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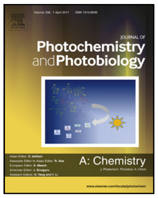

The riboflavin-photosensitized degradation of the *uv*-absorbing azo dye-metabolites Benzidine and *o*-Tolidine. Kinetic and mechanistic aspects

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ABSTRACT

The visible-light-promoted photodegradation of two carcinogenic primary azo-dye metabolites (BZ-C), represented by the colourless water-contaminants Benzidine (BZ) and *o*-Tolidine (OT), was studied in water-methanol solution. It was done in the presence of air and the natural pigment riboflavin (Rf) as a photosensitizer. These conditions reasonably mimic a natural environment scenery.

Both BZ-C interact with the reactive oxygen species (ROS) singlet molecular oxygen ($O_2(^1\Delta_g)$), superoxide radical anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$), all photogenerated *in situ* through energy-transfer and electron-transfer processes. The interaction of BZ-C with $O_2(^1\Delta_g)$ is essentially physical in nature and occurs with a relatively high rate constant value. The effective BZ-C degradation is mainly driven by the remaining photogenerated ROS.

A comparison of the BZ and OT photooxidative-rate with those of the archetypal surface-water-contaminants aniline and phenol indicates that BZ-C degradation occurs in a moderately slower fashion than the reference contaminants, within a common overall time-scale. The primary photoprocesses initiating the Rf-sensitized degradation seems to be quite similar for the four compounds, with the simultaneous operation of Type I and Type II photoprocesses.

In conclusion: the natural Rf- photosensitized degradation of BZ-C appears as a plausible process, as evaluated under simulated environmental conditions.

1. Introduction

It is well known that significant amounts of different synthetic dyes are usual contaminants of surface waters and soils. Consequently their microbiological, thermal and photochemical degradative pathways still being topics of an enormous research interest [1–8].

Azo-dyes are widely used in clothing industry, as it follows for the majority of the industrial demand. Once incorporated to industrial effluents, the dyes can lead to simpler carcinogenic and genotoxic compounds named benzidine-like compounds (BZ-C) [9,10], such as *o*-Tolidine (OT) and the very BZ. These dye metabolites are formed after a reductive cleavage of the azo-group [11,12]. Structural formulae of BZ-C are shown in Scheme 1.

Different authors successfully tried the so-called alternative photodegradation ways for BZ-C compounds, employing artificial *uv*-irradiation. Muneer et al. studied the photocatalitic BZ-C oxidation process under different experimental conditions, whereas S.A. Azin demonstrated that the mechanism of direct *uv*-photodegradation, in different halomethane solvents, occurs through a radical-mediated process [9,10].

BZ-C, coming from industrial wastes, finally appear dissolved or suspended in surface waters. Hence, a thorough knowledge on the photodegradation feasibility of these pollutants in aerated solution and under daylight irradiation, *i.e.* mimicking a natural photochemical decay, constitute a subject of significant relevance.

When colourless water pollutants such as BZ-C do not absorb in the sunlight wavelength, their photodegradation in natural aquatic

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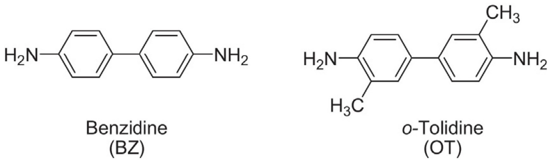


Scheme 1. Structural formulae of Benzidine and *o*-Tolidine.

environments becomes hindered. In this case the process could become feasible in the presence of a photosensitizer, *i.e.* a visible light-absorbing agent that enables the degradation through a sort of catalyzed event. This point sparked our interest on the potential photosensitizing action of riboflavin (Rf, vitamin B2). The vitamin is a naturally occurring pigment present as traces in water courses, lakes and seas [13,14]. It is well known that Rf generates the oxidative species $O_2(^1\Delta_g)$ and $O_2^{\bullet-}$ upon visible light irradiation, with quantum yields of 0.47 and 0.009, respectively [15,16]. On this basis, we have carried out a study on the possible Rf-sensitized photodegradation of BZ and OT in solution. The work was undertaken with two main and parallel purposes: (a) evaluation of the possible fate of industrially relevant aromatic amines, under visible-light irradiation, in the presence of the natural pigment Rf and (b) gaining insight into suitable experimental conditions, towards an eventual design of daylight-mediated degradation devices for accumulated BZ-C.

Finally, in order to establish a sort of degradation-reference pattern, BZ-C sensitized-photodegradation rates were compared to those obtained under similar experimental conditions for the archetypal contaminant model-compounds phenol (PH) and aniline (AN).

2. Materials and methods

2.1. Materials

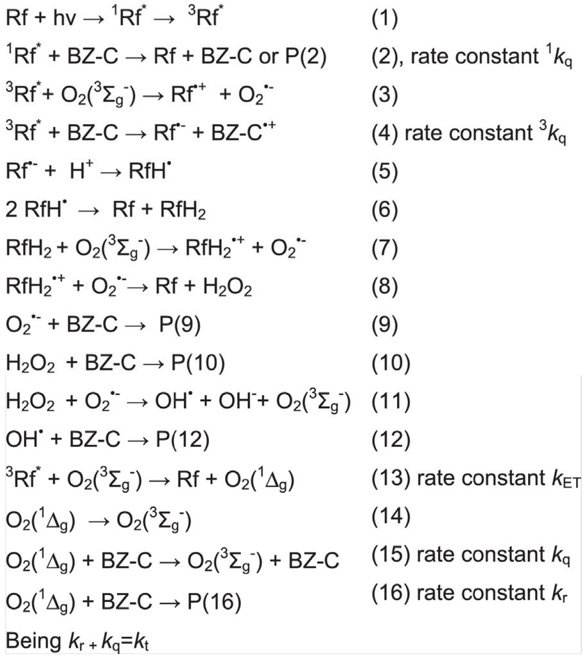
Riboflavin (Rf), Rose Bengal (RB), Benzidine(1,1′-biphenyl-4,4′-diamine), *o*-Tolidine(3,3′-Dimethyl-[1,1′-biphenyl]-4,4′-diamine), from bovine erythrocytes, phenol (PH), aniline (AN), Mannitol, Catalase from bovine liver (CAT), Superoxide Dismutase (SOD), monodeuterated methanol (MeOD) 98% and Deuterium oxide (D_2O) 99.9% were purchased from Sigma Chem. Co. Sodium azide (NaN_3) was from Merck. Furfuryl alcohol (FFA) was from Riedel de Haën. All these substances were used as received. Methanol (MeOH) HPLC quality, was from Sintorgan. All measurements were carried out with freshly prepared solutions and at room temperature. All experiments were carried out employing a mixture H_2O-MeOH (1:1, v/v) as a solvent. H_2O was triply distilled. H_2O and MeOH were replaced by D_2O and MeOD in the determinations of $O_2(^1\Delta_g)$-phosphorescence lifetime in order to enlarge the lifetime of $O_2(^1\Delta_g)$ within the allowed time-window of the time resolved phosphorescence detection (TRPD) apparatus employed.

2.2. Methods

2.2.1. Stationary photolysis

Continuous aerobic photolysis of aqueous solutions of BZ-C with Rf or RB as individual dye-sensitizers were carried out in a home-made photolyzer provided with a 150-W quartz-halogen lamp and a cut-off filter at 400 nm or with a home-made photolyzer composed by six blue light emitting diodes (LEDs, λ_{max} 467 nm, polarization current 30 mA). The compounds BZ, OT, PH and AN did not absorb any incident light.

Ground state absorption spectra were registered in a Hewlett Packard 8452A diode array spectrophotometer.



$Rf + h\nu \rightarrow {}^1Rf^* \rightarrow {}^3Rf^*$ (1)
${}^1Rf^* + BZ\text{-}C \rightarrow Rf + BZ\text{-}C$ or P(2) (2), rate constant ${}^1k_q$
${}^3Rf^* + O_2(^3\Sigma_g^-) \rightarrow Rf^{\bullet+} + O_2^{\bullet-}$ (3)
${}^3Rf^* + BZ\text{-}C \rightarrow Rf^{\bullet-} + BZ\text{-}C^{\bullet+}$ (4) rate constant ${}^3k_q$
$Rf^{\bullet-} + H^+ \rightarrow RfH^\bullet$ (5)
$2\ RfH^\bullet \rightarrow Rf + RfH_2$ (6)
$RfH_2 + O_2(^3\Sigma_g^-) \rightarrow RfH_2^{\bullet+} + O_2^{\bullet-}$ (7)
$RfH_2^{\bullet+} + O_2^{\bullet-} \rightarrow Rf + H_2O_2$ (8)
$O_2^{\bullet-} + BZ\text{-}C \rightarrow P(9)$ (9)
$H_2O_2 + BZ\text{-}C \rightarrow P(10)$ (10)
$H_2O_2 + O_2^{\bullet-} \rightarrow OH^\bullet + OH^- + O_2(^3\Sigma_g^-)$ (11)
$OH^\bullet + BZ\text{-}C \rightarrow P(12)$ (12)
${}^3Rf^* + O_2(^3\Sigma_g^-) \rightarrow Rf + O_2(^1\Delta_g)$ (13) rate constant k_{ET}
$O_2(^1\Delta_g) \rightarrow O_2(^3\Sigma_g^-)$ (14)
$O_2(^1\Delta_g) + BZ\text{-}C \rightarrow O_2(^3\Sigma_g^-) + BZ\text{-}C$ (15) rate constant k_q
$O_2(^1\Delta_g) + BZ\text{-}C \rightarrow P(16)$ (16) rate constant k_r
Being $k_r + k_q = k_t$

Scheme 2. Possible pathways for a Rf-sensitized process in the presence of a hypothetical electron donor (BZ-C), transparent to the photoirradiation wavelength.

The rate constant k_r for the reaction $O_2(^1\Delta_g)$ + BZ-C (process (16) in Scheme 2 , see further Section 3) was determined employing the method described by Scully and Hoigné [17]. The k_r value for BZ-C was deduced using the expression slope/slope$_R$ = k_r [BZ-C]/k_{rR} [R]. k_{rR} is the reactive rate constant for a reference compound R, being slope and slope$_R$ the respective slopes of the first-order plots of BZ-C and R consumption or oxygen consumption by these compounds. The reference employed was FFA, with a pH-independent reported value for k_{rR} of $1.2 \times 10^8\ M^{-1}\ s^{-1}$ [17].

The rates of oxygen uptake (ROU) were measured in H_2O–MeOH (1:1, v/v) solution employing a Orion 97-08 specific oxygen electrode immersed in a 150 ml quartz cell hermetically sealed, containing the solution of the sensitizer plus substrate. The ROU for the different compounds, in the presence of either Rf or RB, were determined by plotting ppm of oxygen consumed upon photoirradiation as a function of photoirradiation time.

The ROU for individual Rf-sensitized solutions of BZ or OT in H_2O–MeOH (1:1, v/v), in the absence and in the presence of SOD; CAT and MAN, were the mean value of three runs not differing more than 3% each other

Rates of Rf consumption in deoxygenated solutions were obtained by plotting the absorbance decay of the 445 nm absorption band of the vitamin as a function of photoirradiation time.

2.2.2. Quenching of Rf electronically excited states

Fluorescence quenching of Rf was determined employing a JASCO FP6200 apparatus with 445 and 525 nm as excitation an emission wavelengths respectively.

In the laser flash photolysis experiments Argon-saturated 0.04 mM Rf aqueous solutions were photolyzed employing the a previously reported apparatus [18]. Briefly: A ns Nd:YAG laser system (Spectron) at 355 nm was the excitation source. A 150 W Xenon lamp was employed as analyzing light and a PTI monochromator coupled to a red-extended photomultiplier (Hamamatsu R666) constituted the detection system. After acquisition and averaging through a digital oscilloscope (Hewlett-Packard 54504A), the signal was transferred, *via* a HPIB parallel interface, to a PC where it was analyzed and stored. The disappearance of ^{3}Rf* was monitored from the first-order decay of the absorbance at 670 nm, allowing the neglection of any possible interference by other species in this spectral zone. The possibility of self-quenching and triplet-triplet annihilation was avoided by determining the triplet decay at sufficiently low Rf concentration (in the range 0.04–0.07 mM) and at low enough laser energy. The Stern-Volmer expression $1/{}^3\tau = (1/{}^3\tau_0) + {}^3k_q$ [BZ-C], was employed to obtain the rate constant ${}^3k_q$, which accounts for the interaction ^{3}Rf* + BZ-C (process (8) in Scheme 2). The experimentally determined ${}^3\tau_0$ and ${}^3\tau$ values represent the respective lifetimes of ^{3}Rf* in the absence and in the presence of BZ-C.

The previously described TRPD device was employed in the evaluation of the overall quenching rate constant of $O_2(^1\Delta_g)$ by BZ-C (k_t, see further Scheme 1 in Section 3) [19]. Upon excitation with the 532-nm emission from a Nd:YAG laser (Spectron) the time evolution of $O_2(^1\Delta_g)$ phosphorescence decay at 1270 nm was detected, after appropriate filtering, using an amplified Judson J16/8Sp Germanium detector. The detection output was connected to a digital oscilloscope and sent to a PC for signal processing. Normally ten-shots averaging were enough to achieve a good signal-to-noise ratio, from which the decay trace was obtained. Air saturated solutions were used in all cases. In these dynamic determinations, solutions of RB in D_2O-MeOD ($Abs_{560} = 0.3$) were employed. The $O_2(^1\Delta_g)$ lifetimes were evaluated in the presence (τ) and in the absence (τ_0) of BZ-C. After plotting $\tau_0/\tau = 1 + k_t\tau_0$ [BZ-C] for different quencher concentrations, k_t values were obtained.

3. Results

3.1. Photoirradiation of BZ-C in the presence of Rf

The photoirradiation of air-saturated aqueous–methanolic solutions of individual 0.038 mM BZ or 0.04 mM OT in the presence of 0.043 mM Rf ($\lambda_{irr} > 400$ nm) produces changes in the region of the electronic absorption bands of the BZ-C and also in absorption bands of the sensitizer. Representative results for BZ are shown in Fig. 1 (main).

In parallel, photoirradiation of similar solutions gave rise to oxygen consumption (quantitative ROU results will be shown further). Both sets of experiments strongly suggest the operation of a Rf-photoinitiated reaction chain with participation of electronically excited states of the sensitizer and/or the involvement of reactive oxygen species (ROS) produced through these excited states.

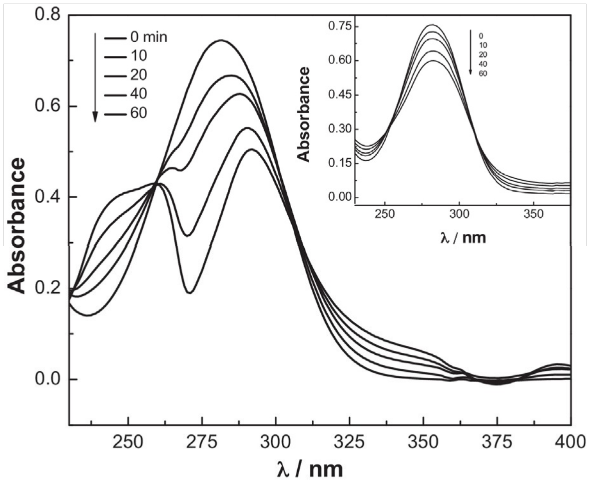


Fig. 1. Changes in the UV-Vis absorption spectra of Rf 0.039 mM plus BZ 0.4 mM, taken *vs.* Rf 0.039 mM upon irradiation ($\lambda_{irr} > 400$ nm) under air-saturated conditions. Inset: Changes in the UV–vis absorption spectra of RB ($A_{550\,nm} = 0.49$) plus OT 0.38 mM, *vs.* RB ($A_{550\,nm} = 0.49$), upon irradiation under the same conditions of the main figure. Numbers at the side of both spectra represent photoirradiation time in minutes. Solvent MeOH–H_2O (1:1, v/v).

Results have been interpreted and discussed on the basis of Scheme 2, already employed by other authors and by ourselves in relation to Rf-sensitized photooxidation of different electron-donating molecules, including environmental contaminants and biologically relevant compounds [20–22].

The scheme constitutes a self-defined sequence of reactions occurring after Rf photoexcitation, in the presence of BZ-C as the electron-releasing species. Eventual products for the respective reaction (n) are represented by $P(n)$ and ground state molecular oxygen is symbolized as $O_2(^3\Sigma_g^-)$.

3.2. Evaluation of ROS-mediated photoprocesses

The rates of oxygen consumption (ROU) for solutions 0.04 Mm BZ or OT in H_2O–MeOH (1:1, v/v) were decreased when measured in the individual presence of 0.1 μM SOD; 2 mM CAT and 1 μg/ml MAN as quantitatively shown in the bars diagram of Fig. 2. The enzymes SOD and CAT produce the dismutation of the species $O_2^{\bullet-}$ and decomposition of H_2O_2 respectively, according to reactions (17) and (18) whereas MAN deactivates the species $OH^\bullet$ (reaction (19)).

$$2\ O_2^{\bullet-} + 2\ H^+ + SOD \rightarrow O_2(^3\Sigma_g^-) + H_2O_2 \tag{17}$$

$$2\ H_2O_2 + CAT \rightarrow 2\ H_2O + O_2(^3\Sigma_g^-) \tag{18}$$

$$OH^\bullet + MAN \rightarrow \text{deactivation} \tag{19}$$

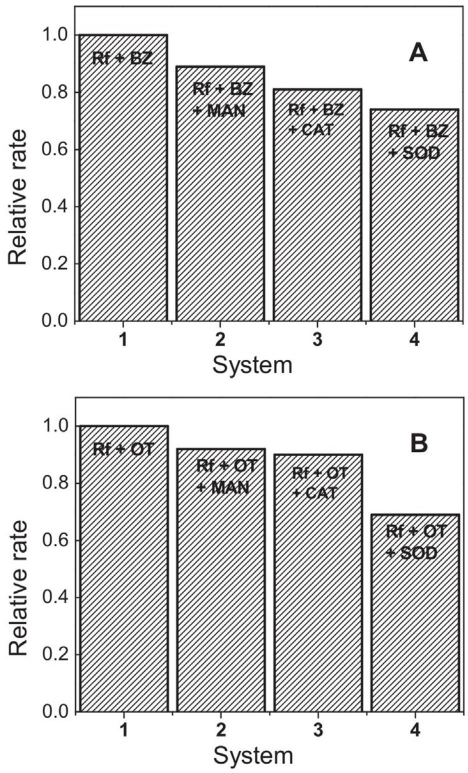


Fig. 2. (A and B) Bars diagrams for the relative rates of oxygen uptake upon 0.04 mM Rf photosensitization in H_2O-MeOH (1:1 v/v) of 0.4 mM BZ (**A**) and 0.4 mM OT (**B**) in the absence (1) and in the presence of 1 μg/ml MAN (2); 2 mM CAT (3) and 0.1 μM SOD (4).

The observed decrease in the rates of oxygen uptake in the presence of the selective ROS interceptors indicates, in principle, the operation of H_2O_2–, $O_2^{\bullet -}$ and $OH^{\bullet}$mediated processes in the overall BZ-C oxidation. This classical set of assays has been usually employed for confirm/reject the participation of the mentioned ROS in a given oxidative process [14,23,24].

3.3. The interaction of BZ-C with Rf electronically excited singlet and triplet states

The individual presence of a both BZ-C, in the mM concentration range, produced a decrease in the rate of Rf consumption in deoxygenated solutions. It is well known that photodegradation of Rf proceeds from its electronically excited singlet and triplet states [25,26]. Hence, a plausible possibility is that the observed degradation of BZ-C could be initiated through different quenching processes of these states, by the substrates.

In the presence of BZ-C ≥ 0.5 mM the quenching of the Rf fluorescence begins to be observable. Through monitoring of the stationary fluorescence intensity of Rf in the presence (I) and in the absence (I_0) of different BZ-C concentrations, the classical Stern-Volmer treatment ($I_0/I = 1 + {}^1K_{SV}$ [BZ-C], with ${}^1K_{SV} = {}^1k_q.{}^1\tau_o$) allows the determination of the Stern-Volmer constant. The rate constant for process (2) is ${}^1k_q$, being $\tau_0 = 5.2$ ns the reported value for the fluorescence lifetime of ${}^1Rf^*$ [27]. A ${}^1k_q$ mean value of 2.5×10^{10} $M^{-1}\,s^{-1}$ for both BZ-C is deduced (Table 1, data not shown). This value indicates that *ca.* 4% of the species ${}^1Rf^*$ will be intercepted by 0.4 mM BZ-C, the maximal concentration employed in the Rf-sensitized experiments. Under these conditions, and in practical terms, any influence of the interaction ${}^1Rf^*$ - BZ-C can be disregarded.

Laser flash photolysis experiments performed on Rf ($A_{355} = 0.36$) deaerated solutions showed a neat decrease of ${}^3Rf^*$ lifetime in the individual presence of BZ or OT, both in the sub-mM concentration range. This fact strongly suggests the occurrence of an interaction between the BZ-C and the long-lived electronically excited species. The graphically determined bimolecular rate constants ${}^{null}k_q$ for the quenching process (reaction 4) are collected in Table 1. See Fig. 3 for the case of BZ.

The transient absorption spectrum of the Rf solution after the laser pulse is coincident with that attributed to the species ${}^3Rf^*$ [20,21], whereas the shape of the spectra obtained in the presence 0.7 mM of BZ or OT are practically identical to those reported for the respective species $BZ^{\bullet +}$ and $OT^{\bullet +}$,with an absorption maximum *ca.* 450 nm. The shoulder at 550 nm corresponds to the residual absorption of the species $RfH^{\bullet}$ (reaction 11) formed after protonation of $Rf^{\bullet -}$ (process (5)) which is produced in the electron-transfer step (3) [20,21]. The presence of the radical cation species $BZ^{\bullet +}$ and $OT^{\bullet +}$ has been already reported in thermal and electrochemical oxidations processes respectively, producing polymeric compounds as final products, in both cases [31,32]. These results are shown in Fig. 3, for BZ as a representative case.

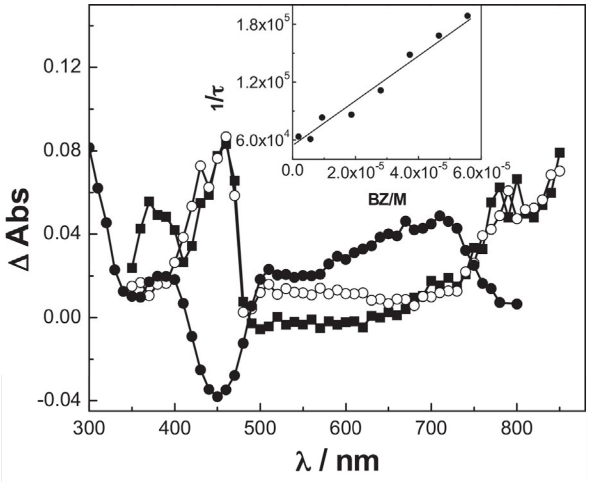


Fig. 3. Transient absorption spectrum of Rf 0.053 mM taken at 5 μs after the laser pulse (●). Transient absorption spectra of Rf 0.053 in the presence of BZ 0.57 mM, after 5 μs the laser pulse (○) and after 50 μs the laser pulse (■). Inset: Stern-Volmer plot for the quenching of ${}^3Rf^*$ by BZ in buffered pH 7 H_2O/MeOH 1:1 (v/v) aerated solution. τ is the lifetime of ${}^3Rf^*$ at different BZ concentrations. All in argon-saturated H_2O–MeOH (1:1, v/v) solution.

3.4. Quenching of $O_2({}^1\Delta_g)$ by BZ-C

As said in the introduction section, Rf photogenerates $O_2({}^1\Delta_g)$ with a quite high $\Phi_\Delta$value [15]. Experiments involving possible $O_2({}^1\Delta_g)$-mediated oxidation of BZ-C were carried out in the presence of the exclusive $O_2({}^1\Delta_g)$-generator dye RB. Rf was not employed in this case in order to avoid interferences by other oxidative species produced in the medium, as above disclosed. RB is the most frequently employed sensitizer in $O_2({}^1\Delta_g)$ reactions, with $\Phi_\Delta = 0.81$ in MeOH [33].

When a solution of RB ($A_{530} = 0.4$) was irradiated in solution with visible light in the presence of BZ-C, modifications in the BZ-C spectral component (Fig. 1, inset), and oxygen consumption were observed.

The IR phosphorescence emission of $O_2({}^1\Delta_g)$, as detected by TRPD experiments was quenched by BZ-C in the sub-mM concentration range, as shown by the Stern-Volmer plot in Fig. 4 for the case of OT. From these data the rate constant k_t for the overall interaction $O_2({}^1\Delta_g)$-BZ-C was independently determined in D_2O-MeOD (Table 1). As indicated in processes (15) and (16) of Scheme 2, the interaction $O_2({}^1\Delta_g)$-BZ-C may be physical and/or chemical (reactive) in nature. The rate constant k_r, accounting for the chemical reaction represented by Eq. (16) was determined for each BZ-C by monitoring oxygen consumption upon photoirradiation of RB–BZ-C mixtures, employing the actinometric method already described (Fig. 4, inset and Table 1) [17].

Table 1
Rate constant values for the fluorescence quenching of Riboflavin (${}^1k_q$); the quenching of triplet excited state of Riboflavin (${}^3k_q$) and reactive and overall quenching of $O_2({}^1\Delta_g)$ (k_r and k_t). Ratio k_r/k_t and relative rates of oxygen consumption upon Rf (RR_{Rf}) and RB (RR_{RB}) photosensitization. All data for BZ-C. Some experimental results and literature values for PH and AN were also included in the table for comparative purposes. Solvent: H_2O–MeOH (1:1, v/v). k_t values were determined in D_2O–MeOD (1:1, v/v) solutions. Errors quoted in the rate constant values correspond exclusively to BZ and OT.

Compound	${}^1k_q 10^{10} M^{-1}s^{-1} \pm 5\%$	${}^3k_q$ $10^9 M^{-1} s^{-1} \pm 10\%$	$k_t 10^8 M^{-1} s^1 \pm 10\%$	$k_r 10^8 M^{-1} s^1 \pm 5\%$	k_r/k_t	RR_{Rf}	RR_{RB}
Benzidine (BZ)	2.0	3.4	2.6	0.008	0.003	0.16	0.009
O-Tolidine (OT)	3.0	2.4	2.2	0.13	0.06	0.32	0.15
Phenol (PH)		0.49 (a) 0.48(b)	0.014 (a)	< 0.01 (c)		0.83	0.02
Aniline (AN)		5.6 (b)	20(d)			1	1

(a) In water, Ref. [28]; (b) In MeOH, Ref. [29];(c) Ref. [17]; (d) In MeOH, Ref. [30].

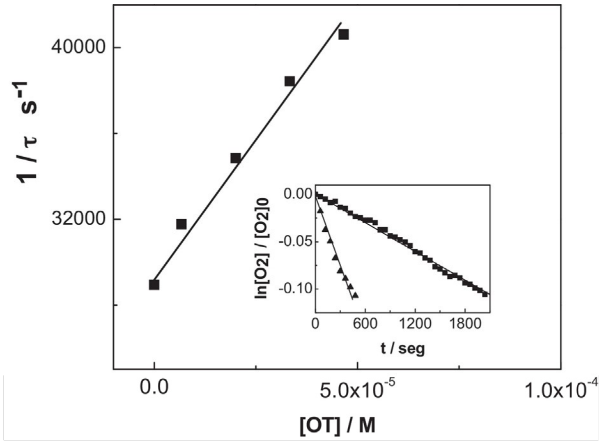


Fig. 4. Stern-Volmer plot for the quenching of $O_2(^1\Delta_g)$ phosphorescence by OT in buffered D_2O/MeOD1:1 (v/v) aerated solution. τ is the $O_2(^1\Delta_g)$ phosphorescence lifetime. *Inset*: First order plots for oxygen uptake in H_2O–MeOH 1:1 (v/v) aerated solution upon photoirradiation (>400 nm) by (■) 0.5 mM OT; (▲) 0.5 mM FFA. $[O_2]_0$ and $[O_2]$ represent the dissolved oxygen concentration at photoirradiation time $t = 0$ and $t = t$, respectively. Sensitizer RB ($A_{550} = 0.5$).

An actual measure for the efficiency of the $O_2(^1\Delta_g)$-mediated oxidative pathway on BZ-C compounds is represented the respective k_r/k_t values shown in Table 1 [34].

3.5. Comparison of BZ-C photooxidation efficiency with the model contaminants PH and AN

As said, the combination of aqueous Rf–BZ-C mixture and visible light, reasonably mimics a potential scenery for contaminated surface waters. On these grounds the photodegradation rate of this system was compared, under identical conditions with that of the archetypal model contaminants PH and AN [35].

The respective ROU in individual photoirradiated solutions 0.5 mM of BZ; OT; PH and AN, in the presence of 0.04 mM Rf were determined in aqueous solution. Again, in order to exclusively search for a $O_2(^1\Delta_g)$-mediated process, analogous experiments were carried out employing RB ($A_{560} = 0.45$) instead of Rf as a sensitizer. The ROU, obtained by monitoring up to 15–20% of the initially dissolved oxygen, was taken as a measure of the overall oxidability for each photoirradiated system. The sequence of the oxidative decay rates in the Rf-sensitized set was AN ~PH > OT > BZ, whereas the sequence upon RB photosensitization was AN > > OT > BZ ~PH. Quantitative results for both systems are shown in Fig. 5A and B.

4. Discussion

4.1. The interaction of BZ-C with different ROS

The spectral changes produced upon photosensitization in BZ and OT solutions indicate the existence of chemical transformations in both substrates. Different mechanistic pathways seem to operate by employing either RB- or Rf-sensitization. An exclusive Type II ($O_2(^1\Delta_g)$-mediated process) prevails in the first case and a composition of Type I (radical-mediated) and Type II mechanisms dominates in the case of Rf-sensitization.

The overall quenching ability of each BZ-C towards $O_2(^1\Delta_g)$ is moderate-to-high, according to the respective k_t values [36]. The accepted mechanism of $O_2(^1\Delta_g)$ quenching by amines, phenols and a couple of electron-donating substrates involves the initial formation of an encounter complex [$O_2(^1\Delta_g)$-substrate] with partial charge-transfer character [34]. From it, the operation of an irreversible electron-transfer process would yield the oxidized

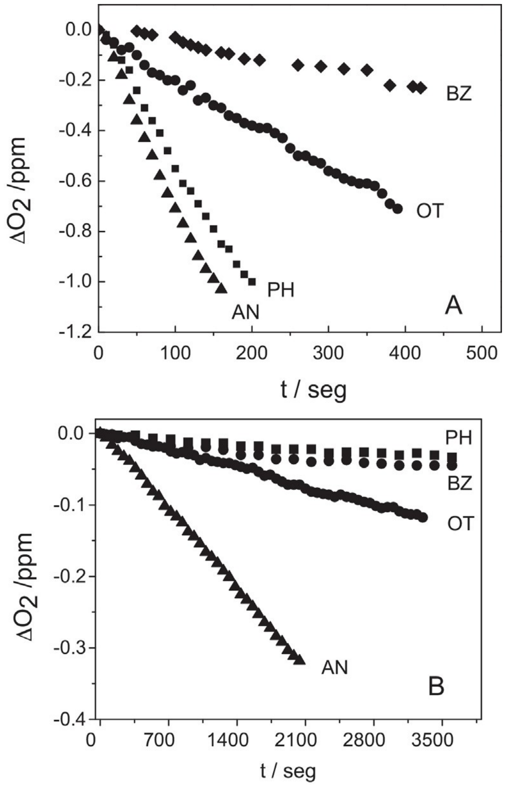


Fig. 5. (A) Oxygen uptake as a function of photoirradiation time ($\lambda_{irr} > 400$ nm) in H_2O-MeOH (1:1, v/v) solutions of 0.038 mM Rf in the individual presence of: 0.4 mM BZ; OT; PH and AN. (B) The same, replacing Rf by RB ($A_{549} = 0.38$).

products. The formation of this complex depends on the electron-donor ability of the substrate, and is favoured by the solvent polarity. In the case of OT, the presence of electron releasing groups in the benzene ring promotes the photooxidation (reaction (16)). Nevertheless the low values observed for the k_r/k_t ratios, especially in the case of BZ, constitutes an expected result in some amine derivatives, where the reversibility of the charge-transfer component within the encounter complex [$O_2(^1\Delta_g)$–Bz-C] precludes an effective oxidation of the substrate [36].

As demonstrated by laser flash photolysis experiments $^3Rf^*$ is quenched by BZ-C (process (4)). This interaction generates the species $RfH^\bullet$ (processes (5) and (6)) that subsequently decays through a disproportionation reaction yielding Rf and fully reduced Rf (RfH_2) (process (6)). In the presence of $O_2(^3\Sigma_g^-)$, RfH_2 is oxidized, giving rise to $RfH_2^{\bullet+}$ and $O_2^{\bullet-}$, and finally producing Rf and H_2O_2 (processes (7) and (8) respectively) [21,37]. The thermodynamic feasibility of the electron-transfer process (reaction (4)) can be estimated by means of the Gibbs free energy for electron transfer, $\Delta_{ET}G_0 = E_{O(BZ\text{-}C/BZ\text{-}C+)} - E_{0(Rf/Rf-)} - E_{Rf^*} + C$ (Eq. (20)) where $E_{0(BZ\text{-}C/BZ\text{-}C+)}$ (0.92 V and 0.875 V for BZ and OT in H_2O respectively) is the standard electrode potential of the donor [38]. $E_{0(Rf/Rf-)}$ represents the standard electrode potential of Rf, in this case the electron acceptor (−0.80 V). E_{Rf^*} is the $^3Rf^*$ energy (2.17 eV), and C is the coulombic energy term (−0.06 V) [39]. From Eq. (20) arises $\Delta_{ET}G_0 = -0.55$ V and − 0.50, indicating that process (4) may be operative. Consequently, the formation of species $O_2^{\bullet-}$ could be incremented (process (4) to (7)) through the above mentioned electron transfer process (4).

Whether process (4) is kinetically competitive with the energy transfer process (13) representing the $O_2(^1\Delta_g)$ generation can be estimated from their respective velocities under work conditions. In other words, the respective products $^{null}k_q$ x [BZ-C] and $k_{ET} \times [O_2(^3\Sigma_g^-)]$ must be compared. The k_{ET} value of process

(13) in MeOH-H_2O (1:1, v/v) is 9.5×10^8 M^{-1} s^{-1} (*i.e.*, 1/9 of the diffusion controlled value) [40] and the respective $^{null}k_q$values are given in Table 1. From this data and considering the same concentrations of dissolved $O_2(^3\Sigma_g^-)$ and BZ-C, the rate value for the generation of $RfH^\bullet$, the initial $O_2^{\bullet-}$ precursor, is between 3.5 and 2.5 times higher the corresponding one for $O_2(^1\Delta_g)$ generation for BZ and OT respectively. In other words, processes (4) and (13) can simultaneously operate, in both BZ-C.

The involvement of $O_2(^1\Delta_g)$ and $O_2^{\bullet-}$ in the photooxidation of the structurally comparable compound 3,3′-diaminobenzidine (DAB) – a fluorescent-labelling systems profusely employed in electron microscopy – and its related complex DAB-Mn^{++} have been published [41,42]. Employing Eosin as a sensitizer, we obtained k_t and k_r values of 11.4×10^8 $M^{-1}s^{-1}$ and 6.2×10^8 $M^{-1}s^{-1}$ respectively for DAB [41]. Steinbeck et al. reported that $O_2(^1\Delta_g)$ is quenched by the DAB-Mn^{++} complex with a k_t value of 1.7×10^8 M^{-1} s^{-1}, and by $O_2^{\bullet-}$ with rate constant vale of 1.6×10^9 M^{-1} s^{-1} [42]. The authors did not search for the eventual involvement of the species H_2O_2 and $OH^\bullet$ in the overall oxidative event.

In the present work, the clear decrease in the rate of oxygen uptake by the photoirradiated Rf - BZ-C solutions, in the individual presence of SOD, CAT and MAN (reactions (16)–(18), Fig. 2)) confirms that $O_2^{\bullet-}$, H_2O_2 and $OH^\bullet$, all *in situ* generated species, participate in the oxidation of each BZ-C (reactions (9), (10) and (12)). Furthermore, kinetic results as indicated by the low kr/kt ratios (Table 1) clearly points to the limited effective oxidation level reached by the species $O_2(^1\Delta_g)$. Hence, it is highly probable that the main degradative pathways in the Rf-sensitized process, might be driven by the composition of several ROS-mediated processes, generated *via* Type I mechanism, and the recombination of the initial radical species.

Regarding the possible by-products, besides the ROS-mediated oxidation ones (steps (9), (10), (12) and (16)), literature reports strongly suggests the formation of polymeric compounds as the final fate for the radical cationic species $BZ^{\bullet+}$ and $OT^{\bullet+}$ (step (4)) [31,32].

4.2. Stability of BZ-C as compared to PH and AN, upon Rf- and RB-sensitization

The set of comparative ROU runs employing PH and AN as reference compounds and Rf as a photosensitizer, allows a rough estimation of BZ-C susceptibility towards degradation by the generated ROS, in a restricted universe of potential water contaminants.

The photodynamic effect on PH and AN is well known [28–30]. The hydroxyl-compound is practically non-reactive towards $O_2(^1\Delta_g)$ at neutral pH 7 although physically deactivates the oxidative species with a moderated rate constant. On the other hand AN effectively quenches $O_2(^1\Delta_g)$ through a dominant chemical fashion (Table 1) [28,29]. This behaviour was evident in the present work from the auxiliary RB-sensitized runs (Fig. 5B). In parallel, both PH and AN quench $^3Rf^*$ through an electron transfer process (see $^{null}k_q$ values in Table 1) [28,29]. Subsequently AN and PH are oxidized by $O_2^{\bullet-}$ produced by reaction (7) (Scheme 1), reported for both substrates as an important degradative step [28,29]. This scenery clearly justifies the high difference observed in the ROU between AN and PH subject to a RB sensitized photoirradiation and the quite similar ROU when RB is replaced by Rf. On the other hand, both BZ-C scarcely react with $O_2(^1\Delta_g)$ being the main degradation pathway due to the participation of the ROS $O_2^{\bullet-}$, $OH^\bullet$ and H_2O_2, all photogenerated after an initial electron transfer process, as already discussed. Within this frame and according to ROU results (Fig. 5A), BZ-C would be 3 to 5 fold less degradable than AN and PH when exposed to an eventual Rf-photosensitized process.

The complexity of natural water systems and the occurrence of simultaneous photoreactions certainty difficult a straightforward extrapolation of the data to field conditions. Nevertheless, the same data indicates, as an overall result, that the effective degradation of BZ-C in the presence of more labile contaminants, could be just a matter of time when the presence of a native photosensitizer, sunlight, and dissolved oxygen is ensured, as currently occurs in natural surface waters. It can be deduced, as an immediate consequence, that confined daylight-irradiated residual waters containing BZ-C could be treated in a simple low-cost reactor, upon addition of a Type I-photosensitizing agent.

5. Conclusions

According to the experimental data, in the presence of Rf as a dye sensitizer and under visible light irradiation, BZ and OT interact with $O_2(^1\Delta_g)$ and the ROS $O_2^{\bullet-}$, H_2O_2 and $OH^\bullet$, in H_2O-MeOH solutions. All oxidative species are generated *in situ* through energy transfer and electron transfer processes by interaction of excited triplet Rf with ground state molecular oxygen and with both BZ-C. The interaction BZ-C - $O_2(^1\Delta_g)$ is essentially physical in nature. Hence, the overall Rf-sensitized photooxidation process seems to be mainly driven by the reaction with the remaining partially reduced ROS.

A comparison of the BZ-C reactivity with well known surface water-contaminants such as AN and PH indicates that BZ-C can suffer a relatively efficient spontaneous photodegradation, under natural environmental conditions. The process occurs in a moderately slower fashion than the corresponding ones for AN and PH within a common overall time-scale.

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References

[1] F. Deniz, E.T. Ersanli, Simultaneous bioremoval of two unsafe dyes from aqueous solution using a novel green composite biosorbent, Microchem. J. 128 (2016) 312–319.

[2] S.K. Sen, S. Raut, P. Bandyopadhyay, S. Raut, Fungal decolouration and degradation of azo dyes: a review, Fungal Biol. Rev. 30 (2016) 112–133.

[3] H. Li, Z. Xiong, X. Dai, Q. Zeng, The effect of perspiration on photo-induced chemical reaction of azo dyes and the determination of aromatic amine products, Dyes Pigments 94 (2012) 55–59.

[4] R. Li, X. Song, Y. Huang, Y. Fang, M. Jia, W. Ma, Visible-light photocatalytic degradation of azo dyes in water by Ag_3PO_4: an unusual dependency between adsorption and the degradation rate on pH value, J. Mol. Catal. A: Chem. 421 (2016) 57–65.

[5] J.M. Chacón, M. Teresa Leal, M. Sánchez, E.R. Bandala, Solar photocatalytic degradation of azo-dyes by photo-Fenton process, Dyes Pigments 69 (2006) 144–150.

[6] C.-C. Liu, Y.-H. Hsieh, P.-F. Lai, C.-H. Li, C.-L. Kao, Photodegradation treatment of azo dye wastewater by UV/TiO2 process, Dyes Pigments 68 (2006) 191–195.

[7] A. Rostami-Vartooni, M. Nasrollahzadeh, M. Salavati-Niasari, M. Atarod, Photocatalytic degradation of azo dyes by titanium dioxide supported silver nanoparticles prepared by a green method using Carpobrotus acinaciformis extract, J. Alloys Compd. 689 (2016) 15–20.

[8] C. Fernández, M. Pilar Callao, M. Soledad Larrechi, UV–visible-DAD and 1H-NMR spectroscopy data fusion for studying the photodegradation process of azo-dyes using MCR-ALS, Talanta 117 (2013) 75–80.

[9] S.A. Azim, Photo-degradation and emission characteristics of benzidine in halomethane solvents, Spectrochim. Acta A 56 (2000) 127–132.

[10] M. Muneer, H.K. Singh, D. Bahnemann, Semiconductor-mediated photocatalysed degradation of two selected priority organic pollutants,

benzidine and 1,2-diphenylhydrazine, in aqueous suspension, Chemosphere 49 (2002) 193–203.

[11] K.-T. Chung, C.E. Cerniglia, Mutagenicity of azo dyes: structure–activity relationships, Mutat. Res. 277 (1992) 201–220.

[12] R. Ouellet-Hellstrom, J.D. Rench, Bladder cancer incidence in Arylamine workers, J. Occup. Environ. Med. 38 (1996) 1239–1247.

[13] C.A. Banassi, E. Scoffone, G. Galiazzo, G. Iori, Proflavine-sensitized photooxidation of tryptophan and related peptides, Photochem. Photobiol. 6 (1967) 857–866.

[14] J.P. Escalada, A. Pajares, J. Gianotti, W.A. Massad, S. Bertolotti, F. Amat-Guerri, N. A. Garcia, Dye-sensitized photodegradation of the fungicide carbendazim and related benzimidazoles, Chemosphere 65 (2006) 237–244.

[15] J.N. Chacon, J. McLearie, R.S. Sinclair, Singlet oxygen yields and radical contributions in the dye-sensitised photo-oxidation in methanol of esters of polyunsaturated fatty acids (oleic, linoleic, linolenic and arachidonic), Photochem. Photobiol. 47 (1988) 647–656.

[16] C.M. Krishna, S. Uppuluri, P. Riesz, JSZ Jr., D. Balasubramanian, A study of the photodynamic efficiencies of some eye lens constituents, Photochem. Photobiol. 54 (1991) 51–58.

[17] F.E. Scully, J. Hoing, Rate constants for the reaction of singlet oxygen with phenols and other compounds in water, Chemosphere 16 (1987) 694–699.

[18] S. Criado, A. Pajares, J. Gianotti, G. Stettler, J.P. Escalada, S. Bertolotti, F. Amat-Guerri, N.A. Garcı#x0301;a, Kinetic study of the riboflavin-sensitised photooxygenation of two hydroxyquinolines of biological interest, J. Photochem. Photobiol. B 71 (2003) 19–25.

[19] M. Neumann, N.A. Garc¡a, Kinetics and mechanism of the light-induced deterioration of lemon oil, J. Agric. Food Chem. 40 (1992) 957–960.

[20] H. Görner, Oxygen uptake induced by electron transfer from donors to the triplet state of methylene blue and xanthene dyes in air-saturated aqueous solution, Photochem. Photobiol. Sci. 7 (2008) 371–376.

[21] C.-Y. Lu, W.F. Wang, W.Z. Lin, Z.H. Han, S.D. Yao, N.Y. Lin, Generation and photosensitization properties of the oxidized radicals of riboflavin: a laser flash photolysis study, J. Photochem. Photobiol. B 52 (1999) 111–116.

[22] J.P. Escalada, A. Pajares, J. Gianotti, A. Biasutti, S. Criado, P. Molina, W. Massad, F. Amat-Guerri, N.A. García, Photosensitized degradation in water of the phenolic pesticides bromoxynil and dichlorophen in the presence of riboflavin, as a model of their natural photodecomposition in the environment, J. Hazard. Mater. 186 (2011) 466–472.

[23] E. Silva, A.M. Edwards, D. Pacheco, Visible light-induced photooxidation of glucose sensitized by riboflavin, J. Nutr. Biochem. 10 (1999) 181–185.

[24] E. Silva, L. Herrera, A.M. Edwards, J. de la Fuente, E. Lissi, Enhancement of riboflavin-mediated photo-oxidation of glucose 6-phosphate dehydrogenase by urocanic acid, Photochem. Photobiol. 81 (2005) 206–211.

[25] P.F. Heelis, The photophysical and photochemical properties of flavins (isoalloxazines), Chem. Soc. Rev. 11 (1982) 15–39.

[26] P.F. Heelis, F. Muller, The Photochemistry of flavins, Chemistry and Biochemistry of Flavoenzymes, CRC Press, 1991, pp. 171–193.

[27] A.W. Varnes, R.B. Dodson, E.L. Wehry, Interactions of transition-metal ions with photoexcited states of flavines. Fluorescence quenching studies, J. Am. Chem. Soc. 94 (1972) 946–950.

[28] E. Haggi, S. Bertolotti, N.A. Garc¡a, Modelling the environmental degradation of water contaminants. Kinetics and mechanism of the riboflavin-sensitized photooxidation of phenolic compounds, Chemosphere 55 (2004) 1501–1507.

[29] M. Barra, B. VanVeller, C. Verberne, Absolute rate constants for 5-deazariboflavin triplet excited state in one-electron transfer processes, J. Photochem. Photobiol. A 225 (2011) 113–116.

[30] N. Miyoshi, G. Tomita, Fluorescein-photosensitized furan oxidation in methanolic and reversed micellar solutions. Part II: Kinetic analysis, Z. Naturforsch., B: J. Chem. Sci. 35 (1980) 107–111.

[31] G.M. do Nascimento, P.S.M. Barbosa, V.R.L. Constantino, M.L.A. Temperini, Benzidine oxidation on cationic clay surfaces in aqueous suspension monitored by in situ resonance Raman spectroscopy, Colloids Surf., A 289 (2006) 39–46.

[32] M.-L. Liu, J. Luo, Y.-Y. Zhang, S.-Z. Yao, In situ piezoelectric infrared spectroelectrochemistry for study of electrochemical oxidation of o-Tolidine, Chin. J. Anal. Chem. 36 (2008) 1172–1176.

[33] F. Amat-Guerri, M.M.C. López-González, R. Martínez-Utrilla, R. Sastre, Singlet oxygen photogeneration by ionized and un-ionized derivatives of Rose Bengal and Eosin Y in diluted solutions, J. Photochem. Photobiol. A 53 (1990) 199–210.

[34] N.A. García, Singlet molecular oxygen-mediated photodegradation of aquatic phenolic pollutants. A kinetic and mechanistic overview, J. Photochem. Photobiol. B 22 (1994) 185–196.

[35] M.M. Halmann, Photodegradation of Water Pollutants, CRC Press, 1995.

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

[37] C. Lu, G. Bucher, W. Sander, Photoinduced interactions between oxidized and reduced lipoic acid and Riboflavin (Vitamin B2), ChemPhysChem. 5 (2004) 47–56.

[38] L.F. Oldfield, J.O.'M. Bockris, Reversible oxidation-reduction reactions of aromatic amines, J. Phys. Chem. 55 (1951) 1255–1274.

[39] G. Porcal, S.G. Bertolotti, C.M. Previtali, M.V. Encinas, Electron transfer quenching of singlet and triplet excited states of flavins and lumichrome by aromatic and aliphatic electron donors, Phys. Chem. Chem. Phys. 5 (2003) 4123–4128.

[40] M. Koizumi, S. Kato, N. Mataga, T. Matsuura, I. Isui, Photosensitized reactions, Kagakudogin (1978).

[41] J.E. Natera, W.A. Massad, F. Amat-Guerri, N.A. García, Elementary processes in the eosin-sensitized photooxidation of 3,3′-diaminobenzidine for correlative fluorescence and electron microscopy, J. Photochem. Photobiol. A 220 (2011) 25–30.

[42] M.J. Steinbeck, A.U. Khan, W.H. Appel, M.J. Karnovsky, The DAB-Mn^{++} cytochemical method revisited: validation of specificity for superoxide, J. Histochem. Cytochem. 41 (1993) 1659–1667.