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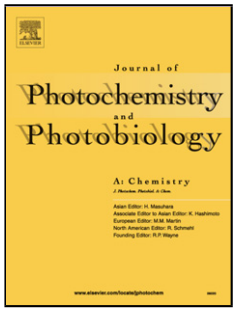

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Riboflavin sensitized photodegradation of Furaneol in a β-cyclodextrin complex

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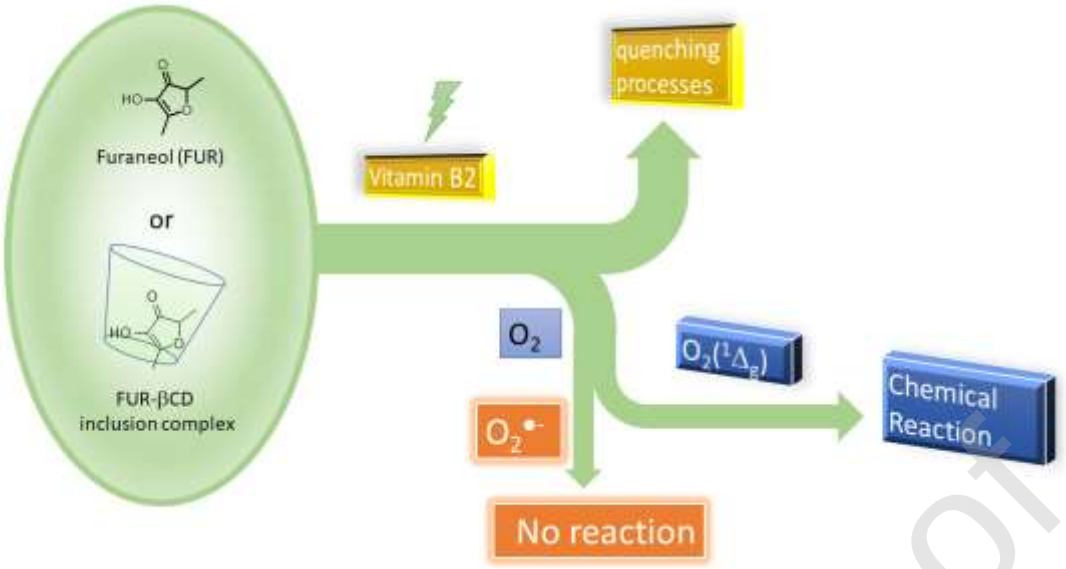
Graphical Abstract



Highlights

- Photochemical reaction of Riboflavin and Furaneol was studied in homogeneous and β-cyclodextrin microheterogeneous media under day-light conditions
- Experimental and theoretical results showed the formation of an inclusion complex of Furaneol in β-cyclodextrin
- The presence of β-cyclodextrin produces an increase in the rate of sensitized degradation of FUR by Rf.

Abstract

A study on the photodegradation of the widely employed flavoring compound Furaneol (FUR), in the presence of vitamin B_2 (Riboflavin, Rf) was carried out, in aqueous and microheterogeneous media. The system as a whole allows the evaluation of FUR stability, since the vitamin is an endogenous natural photosensitizer. In a visible-light-irradiated solution of Rf plus FUR, a complex mechanism of competitive reactions occurs. FUR quenches the singlet (2.7 x 10^9 $M^{-1}s^{-1}$) and triplet (6.0 x 10^8 $M^{-1}s^{-1}$) Rf electronically excited states and reacts with singlet molecular oxygen ($O_2(^1\Delta_g)$) generated from the triplet excited state of the sensitizer, with a reactive rate constant of 1.97 x 10^7 $M^{-1}s^{-1}$. In the oxidation process, two oxygen molecules are incorporated. In the presence of β-cyclodextrin (βCD), experimental and theoretical results show the formation of an inclusion 1:1 complex of FUR with the oligosaccharide. The Rf-photosensitized oxidation rates of FUR were higher in the microheterogeneous medium, probably due to the incorporation of FUR in the non-inclusion complex between Rf and βCD which may produce $O_2(^1\Delta_g)$ near the oxidizable substrate. The overall result of this study demonstrates that visible-light exposure of formulations containing Rf and FUR may cause significant oxidation of the flavoring agent.

Keyboards: Furaneol; Riboflavin; Photodegradation; β-cyclodextrin

1. Introduction

Furaneol (FUR) is an organic flavoring compound, naturally present in a wide variety of ripe fruits and dairy products.[1–4] It has a low olfactory detection threshold, and is capable of imparting a delightful fruity aroma. FUR is produced by chemical synthesis and widely employed as an industrial additive in food, medicinal, and cosmetic formulations.[2,5] FUR is a 3(2*H*)-furanone, a chemical structure with anti-oxidative as well as pro-oxidative properties.[6] FUR presents an antioxidant activity against lipid peroxidation or superoxide radicals in lens tissue, which contributes to inhibiting the onset of spontaneous cataract.[6,7] On the other hand, significant pro-oxidant properties of FUR in foods have been reported.[6,8]

To increase the permanency of FUR fragrance in commercial preparations, manufacturers often incorporate complexing agents. Cyclodextrins (CDs) are among the most used compounds to retain volatile guests and reduce their volatility.[9,10] These naturally occurring cyclic oligosaccharides of 1,4-linked α-D-glucose, formed by the bacterial degradation of starch, possess a relatively hydrophobic central cavity and a hydrophilic outer surface.[9] The described structural characteristics and the low manufacturing costs make CDs appropriate candidates to associate with industrial flavoring substances.[11,12] Although FUR is transparent to visible light, it could be degraded by ultraviolet light, or by photosensitized processes induced by visible light irradiation of photosensitizers present in commercial formulations. Among these photosensitizers is Riboflavin (Rf), a visible-light absorbing vitamin with well-known photosensitizing properties,[13–15] abundant in meat, beer, wine, dairy products, and topical commercial products.[16–18] For example, different studies have reported the Rf-photosensitization of antioxidants, flavorings, or

food-related substances.[19–22] Nevertheless, no previous study has reported the Rf-photosensitization of FUR in homogeneous media or CDs solutions. FUR, Rf, and β-cyclodextrin (βCD) coexist in a wide variety of formulations. Previous researchers have established the formation of a βCD inclusion complex with FUR [12] . With respect to the interaction of βCD with Rf, both the formation of an inclusion complex [23,24] and a non-inclusion complex (Rf-βCD) [25] were postulated. Moreover, it has been demonstrated that CDs can modify the photodegradation rate of some molecules.[26] This scenario constitutes an interesting starting point to know in depth about the possible degradation of FUR, in the presence of Rf in homogeneous and β-cyclodextrin (βCD) microheterogeneous media under day-light conditions.

2. Materials and Methods

2.1 Chemicals and materials

Furaneol (4-Hydroxy-2,5-dimethyl-3-furanone, FUR), Riboflavin (Rf), Rose Bengal (RB), furfuryl alcohol (FFA), and furfuryl acetate (FAc), deuterium oxide 99.9% (D_2O), β-cyclodextrin (βCD), and superoxide dismutase (SOD) from bovine erythrocytes, were purchased from Sigma-Aldrich (USA). All chemicals were used without further purification. Water was triple distilled. Methanol, HPLC grade, was obtained from Sintorgan. All experiments were carried out at room temperature, using freshly prepared solutions.

2.2 Spectroscopy

Absorption spectra were carried out in an Agilent 8453 diode array spectrophotometer.

Fluorescence spectra were measured with a Spex Fluoromax spectro-fluorometer at 25 ± 1 °C in air-equilibrated solutions. Fluorescence lifetimes were determined by a time-correlated single-photon counting technique (SPC) on an Edinburgh FL-900CD instrument, equipped with a blue LED (PicoQuant PLS 450).

2.3 Photolysis experiments

Steady-state aerobic photolysis of aqueous solutions of Rf or RB plus FUR was carried out in a customized photolysis device that uses two Light Emitting Diodes (LED) as the excitation source.[27] Blue (λmax = 467 nm) or green (λmax = 510 nm) LEDs for Rf- and RB-sensitization were employed, respectively.

2.4 Quenching of Rf electronically excited states

Fluorescence quenching was studied in methanol (due to the low solubility of FUR in water). The bimolecular rate constant for quenching of Rf singlet excited state (^{1}Rf*) by FUR (^1k_q) was determined using classical Stern–Volmer plots of $^1\tau_0/\ ^1\tau$ (eq. 1)

$$^1\tau_0/\ ^1\tau = 1 + {}^1k_q\,\tau_0\,[\mathrm{FUR}] \qquad \text{eq. 1}$$

where $^{1}\tau$, and $^{1}\tau_0$ are the lifetimes for Rf fluorescence in the presence and absence of FUR, respectively. In all cases, the lifetime of Rf fluorescence decay was fitted by a monoexponential decay.

Steady-state Rf fluorescence quenching by FUR data was treated using the modified Stern-Volmer equation, eq. 2: [14,28]

$$I_0/I = (1+K_D[Q])(1+Kas[Q]) \qquad \text{eq. 2}$$

where the constant K_D ($K_D = {}^{1}k_q\,{}^{1}\tau_0$) considers the dynamic component of fluorescence quenching.

Transient absorption spectra in a flow system under Argon-saturated 0.04 mM Rf aqueous solutions were determined, using a flash photolysis equipment previously reported.[27,28] Usually, the signal transient at each wavelength was an average of five laser shots. Riboflavin triplet excited state (^{3}Rf*) signal was monitored at 670 nm. ^{3}Rf* decay was determined at low concentrations of Rf and low enough laser energy to avoid self-quenching and triplet-triplet annihilation. For the determination of the rate constant for the interaction of ^{3}Rf*–FUR ($^{3}k_q$), the Stern–Volmer expression (eq. 3) was employed.

$$1/{}^{3}\tau = (1/{}^{3}\tau_0) + {}^{3}k_q[FUR] \qquad \text{eq. 3}$$

where $^{3}\tau$ and $^{3}\tau_0$ are the measured lifetimes of ^{3}Rf* in the presence and absence of FUR, respectively.

2.5 Reaction of FUR with singlet oxygen

In order to determinate the contribution of singlet oxygen ($O_2(^1\Delta_g)$) in FUR degradation, RB as a sensitizer was used. RB has a reported $O_2(^1\Delta_g)$ generation quantum yield of 0.75 [29] and has been widely used for this type of determinations.[27,28]

The total quenching rate constant for $O_2(^1\Delta_g)$ deactivation by FUR (k_t) was measured by time-resolved phosphorescence detection (TRPD) equipment.[27] Air-saturated solutions were employed in all cases. D_2O instead of H_2O was used as a solvent, to enlarge the lifetime of $O_2(^1\Delta_g)$ within the temporal range.[30] The $O_2(^1\Delta_g)$ lifetime was evaluated in the presence (τ), and absence (τ_0) of FUR, and the data were plotted as a function of concentration, according to a simple Stern–Volmer treatment ($1/\tau = 1/\tau_0 + k_t$ [FUR]).

The reactive rate constant, k_r, for the reaction of $O_2(^1\Delta_g)$ with FUR was determined by a comparative method already reported.[31] k_r was calculated from the expression $slope/slope_R = k_r$ [FUR]/k_{rR} [R], where slope and $slope_R$ denote slopes of a pseudo-first-order plot of FUR consumption or a reference compound (R) under RB-sensitized irradiation. Assuming the reaction of $O_2(^1\Delta_g)$ with FUR is the only way of O_2 consumption, with a 1:1 stoichiometry, the ratio of the first order slope of O_2-uptake by FUR and R, yields k_r/k_{rR}. FFA as the reference compound was used with a reported k_r value of 1.2×10^8 M^{-1} s^{-1}.[30]

Experiments of O_2-uptake was monitored with a polarographic 97-08 Orion electrode and carried out in customized photolysis equipment, as previously reported.[27] Briefly, the irradiation source was a 150 W quartz-halogen lamp, and a cut-off filter of 400 nm was used to guarantee RB was the only absorbing specie.

Rates of FUR photo-consumption from absorbance decrease at 287 nm were obtained, upon RB-sensitized photolysis using two green LED as excitation source in the system described previously. In these experiments, FAc was used as reference. FAc has a reported k_r value of 5.5 x 10^7 $M^{-1}s^{-1}$.[32]

2.6 Interaction βCD-FUR

The association constant (K_{in}) between βCD and FUR was determined by UV-Visible absorption spectroscopy. FUR maximum absorbance changes were monitored as a function of the concentration of β-CD increment. The FUR concentration ([FUR]) remained constant through the experience. Employing this experimental data and using the Benessi-Hildebrand method (eq. 4), the association constant value was calculated:

$$\frac{1}{A} = \frac{1}{\varepsilon[\mathrm{FUR}]_0 K_{in}[\beta\mathrm{CD}]} + \frac{1}{\varepsilon[\mathrm{FUR}]_0} \quad \text{eq. 4}$$

where: A is FUR absorbance after each βCD addition, $[FUR]_0$ is FUR concentration, kept constant, K_{in} is the association constant, [βCD] is the βCD concentration and ε is the complex molar extinction coefficient.[33,34]

2.7 Molecular Modeling

The inclusion of FUR in the βCD cavity was simulated with the semiempirical PM6 model,[35] following a known procedure.[36] The initial geometry of βCD was built from crystallographic data.[37] Two possible orientations were considered for the inclusion complex formation: the “Head-Up” orientation in

which FUR points toward the primary OH of βCD and the “Head-Down” orientations, with FUR facing the secondary OH groups of βCD. A total of 13 structures for each orientation were calculated, passing the drug completely through the βCD cavity. The PM6 optimized geometries of minimum energy in each orientation were further optimized using the DFT B3LYP/6-311G(2d,2p) level of theory. The solvent effect was simulated using the universal solvation model SMD.[38] The nature and strength of relevant intermolecular interactions between FUR and βCD were analyzed with the Quantum Theory Atoms in Molecules (QTAIM) methodology,[39] using a wavefunction generated at B3LYP/6-311G(2d,2p) level of theory. All the calculations were performed with GAUSSIAN 09 [40] and Multiwfn [41] software packages.

3. Results

3.1 Riboflavin sensitized photodegradation of FUR

Blue light irradiation of a FUR (0.1 mM) + Rf (0.03 mM) aqueous solution produced meaningful spectral changes in the electronic absorption spectrum of FUR (Figure 1, main). The experiment was carried out in such a way that only the sensitizer absorbs blue LED radiation, λ_{max}= 467 nm (Figure S1). In the same run, O_2 consumption was observed. Since O_2-consumption can be due to reaction of ROS generated by interaction of ^{3}Rf* with FUR or with Rf itself, a control experiment was carried out by irradiation of Rf solution alone (Figure 1, inset). No changes in oxygen concentration were observed, suggesting that reaction of ROS with FUR is the main route for O_2-consumption.

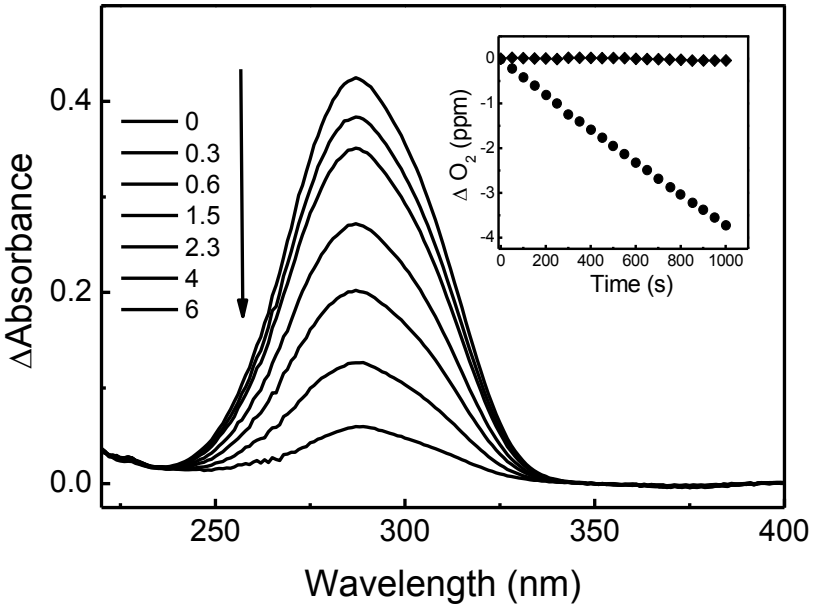
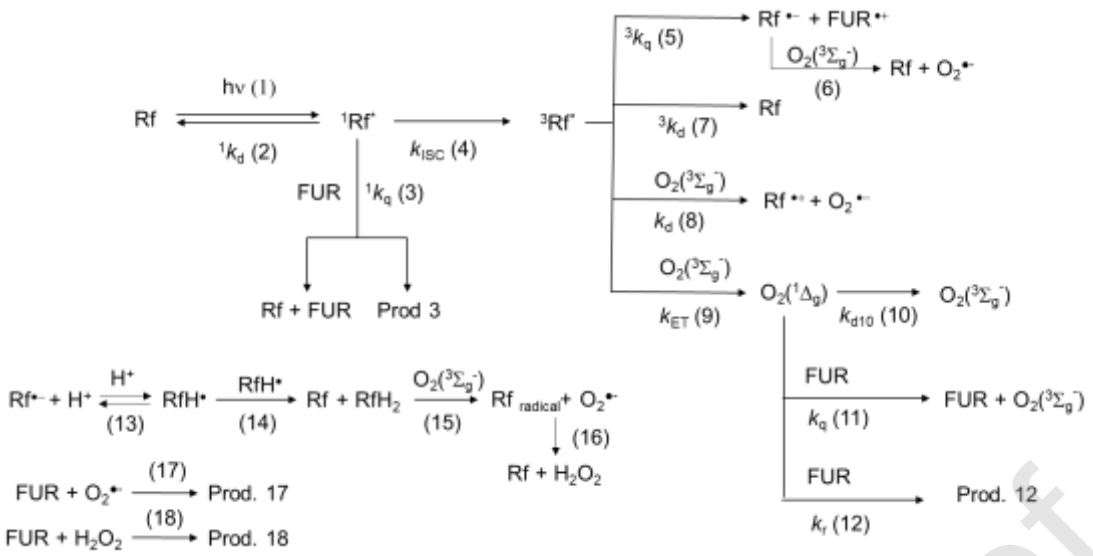


Figure 1: Difference absorption spectra of aqueous solutions [FUR] = 0.1 mM + [Rf] = 0.03 mM vs. [Rf] = 0.03 mM after different photoirradiation times. Numbers in the spectrum correspond to photolysis time in minutes. Inset: Oxygen uptake as a function of photoirradiation time with blue-light: (♦) Rf (0.03 mM) and (●) FUR (0.1 mM) + Rf (0.03 mM).

These results strongly suggest the participation of Rf electronically excited states (Rf*) and/or reactive oxygen species (ROS) photogenerated by the vitamin in the degradation of FUR. It is known that Rf + light is capable to degrade compounds belong to different chemical family-like phenols [42] , bisphenols [43] , catecholamines [27] , benzimidazoles [44] . In all the mentioned cases Rf presents a reaction scheme that may contain the reactions described in Scheme 1.



Scheme 1: Reaction mechanism pathway for visible light irradiation of furaneol (FUR) in the presence of riboflavin (Rf) in aqueous solution.

Results were interpreted and discussed based on Scheme 1. Briefly, vitamin B2 absorbs in the visible spectrum and initially yields ^{1}Rf* and produces ^{3}Rf* through efficient intersystem crossing (process (4)) with a quantum yield of 0.67.[13] The ^{3}Rf* has a strong oxidation potential (E ≈+1.7 V),[13] which may produce substrate degradation *via* electron transfer (process (5)). Moreover, ^{3}Rf* can react with O_2 in the ground state (process (8)), giving rise to the formation of ROS such as superoxide radical anion ($O_2^{\bullet-}$, process (8)) and singlet oxygen (process (9)) with known quantum yields of 0.009 and 0.49 respectively.[16,28]

As can be inferred from photolysis experiments, Rf electronically excited states and different ROS may be involved in FUR degradation. Thus, the following set of experiments were carried out in order to elucidate the photodegradation mechanism.

3.2 Reaction of Rf with FUR*

FUR quenches the fluorescence emission of the singlet-excited state of Rf, reducing fluorescence intensity but maintaining the shape of the emission band. Simultaneously, Rf fluorescence lifetime decay in the absence (${}^1\tau_0$) and presence (${}^1\tau$) of FUR was determined by SPC technique. Figure 2 (inset A) shows the respective Stern-Volmer plots obtained from steady-state and time-resolved measurements. The steady-state Rf fluorescence quenching exhibits a positive curvature, whereas the plot for time-resolved Rf fluorescence quenching data is linear. This kind of Stern-Volmer plots appears when a fluorescent compound is simultaneously quenched by dark association of its ground state (*Kas*, process (19)) and by collisional deactivation of its excited singlet state (process (3), Scheme 1).

$$Rf + FUR \rightleftharpoons [Rf\cdots FUR] \qquad (19)$$

According to previous works,[14,28] this kind of system can be treated using the modified Stern-Volmer, equation 2 (see section 2.4). The dynamic component of fluorescence quenching by time-resolved experiments was independently determined. From inset A of Figure 2, a K_D value of 15.5 ± 0.3 M^{-1} was found for FUR. Using the obtained ${}^1\tau_0$ value of 5.73 ns, which is in concordance with the published fluorescence lifetime in methanol,[28,45] ${}^1k_q$ = (2.7 ± 0.1) x 10^9 M^{-1} s^{-1} was calculated for FUR (Table 1). Using the formerly mentioned values, and a non-linear least-square fitting of eq. 2, an association constant of *Kas* = 1.8 ± 0.2 M^{-1} was obtained.

The ^{3}Rf* lifetime was reduced by FUR presence, demonstrating the occurrence of an interaction between them (Figure 2, inset B). Analysis of the triplet quenching using the expression $1/^3\tau = (1/^3\tau_o) + {}^3k_q$ [FUR] was carried out, and a value for the bimolecular rate constant of ^3k_q (6.0 ± 0.2) x 10^8 M^{-1} s^{-1} was obtained (Table 1), as shown in the inset B of Fig. 2.

Table 1: Quenching rate constant for singlet (^1k_q) and triplet excited state (^3k_q) of Rf by FUR in homogenous and 0.01 M βCD. Reactive (k_r) and overall (k_t) quenching of $O_2(^1\Delta_g)$, RB was used as photosensitizer for $O_2(^1\Delta_g)$ measurements.

	^1k_q ($M^{-1}s^{-1}$) x 10^{-9} $M^{-1}s^{-1}$	**^3k_q ($M^{-1}s^{-1}$) x 10^{-8}**	**k_t ($M^{-1}s^{-1}$) x 10^{-7} (b)**	**k_r ($M^{-1}s^{-1}$) x 10^{-7}**
homogeneous media	2.7 ± 0.1 (a)	6.0 ± 0.2	2.17 ± 0.03	1.97 ± 0.04 4.1 ± 0.1 (c)
0.01 M βCD aqueous solution	1.89 ± 0.05	ND	3.7 ± 0.4	3.92 ± 0.05

(a) in methanol; (b) in D_2O; (c) measured by O_2-consumption

Transient absorption spectra were measured to evaluate the interaction between FUR and ^{3}Rf*. Figure 2 displays the transient spectrum for 0.03 mM Rf solution at 4 and 20 μs after the laser pulse. Also, Figure 2 shows the transient spectra of the same solution plus FUR 1 mM, under this condition 96% of ^{3}Rf* is quenched by FUR.

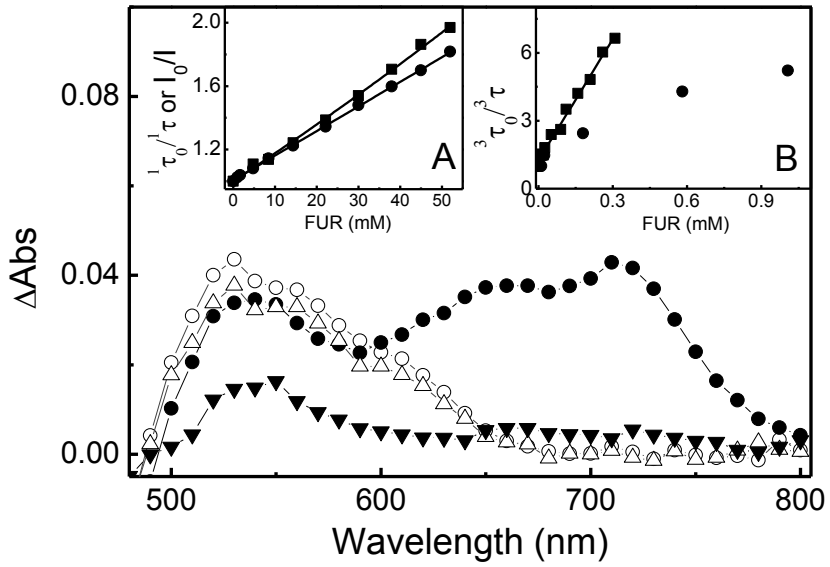


Figure 2: Transient absorption spectrum of Rf (0.03 mM) taken 4 (●) and 20 (▼) microseconds after the laser pulse, respectively. Transient absorption spectra of Rf (0.03 mM) + FUR (1 mM), 4 μs after the laser pulse (○), and 20 μs after the laser pulse (◊). Inset A: Stern-Volmer plots for steady-state (■) and time-resolved (●) Rf fluorescence quenching by FUR. Inset B: Stern-Volmer plot for ^{3}Rf* quenching by FUR: (■) in aqueous solution and (●) in micro-heterogeneous media of βCD (10 mM).

3.3 Reaction of FUR with photogenerated ROS

Visible light irradiation of RB (Abs_{549}= 0.39) + FUR (0.1 mM) vs. RB (Abs_{549}= 0.39) in aqueous solution causes changes in FUR absorption intensity (Figure 3, main). This experiment demonstrates the possible existence of an interaction FUR-$O_2(^1\Delta_g)$, which may be physical (k_q, reaction (11)) and/or chemical (k_r, reaction (12)), see Scheme 1. Rate constants k_q and k_r were determined using RB as the sensitizer. As mentioned in section 2.5, RB is an exclusive $O_2(^1\Delta_g)$

generator, with a quantum yield Φ_Δ of 0.75.[29] The election of this photosensitizer makes it possible to discard the reaction of FUR with other ROS generated from ^{3}Rf*.

For overall quenching rate constant value determination k_t ($k_t = k_q + k_r$), $O_2(^1\Delta_g)$ phosphorescence was quenched by FUR in sub-mM concentration range (Figure 3, inset). Through a Stern–Volmer treatment, a k_t value of (2.17 ± 0.03) x 10^7 $M^{-1}s^{-1}$ was determined in D_2O (Table 1). The deuterated solvent was used in order to increase $O_2(^1\Delta_g)$ phosphorescence lifetime. This result confirms the interaction between $O_2(^1\Delta_g)$ and FUR. The reactive rate constant was measured by two different experiments: (a) by monitoring the decrease in oxygen concentration by O_2-uptake measurements (Figure 4, main), and (b) by FUR degradation measured by UV–Vis absorption spectroscopy (Figure 4, inset). Rate constant values k_r of (4.1 ± 0.1) x 10^7 $M^{-1}s^{-1}$ and (1.97 ± 0.04) x 10^7 $M^{-1}s^{-1}$ were obtained through O_2 and FUR consumption in aqueous solution, respectively (see Table 1).

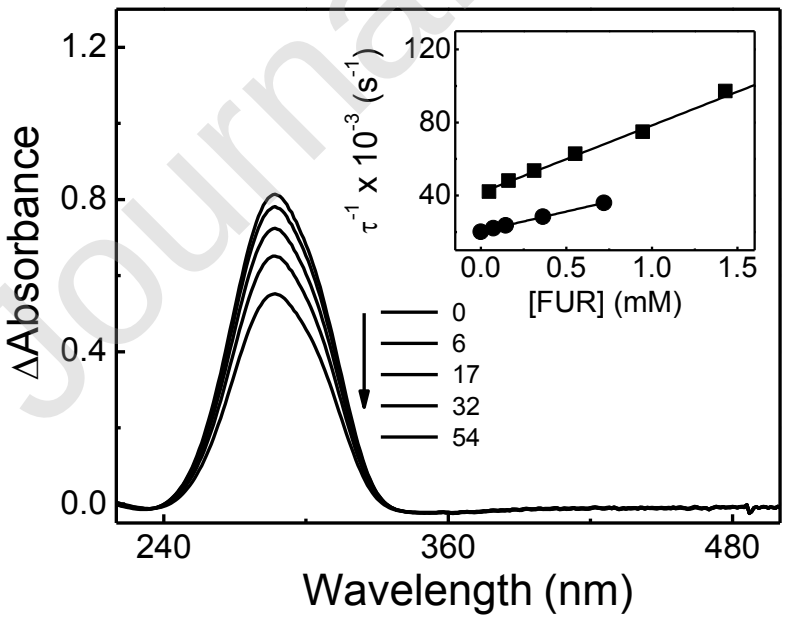


Figure 3: Difference absorption spectra of aqueous RB (Abs_{549}= 0.39) + FUR (0.1 mM) vs RB (Abs_{549}= 0.39) upon irradiation at 510 nm under air-saturated conditions. Numbers in the spectrum represent the irradiation time in minutes. Inset: Stern–Volmer plot for quenching of $O_2(^1\Delta_g)$ phosphorescence by FUR in (●) D_2O and (■) βCD (10 mM) in D_2O.

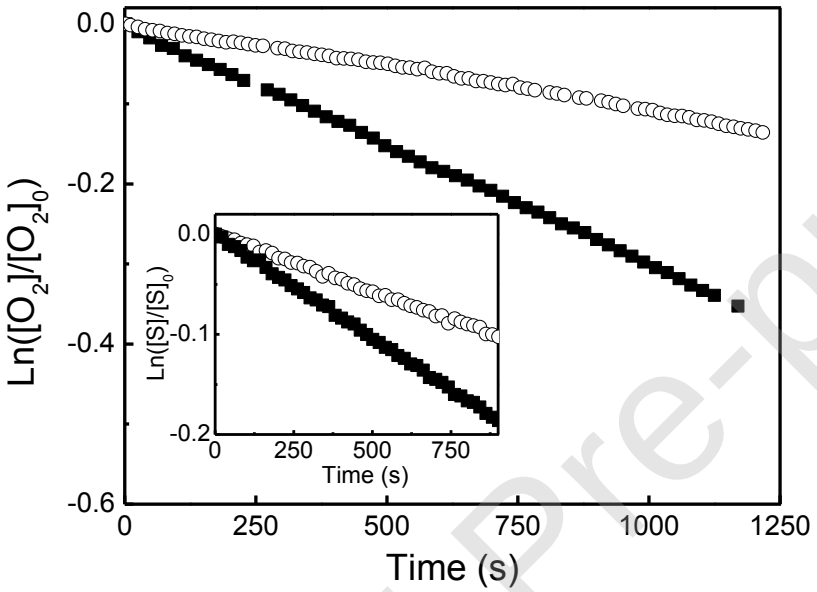


Figure 4: First order plots for O_2-uptake in aerated solution upon visible-ligth irradiation by (○) FUR (0.5 mM) and (■) Furfuryl Alcohol (0.5 mM). Sensitizer: RB (Abs_{549}= 0.28). Inset: First order plots of FUR consumption (measured by UV–vis spectroscopy) of aqueous solutions of RB (Abs_{549}= 0.39) containing (○) FUR (0.1 mM) or (■) FAc (0.07 mM) upon visible-light irradiation.

To assess the participation of $O_2^{\bullet-}$ in FUR photosensitized degradation mechanism, Rf sensitized photolysis assays were performed in the presence of 0.075 nM superoxide dismutase enzyme (SOD). SOD is a specific $O_2^{\bullet-}$

quencher (reaction (20)) and it has been frequently employed to confirm/discard $O_2^{\bullet-}$ participation in oxidative reactions.[14,43]

$$2\ O_2^{\bullet-} + 2\ H^+ \xrightarrow{SOD} H_2O_2 + O_2 \qquad (20)$$

No changes in the FUR degradation rate in the presence and the absence of SOD were observed (data not shown). This outcome disproves the $O_2^{\bullet-}$ relevance in the aforementioned mechanism.

3.4 Effect of βCD in FUR aqueous solution

The gradual addition of βCD to a FUR aqueous solution caused changes in the FUR UV-visible absorption spectrum. As shown in Figure S2, when β-CD was added to an aqueous solution of FUR, an increase at 286 nm absorption maximum was observed. Moreover, a slight bathochromic shift of the entire absorption band was detected. These results suggest the formation of the already reported [11] inclusion complex between the oligosaccharide and the flavoring agent. A stability constant value K_{in} = 6.8±0.6 M^{-1} (reaction (21)) for the species FUR-βCD was determined employing the Benesi Hildebrand model for a 1:1 complex. This stoichiometry is coherent with the observed linearity of the fitted experimental data (please see Fig. S2, inset).

$$FUR + \beta CD \rightleftharpoons FUR\text{-}\beta CD \qquad (21)$$

3.5 Molecular Modeling of the FUR-βCD inclusion complex

The FUR-βCD inclusion complex molecular structure employing semiempirical and DFT calculations was investigated. The stabilization energy (ΔE) of FUR-

βCD formation in both orientations was calculated at the PM6 level of theory with equation 5.

$$\Delta E = E_{complex} - \left(E_{furaneol} + E_{\beta CD}\right) \qquad \text{eq. 5}$$

The ΔE values are reported in Table 2. The complex denoted as Head-Down is more stable than the Head-Up by 10 kJ mol^{-1}. This energy difference slightly decreases when it is calculated with the B3LYP/6-311G(2d,2p) level of theory in the gas phase and becomes significantly lower (≈ 3 kJ mol^{-1}) when the solvent (water) is considered in the calculations. The SMD/B3LYP/6-311G(2d,2p) optimized geometry of the FUR-βCD complex in Head-Down orientation is illustrated in Figure 5.

Table 2. Stabilization energies (in kJ mol^{-1}) of furaneol inclusion complexes with βCD calculated at different levels of theory.

FUR-βCD	ΔE_{PM6}	ΔE_{B3LYP}(gas phase)	ΔE_{B3LYP}(SMD)
Head Up	-77.87	-84.12	-24.72
Head Down	-87.20	-92.36	-27.43

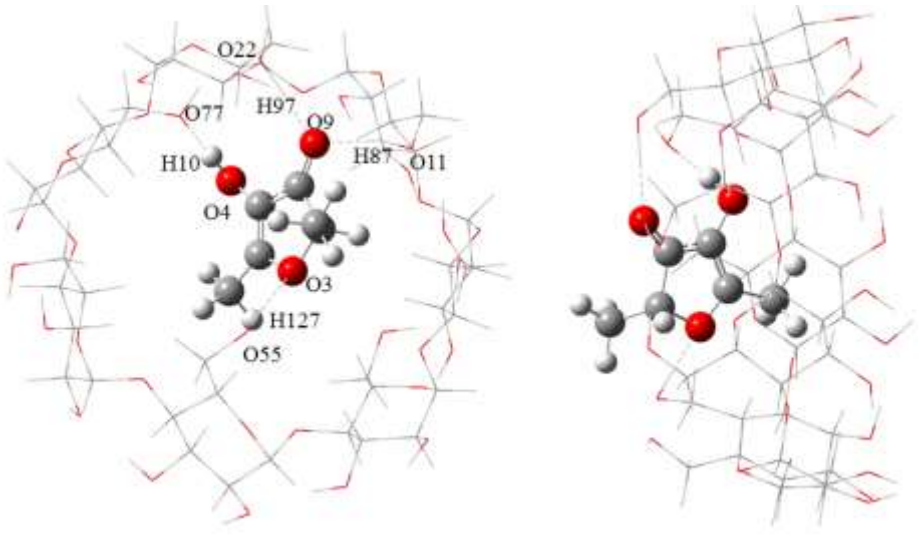


Figure 5: Molecular structure of the most stable FUR-βCD inclusion complex calculated at the SMD/B3LYP/6-311G(2d,2p) level of theory

The inclusion of FUR inside the βCD cavity facilitates the formation of several intermolecular H-bonds between guest and host. Two H-bonds are formed between the carbonyl of FUR and two primary hydroxyl groups of βCD (bond lengths of 1.881 Å and 1.909 Å and bond angles of 177° and 162°, respectively). Other H-bonds are observed between the hydroxyl group and heterocyclic oxygen atom of the guest drug with two primary hydroxyls of βCD. These H-bonds are depicted in Figure 6 and their bond lengths and angles are reported in Table S1.

The strength of intermolecular H-bonds of this inclusion complex can be analyzed with QTAIM theory. In this method, any interaction between two atoms is characterized by bond critical points (BCP). In addition, topological parameters of these BCP, such as electron density ρ(r) and its Laplacian $\nabla^2\rho$, can be used to elucidate the interaction nature. The ρ(r) values of

intermolecular H-bonds typically are in the range of 0.002−0.04 au while $\nabla^2\rho$ values are within the 0.024−0.139 au range.[46] The BCPs of FUR-βCD complex are shown in Figure 6 and the topological parameters ρ(r) and $\nabla^2\rho$ associated with the intermolecular H-bonds are reported in Table S1. These parameters indicate that H-bond between the hydroxyl group of FUR and OH groups of βCD is particularly strong. The strength of other H-bonds is also considerably high, suggesting that these interactions increase inclusion complex stability.[47]

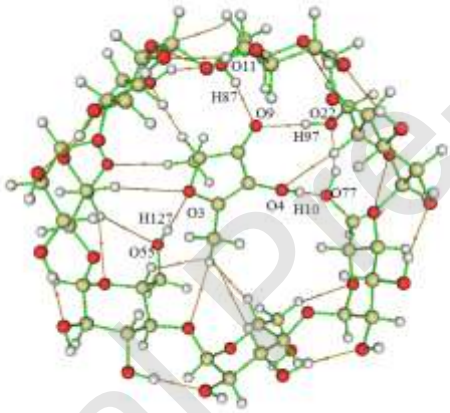


Figure 6: Molecular graph of the furaneol:βCD inclusion complex generated with a wavefunction calculated at B3LYP/6-311G(2d,2p) level of theory. Color references: Red = oxygen, green = carbon and white = hydrogen YP/6-311G(2d,2p) level of theory.

3.7 Sensitized photodegradation of FUR in βCD

In order to evaluate the effect of inclusion complex in photosensitized degradation of FUR, experiments of FUR consumption in presence of βCD using Rf and RB as photosensitizers were performed. These determinations

were made in identical conditions, as used in aqueous media (in βCD absence). Briefly: βCD (10 mM) was added to aqueous solutions of FUR (0.5 mM). The FUR concentration used was 20-times lower than the concentration of βCD. Under this condition, and according to the determined K_{in}, *ca*. 30% of FUR molecules remain free in solution during the photolysis experiments

Figure 7 presents the relative rates of FUR photodegradation by different systems containing Rf as dye sensitizer. Therefore, it can be assured that βCD produces a clear increase in FUR degradation rate. It could be attributed to FUR reaction with Rf-excited states or with ROS photogenerated by Rf and/or *Rf-βCD (Figure S3). The addition of SOD does not change the FUR degradation rate (Figure 7), the same result as in the absence of βCD. Moreover, it was observed that this cyclic oligosaccharide is capable of increasing the rate of Rf degradation itself, which inevitably occurs during its irradiation and may be evidenced by a drop in absorption band centered at 449 nm (see Figure S3, inset).

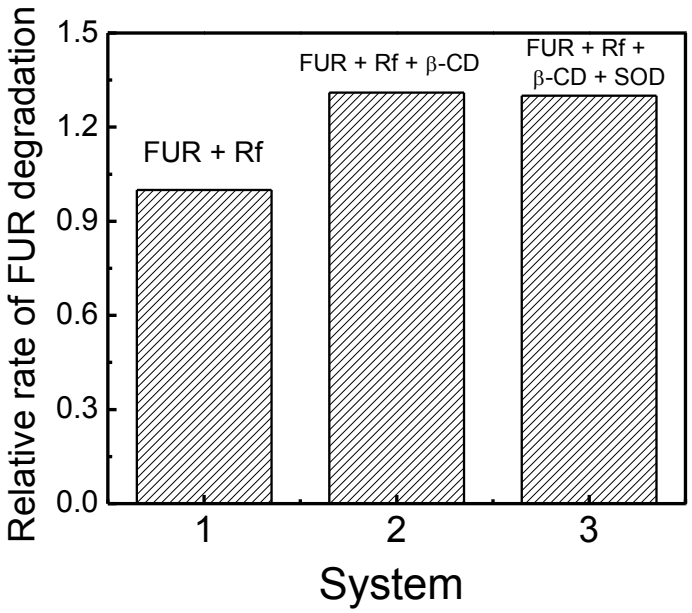


Figure 7: Bars diagram for the relative rates of FUR photo-degradation by different systems containing Rf as dye sensitizer: (1) system I: 0.044 mM Rf + 0.5 mM FUR; (2) system I + 10 mM βCD; (3) system I + 10 mM βCD + 10 nM SOD.

Regarding the interaction between FUR and ^{1}Rf* in βCD microheterogeneous media, a value of (1.89±0.05) x10^9 $M^{-1}s^{-1}$ was determined by means of measurement of time-resolved fluorescence quenching of ^{1}Rf* by FUR in 0.01 M of βCD (Table 1, Figure S4).

With the purpose of studying the βCD effect in the ^{3}Rf* quenching by FUR, the laser flash photolysis technique was again employed. As shown in the inset B of Figure 2, $^3\tau_0/^3\tau$ relationship at different [FUR] shows a curvature in the Stern-Volmer graph (see Table 1).

To assess the role of $O_2(^1\Delta_g)$, k_r and k_t measurements were done in βCD (10 mM). These values were determined from substrate consumption and

$O_2(^1\Delta_g)$ phosphorescence respectively, using RB as a sensitizer. In this media, values of k_r = (3.92±0.05) x 10^7 $M^{-1}s^{-1}$ (Figure S5) and k_t = (3.7±0.4) x 10^7 $M^{-1}s^{-1}$ (Figure 3, inset) were obtained respectively (Table 1).

4. Discussion

4.1 Homogeneous medium

Regarding the dark interaction Rf-FUR, steady-state fluorescence studies confirm that FUR is associated with Rf in the ground state. This dark complex possesses an association constant (*Kas* =1.8 ± 0.2 M^{-1}, process (19)). In addition to this, a ^1k_q = 2.7 10^9 M^{-1} s^{-1} value for the dynamic component of ^{1}Rf* quenching by FUR was obtained by fluorescence lifetime experiments. These results are in agreement with other studies, which showed that Rf forms dark complexes with a variety of low molecular weight organic molecules.[14,28]

According to fluorescence results, a FUR concentration similar to those that used in Rf sensitized photolysis, causes a decrease in ^{1}Rf* lifetime lower than 2%. Hence, under this condition, all effects originated from the interaction of FUR with Rf-excited states could be exclusively assigned to interaction with ^{3}Rf*. Rf-transient spectrum immediately after the laser pulse is similar to that found in previous research.[48] While Rf-transient spectrum obtained in presence of 1 mM FUR is analogous to that informed for $RfH^{\bullet}$ species.[28,48] This result is consistent with an electron transfer process from FUR to ^{3}Rf* producing FUR radical cation ($FUR^{\bullet+}$) and Rf radical anion ($Rf^{\bullet-}$), respectively. At pH 7, the neutral Rf radical ($RfH^{\bullet}$, pKa = 8.3) would be formed after protonation of the species $Rf^{\bullet-}$ (reaction (13)). [14,28]

As proposed in Scheme 1, ^{3}Rf* can be quenched by ground-state molecular oxygen dissolved in the solution generating $O_2^{\bullet-}$ (process (8)). Also, the $O_2^{\bullet-}$ production may be increased by an electron transfer reaction (process (5)), generating $Rf^{\bullet-}$ followed by reaction with O_2 (process (6)). An other way includes $Rf^{\bullet-}$ which can be quickly protonated (process (13)) and later produces $O_2^{\bullet-}$ (reactions (14) and (15)).[16,28] However, O_2-uptake experiments in presence of SOD allow to discard $O_2^{\bullet-}$ reaction with FUR.

Besides $O_2^{\bullet-}$ generation, the reaction of ^{3}Rf* with O_2 can generate $O_2(^1\Delta_g)$ (process (9)), and this ROS may react with FUR *via* physical (process (11)) and/or chemical (process (12)) quenching process. As a substituted furanone, FUR is a good $O_2(^1\Delta_g)$ quencher.[49] FUR quenches $O_2(^1\Delta_g)$ with rate constants of 1.97 x 10^7 $M^{-1}s^{-1}$ and 2.17 x 10^7 $M^{-1}s^{-1}$ for k_r and k_t, respectively. It is interesting to note that the k_r value determined by oxygen consumption (4.1 x 10^7 $M^{-1}s^{-1}$) is twice as large as obtained by substrate consumption.[50] Assuming that reaction of $O_2(^1\Delta_g)$ with the quencher is the only way for oxygen uptake, this result could indicate that two oxygen molecules react with one FUR molecule in the $O_2(^1\Delta_g)$ reaction represented by step (12), Scheme 1. This finding is consistent with the research published by Chen *et al.*, [51] reporting to 2-oxopropyl 2-acetoxypropionate as one of the final degradation products of FUR by $O_2(^1\Delta_g)$.

In addition, k_r/k_t ratio evaluation represents an approach to the fraction of overall quenching that results in a chemical reaction. In this case, the high value for the quotient $k_r/k_t \sim 0.91$ exhibited by FUR, implies that the interaction of $O_2(^1\Delta_g)$ is mainly reactive in nature.

4.2 Microheterogeneous medium

After βCD addition to the Rf + FUR system, an additional series of new interactions must be taken into account. FUR forms a 1:1 inclusion complex with βCD (reaction (21)) (see Figure S2). The FUR-βCD inclusion complex was confirmed and its molecular structure was studied by computational methods. The ΔE values for Head-Down and Head-Up complexes indicate that the formation of both orientations is favorable in aqueous solutions, and both orientations have similar stabilities in this environment (log K = 4.81 for Head Down and log K = 4.33 for Head Up).

Concerning to the Rf-βCD interactions, this system has been studied by Bispo de Jesus *et. al.*[25] These authors reported a non-inclusion complex with an association constant of 39.7 (process (22)):

$$Rf + \beta\text{-CD} \rightleftharpoons Rf\text{-}\beta CD \qquad (22)$$

Rf-βCD formation is attributed to hydrogen bonding between the flavonoid and the external rim of βCD.[25] This association does not significantly affect Rf singlet photophysical properties, the fluorescence lifetime and emission spectra of Rf in water and βCD are almost the same. Whereas the T–T absorption of Rf in βCD is similar in both media (water and βCD), with additional bleaching around 380 nm which is assigned to the absorption due to the formation of H-bonded complex of Rf with the external hydroxyl groups of βCD in ground state.[52]

Concerning βCD effect in Rf-photosensitized degradation of FUR, the presence of βCD increases by 30% the Rf photosensitized degradation rate of FUR (see Figure 7). This rise in FUR rate consumption can be attributed to new reactive species present in βCD medium (Rf-βCD excited states and/or FUR-βCD inclusion complex) and/or changes in the quenching rate process in aqueous media.

As regards the interactions between FUR with Rf-excited states in βCD, a value of 1.89 x 10^9 $M^{-1}s^{-1}$ for the quenching constant of ^{1}Rf (free o bounded) by FUR was determined. This value is slightly smaller to measured in homogeneous medium 2.7 x 10^9 M^{-1} s^{-1}. In respect of ^{3}Rf-βCD* quenching by FUR (process (28) and (29)), the Stern-Volmer graph (see Figure 2, inset B) shows a negative curvature, and the quenching degree is lower in a βCD medium when is compared to a homogeneous medium. Similar results were reported by Gatica *et. al.*[26] where the effect of βCD produced a decrease in Rf sensitized degradation by Niclosamide. This can be attributed to two main factors: a) a decrease in collision probability between Rf and FUR because of the encapsulation effect; b) the stabilization of radicals formed in the process. If these radicals are not properly stabilized a back electron transfer can occur, and regenerate ^{3}Rf* and FUR. Both effects could simultaneously cooperate in a way as a synergistic effect decreasing the quenching rate constant ^3k_q accounting for the effective electron transfer process.

In the matter of FUR or FUR-βCD reaction with $O_2(^1\Delta_g)$, RB was used as a sensitizer in the same way that was done in a homogeneous medium. After βCD addition to RB + FUR solution, a 99 % and 70% increase in k_r and k_t

values were respectively observed, compared to the same values in a homogenous medium. Furthermore, k_r/k_t values were close to unity in both media. These results indicate that FUR-βCD formation produces a rise in the reaction rate (higher k_r and k_t values) of FUR with $O_2(^1\Delta_g)$.

As in aqueous medium, the possible $O_2^{\bullet-}$ reaction with FUR-βCD was studied. As shown in Figure 7, SOD enzyme does not change FUR rate consumption allowing discard the reaction between $O_2^{\bullet-}$ and FUR either free or included in βCD cavity.

Neckers and Paczkowski [53] have illustrated different systems where the covalent bonding of the photosensitizer to βCD (βCD-Dye) produces an increase in the photooxidative process. This is attributed to the inclusion of the substrate in βCD-Dye complex, which produces an increase in the local concentration of the substrate to be oxidized.[53,54] The comparison between the overall FUR photodegradation efficiency by Rf-sensitized in aqueous and βCD media respectively, indicates that the reaction of FUR with $O_2(^1\Delta_g)$ is the main pathway to FUR degradation in both media. This enhancement may be attributed to the formation of a ternary complex, constituted by the reported non-inclusion complex between Rf and βCD [25] , where FUR can be hosted in βCD cavity. This system would be producing $O_2(^1\Delta_g)$ near the substrate to be oxidized.

5. Conclusions:

Rf-sensitized photoirradiation of FUR in aqueous solution produces a series of changes in FUR molecule and Rf itself. Kinetic and mechanistic data analysis indicates the involvement of $^3Rf^*$ and $O_2(^1\Delta_g)$ in photodegradation of FUR. Regarding $O_2(^1\Delta_g)$ reaction with FUR, results suggested that the quenching of the oxidative species is mainly reactive, and a kinetic analysis suggest that each FUR molecule incorporates two oxygen molecules in the process.

On the other hand, FUR forms an inclusion complex with βCD in aqueous solution. This causes an increase in the rate of sensitized degradation of FUR by Rf in βCD media, probably due to the inclusion of FUR in the complex Rf-βCD which produces the reactive specie $O_2(^1\Delta_g)$ in the proximity of the oxidizable substrate.

Results show that FUR under environmental light exposure in the presence of naturally occurring photosensitizers, such as Rf, can cause irreversible phototransformations in the flavoring agent. This fact represents an undesirable property for food, cosmetic, and topical formulation components.

Author Contributions

- Carolina Gambetta and Agustina Reynoso conducted the experiments.
- Matías I. Sancho performed the theoretical calculation.
- José Natera, Paulina Montaña and Walter Massad planned the different experiment and collaborated with the preparation of the manuscript.
- Walter A. Massad organized the final discussion, wrote the manuscript and supervised the project.

All authors discussed on the results and commented on the manuscript. All authors have given approval to the final version of the manuscript

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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