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The employment of a removable chitosan-derivatized polymeric sensitizer in the photooxidation of polyhydroxylated water-pollutants

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Abstract

The known $O_2(^1\Delta_g)$-sensitizer system Chitosan bounded Rose Bengal (CH-RB), with Rose Bengal (RB) immobilized by irreversible covalent bonding to the polymer Chitosan (CH), soluble in aquous acidic medium, was employed in the photodegradation of three tri-hydroxy benzene water-contaminants (THBs). The system sensitizes the $O_2(^1\Delta_g)$-mediated photodegradation of THBs by a process kinetically favored, as compared to that employing free RB dissolved in the same solvent. Additionally the free xanthene dye, degradable by $O_2(^1\Delta_g)$ through self-sensitization upon prolonged light-exposure, is considerably protected when bonded to CH-polymer. The polymeric sensitizer, totally insoluble in neutral medium, can be removed from the solution after the photodegradative cycle by precipitation through a simple pH change. This fact constitutes an interesting aspect in the context of photoremediation of confined polluted waters. In other words, the sensitizing system could be useful for avoiding to dissolve dyestuffs in the polluted waters, in order to act as conventional sunlight-absorbing dye-sensitizers.

In parallel the interaction CH - $O_2(^1\Delta_g)$ in acidic solution was evaluated. The polymer quenches the oxidative species with a rate constant 2.4×10^8 $M^{-1}s^{-1}$ being the process mostly attributable to a physical interaction. This fact promotes the photoprotection of the bonded dye in the CH-RB polymer.

Abbreviations
1,2,3THB,pyrogallol
1,2,4THB, 1,2,4-Trihydroxybenzene
1,3,5THB, Phloroglucinol
CH, Chitosan
CH-RB, Chitosan-RB polymer
D_2O, Deuterium oxide
EDAC, N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride
FDA, Food and Drug Administration
FFA, Furfuryl alcohol
GRAS, Generally Recognized as Safe
MeOH, methanol

NaN_3, Sodium azide
$O_2(^1\Delta_g)$,Singlet molecular oxygen
PN, Perinaphthenone
RB,Rose Bengal
ROS,Reactive oxygen species
THBs, tri-hydroxy benzene derivatives
TRPD, Time resolved phosphorescence detection

Introduction

Significant amounts of biocides are frequent contaminants of soils and surface waters (1) and their use in agriculture is normally conditioned by their persistence in the environment. As a consequence, the biological, thermal and photochemical pathways in the degradation of these contaminants are topics of an enormous research interest.

The photoinduced degradation in aerated water solution and under sunlight irradiation has been particularly tested with different degree of success, and the reactions involved have been studied in order to gain insight into the natural photochemical decay of all these substances. (2,3) The use of organic photocatalysts, especially in the UV range, constitutes an interesting alternative in the oxidation of pollutants. Important findings in this area has been recently reviewed by Marin et al. (4)

When the contaminant biocide does not absorb visible light, its decomposition in a natural aquatic environment can be carried out as well through its reaction with reactive oxygen species (ROS), if any colored compound is present in the medium. This compound, named photosensitizer, promotes the so called sensitized photo-degradative and in particular photo-oxidative reactions, that operate upon light absorption in the wavelength range of irradiation close to sunlight, in the presence of molecular oxygen. The overall process can be simplified by employing specific dye-sensitizers. The synthetic dye Rose Bengal (RB) is a sensitizer that generates singlet molecular oxygen ($O_2(^1\Delta_g)$) with high efficiency (5,6). On the other hand, this xanthenic dye and similar $O_2(^1\Delta_g)$-generators could be used for a non-natural photochemical degradation of pollutants, in confined aqueous solutions and under controlled conditions. After an adequate photooxidative treatment, remediated waters could be discharged as waste waters with considerable diminution in their contamination risks. Nevertheless, at this point an essential problem remains: It is related to the difficulties in separation the intense colored photosensitizer from the reaction mixture at the end of the photooxidative cycle. Obviously these problems could be overcome by employing a removable heterogeneous system, with the photosensitizer covalently attached to an insoluble polymer chain. (7)

In the present work we employed a sensitizer system already described in the literature, in which the dye RB was immobilized by covalent bonding to the polymer chitosan (CH). (8) Similar systems employing other xanthene dyes (9), porphyrins and phthalocyanines, (10) immobilized on CH films have been tested for drug delivery and water disinfection.

The oxidizable target molecules we utilized in this work for modeling the water contaminants were three trihydroxybenzene derivatives (THBs). Due to their profuse use with industrial purposes (11,12,13) THBs have been frequently found as contaminants in waste-waters. (14)

Chitosan (β-(1-4)-2-amino-2-deoxy-β-D-glucose,) is the second most naturally abundant polysaccharide next to cellulose, considered as GRAS (Generally Recognized as Safe) by Food and Drug Administration (FDA). (15,16) Due to the hydroxyl and amino groups on its backbones, CH is a willing molecule that can be relatively easy modified by various methods. The solubility of CH in aqueous solution is highly dependent on pH (See Scheme 1 for a structural representation of CH). The polymer can be dissolved in aqueous solution at acidic pH, whereas it is totally insoluble in neutral medium (17). We took advantage of this property in the present work: The degradative photolysis of the pollutant was carried out at acidic pH employing the soluble chitosan-RB polymer (CH-RB). Following the polymer can be removed after the photodegradative cycle, by means of a simple change in the pH value of the solution.

The present contribution mimics, as closely as possible, a scenery consisting in a confined polluted water in the presence of oxygen, visible light and a removable RB-derivative as a dye-sensitizer. Under these conditions the photosensitizing efficiency of the soluble polymer CH-RB and its ulterior extraction as a solid after the oxidative run was tested. The work constitutes a comparative study with the simple and conventional system represented free RB in solution. Besides, several properties of the very CH as quencher of $O_2(^1\Delta_g)$ under work conditions were investigated.

Materials and Methods

Materials

Chitosan of low molecular weight, deuterium oxide 99.9% (D_2O), Perinaphthenone (PN), N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) were purchased from Sigma Chem. Co. (St. Louis, MO, USA). Rose Bengal (RB), furfuryl alcohol (FFA), Phloroglucinol (1,3,5-THB), 1,2,4-THB, and Pyrogallol (1,2,3-THB) were bought from Aldrich (Milwaukee, WI, USA). Acetic acid and sodium hydroxide were from Cicarelli (Buenos Aires, Argentina). Methanol HPLC grade was from Sintorgan (Buenos Aires, Argentina). All these chemicals were used as received. Water was triply distilled. Buffered aqueous solutions were prepared, from acetic acid and sodium hydroxide (18). All measurements were made at room temperature.

Methods

CH -RB was synthesized according to the procedures described in the works of Mansuri *et al.* and De Campos *et al.* (19,9) We dissolved 1 g of CH (70% deacetylation degree) in 80 mL of acetic acid (1% v/v) and the pH was adjusted to 5.9 by dropping a concentrated NaOH solution. Following, water was added to this solution to make the final volume of 100 mL. Separately, 46 mg of RB were dissolved in ethanol and 47 mg of EDAC were added. This mixture was allowed to react for 20 minutes. Thereafter, both solutions were mixed up together and the resulting solution was brought to pH 5.9 by drop wise addition of diluted aqueous NaOH. Then the solution was stirred at room temperature in the dark for 18 h. The resulting polymer was precipitated by the addition of NaOH up to a pH = 8.5 and washed successively with ethanol and the precipitate was then redissolved in acetic acid. CH-RB. The polymer was isolated by dialysis (cellulose membrane 12,400 Da, Sigma-Aldrich) against water (3 days) and then against a phosphate buffer (pH = 7.4) for 2 days. Then the polymer was precipitated by adding a sodium hydroxide solution up to pH = 8.5; the precipitate was filtered and washed with methanol.

All steady-state photolysis were carried out at wavelengths > 400 nm (cut-off filter) employing a home-made photolyzer provided with a 150 W quartz-halogen lamp. Ground state absorption spectra were registered employing a Hewlett Packard 8452A diode array spectrophotometer.

The total quenching rate constant of deactivation of the $O_2(^1\Delta_g)$ by THBs or CH (k_t, see further reactions [1] and [2], being Q = CH or Q = THBs) was determined using a system previously reported. (20) Briefly, it consisted a Nd:Yag laser (Spectron) as the excitation source. The emitted ($O_2(^1\Delta_g)$) phosphorescence at 1270 nm was detected at right angles using an amplified Judson J16/8SP Germanium detector, after having passed through the appropriate filters. The output of the detector was coupled to a digital oscilloscope and to a personal computer to carry out the signal processing. Usually, 10 shots were needed for averaging so as to achieve a good signal to noise ratio, from which the decay curve was obtained. Air equilibrated solutions were employed in all cases.

The concentration of the sensitizer (RB) was *ca.*0.02 mM. D_2O was used in the dynamic determinations, instead of H_2O as solvent, in order to enlarge the lifetime of $O_2(^1\Delta_g)$.(21)

In kinetic terms the interaction of $O_2(^1\Delta_g)$ with a given quencher Q can be depicted by reactions [1] and [2] representing respectively the physical and reactive deactivation of the excited oxidative species, where $O_2(^3\Sigma_g^-)$ symbolizes ground state oxygen. The addition of the rate constants k_q and k_r, is generically known as k_t, the overall rate constant for the quenching of $O_2(^1\Delta_g)$.

$$O_2(^1\Delta_g) + Q \longrightarrow \text{Products} \qquad \text{rate constant } k_r \quad [1]$$

$$O_2(^1\Delta_g) + Q \longrightarrow O_2(^3\Sigma_g^-) + Q \qquad \text{rate constant } k_q \quad [2]$$

For the determination of k_t (reactions (1) and (2)), the $O_2(^1\Delta_g)$ lifetimes were evaluated in the presence (τ) and in the absence (τ_0) of the quencher and the data were plotted according to a Stern-Volmer treatment (Equation [3]).

$$\tau_0/\tau = 1 + k_t\,\tau_0\,[Q] \qquad [3]$$

The reactive rate constant k_r for the reaction of Q with $O_2(^1\Delta_g)$ (reaction [2]), was determined using the method described by Scully and Hoigné (22) (Equation II), for which the knowledge of the reaction rate constant for the photo-oxidation of a reference compound R is required:

$$\text{Slope} / \text{slope}_R = k_r\,(Q) / k_{rR}\,(R) \qquad [4]$$

Where slope and slope_R are the respective slopes of their first-order plots of oxygen consumption by Q and by a reference compound R (each at the same concentration) under photoirradiation with visible-light in the presence of dye sensitizers. Assuming that the reaction of $O_2(^1\Delta_g)$ with the quencher is the only possible pathway of oxygen consumption, the ratio of the first order slopes of oxygen uptake by the substrate and the reference compound yields k_r/k_{rR}. The reference was FFA, with a reported pH-independent k_{rR} value of 1.2 x 10^8 M^{-1} s^{-1}. (2) Rose Bengal and PN were employed as dye-sensitizers in the determination of k_r values for THBs and CH respectively. The rates of oxygen consumption were determined with the specific oxygen electrode Orion 97-08. The experimental array has been already described. (23)

The photodegradation rates of aqueous solutions of RB and CH-RB were determined by monitoring the absorbance decrease in their respective absorption *maxima* in the visible spectral region, as a function of photoirradiation time.

For the evaluation of the percent of recovery of the solid polymer after dissolution in acidic aqueous solution, the following experiment was performed: 0.5 g CH-RB was dissolved in 50 ml of pH 4.75 buffered aqueous solution. and stirred during 15 min. The solution was neutralized by dropping a 0.5 M NaOH solution and the appearance of the precipitate CH-RB was observed. The mixture was stored for 12 hs in the dark and the solid fraction was quantitatively collected by vacuum filtration and washed three times with 15 ml methanol. The solid was maintained in the dark at RT for 12 hs and then in a stove at 80°C for 30 min. After 2 hs the solid was weight up to constant value. The experiment was made by triplicate and the mean value obtained for the recovery of the solid material was 98%.

The pH/pD values were controlled with a MP220 Mettler-Toledo pH-metter. The pD value was calculated from the relation: pD = pH reading + 0.4. (24)

Results and discussion

Preparation of Chitosan-Rose Bengal polymer and stability of the polymer upon sensitized photoirradiation.

The polymer CH-RB, with a pinky powder aspect, was obtained by the amide linkage between the carboxylic group of the dye and the amino group of the polysaccharide, the presence of carbodiimide EDAC (Scheme 2). The mechanism, as described by Nakayima and Ikada, (25) includes the formation of carboxylicanhydride as intermediate; a step that is favored in the pH range 3.5-4.5.

The polymer was soluble in acidic water. We tested total dissolution up to 2% w/w. The absorption spectrum of CH-RB is significantly red shifted as compared to the absorption spectrum of free RB under identical experimental conditions, as shown in Fig.1, inset. This characteristic has been considered as an evidence for the attachment of the dye chromophore to the polymer. (8) The ratio of the absorbances at the absorption maxima $A_{\mathrm{I}\ (561\ \mathrm{nm})}/\ A_{\mathrm{II}\ (523\ \mathrm{nm})}$ = 1.58 (see spectrum “a” in Figure 1, inset), very close to the value of 1.6 reported in the literature for a well characterized CH-RB. (8)

For this compound, a value of the extinction coefficient ε_{561} = 45,000 $\mathrm{cm^{-1}\ M^{-1}}$ has been employed. (8) We used this data to evaluate a value of 0.38 for the percentage of binding of the dye to CH polymer (the content of RB as a molar ratio with respect to the glucosamine unit of CH). The employed composition of the polymer conjugates determine several properties: (a) Being *ca*. 70% of the Nacetylglucosamine units of chitin deacetylated, this proportion enables a solubility window for the CH-RB polymer that warrants adequate light absorption. (b) The above mentioned percentage of binding dye-polymer is high enough to generate $O_2(^1\Delta_g)$ upon adequate photoirradiation and low enough to avoid self-quenching of RB excited states by the ground state of the dye.

Regarding the photostabiliry of the CH-RB, it is known that dissolved RB is photolabile. There are a large number of publications about the photopromoted processes/degradation of RB under oxidative and reducing conditions in aerobic and deoxygenated media. (26,27,28). Nevertheless no information about possible oxidation products was given in those papers. Semiconductor catalysis has also been employed for RB photodestruction. The sensitized oxidation leads to mineralization. (29) The kinetics of RB degradation was studied by means of oxygen photoconsumption measurements. The reaction was first order in dye concentration, and the apparent order with respect to oxygen depends on the oxygen/dye ratios. The stoichiometry of the reaction resulted approximately 2 oxygen molecules consumed per molecule of RB, and reaction intermediates were detected. (30). A rate constant $k_t = 2 \times 10^7\ M^{-1}s^{-1}$ for the $O_2(^1\Delta_g)$–mediated process has been reported for RB in MeOH (31). The photodegradation rate of the dye can be monitored by decrease of the absorbance maximum as a function of photoirradiation time. In order to evaluate the stability of the dye bonded to the polymer in CH-RB, individual solutions of CH-RB and the free RB with matched absorbances at the irradiation wavelength were photolyzed with light of 550 ± 10 nm. Results are shown in Figure 1, main. As can be clearly seen the photodegradation rate of the polymer-bonded RB is *ca*. five times lower than the free dye, under the described conditions. Assuming a similar fraction of absorbed light for both compounds, a plausible reason for the mentioned difference may due to the presence of the polysaccharide moiety in the CH-RB derivative exerting a sort of protection on the $O_2(^1\Delta_g)$-mediated degradation of the bonded dye. Then this possibility was evaluated as follows.

The interaction Chitosan - $O_2(^1\Delta_g)$

All experiments involving possible $O_2(^1\Delta_g)$-mediated processes on CH-RB at pH/pD 4.5 were carried out employing the exclusive $O_2(^1\Delta_g)$-generator dye PN. It is one of the sensitizers most frequently used in $O_2(^1\Delta_g)$ reactions with a reported quantum yield for the production of the oxidative species of *ca.* 1 in aqueous solution. (32)

The presence of CH (70% deacetylation degree) in the sub-mM concentration range quenches the IR phosphorescence emission of $O_2(^1\Delta_g)$, as detected by TRPD experiments. A rate constant k_t = 2.4 (±0.2) $\times 10^8$ $M^{-1}s^{-1}$ for the overall interaction $O_2(^1\Delta_g)$-CH was independently determined in pD 4.75 D_2O through a Stern-Volmer treatment (Figure 2, main). This experiment unambiguously demonstrates the existence of this interaction, which may be reactive in nature (reaction [1]) and/or a mere physical process (reaction [2]).The k_t values, as determined by TRPD, do not depend on the type of sensitizer or on potential interactions of the substrate with excited states of the sensitizer involved in the $O_2(^1\Delta_g)$ generation. An inspection on the CH chemical structure, looking for recognized $O_2(^1\Delta g)$-quenchers, only identifies the primary amino groups as potential candidates. The reported k_t values for such compounds are in the range of 4 x10^5 $M^{-1}s^{-1}$ as a mean value. (2) Nevertheless, considering that CH is formed by *ca.* 1300 glucosamine units, *i.e.*1300 primary amino groups, the k_t value of 5x10^8 $M^{-1}s^{-1}$ roughly matches a value that would correspond to the approximate bulk concentration of primary amino groups in the polymer aqueous solution.

A rate constant value value k_r = 2.7 (±0.1) x10^7 $M^{-1}s^{-1}$ for CH (reaction [1]) was determined in pH 4.75 aqueous solution by monitoring oxygen consumption upon photoirradiation of PN-CH mixtures, following a described method.(22) The calculated k_r/k_t = 0.11 ratio represent a measure of the efficiency of the degradation pathway of CH *via* reaction with $O_2(^1\Delta_g)$. The relatively low k_r/k_t value obtained for CH indicates, as a final result, the elimination of the oxidative species $O_2(^1\Delta_g)$ without appreciable loss of the scavenger. This behaviour could explain the observed protective effect on the degradability of the polymer-bonded RB, through a physical quenching of $O_2(^1\Delta_g)$ exerted by the polymeric CH moiety.

The main activity of CH as a physical quencher of $O_2(^1\Delta_g)$ is also expected and characteristic for compounds with a prevalent presence of primary amino groups. Je and Kim, Pasanphan and Chirachanchai, among other researchers, investigated on the antioxidant properties of CH and CH-based derivatives against different ROS, including superoxide anion, hydroxyl radicals and hydrogen peroxide.(33,34) The topic has recently been reviewed by Luo and Wang and no reports on the activity of the polymer towards $O_2(^1\Delta_g)$ were included. (17) In this context and acording to our knowledge, this is the first quantitative report on the interaction between the polymer dissolved in aqueous solution and photogenerated $O_2(^1\Delta_g)$.

Degradation of THBs employing CH-RB photosensitization

In a previous paper we reported a kinetic study on the RB-sensitized photodegradation of 1,2,4-THB; 1,2,3-THB and 1,3,5-THB in pH 2 aqueous solution. (35) THBs were photodegraded through a $O_2(^1\Delta_g)$-mediated photooxidative process, as depicted by reaction (1). Possible photoproducts are quinonic compounds, typically formed in $O_2(^1\Delta_g)$-mediated reactions of phenols. (14). Hydroxyquinonic derivatives have been also reported as photoproducts in the direct photolysis of aqueous THBs in the presence of oxygen. (36)

The photoirradiation of the mixture CH-RB (0.052% w/v) plus 0.2 mM 1,2,4-THB; 1,2,3-THB and 1,3,5-THB in pH 4.75 water (>350 nm, cut off filter) produced neat changes in the absorption spectra of the THBs. In Figure 3 is shown the case of 1,2,4-THB employing the sensitizer CH-RB, as a typical example. No spectral changes were observed when the same photoirradiation was performed in the absence of CH-RB for any of the THBs .

In parallel, photoirradiation of the same solutions under identical experimental conditions gave rise to oxygen consumption. The rate of Oxygen uptake was neatly decreased in the presence of NaN_3 0.6 mM, as shown in Figure 4, inset. The salt is a well known $O_2(^1\Delta_g)$ scavenger, with a reported k_t value of 2.08×10^9 $M^{-1}s^{-1}$. It is profusely employed to confirm/discard the involvement of the oxidative species in a given photoprocess. (2) This result in addition to the reported value of 0.83 for the quantum yield of $O_2(^1\Delta_g)$ generation by the polymer CH-RB, (8) strongly suggest that the observed degradation of THBs is due to a $O_2(^1\Delta_g)$-mediated photooxidative process, similar to the already described for THBs, photosensitized by free RB. (35). However, a contribution from hydroperoxyl radical ($HO_2^\bullet$) to the overall oxidative process should not be disregarded. It is known that photoexcited RB may, in a minor extent, generate the species superoxide radical anion ($O_2^{\bullet-}$) that is protonated in acidic medium to form the species $HO_2^\bullet$ which is easily reduced by many aromatic electron donors. (37,38)

Figure 4 shows the first order plot employed for the determination of the respective k_r values of THBs, resulting 0.68 x 10^8 $M^{-1}s^{-1}$; 1.44 x 10^8 $M^{-1}s^{-1}$ and 1.00 x 10^8 $M^{-1}s^{-1}$ for 1,2,3-THB 1,3,5-THB and 1,2,4-THB. These k_r values lie within the expected order of magnitude of those for poly-hydroxybenzenes in acidic/neutral aqueous solutions reported by other authors. (14,39,40)

In Figure 5 both the k_r values obtained in pH 4.75 water, employing CH-RB as a photosensitizer, and those already reported in pH 2 water employing the free dye, (35) are compared by means of a logarithmic bars diagram. As can be seen, in all cases the rate constants determined employing the polymeric sensitizer are appreciably higher than the corresponding ones for the system with free RB as a sensitizer.

The reported quantum yield of ca. 0.83 by CH-RB is *ca*. 7% higher than that of the free dye. (8) In principle this factor does not appear as the only responsible for the remarkable photodegradation-rate increase exerted by the polymer-bonded RB sensitization. On the other hand, this effect contrasts to some degree with the above mentioned photoprotection exhibited by the polymer against $O_2(^1\Delta_g)$ attack.

We think that the increase in the k_r values could be due to an increase in the local concentration of the THBs nearby the sites of generation of the oxidative species. In that case the $O_2(^1\Delta_g)$ diffusion to the reaction site -one of the limiting steps in the whole photosensitization/photooxidation process- is minimized as compared to an scenery of homogeneous distribution of the oxidizable substrate. Concomitantly, the physical quenching of the oxidiative species by the primary amino groups of the polymer bone, should be also decreased. A similar model has been already employed by Zhang *et al.* to interpret the efficiency increase in $O_2(^1\Delta_g)$-mediated reactions by comparing free hematoporphyrin and hematoporphyrin -doped silica nanoparticles as photosensitizers with an anthracene derivative as oxidizable target. (41).

Finally, a significant increase in the k_r values due to ionization of the OH groups of the THBs on going from pH 2 (homogeneous sensitizer) to pH 4.75 (heterogeneous sensitizer) must be disregarded. (14). The reported pK values for the first ionization of THBs are, in all cases, higher than 7. (35,42).

Recuperation of the solid polymer after dissolution.

The evaluation of the percent of recovery of the solid polymer bonded to RB was performed by dissolving 0.5 g CH-RB in 50 ml of pH 4.75 aqueous solution. An absorbance value of 0.9 was registered in the spectral maximum at 560 nm for a dilution 1:10 of this solution *vs.* the pH 4.75 aqueous solution . The operation was repeated after 3 hs storage of the concentrated solution in the dark under continuous stirring, and the original absorbance value was maintained. The whole procedure, described in the experimental section, was made by triplicate with a mean value of 97.9 + 0.2. A mean absorbance value in the wavelength range 550-570 nm, determined for a dilution 1:10 v/v of the supernatant fraction, was lower than 0.01.This result demonstrates that in practise the loss of RB bonded to the polymer is negligible.

Final remarks

Results in the present contribution demonstrates that the polymer CH-RB can photosensitize the $O_2(^1\Delta_g)$-mediated degradation of THBs in acidic aqueous solution. In this particular case the photodegradative process is kinetically favored as compared to the conventional one, employing RB straightforwardly dissolved in aqueous solution. Additionally the sensitizer, degradable by $O_2(^1\Delta_g)$ through self-sensitization upon prolonged light- exposure, is considerably protected when bonded to CH-polymer. The polymeric sensitizer can be removed from the solution after simple neutralization.

Scheme and Figure captions

Scheme 1.
Chemical structure of Chitosan

Scheme 2.

Chemical structure of the polymer Chitosan-Rose Bengal

Figure 1.
Absorbance changes as a function of photoirradiation time in solutions of CH-RB (a) and RB (b) with matched absorbances at 550 nm, in pH 4.75 aqueous solutions, monitored at 561 and 548 nm respectively. Inset: Normalized electronic absorption spectra of the polymer CH-RB (a) and RB (b) in their respective maxima, in pH 4.75 aqueous solution. A_I and A_{II} are the absorbance values for the maximum and the shoulder of a solution of CH-RB in pH 4.75 aqueous solution.

Figure 2.
Stern-Volmer plots for the quenching of $O_2(^1\Delta_g)$ phosphorescence by CH in pD 4,75 aqueous solution. τ and τ_0 are the respective $O_2(^1\Delta_g)$ phosphorescence lifetimes in the presence and in the absence of CH. Inset: first order plots for oxygen uptake in pH 4,75 aqueous solution by CH (a) and FFA (b). RB (A_{548} = 0.5) as a sensitizer.

Figure 3.
Changes in the UV-Vis absorption spectrum of a pH 4.75 aqueous solution of 1,2,4-THB plus CH-RB (A_{561} = 0.6) *vs*. CH-RB (A_{561} = 0.6). Inset: Absorbance decrease of the described system as a function of photoirradiation time, monitored at 291 nm.

Figure 4.
First order plots for oxygen uptake in pH 4,75 aqueous solution by 1,2,3-THB (a); 1,2,4-THB (b); 1,3,5-THB (c) and FFA (d) photosensitized by CH-RB (0.052% w/v). Inset: oxygen consumption as a function of photoirradiation time by the following pH 4,75 aqueous solutions: CH-RB (0.052% w/v) plus 0.5 mM 1,3,5-THB (b) and CH-RB (0.052% w/v) plus 0.5 mM 1,3,5-THB plus 0.6 mM sodium azide (a).

Figure 5.
Values of log k_r for 1,2,3-THB; 1,2,4-THB and 1,3,5-THB in pH 4,75 aqueous solution photosensitized by CH-RB (lined bars) and the same at pH 2, photosensitized by RB (empty bars).

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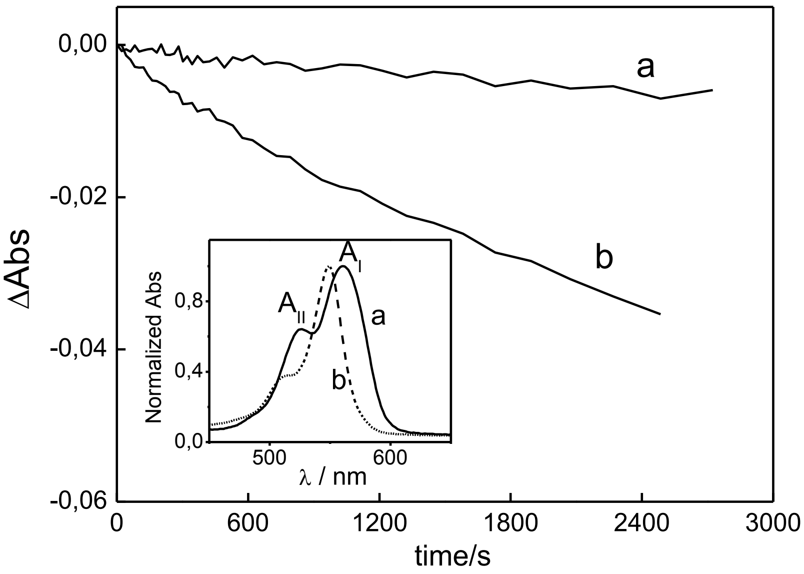


Figure 1

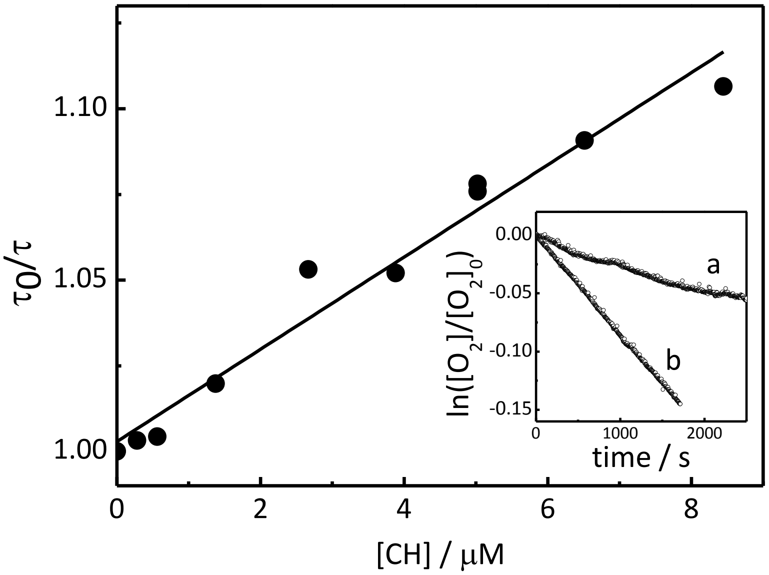


Figure 2

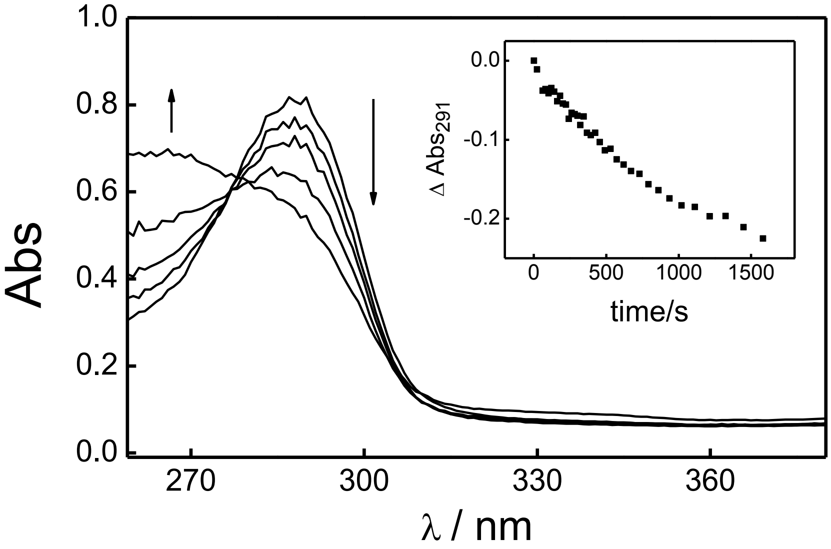


Figure 3

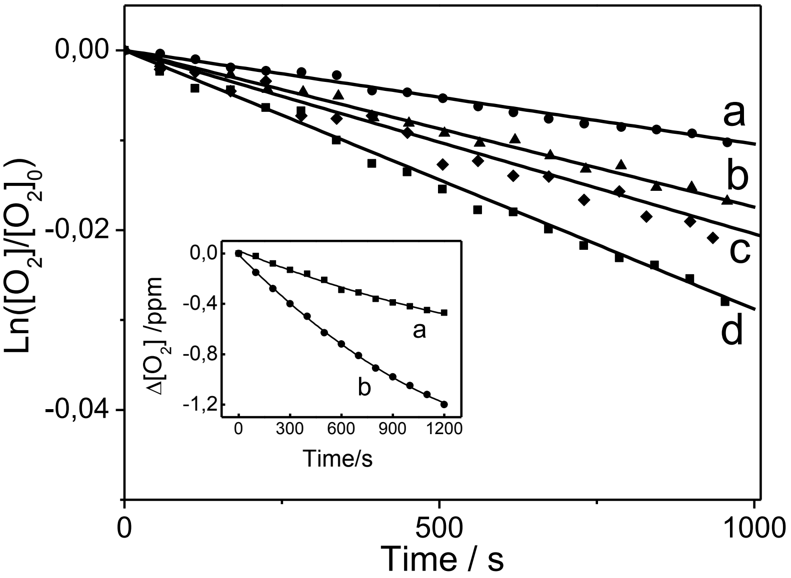


Figure 4

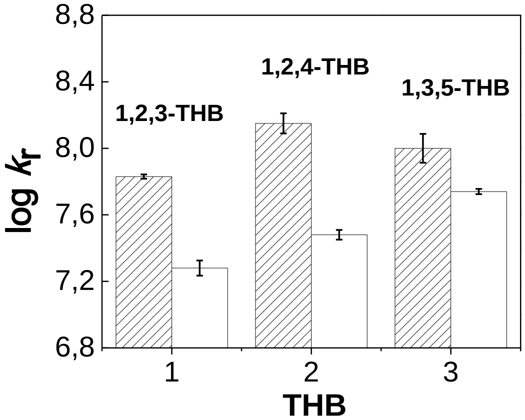
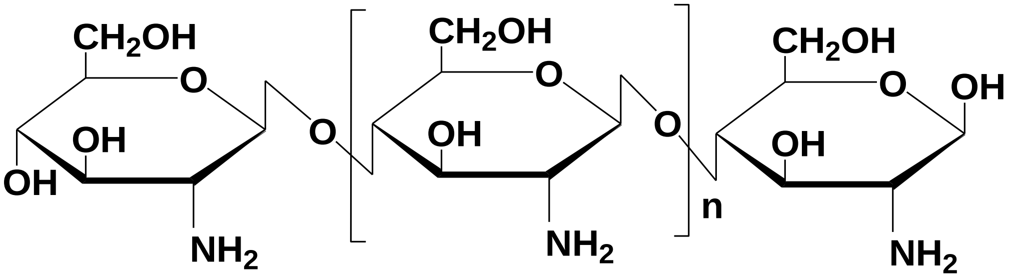
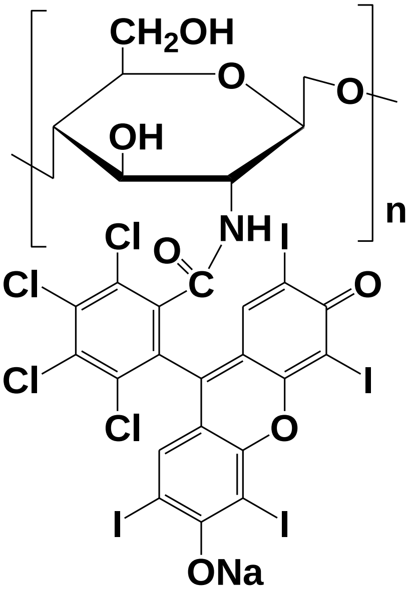


Figure 5



Scheme 1



Scheme 2