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The effect of 2-hydroxypropyl-β-cyclodextrin on the excited triplet state of promazine and chlorpromazine

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ABSTRACT

Chlorpromazine hydrochloride (CPZ) is a widely used anti-psychotic drug that induces skin photosensitization and photoallergy response after systematic use or topical applications. The photoallergic mechanism is still unknown. However, it has been proposed that the triplet excited state (^{3}CPZ*) could participate in the photodamaging effects. In this work, we report the photophysical properties of the triplet excited state of CPZ and its parent derivative promazine hydrochloride (PZ) in the presence of 2-hydroxypropyl-β-cyclodextrin (HPC). Absorption measurements indicate that PZ and CPZ form an inclusion complex with HPC through a 1:1 stoichiometry. The equilibrium constant at 25 °C is $(2.55 \pm 0.09) \times 10^3\ M^{-1}$ and $(3.27 \pm 0.07) \times 10^3\ M^{-1}$ for PZ and CPZ, respectively. The CPZ and PZ triplet excited state properties changed in the presence of HPC. The triplet lifetime increases with HPC concentration that is related to the amount of drug bound. In addition, the triplet intersystem crossing quantum yield was determined to be 0.45 and 0.17 for PZ and CPZ, respectively, when more than 95% of the drug molecules are bound to HPC. Altogether, these results suggest that the microenvironment plays a crucial role in the ^{3}CPZ* and ^{3}PZ* properties and thus it can modulate their photosensitizing effects.

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1. Introduction

Phenothiazines derivatives (PH) have been used since the 1950s as tranquilizers and antipsychotics and have promising anti-infective and anti-cancer activities [1–5]. The biological activity of these drugs depends on their physicochemical properties, the type of target tissue acted on, and the presence of functional groups on the phenothiazine structure [6]. Biologically relevant 2-substituted PH derivatives are also known to induce photosensitization of the skin after systematic use or topical applications. One of the well known PH derivatives is CPZ (Fig. 1).

The photochemistry of CPZ is very complex and many factors can influence the light induced outcome. The factors include the excitation wavelength, the polarity of the microenvironment, the light intensity, and the pH. Upon irradiation with UV, CPZ yields its cation radical (photoionization), the neutral promazinyl radical plus chlorine atom (homolytic cleavage), and a sulfur-centered peroxy radical [7]. Although the radical cation is probably responsible for some of the observed *in vitro* phototoxic effects of this drug, it seems unlikely that it is involved in phototoxicity *in vivo*, since photoionization occurs through a biphotonic processes and is solvent and wavelength dependent [8,9]. Irradiation of CPZ at wavelength of 330 nm leads to the formation of a neutral radical that can undergo oxidation by molecular oxygen to promazine superoxide. The neutral radical can also react with other neutral radical or CPZ molecule and form dimers and higher molecular weight species [10].

Chlorpromazine has a high intersystem crossing quantum yield and a long triplet lifetime in organic solvents [11]. In aqueous solutions, on the other hand, the values of these properties decrease [8]. For instance, the excited singlet state has a low fluorescence quantum yield ($\Phi_f < 0.10$) with a short lifetime ($\tau_f < 3$ ns) [8,11]. These properties make the ^{3}CPZ* a good candidate for excited state reactions. The well known induced photosensitization side effects of the PH derivatives, specifically CPZ, implies that its triplet excited state (^{3}CPZ*) is playing a key role [8,11]. Moreover, if the magnitude of the photosensitization side effects is somehow related to the triplet state, then microenvironment properties could modulate the net phototoxic result.

Abbreviations: BP, benzophenone; CDs, cyclodextrins; CPZ, chlorpromazine hydrochloride; DFT, Density Functional Theory; HPC, 2-hydroxypropyl-β-cyclodextrin; PBS, phosphate buffer solution; PH, phenothiazine; PZ, promazine hydrochloride; TCA, tricyclic antidepressants; TX, thioxanthone.

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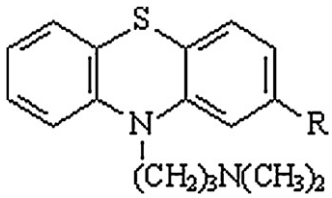

Fig. 1. Molecular structures of CPZ (R = Cl) and PZ (R = H).

Cyclodextrins (CDs) have been employed for fundamental studies related to host–guest interactions as well as for practical applications [12–15]. The hydrophobic cavity of CD was shown to be capable of modifying the photoreactivity of a large variety of molecules and to attenuate the toxic effects of several drugs *in vitro* [15,16]. For example, CD has been used in pharmaceutical formulations to enhance PH solubilization [17], suppress CPZ hemolysis [14,18–21], and reduce the PZ and CPZ phototoxic effects [17,22–25]. This reduction in phototoxicity could be related to a decrease in the photodecomposition quantum yield, changes in the destruction mechanism, and/or changes in the photophysical properties of the molecules. Indeed, complexation of many drug molecules with CD derivatives has shown a marked effect on the photochemistry of these molecules [15]. Moreover, the association to CD via non-covalent binding can represent a useful model to mimic the interaction of the drug with proteins that have hydrophobic biological sites [26]. The CDs, in particular β-CD, have limited aqueous solubility (1.85 g/100 mL at 25 °C) [27]. 2-hydroxypropyl cyclodextrin (HPC) is obtained by treating a base-solubilized solution of β-CD with propylene oxide, resulting in an isomeric system that has an aqueous solubility well higher than 60 g/100 mL. In the present work, HPC was selected because of its higher solubility. The CPZ and PZ concentrations used for laser flash photolysis are in the 0.40–0.90 mM range. Thus, the HPC highest concentration 0.10 M will ensure that for laser flash studies an excess of CD exists. In consequence, at the moment of laser excitation most of the drugs are complexed.

In this work, we report the effect of HPC in the CPZ and PZ triplet excited state properties. The results show that the formation of inclusion complexes has a large effect on the triplet intersystem crossing quantum yield and triplet lifetime. To our best knowledge, this is the first report regarding the CPZ and PZ triplet excited state properties in the presence of CD.

2. Materials and methods

2-Hydroxypropyl cyclodextrin (HPC, M_{ave} = 1590 at 0.60 mole substitution), benzophenone (BP), PZ, CPZ, and all other reagents and solvents were purchased from Sigma–Aldrich (USA) and used without further purification. Phosphate saline buffer solution (PBS, Na_2HPO_4/NaH_2PO_4) with analytical concentration of 10 mM at pH = 7.40 were used in all experiments. CPZ and PZ free base were prepared by addition of NaOH to an aqueous solution of the protonated drug and then extracting with diethyl ether. All gases were purchased from Air Products (Humacao, P.R.).

2.1. Absorption measurements

Absorption spectra were taken with a HP 8453 UV–vis photodiode array spectrophotometer. The equilibrium binding constants between the drugs and HPC were determined by titration of the drugs with aliquots of HPC (stock solution 0.100 M in 10 mM phosphate buffer, pH 7.40) at a constant drug concentration. The thermodynamic equilibrium constant was determined by nonlinear regression of the absorbance changes using the Solver option in the Microsoft Excel software. The goodness of fit was defined by the minimization of the sum of square of the residuals between the calculated and observed values of the absorbance change for all experimental points. The uncertainties in the fitting parameters were determined using the Excel Solvstat macro [28].

2.2. Nanosecond transient absorption spectroscopy

The CPZ and PZ analytical concentration for laser induced transient absorption spectroscopy experiments were kept below 0.90 mM, much lower than the critical micelle concentration (~3 mM) [29]. This concentration allows us to minimize the contribution of higher aggregates to the transient absorption spectrum. The nanosecond spectrokinetic system has been described elsewhere [30,31]. Briefly, the 355 nm harmonic of the Nd/YAG Continuum Surelite II Laser was used for sample excitation with the following standard conditions: pulse duration = 5 ns, repetition rate = 10 Hz, laser irradiation area = 0.30 cm^2, and maximum pulse energy = 20 mJ/cm^2. The laser actinometry was measured using the benzophenone triplet–triplet absorption (^{3}BP*) at 520 nm in acetonitrile (Φ_T = 1.00, ε_T = 6500 M^{-1} cm^{-1} [32] or thioxanthone (^{3}TX*) at 635 nm in acetonitrile (Φ_T = 0.66, ε_T = 22,000 M^{-1} cm^{-1} [33]. Transient absorption spectra were taken using a 1 cm^2 flow-through cell connected to a relatively larger reservoir to minimize photodegradation. The kinetics at a single wavelength were determined using static samples, for which no more than five laser pulses were averaged to avoid sample degradation. The quantum yields of triplet formation (Φ_T) were determined using the comparative actinometry method for ^{3}BP* at 520 nm absorbance in nitrogen-saturated acetonitrile solutions as reference [31].

2.3. Density Functional Theory (DFT) calculations

The geometry pre-optimizations were performed in vacuo with the PM3 semiempirical method using the Polak-Ribiere conjugated gradient protocol (1×10^{-5} convergence limit, 0.01 kcal/Å mol RMS-limit). The final optimizations were performed with DFT [B3LYP/6-31G(d) OPT SCRF = (PCM)] using Gaussian 03 at High Performance Computer facility (UPR Río Piedras). All conformational and thermodynamics parameters were obtained with a DFT single point calculation. The enthalpy of the complex formation was obtained as the difference of the formation enthalpies: $\Delta H\,(\text{kcal/mol}) = \Delta H_f^{complex} - \Delta H_f^{CD} - \Delta H_f^{CPZ}$.

3. Results and discussion

3.1. Ground state equilibrium constant

The absorption spectra of CPZ and PZ have been well characterized and consist of a band with a high molar absorption coefficient (>10^4 M^{-1} cm^{-1}) at 254 nm, indicative of its π–π* character localized in the heterocyclic system, and a broad long wavelength absorption band attributed to the n–π* transition [11]. The equilibrium binding constants between PZ or CPZ and HPC were determined in aqueous solution by titration. This information is relevant for the laser flash photolysis experiments (Section 3.2) which requires that more than 90% of the drug molecules being bound to HPC at the moment of laser excitation. The titration of 31.4 μM CPZ solution with HPC is shown in Fig. 2 as an example. Fig. 2A shows the absorption spectrum at different HPC concentrations. A hypochromic effect is observed at 254 and 308 nm as the HPC concentration increases. In addition, the band with maximum at 254 nm shows a red shift with increasing HPC concentration. The titration results, in a more clear perspective, are also presented as the difference of the spectrum of each addition of HPC and the spectrum of CPZ alone in Fig. 2B. Negative peaks are related to a

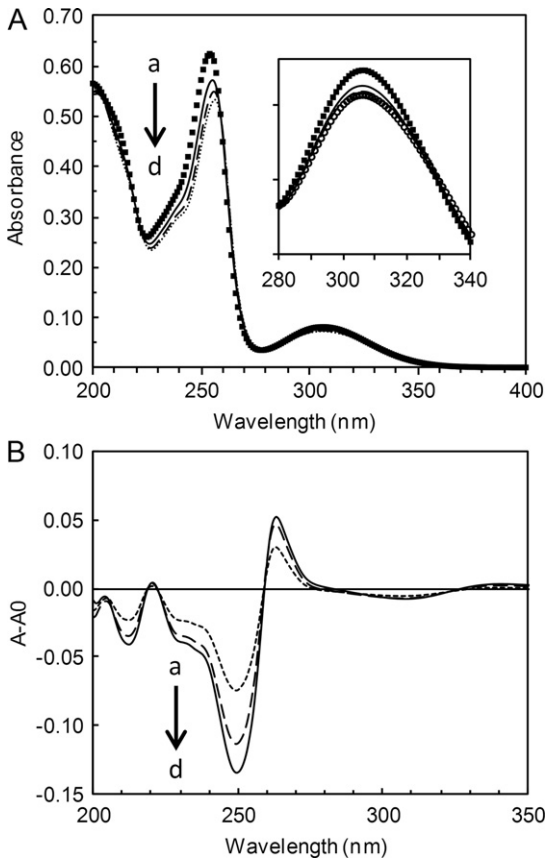

Fig. 2. Spectrophotometric titration of 31.4 μM CPZ with increasing concentration of HPC in PBS 7.40. (A) Absorption spectra at various concentrations of HPC in PBS 7.40: (a) 0.00, (b) 0.33, (c) 0.99, and (d) 5.08 mM. (B) Difference absorption spectra generated by subtracting the spectrum collected at each HPC concentration from the CPZ spectrum alone.

hypochromic effect ($\lambda \approx 254$ nm) while positive peaks results from the bathochromic shift of the band. In addition, an isosbestic point at 259 nm suggests that the equilibrium between CPZ and HPC can be described with a 1:1 stoichiometry. These absorbance changes upon CD addition have been attributed to the formation of an inclusion complex between promazine and α, β, and γ CDs [18,22]. Furthermore, the dependence of the absorption of the drug on the HPC concentration can be analyzed by the Benesi–Hildebrand linear regression, which provides information on the equilibrium constant (K_{eq}) and the stoichiometry of the complex [34]. Double reciprocal plot [$1/(A-A_0)$ versus $1/C_{HPC}$] or [$1/(A-A_0)$ versus $1/C_{HPC}^2$] should result in a linear relationship for the case of 1:1 or 1:2 stoichiometry, respectively. In these relationships, the A and A_0 are the absorbance at a particular wavelength in the presence and absence of HPC, respectively, and C_{HPC} is the molar analytical concentration of HPC. For CPZ, a 1:1 ($R^2 = 0.999$) stoichiometry results in better correlation coefficient than for 1:2 ($R^2 = 0.945$). From the slope and intercept of the 1:1 case, the K_{eq} was determined to be $2.9 \times 10^3\ M^{-1}$. For PZ, the double reciprocal plot for 1:1 stoichiometry results in a R^2 of 0.999, and a $K_{eq} = 2.2 \times 10^3\ M^{-1}$. Previous reports established either a 1:1 or 1:2 stoichiometry resulting in ambiguous characterization of the complex [35]. Our results are in agreement with those reports indicating a 1:1 equilibrium between CPZ and β-CD [13,26]. Although the Benesi–Hildebrand method has been used to determine the inclusion equilibrium constant for different drugs, the linear double reciprocal plots suffers from a highly biased weighting of points [36]. Therefore, we proposed here a non-linear regression and global analysis for quantitative purposes.

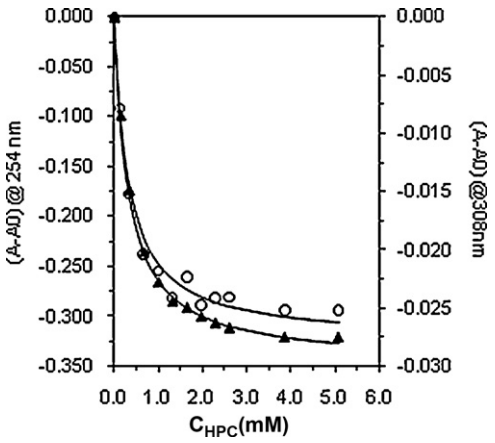

Fig. 3. Binding isotherm at 25 °C of 31.3 μM CPZ in the presence of increasing concentration of HPC in PBS 7.4 measured at (▲) 254 and (◯) 308 nm. The solid lines correspond to the non-linear global regression of Eq. (1).

The K_{eq} values were also determined using the Beer–Lambert's law and mass balance equations to the following equation, which assumes a 1:1 stoichiometry,

$$A - A_0 = \left(\frac{\Delta\varepsilon}{2}\right)\left[\left(C_D + C_{HPC} + \frac{1}{K_{eq}}\right) - \sqrt{\left(C_D + C_{HPC} + \frac{1}{K_{eq}}\right)^2 - (4C_D C_{HPC})}\right] \quad (1)$$

where A, A_0 and C_{HPC} has been defined above, $\Delta\varepsilon$ is the difference in the molar absorptivity of the complex ($\varepsilon_{complex}$) and PZ (ε_{PZ}) or CPZ alone (ε_{CPZ}), and C_D is the molar analytical concentration of CPZ or PZ. The ε_{252}^{PZ} and ε_{302}^{PZ} were obtained from calibration curves and fixed with values of $(2.04 \pm 0.03) \times 10^4$ and $(2.72 \pm 0.01) \times 10^3\ M^{-1}\ cm^{-1}$, respectively. For CPZ, the fix values are $\varepsilon_{254}^{CPZ} = (2.70 \pm 0.03) \times 10^4$ and $\varepsilon_{308}^{CPZ} = (3.50 \pm 0.06) \times 10^3\ M^{-1}\ cm^{-1}$, respectively. Global fitting of Eq. (1), was performed using the absorbance changes at 254 and 308 nm and defining the fitting variables as the $\varepsilon_{complex}$ at 254 and at 308 nm, with a common K_{eq}. This result in a K_{eq} of $(3.27 \pm 0.07) \times 10^3\ M^{-1}$ for CPZ, see Fig. 3. In addition, the corresponding values for $\varepsilon_{complex}^{254}$ and $\varepsilon_{complex}^{308}$ are $(1.69 \pm 0.01) \times 10^4$ and $(2.61 \pm 0.03) \times 10^3\ M^{-1}\ cm^{-1}$, respectively. The analysis of PZ was obtained using only the absorbance change at 252 nm, because the changes at 302 nm are very small introducing higher errors. The results for PZ are $K_{eq} = (2.55 \pm 0.09) \times 10^3\ M^{-1}$ and $\varepsilon_{complex}^{252} = (1.42 \pm 0.01) \times 10^4\ M^{-1}\ cm^{-1}$. The magnitude of the K_{eq} is in agreement to previous reports for the inclusion complex between CPZ or PZ with other β-CDs derivatives [15,26,35]. In addition, the higher K_{eq} for CPZ is in agreement with the observation that the chlorine atom increases the affinity of the drugs towards CD [7].

Density Functional Theory (DFT) calculations were performed in order to characterize the CPZ-CD complexes, based on a 1:1 stoichiometry (Fig. 4). The calculations were carried out using β-CD as the host because the exact structure of HPC is unknown [37]. The synthesis of HPC results in a random pattern substitution of the hydrogen atoms in β-CD by the hydroxypropyl groups. This alkylation products mixture cannot be separated into its constituents. Moreover, CDs have large size and conformational freedom and the use of ab initio methods is quite problematic, even when symmetry conditions are imposed [38].

The DFT results shows that, depending on the interacting terminals of CPZ (head, H or tail, T) and the OH-density of β-CD (high density, Hi or low density, Lo), there are four possible complexes (HiH, HiT, LoH, and LoT). The DFT results predict that the stability of the complexes depend on these two parameters and on the protonation state of CPZ. The most stable CPZ complexes are formed

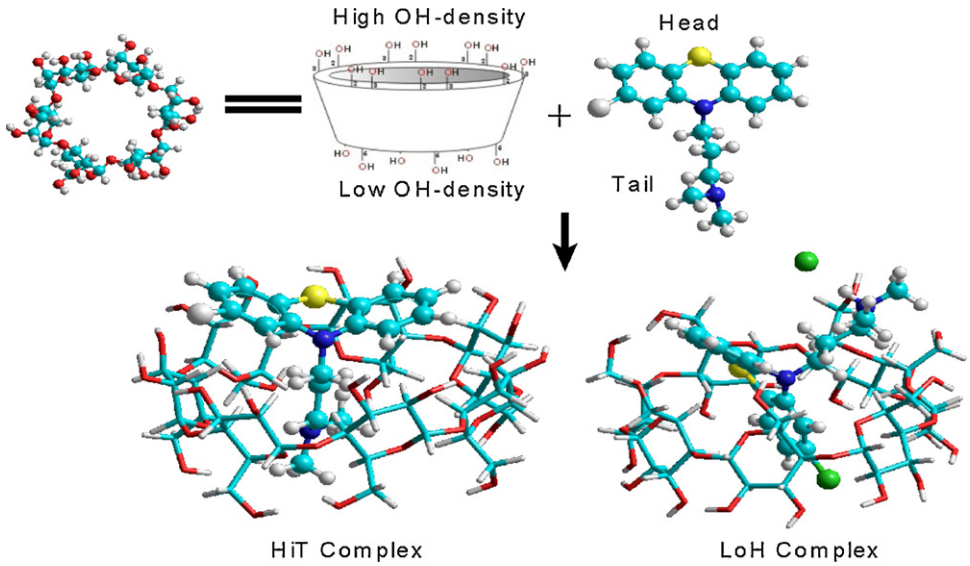

Fig. 4. Insertion complexes of CD and CPZ showing the complexes HiT of CPZ free base and LoH of CPZ. The size of β-CD is shown with a smaller rendering for clarity.

Table 1
Formation enthalpy of CPZ, CPZ-HCl, β-CD and their insertion complexes.

Molecule	CD OH-density	CPZ orientation	ΔH_f (kcal/mol)		$\Delta H_{complex}$ (kcal/mol)	
			Protonated	Free base	Protonated	Free base
CPZ			78.46	71.84		
β-CD			−1406.95	−1406.95		
Complex	High	Head	−1297.92	−1336.40	30.57	−1.29
		Tail	−1327.77	−1338.97	0.72	−3.86
	Low	Head	−1333.72[a]	−1332.87	−5.23	2.24
		Tail	−1331.94	−1334.49	−3.45	0.62

[a] If the Cl-atom is forced to stay outside the CD-cavity: −1330.95 kcal/mol.

with the low density terminal of β-CD, while the free base complexes are more stable if formed at the high density OH-terminal (Table 1). For instance, the two most stable complexes are HiT for free base and LoH for CPZ (Fig. 4). The "head-complex" of CPZ-HCl includes the insertion of the Cl-atom into the β-CD cavity. If the Cl-atom is forced to stay in the outside, the complex is destabilized by ~3 kcal/mol. Besides, in the LoH-complex, the host molecule is closer to β-CD than in the corresponding head complex of the free base. This and the difference in the orientation between CPZ (head) and its free base (tail) are explained in terms of the polarity of the guest molecule. The dipole moment of CPZ is only 1.8 D, which increases to a value of 4.3 D on protonation. Indeed, this is why the solubility of chlorpromazine is very low at pH values below 8.0.

3.2. Nanosecond laser flash photolysis

Many reports described the ground state interaction between CPZ or PZ with several CD derivatives. However, less is known on the effect of CD on the CPZ or PZ triplet excited state (^{3}CPZ* or ^{3}PZ*). This is relevant to the understanding of the CPZ and PZ photodamaging effects. The excited singlet state of these drugs has short lifetime ($\tau_f < 3$ ns) and low fluorescence quantum yield ($\Phi_f < 0.10$) [11]. On the other hand, the excited triplet state has a long lifetime and high intersystem crossing quantum yield in organic solvents. The most dramatic effect is observed for CPZ which has a short triplet lifetime ($\tau_T < 100$ ns)) in aqueous solution [11]. Consequently, it is expected the triplet state to live long *in vivo* and to play a key role in the photosensitizing effects of the drugs. If, somehow, the ^{3}CPZ* or ^{3}PZ* properties change upon formation of the inclusion complex, then photosensitizing effects could also be affected. We determined the nanosecond laser induced transient absorption spectra of CPZ and PZ in the absence and presence of HPC. The 355 nm laser induced transient absorption spectrum of a nitrogen-saturated PZ (0.84 mM) PBS 7.40 solution in the presence of 0.97 mM HPC at laser energy of 5 mJ/pulse is shown in Fig. 5. Under these conditions and using the K_{eq} of 2.55×10^3 M^{-1}, more than 85% of the drugs form a complex with HPC at the moment of laser excitation. The transient

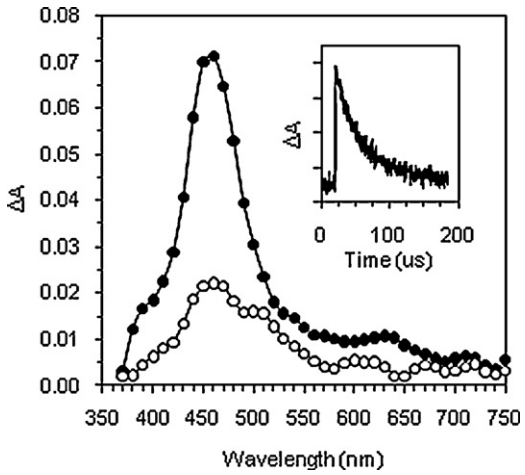

Fig. 5. Nanosecond laser (E = 5.0 mJ/pulse) induced transient absorption spectra of a nitrogen-saturated 0.87 mM PZ in PBS 7.40 in the presence of 0.94 mM HPC at 4.00 μs (●), and 50.00 μs (○) after the laser pulse. Inset: kinetic trace at 470 nm.

absorption spectra show a broad band with maximum at 470 nm. The kinetic trace at 470 nm shows a single exponential decay with a rate constant of $(2.86 \pm 0.04) \times 10^4\ s^{-1}$. The presence of oxygen decreases the transient decay lifetime ($\tau < 0.50\ \mu s$). These results are in agreement to the previous characterization of this transient species attributed to be the $^3PZ^*$ [8,11,39].

In general, the laser induced transient absorption spectra of CPZ in PBS 7.40 are more complex than for PZ [8,11]. The laser intensity has a major impact on the CPZ transient absorption spectra. It has been observed that, under high laser intensity ($I > 12\ mJ/cm^2$), the number of transient species increases due to second order effects. Photoionization and its corresponding radical cation formation are the major transient populated species [8,11,39].

In the present work, the results in the absence of HPC are in complete agreement with previous reports. Nanosecond induced transient absorption spectra in nitrogen-saturated CPZ/PBS 7.40 solution results in photoionization and radical cation formation, and no formation of $^3CPZ^*$ was observed at times longer than 100 ns. However, when HPC (40.0 mM) is present the transient absorption of CPZ (0.40 mM) in nitrogen-saturated PBS 7.40 and laser energy/pulse of 14.2 mJ/cm^2 shows contribution from two main transient species with different lifetimes. At 197 ns after the laser pulse, the transient spectrum has major contribution of the $^3CPZ^*$ with maximum at 470 nm, and a longer lived species. The transient spectrum corresponding to 9.0 μs after the laser pulse has contribution only from the longer lived transient species with maximum at 520 nm, characteristic of radical the cation [8,11,39]. It is important to note that the transient absorption spectra were measured using a flow cell. These conditions are not ideal for quantitative determination of the transient species lifetime, because low oxygen removal efficiency. Nevertheless, the contribution of the $^3CPZ^*$ in PBS 7.40 when HPC is present has a major implication for *in vivo*. In addition, the fact that $^3CPZ^*$ cannot be seen at times longer than 100 ns in aqueous solution without HPC means that the inclusion complex stabilizes the excited state of the drugs [9,39].

To study the effect of HPC on the $^3PZ^*$ and $^3CPZ^*$ lifetime, kinetic traces were measured at 460 nm with increasing HPC concentration. To minimize nonlinear photon processes, all experiments were performed at low laser energies ($E < 10$ mJ/pulse) and static samples were used for better degasification. It was found that the triplet decay has a longer lifetime in the presence of HPC for both, PZ and CPZ, see Fig. 6. In addition, the value of the observed decay constant is related to the amount of PZ or CPZ encounter as complex with HPC. Thus, as the HPC increases, a greater amount of the drug forms an inclusion complex and its triplet decay lifetime increases. These results suggest that HPC provides a cavity with lower number of water molecules and a hydrophobic character. It has been proposed that water quenches the $^3CPZ^*$ by a proton mechanism [8,11]. This is in agreement with reports on the increase photostability or decrease photosensitization of CPZ in the presence of different CDs [23–25,40]. It is apparent that $^3CPZ^*$ induced photosensitization is a function of the microenvironment. To verify if the microenvironment modulates the $^3CPZ^*$ properties, we measured its laser induced transient absorption spectra in hexane, Fig. 7. The laser induced transient absorption spectra of CPZ free base in hexane results in a broad band with maximum at 470 nm. This band decays with a lifetime of 32 μs. Furthermore, steady state photolysis in hexane reveals that CPZ is more photostable than in alcohols (unpublished results). Thus, in non-polar solvents, photodehalogenation from $^3CPZ^*$ is inhibited and the neutral radical does not form. This is agreement with the DFT results where the "head-complexes" includes the insertion of the Cl-atom into de CD-cavity. In this orientation, the Cl-atom is protected and the $^3CPZ^*$ cannot be deactivated by the solvent protons. Therefore, it should be longer-lived than in PBS. However, if the dehalogenation processes is favored by transient intermediates stabilization, then

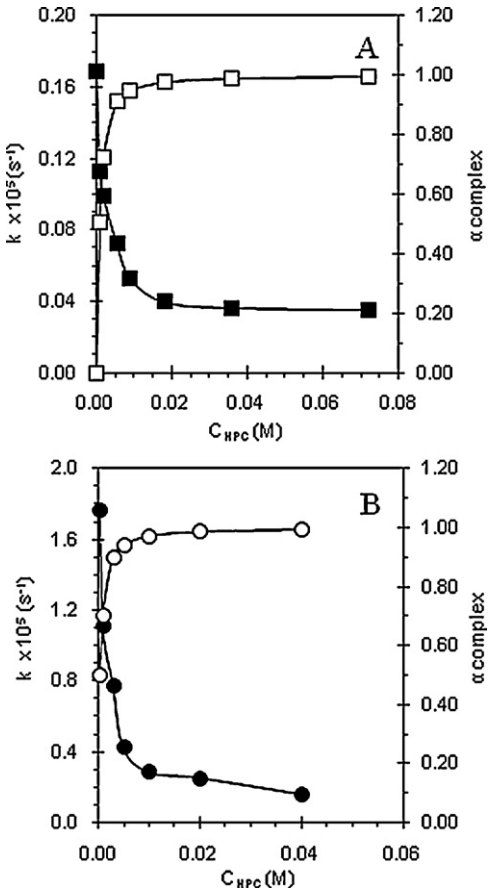

Fig. 6. The effect of HPC on the triplet decay rate constant at 460 nm of (A) PZ (0.87 mM), and (B) CPZ (0.40 mM) in aqueous nitrogen-saturated solution. The filled symbols correspond to the observed first order decay rate constant. The open symbols corresponds to the fraction of PZ or CPZ present as drug-HPC complex just before laser excitation.

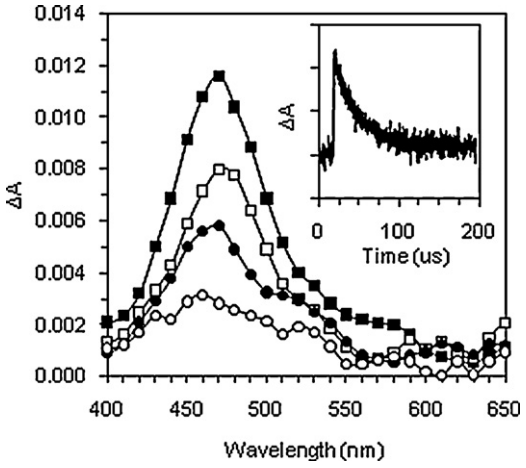

Fig. 7. Transient absorption spectra of CPZ free base (0.168 mM) in nitrogen-saturated hexane at 1.98 μs (■), 9.90 μs (□), 15.80 μs (●), and 27.7 μs (○) after the laser pulse. The laser energy per pulse is 1.34 mJ/cm^2. Inset: kinetic decay trace at 470 nm that follows a single exponential function: $\Delta A = 8.8 \times 10^{-4} + (9.8 \times 10^{-3}) \times \exp(-3.06 \times 10^4 \times t)$.

radical induced photodamage could occurred. This further proves that the microenvironment plays a key role in the $^3CPZ^*$ deactivation processes.

The Φ_T was determined using the comparative method. This value provides information about the population of molecules that

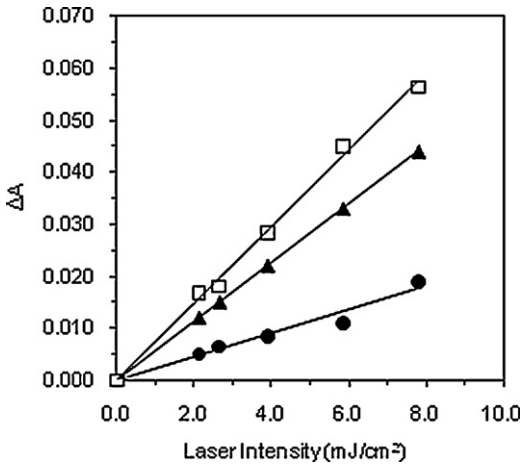

Fig. 8. Transient absorption signal at 470 nm at the end of the 355 nm laser excitation pulse for 0.40 mM of (●) CPZ, and (□) PZ with 40.0 mM of HPC in PBS 7.40 under anaerobic conditions. The laser actinometry was determined using the ^{3}BP* in nitrogen-saturated acetonitrile at 520 nm (▲). The solutions have similar ground state absorbance at 355 nm.

reach the triplet excited state after excitation. This property is relevant because the higher its value the higher the probability for this state to participate in photodamaging reactions. For the quantification of Φ_T, the analytical concentrations are 40.0 mM and 0.40 mM for HPC and PZ or CPZ, respectively. These conditions ensured that more than 95% of PZ or CPZ exists as complex with HPC at the moment of laser excitation. Fig. 8 shows the ^{3}CPZ*, ^{3}PZ*, and ^{3}BP* transient absorbance at the end of the laser pulse as a function of laser energy per pulse.

From the slopes of each plot, the Φ_T was determined to be 0.45 ± 0.06 and 0.17 ± 0.05 for PZ and CPZ, respectively. The difference in Φ_T between PZ and CPZ may correspond to differences in the triplet state deactivation mechanism. Also, this value was calculated using the molar extinction coefficient of the molecules in organic solvents, assuming that it does not change with solvent. However, the fact that HPC stabilizes the ^{3}PZ* and ^{3}CPZ* relative to aqueous solution in the absence of the CD could have implications for the *in vivo* situation. The interaction of CPZ with blood components influences its bioavailability and can affect the function of several biomolecules [10,41,42]. In addition, it have been demonstrated in this work that a key component in the drug's triplet state properties is the microenvironment where the drug resides. Antidepressives drugs are known to be amphiphilic compounds. Therefore, the drug localization in the cell could modulate their photophysical properties.

4. Conclusions

The triplet excited state properties of PZ and CPZ were determined in the presence of HPC. These studies show that for PZ and the biologically relevant drug CPZ, their triplet excited state lifetime and yields are strongly dependent on the microenvironment. Altogether, these results could be correlated to the known CPZ photosensitivity. It can be established that binding of CPZ to hydrophobic sites in proteins could result in triplet excited state derived photodamage because of its higher Φ_T and lifetime values. In addition, a CPZ induced photoallergic response could be initiated by transient species derived from its triplet state. If the binding site of macromolecules could stabilize radical formation then covalent modification could occur. Also, these results also explain the observed reduced photosensitivity of CPZ when CD is present. The drug distributes between CD and proteins, decreasing the photosensitizing effects in the biological macromolecules.

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