

THE BIPHOTONIC PHOTOIONIZATION OF CHLORPROMAZINE DURING CONVENTIONAL FLASH PHOTOLYSIS: SPIN TRAPPING RESULTS WITH 5,5-DIMETHYL-1-PYRROLINE-N-OXIDE

ROBERT D. HALL*, GARRY R. BUETTNER† and COLIN F. CHIGNELL‡
Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences,
P.O. Box 12233, Research Triangle Park, NC 27709, USA

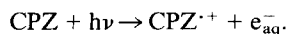
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Abstract—A novel combination of conventional flash photolysis and electron spin resonance (ESR) spin-trapping has been used to demonstrate that photoionization of chlorpromazine (CPZ), and the concomitant production of hydrated electron, occurs through a stepwise biphotonic mechanism during conventional flash photolysis at wavelengths above 290 nm. The production of hydrated electron in the flash photolysis experiment has been monitored and quantified through the use of the spin trapping agent 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO). The effects of nitrous oxide, varying concentrations of CPZ and DMPO, and a range of flash intensities on the ESR spectra of the observed spin adducts of DMPO are discussed. The use of ESR spin trapping to monitor hydrated electron yields in flash photolysis experiments has the potential to permit the use of a much wider range of flash intensities than is typically possible with conventional optical experiments. Thus, there is a greater possibility of distinguishing between monophotonic and biphotonic processes.

INTRODUCTION

It has been known for many years that chlorpromazine (2-chloro-*N,N*-dimethyl-10H-phenothiazine-10-propanamine, CPZ§) photoionizes in aqueous solution under ultraviolet irradiation (Lagercrantz, 1962; Borg and Cotzias, 1962; Iwaoka and Kondo, 1974; Navaratnam *et al.*, 1978; Motten *et al.*, 1985; Buettner *et al.*, 1989). Irradiation with a variety of light sources has demonstrated this phenomenon: Iwaoka and Kondo (1974) used unfiltered light from mercury flash lamps; Navaratnam *et al.* (1978) used 347 nm laser flash photolysis; Motten *et al.* (1985) used monochromatic 266 nm steady-state irradiation; and Buettner *et al.* (1989) have used 266 and 355 nm laser flash photolysis.

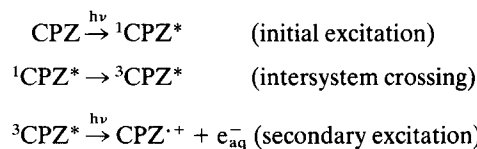
As a result of the earlier optical studies, it has generally been assumed (Iwaoka and Kondo, 1974; Navaratnam *et al.*, 1978; Bensasson *et al.*, 1983) that CPZ photoionizes by a monophotonic process (i.e. a single-photon mechanism) to produce the cation radical and hydrated electron,



However, an electron spin resonance (ESR) spin

trapping study by our research group (Motten *et al.*, 1985) yielded the startling result that at the relatively low intensities characteristic of monochromatic light from conventional steady-state xenon lamps, the spin adduct obtained from the reaction of 5,5-dimethylpyrroline-*N*-oxide (DMPO) with hydrated electron was only observed during excitation of CPZ at wavelengths below 290 nm. Therefore, we suggested that a biphotonic (i.e. two-photon) mechanism was required to account for the production of hydrated electron observed with irradiation above 290 nm.

In an attempt to further clarify the situation, we carried out a detailed study of the photoionization of CPZ during laser flash photolysis at 355 nm (Buettner *et al.*, 1989). Our computer simulation of experimental results obtained from laser flash photolysis strongly supported a stepwise (Scaiano *et al.*, 1988) biphotonic mechanism for photoionization of CPZ via a triplet excited state of CPZ:



Our computer analysis of the excitation processes originating in the $S_0 \rightarrow S_1$ electronic transition of CPZ also led us to conclude that the photoionization of CPZ can show monophotonic behavior during laser flash photolysis over a significant range of laser flash intensities. Similar studies have been carried out with other photoionizing chemicals (Alkaitis *et al.*, 1975; Lachish *et al.*, 1976; Pileni *et al.*, 1975;

*Present address: SYVA Company, 900 Arastradero Road, Palo Alto, CA 94303, USA.

†Present address: The University of Iowa, College of Medicine, EMRB 68, Iowa City, IA 52242, USA.

‡To whom correspondence should be addressed.

§Abbreviations: CPZ, chlorpromazine; DMPO, 5,5-dimethyl-1-pyrroline-*N*-oxide; ESR, electron spin resonance; *P, 10-[3-dimethylamino-propyl]-10H-phenothiazin-2-yl radical; PMT, photomultiplier tube.

Pillof and Albrecht, 1968).

The objective of the present paper was to resolve the conflicting experimental results and discrepancies in mechanistic interpretation between Iwaoka and Kondo (1974), Navaratnam *et al.* (1978) and Buettner *et al.* (1989). In the process, we developed a novel method for studying photoionization that combines the techniques of flash photolysis and ESR spin trapping.

MATERIALS AND METHODS

The CPZ was obtained from Sigma Chemical Co. (St Louis, MO). We have used the extinction coefficient $4030 \text{ M}^{-1} \text{ cm}^{-1}$ at 308 nm for CPZ (Buettner *et al.*, 1989). The DMPO was from Aldrich Chemical Co. (Milwaukee, WI) and was purified on activated charcoal and stored at -20°C as an aqueous 1 M solution until used (Buettner and Oberley, 1978). The absorption spectrum of DMPO has no significant absorption in the wavelength range for the $S_0 \rightarrow S_1$ transition of CPZ. However, DMPO absorption ($\lambda_{\text{max}} = 230 \text{ nm}$, $\epsilon_{230} = 7700 \text{ M}^{-1} \text{ cm}^{-1}$) should be considered in the study of other compounds by this approach. The $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was analytical reagent grade from Mallinckrodt (St Louis, MO) and the $\text{CoSO}_4 \cdot \text{XH}_2\text{O}$ was technical grade from Aldrich Chemical Co.

Flash photolysis. Flash photolysis experiments were performed with a FP1000 Flash Photolysis system from Photochemical Research Associates, Inc. (Ontario, Canada). This system was utilized with xenon flash lamps flashed with a maximum power limit of 20 W. The average pulse width was given by the manufacturer as 1.8 μs FWHM. In practice, the pulse width was estimated as $<4 \mu\text{s}$ and it did not appear to vary between the lowest and highest power settings used in our experiments.

In order to obtain the transient absorption spectra for flash photolyzed CPZ in aqueous solution, a large reservoir of the solution was continuously bubbled with nitrogen (or other gases where relevant), and drawn through tygon tubing into a single-jacket, 10 cm pathlength, cylindrical cuvette from Hellma Cells, Inc. (Jamaica, NY). The annular jacket surrounding the sample contained water or a filter solution composed of 192 g/L $\text{CoSO}_4 \cdot \text{XH}_2\text{O}$, 80 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 1 M H_2SO_4 . The filter solution was very stable to repeated flashing at high flash intensities and the time between flashing was long enough to maintain the solutions at room temperature, usually 20°C . Sample solutions were only flashed a single time before replacing them with fresh solution. Transient decay data were acquired with a DL902 Transient Recorder from Data Laboratories Limited (Surrey, England). Typically, the transient recorder was set to acquire 2048 points with a 1 μs time increment. An initial baseline was obtained by setting the FP1000 to open the shutter of the monitoring lamp 100 μs before flashing. The input amplifier of the transient recorder and the output voltage of the photomultiplier were carefully matched so that the maximum digitalized voltage of the recorder corresponded to the output from the (negatively biased) PMT when the shutter was closed, and so that the output corresponding to the transmittance of the solution prior to flashing could be recorded. Thus, the absorbance at any time, A_t , could be calculated as

$$A_t = \log \frac{V_0}{V_0 - V_t}$$

where V_0 represents the average digitalized PMT voltage prior to flashing the sample and V_t is the digitalized voltage at time, t . At the present time, the digitalized voltages are sent from the transient recorder to an Apple IIe computer and are then transferred to a VAX/VMS computer system

along with other instrumental parameters and sample information. Absorbance calculations and curve-fitting were carried out with PASCAL programs written for the VAX/VMS operating system or with AMIGABASIC Programs (Microsoft Corp.) on an AMIGA 1000. A non-linear least-squares methodology developed by Marquardt (Bevington, 1965) was used for curve-fitting routines.

Actinometric calibration of a variety of voltage settings on the PRA flash photolysis instrument was performed by flashing 0.14 M potassium ferrioxalate solutions with filter solutions or water in the annular jacket of the cylindrical cuvette. The flashed ferrioxalate solutions were then assayed for Fe(II) by monitoring the absorbance of the Fe(II)-1,10 phenanthroline complex at 510 nm (Gordon and Ford, 1972). The shape of the actinometric curve obtained by this method did not depend upon the type of filter solution used in the jacket, although the calculated photon flux varied according to the solution transmittance. This suggests that the energy distribution of the flash within the wavelength band selected by the filter solution (optimized for the S_1 absorption band of CPZ) does not change significantly over the range of voltage settings used in this study.

Using an extinction coefficient of $1.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ for the Fe(II)-1,10 phenanthroline complex at 510 nm, we estimated that at the maximum instrument setting (9.8 kV), the flash lamps delivered 8.6×10^{18} photons to the jacketed cuvette containing the filter solution described above. All references to flash intensity in this paper are given in percent of this maximum number of photons as measured by the ferrioxalate method.

Electron spin resonance. Electron spin resonance measurements were made on a Bruker ESP-300 system (Billerica, MA). The outlet of the central chamber of the cylindrical cuvette in the PRA flash photolysis instrument was connected to the inlet of a standard ESR flat cell for the TM cavity with small polyethylene (ID 1.2 mm) tubing less than 1 m in length. The input to the cylindrical cuvette was connected to a loosely covered sample reservoir, which was continuously bubbled with nitrogen during the experiment. The output from the flat cell was connected to a Gilford Rapid Sampler (Model 2443-A, Gilford Instrument Laboratories, Oberlin, OH).

In a typical experiment, the cylindrical cuvette was alternatively flushed with nitrogen and fresh sample and then filled with fresh solution. The flash intensity control was then set to the desired voltage (3–9.8 kV) and the sample was flashed. Finally, half (*ca* 4 mL) of the flash photolyzed sample was pulled into and through the flat cell while drawing nitrogen from the sample reservoir. The ESR spectral measurement was then initiated. It was possible to reduce the time between flashing and initiation of the spectral measurement to less than 20 s, while the DMPO/H spin adduct had a 40 min half-life under our experimental conditions (unpublished result). Thus, we lost very little ESR signal during the transfer operation and spectral scanning. Solutions were not flashed more than once. Flash photolysis of CPZ alone or DMPO alone gave no observable ESR spectra.

The ESR simulations of the spin adduct spectra were performed on a Hewlett-Packard 9835B computer (Palo Alto, CA). In most of the experiments described here, the median value of data obtained in three ESR experiments was used to construct figures.

All absorption and transmittance spectra were obtained with a Gilford Response spectrophotometer (Oberlin, OH).

RESULTS

The objective in the present study was to examine the mechanism by which excitation of the $S_0 \rightarrow S_1$

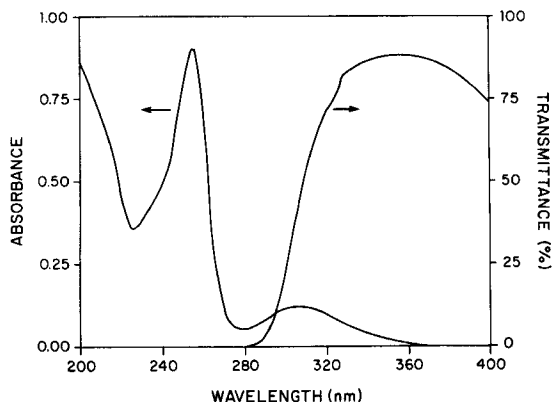


Figure 1. Absorption spectrum of 28 μM CPZ in 10 mM sodium phosphate buffer, pH 6.0, and transmittance spectrum of the filter solution used in the flash photolysis experiments. The pathlengths were, respectively, 10 and 3 mm. The filter solution was composed of 192 g/L $\text{CoSO}_4 \cdot \text{XH}_2\text{O}$, 78 g/L $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, and 1 M H_2SO_4 .

electronic transition in CPZ leads to its photoionization. Therefore, we prepared a filter solution that would allow wavelengths greater than 290 nm to reach the sample solution during flash photolysis but would block wavelengths below 290 nm and above 440 nm. Figure 1 shows that the filter solution did not transmit light below 290 nm. However, the transmittance of the filter solution varied from 5 to 10% between 460 and 560 nm. Thus, further excitation of any transient species absorbing between 440 and 600 nm could occur, although the filter solution was designed to minimize as much as possible excitation or bleaching of the cation radical of CPZ.

A variety of transient absorbing species (e_{aq}^- , $^3\text{CPZ}^*$, $\text{CPZ}^{\cdot+}$) have been observed in flash photolysis studies of CPZ (Iwaoka and Kondo, 1974; Navaratnam *et al.*, 1978; Buettner *et al.*, 1989). The transient absorption spectra that we observed in nitrogen-saturated solutions of CPZ in 10 mM sodium phosphate at pH 6.0 following flash photolysis (at 88.5% of maximum intensity) are shown in Fig. 2. The spectral shape and evolution of these spectra† are consistent with the results in the microsecond to millisecond time domain described by both Navaratnam *et al.* (1978) (exciting CPZ with laser light at 347 nm) and Buettner *et al.* (1989) (exciting CPZ with laser light at 355 nm). Thus, the broad band excitation of CPZ carried out under our present flash conditions does not appear to affect the composition of the transient spectra observed in the visible spectrum.

Iwaoka and Kondo (1974) also used a conventional flash photolysis system but they irradiated

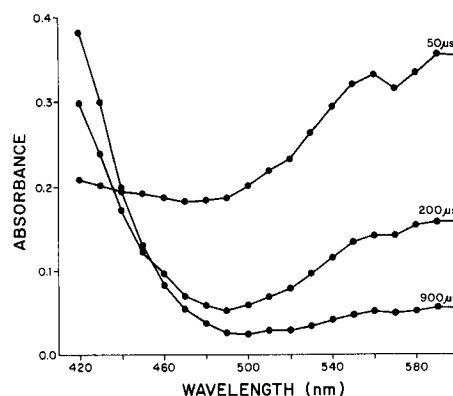


Figure 2. Transient absorption spectra of a N_2 -saturated solution of CPZ in 10 mM sodium phosphate buffer, pH 6.0, at 50, 200 and 900 μs after the beginning of the flash. The absorbance pathlength was 10 cm. The initial concentration at CPZ was 28 μM . The flash intensity was 88.5% of the instrument maximum.

CPZ with the 254 nm line of a mercury lamp. Although we tried to reproduce the sample conditions described in their paper (*e.g.* using oxygenated and de-aerated, unbuffered solutions of CPZ), we did not observe the characteristic 510 nm spectrum of the cation radical in the microsecond or millisecond time domain. Replacing the optical filter solution with distilled water to allow excitation of CPZ through the S_2 absorption band also yielded no significant changes in the recorded spectrum.

When CPZ in nitrogen-saturated 10 mM sodium phosphate buffer was flash photolyzed in the presence of DMPO, an ESR spectrum like that in Fig. 3(a) was obtained. The simulation of the ESR spectrum shown in Fig. 3(b) suggests that three species are present:

- (1) The electron or hydrogen atom-adduct of DMPO (DMPO/H);
- (2) the hydroxyl radical-adduct (DMPO/OH); and
- (3) a carbon-centered adduct of DMPO (DMPO/P) having hyperfine splitting constants identical to those for the radical resulting from the dechlorination of CPZ, as identified by Motten *et al.* (1985).

The predominant species is DMPO/H , as shown in the legend for Fig. 3. The ratio of detected species did not change significantly with CPZ or DMPO concentration (see below). These results are consistent with those found by Motten *et al.* (1985) for CPZ and promazine and by Hoebeke *et al.* (1986) for tryptophan during steady-state irradiation of these compounds.

The DMPO/H species can arise through the reaction of DMPO with a hydrogen atom or hydrated electron [$\text{DMPO} + e_{\text{aq}}^- + \text{H}^+ \rightarrow \text{DMPO}/\text{H}$; Sargent and Gardy (1976)]. Therefore, to distinguish between these two possibilities we repeated the

† Navaratnam and co-workers (1978) have suggested that the transient appearing near 570 nm is formed by reaction of $\text{CPZ}^{\cdot+}$ with the promazinyl radical, $\cdot\text{P}$.

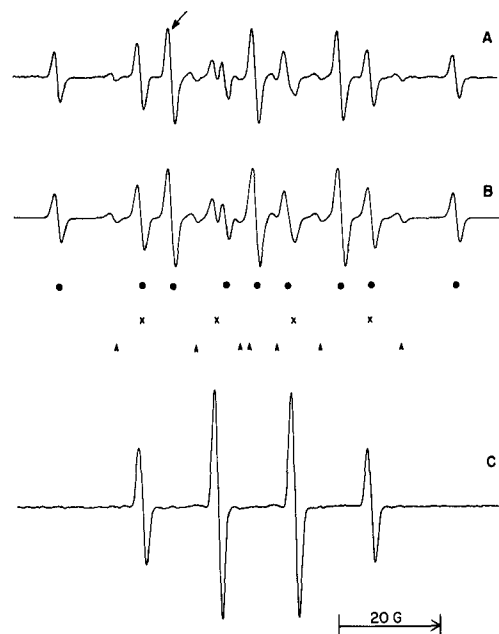
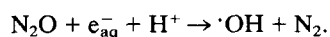


Figure 3. Electron spin resonance spectra of flash photolyzed solutions of CPZ and DMPO. The solution contained $29 \mu\text{M}$ CPZ and 0.2 mM DMPO. A flash at 88.5% of maximum intensity was used to obtain the spectrum. (A) Nitrogen-saturated solution; (B) simulation of A. The model has three species: ●, DMPO/H (i.e. $e_{\text{aq}}^- + \text{H}^+$), [$a^{\text{N}} = 16.53 \text{ G}$, $a^{\text{H}}(2) = 22.55 \text{ G}$]; ×, DMPO/OH, ($a^{\text{N}} = 14.92 \text{ G}$, $a^{\text{H}} = 14.98 \text{ G}$); ▲, DMPO/P, ($a^{\text{N}} = 15.97 \text{ G}$, $a^{\text{H}} = 24.23 \text{ G}$) in a concentration ratio of 11:4:1, respectively. See Buettner (1987) for comparison of hyperfine splitting constants of these species. (C) Nitrous oxide-saturated solution showing only DMPO/OH. The instrument settings for both (A) and (C) were: modulation amplitude, 1.4 G; receiver gain, 2.5×10^5 ; time constant, 0.33 s; field sweep, 0.6 G s^{-1} ; power, 20 mW. The arrow indicates the line of DMPO/H used for the signal amplitude measurements of Figs. 4–6.

experiments with nitrous oxide-saturated solutions of CPZ instead of nitrogen-saturated solutions. In this case we obtained only the hydroxyl radical adduct, DMPO/OH. This is consistent with the hydrated electron, produced during the initial photoionization of CPZ, reacting with N_2O to give $\cdot\text{OH}$ (Bielski and Gebicki, 1977):



We obtained the predicted substitution of DMPO/OH for DMPO/H in N_2O -saturated solutions of CPZ with concentrations of DMPO as low as $0.2 \mu\text{M}$. Thus, the DMPO/H adduct appears not to be obtained through direct reactions between DMPO and excited state CPZ but rather through the reaction of DMPO with the hydrated electron.

Nitrogen-saturated solutions of $29 \mu\text{M}$ CPZ were flash photolyzed in the presence of different concentrations of DMPO in order to establish the minimum concentrations of DMPO necessary to efficiently capture the hydrated electron. The results in Fig.

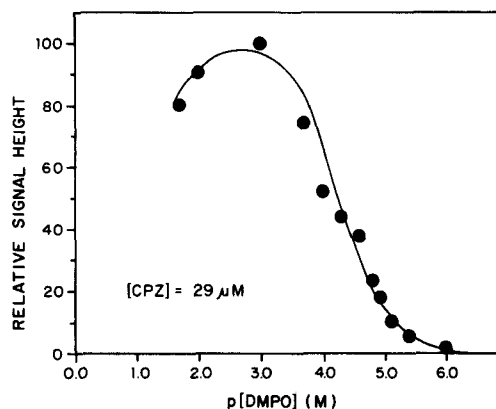


Figure 4. Dependence of ESR signal intensity of DMPO/H upon the initial concentration of DMPO, following flash photolysis of a nitrogen-saturated solution of $29 \mu\text{M}$ CPZ. The buffer was 10 mM sodium phosphate, pH 6.0 and the flash intensity was set at 88.5% of the instrument maximum. Midpoint occurs at about $50 \mu\text{M}$ DMPO. The curve is a non-linear least squares fit of the data to Eq. (1). See text for details. $p[\text{DMPO}] = -\log [\text{DMPO}]$.

4 indicate the presence of a maximum scavenging efficiency at about 1.0 mM DMPO and a titration midpoint at about $50 \mu\text{M}$ DMPO. Above 1.0 mM DMPO, the ESR signal intensity of DMPO/H begins to drop. It is possible that at higher concentrations DMPO may quench a fraction of the excited-states of CPZ, precluding further excitation (see Discussion). Absence of buffer did not affect signal intensity or composition of the ESR spectrum.

A number of researchers have noted the strong surfactant characteristics of phenothiazines, including CPZ (Forrest *et al.*, 1984; Salhab and Dujovne, 1986). Although the optical geometry of the cuvette and the absorption characteristics of CPZ forced us to work at concentrations of CPZ well below those for which significant surface activity has been shown, we studied the intensity of the ESR signal obtained by flash photolyzing different concentrations of CPZ in nitrogen-saturated solutions containing the same concentration of DMPO. The results in Fig. 5 demonstrate that the ESR signal intensity of the DMPO/H adduct is proportional to the initial concentration of CPZ, within the experimental error of the experiment. These results suggest that surfactant characteristics of CPZ were not a factor in our experiments.

Figure 6 demonstrates the dependence between the ESR signal intensity of DMPO/H and flash intensity at a number of flash intensities that we have defined quantitatively by ferrioxalate actinometry. It is readily seen that the ESR signal intensity is linear with the *square* of the flash intensity. It should be noted that the experimental data have been obtained over an order-of-magnitude range of flash intensities. Thus, to a good approximation the data satisfy a quadratic relationship.

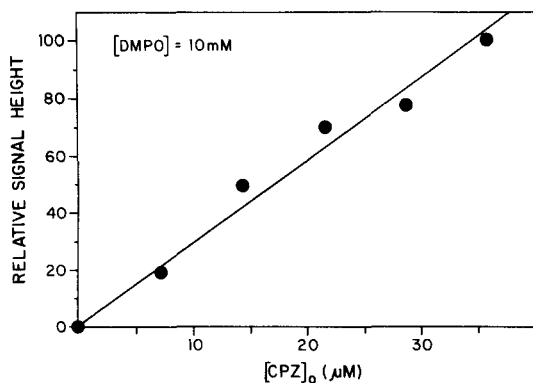


Figure 5. Dependence of the ESR signal intensity of DMPO/ \cdot H upon the initial concentration of CPZ. The DMPO concentration was 10 mM, the buffer was 10 mM sodium phosphate, pH 6.0 and the flash intensity was set at 88.5% of the instrument maximum. Solutions were nitrogen-saturated.

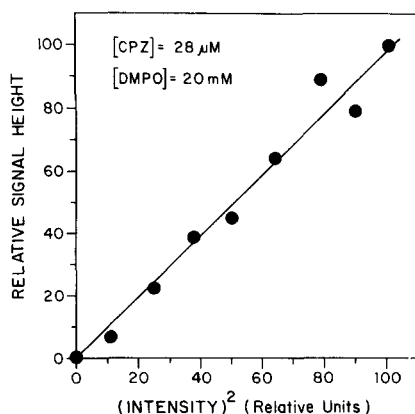


Figure 6. The ESR signal intensity of DMPO/ \cdot H versus the square of flash intensity, in relative actinometric units, observed in flash photolyzed, nitrogen-saturated solutions of 28 μ M CPZ and 20 mM DMPO. The buffer was 10 mM sodium phosphate, pH 6.0.

DISCUSSION

The results of the present study are consistent with those obtained by Buettner *et al.* (1989) and further support the notion proposed by Motten *et al.* (1985) of a stepwise biphotonic mechanism of photoionization of CPZ consequent upon $S_0 \rightarrow S_1$ excitation of the molecule. Certainly, these results are subject to the qualification that the excitation process initiated by conventional flash lamps, with their long ($> 2 \mu$ s) pulse widths and polychromatic light, may be very different from that initiated by laser pulses. However, the excellent quadratic dependence of the ESR signal intensity of the DMPO/ \cdot H adduct upon flash intensity suggests that one or more of the excited states of CPZ are strongly susceptible to photoionization through absorbance of a second photon.

As described in detail in Buettner *et al.* (1989), for moderate flash intensities, the initial concen-

tration of the hydrated electron arising from photoionization of CPZ can be given by $[e_{aq}^-] = kI_p^n$, where k is a constant encompassing the specified experimental conditions, I_p is the pulse intensity, and n is an integer that will be 1 for a monophotonic process and 2 for a biphotonic process. At higher intensities, n may be less than 2 for a stepwise biphotonic process. Our results show that essentially all e_{aq}^- generated in our experiments are captured as DMPO/ \cdot H. Thus $[\text{DMPO}/\cdot\text{H}] = kI_p^n$, and the concentration of DMPO/ \cdot H observed in a stepwise biphotonic process, using moderate flash intensities, will have a quadratic dependence on I_p , *i.e.* $n = 2$.

The dependence of the spin adduct ESR signal intensity on initial DMPO concentration can be understood by recognizing that two different effects of the spin trap may be competing with each other. At lower concentrations, DMPO competes with CPZ (Navaratnam *et al.*, 1978) for e_{aq}^- , but at higher concentrations ($> 1 \text{ mM}$) the spin trap probably directly quenches one or more excited states of CPZ. Thus, the ESR signal intensity, A , obtained at constant CPZ concentration (29 μ M) as a function of DMPO concentration can be described as follows:

$$A = \frac{A_0 \cdot k_D [\text{DMPO}]}{k_{\text{H}_2\text{O}} + k_C \cdot [\text{CPZ}] + k_D \cdot [\text{DMPO}]} \times \frac{1}{1 + K_q \cdot [\text{DMPO}]} \quad (1)$$

where $k_{\text{H}_2\text{O}}$ is the rate of disappearance of e_{aq}^- in buffer, k_C and k_D are the rate constants for the capture of e_{aq}^- by CPZ and DMPO, respectively, K_q is the excited-state Stern-Volmer quenching constant (Birks, 1970) of the singlet or triplet state of CPZ, and A_0 is the maximum ESR signal intensity initially produced by the flash in the absence of quenching.

The curve in Fig. 4 is the non-linear least-squares fit of the data (see Materials and Methods), assuming $k_D = 6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (Buettner *et al.*, 1989), and solving for A_0 , K_q and the quantity $k_{\text{H}_2\text{O}} + k_C \cdot [\text{CPZ}]$. The fitting iteration converged slowly, but demonstrated a definite X^2 minimum at $A_0 = 102$, $k_{\text{H}_2\text{O}} + k_C \cdot [\text{CPZ}] = 0.38 \pm 0.02 \mu\text{s}^{-1}$ and $K_q = 12 \pm 0.6 \text{ M}^{-1}$. For $k_D = 1.2 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ (Hoebeke *et al.*, 1986), better convergence was noted and the final parameter values were $A_0 = 101$, $k_{\text{H}_2\text{O}} + k_C \cdot [\text{CPZ}] = 0.78 \pm 0.02 \mu\text{s}^{-1}$ and $K_q = 12 \pm 0.02 \text{ M}^{-1}$.

Although it has been possible to obtain a good fit of the experimental points by assuming a single quenching reaction (hence a single Stern-Volmer term rather than a product of terms), it is not possible to conclude with certainty whether DMPO quenches the singlet or triplet excited state of CPZ. Nevertheless, we have calculated the bimolecular rate constant associated with the singlet excited state of CPZ through the use of the fluorescence lifetime

for CPZ reported in Buettner *et al.* (1989). The value of K_q obtained, $2.4 (\pm 1.2) \times 10^{10} M^{-1} s^{-1}$, is consistent with diffusion-controlled quenching of that state.

Navaratnam *et al.* (1978) and Buettner *et al.* (1989) have estimated k_C to be $1.9 \times 10^{10} M^{-1} s^{-1}$ and $1.7 \times 10^{10} M^{-1} s^{-1}$, respectively. Unfortunately, the uncertainty in k_D does not allow us to calculate k_{H_2O} , although the product $k_C \cdot [CPZ] = 0.52 \mu s^{-1}$, calculated using the average reported value of k_C and the concentration of CPZ used in this study, falls within the range of values fitted for the term $k_{H_2O} + k_C \cdot [CPZ]$. Our transient flash photolysis spectra, although quite consistent with those obtained with laser flash photolytic techniques by Navaratnam *et al.* (1978) and Buettner *et al.* (1989), do not agree with those reported by Iwaoka and Kondo (1974). At the same time, both Navaratnam and co-workers (1978) and Iwaoka and Kondo (1974) have claimed evidence for monophotonic photoionization of CPZ. We have already presented evidence obtained by laser flash photolysis that photoionization of CPZ occurs through a biphotonic process and we have tried to address the discrepancies between our work and that of Navaratnam *et al.* (1978). We have now presented further evidence, using conventional flash photolysis, that the photoionization of CPZ easily occurs through a biphotonic process. We suggest that part of the explanation for the experimental results of Iwaoka and Kondo (1974) may lie in the fact that they irradiated CPZ with the intense 254 nm line of a mercury lamp through its S_2 transition, where the absorption coefficient is four to five times larger than that of the S_1 transition. Lachish *et al.* (1976) have pointed out that pseudo-monophotonic behavior can be expected in cases where the product of photon density and absorption cross-section becomes too large. One consequence of excessive irradiation would be that ground-state depletion of CPZ could remove a potential reactant of $CPZ^{\cdot+}$, thus extending the lifetime of the radical. This could be an interesting area for further work.

Although ESR spin trapping and flash photolysis techniques have been combined before (Ito and Matsuda, 1984), we believe that this is the first application of this methodology to the study of mechanisms of photoionization. Here, the integrative nature of the spin trapping process permits the investigation of light-dependent phenomena over a large range of pulse intensity. In addition the absolute yields e_{aq}^- can be determined using standard ESR analytical techniques (Poole, 1983), so direct comparisons can be made between ESR and optical data in cases where concomitant optical information can be obtained. In the present instance, we have been able to establish firmly the quadratic response of the photoionization of CPZ to the intensity of light in the flash photolysis of this drug. By comparison, it has been difficult to reduce flash intensities

to less than 30% of maximum intensity without serious degradation of initial absorbance information obtained by optical analysis (*cf.* Iwaoka and Kondo, 1974; Navaratnam *et al.*, 1978; Buettner *et al.*, 1989). The 'stabilization' of e_{aq}^- by converting it into a relatively long lived and easily detectable product, DMPO/H, offers many new possibilities in flash experiments. The potential for applying laser flash photolysis and spin trapping together is evident, where it will be possible to flash samples directly in the flat cell in the ESR instrument.

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