized photo-oxygenation of tryptophan alkyl esters, J. Photochem. Photobiol. B: Biol. 34 (1996) 79–86.

[23] C. Schweitzer, R. Scmidt, Physical mechanisms of generation and deactivation of singlet oxygen, Chem. Rev. 103 (2003) 1685–1757.

[24] I. Gutiérrez, S.G. Bertolotti, M.A. Biasutti, A.T. Soltermann, N.A. García, Quinones and hydroxyquinones as generators and quenchers of singlet molecular oxygen, Can. J. Chem. 75 (1997) 423–428.

[25] R. Schmidt, C. Tanielian, R. Dunsbach, C. Wolff, Phenalenone a universal reference compound for the determination of quantum yields of singlet oxygen $O_2(^1\Delta_g)$ sensitization, J. Photochem. Photobiol. A: Chem. 79 (1994) 11–17.

[26] M. Della Greca, A. Fiorentino, M.R. Iesce, M. Isidori, A. Nardelli, L. Previtera, F. Temussi, Identification of Phototransformation products of prednisone by sunlight: toxicity of the drug and its derivatives on aquatic organisms, Environ. Toxicol. Chem. 22 (2003) 534-539.

[27] S. L. Murov, I. Carmichael and G. L. Hug (1993) *Handbook of Photochemistry*, 2nd edn, p. 293. Dekker, New York.

[28] D. Ren, B. Huang, D. Xiong, H. He, X. Meng, X. Pan, Photodegradation of 17αethynylestradiol in dissolved humic substances solution: Kinetics, mechanism and estrogenicityvariation, J. Environm.Sci. (2016) *In press*. DOI:10.1016/j.jes.2016.03.002

[29] A. Ricci, E. Fasani, M. Mella, A. Albini, Noncommunicating Photoreaction Paths in Some Pregna-1,4-diene-3,20-diones, J. Org. Chem. 66 (2001) 8086-8093.

[30] D. Gryglik, M. Olak, J.S. Miller, Photodegradation kinetic of androgenic steroids boldenone and trenbolone in aqueous solution, J. Photochem. Photobiol. A: Chem. 212 (2010) 14-19.

[31] S. Foote, T.Y. Ching, Chemistry of singlet oxygen XXI. Kinetic of bilirubin photooxygenation, J. Am. Chem. Soc. 97 (1975) 6209–6214.

[32] F. Wilkinson, W.P. Helman, A.B. Ross, Rate constant for the decay and reaction of the lowest electronically excited state of molecular oxygen in solution. An extended and revised compilation, J. Phys. Chem. Ref. Data 24 (1995) 663–1021.

[33] D.C. Neckers, Rose Bengal. Review. J. Photochem. Photobiol. A. 47 (1989) 1-29.

Photochemical & Photobiological Sciences Accepted Manuscript

[34] I. Fridovich, Superoxide radicals, superoxide dismutases and the aerobic lifestyle, Photochem. Photobiol.28 (1978) 733-741.

[35] R.M. Baxer, J.H. Carey, Evidence for photochemical generation of superoxide ion in humic water, Nature 306 (1983) 575–576.

[36] P.G. Zang, H.P. Misra, Superoxide radicals generated during the autooxidation of 1-methyl-4-phenyl-2,3-dihydropyridinium perchlorate, J. Biol. Chem. 267(1992) 17547–17552.

[37] I.B. Afanas'ev, Superoxide Ion Chemistry and Biological Implications, 1º Edition, CRC Press, Boca Ratón (Eds.) (1989).

[38] C. Gambetta, W.A. Massad, A.V. Nesci, N.A. Garcia, Vitamin B2-sensitized degradation of the multifunctional drug Evernyl, in the presence of visible light – microbiological implications, Pure Appl. Chem. 87 (2015) 997–1010.

[39] G.V. Buxton, C.L. Greenstock, W.P. Helman, A.B. Ross, Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals ($^\bullet$OH /$^\bullet$O$^-$) in aqueous solution. J. Phys. Chem. Ref. Data 17 (1988) 513–886.

[40] E. Reynoso, M.B. Spesia, N.A. García, M.A. Biasutti, S. Criado, Riboflavin-sensitized photooxidation of Ceftriaxone and Cefotaxime. Kinetic study and effect on *Staphylococcus aureus*. J. Photochem. Photobiol. B: Biol. 142 (2015) 35–42.

[41] F.H. Li, K. Yao, W. Y. Lv, G.G. Liu, P. Chen, H. P. Huang, Y. P. Kang, Photodegradation of Ibuprofen under UV–Vis Irradiation: Mechanism and Toxicity of Photolysis Products, Bull. Environ. Contam. Toxicol. 94 (2015) 479–483.

[42] J.H. Baxendale, J.A. Wilson, The photolysis of hydrogen peroxide at high light intensities, T. Faraday Soc. 53 (1957) 344-356.

[43] A. Goi, M. Trapido, Hydrogen peroxide photolysis, Fenton reagent and photo-Fenton for the degradation of nitrophenols: a comparative study, Chemosphere 46 (2002) 913-922.

[44] S. Goldstein, D. Aschengrau, Y. Diamant, J. Rabani, Photolysis of Aqueous H_2O_2: Quantum Yield and Applications for Polychromatic UV Actinometry in Photoreactors, Environ. Sci. Technol. 41 (2007) 7486-7490.

[45] T.A. Mensah, C.T. Ho, S.S. Chang, Products identified from photosensitized oxidation of selected furanoid flavor compounds, J. Agric. Food Chem. 34 (1986) 336-338.

[46] G. Miolo, S. Caffieri, D. Dalzoppo, A. Ricci, E. Fasani, A. Albini, Photochemistry

and Phototoxicity of Fluocinolone 16,7-Acetonides,Photochem.Photobiol. 81 (2005)291-298.

[47] G. Miolo, S. Caffieri, D. Dalzoppo, F. Gallocchio, E. Fasani, G.M.J. Beyersbergen van Henegouwen, Photoactivation of corticosteroids in UV-B exposed skin, J. Photochem. Photobiol. B: Biol. 103 (2011) 35-41.

[48] D.I. Schuster, D.A. Dunn, Kinetic properties of the triplet excited state of a linearly conjugated steroid dienone studied by laser flash photolysis, J. Photochem. 28 (1985) 413-418.