

THE STEPWISE BIPHOTONIC PHOTOIONIZATION OF CHLORPROMAZINE AS SEEN BY LASER FLASH PHOTOLYSIS

GARRY R. BUETTNER¹, ROBERT D. HALL¹, COLIN F. CHIGNELL^{1,†} and ANN G. MOTTEN²

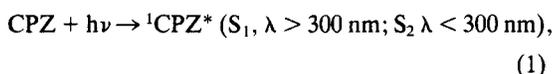
¹Laboratory of Molecular Biophysics, National Institute of Environmental Health Sciences, P.O. Box 12233, Research Triangle Park, NC 27709, USA and ²Department of Chemistry, Duke University, Durham, NC 27706, USA

(Received 29 August 1988; accepted 6 October 1988)

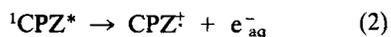
Abstract—It is generally accepted that both promazine (PZ) and chlorpromazine (CPZ) photoionize monophotonically to their respective cation radicals and the corresponding hydrated electrons. It is also supposed that this photoionization has a role in the phototoxic effects of these drugs. However, using laser flash photolysis, we have observed that photoionization of CPZ during S₁ excitation (λ > 300 nm) is a stepwise biphotonic process. In the case of PZ our flash photolysis results are less clear-cut, but are consistent with stepwise biphotonic photoionization for S₁ excitation. We demonstrate, using computer simulation of the intramolecular kinetics, that the estimated triplet state lifetime of CPZ is sufficiently long (23 ns at room temperature) to account for the apparent monophotonic photoionization that has been observed by others at high light intensities and short pulse times. Our laser flash photolysis results also suggest that the photo-ionization mechanism of PZ and CPZ is wavelength-dependent. Both drugs exhibit apparent monophotonic photoionization when they are excited at 266 nm under conditions of laser pulse width and intensity similar to those at 355 nm. We suggest that photoionization is not an important mechanism in the observed phototoxic and photoallergic effects of PZ and CPZ in sunlight.

INTRODUCTION

Phenothiazine drugs have long been used for the treatment of psychiatric disorders, but these clinically important drugs, particularly chlorpromazine, often elicit undesirable side effects that include photoinduced ocular complications and phototoxic and photoallergic reactions. The active intermediates for both photosensitization (Harber and Baer, 1969) and ocular effects (Zelickson and Zeller, 1964) may be free radical photoproducts (Chignell *et al.*, 1985). The free radical photochemistry of chlorpromazine (CPZ)‡ is complex (Motten *et al.*, 1985). After the initial absorption of a photon,



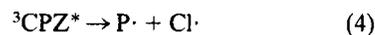
this singlet state could photoionize directly,



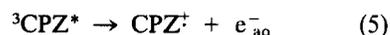
or, relax to a triplet,



The triplet could: (a) dechlorinate,



(b) photoionize directly,



or, (c) photoionize by absorption of another photon,



It is not well understood how CPZ and PZ photoionize to produce hydrated electrons and the corresponding radical cations, CPZ^{·+} and PZ^{·+}.

The photoionization of CPZ and PZ has been studied using flash photolysis by Iwaoka and Kondo (1974) and Navaratnam *et al.* (1978). Using conventional flash photolysis, Iwaoka and Kondo (1974) observed the photoformation of CPZ^{·+}, which was consistent with the previous steady state photochemical studies of Lagercrantz (1962), Borg and Cotzias (1962), and Piette and Forrest (1962). However, conventional flash photolysis cannot be used to directly observe the hydrated electron because its lifetime is short in solution. Using laser flash photolysis, Navaratnam *et al.* (1978) directly observed and identified the excited triplet states of PZ and CPZ, their cation radicals, and the hydrated electrons produced by their photoionization. These authors observed a linear change with flash intensity in the optical density due to e_{aq}⁻, which led them to conclude that photoionization was monophotonic for both molecules. In a similar study of phenothiazine solubilized in micelles, Alkatis *et al.* (1975) concluded that 347 nm laser excitation results in monophotonic photoionization.

†To whom correspondence should be addressed.

‡Abbreviations: CFKR, Center for Fast Kinetic Research; CPZ, chlorpromazine; CPZ*, an excited state of CPZ; CPZ^{·+}, chlorpromazine radical cation; DMPO, 5,5-dimethylpyrrolidine-N-oxide; ESR, electron spin resonance; P[·], 10-[3-(dimethylamino)-propyl]-10H-phenothiazin-2-yl; PZ, promazine; PZ^{·+}, promazine radical cation; R_e, ε_{T1→P1}/ε_{S0S1}; S₁, the first excited singlet state; S₂, the second excited singlet state.

When we employed the ESR spin trapping technique in steady state experiments to study the free radical photolysis pathways of PZ and CPZ (Motten *et al.*, 1985), we detected e_{aq}^- only for the S_2 excitation of CPZ. We observed no e_{aq}^- during S_1 excitation, and photolysis of PZ yielded no evidence for e_{aq}^- whether excitation was into S_1 or S_2 . We concluded that photoionization of CPZ must be a stepwise biphotonic† process during S_1 excitation and a monophotonic process during S_2 excitation. A similar wavelength dependence has been observed for the photoionization of tyrosine by Pileni *et al.* (1975) who postulated a monophotonic mechanism for $\lambda < 270$ nm and a stepwise biphotonic mechanism for $\lambda > 270$ nm.

We have previously argued in the case of PZ that for excitation into both S_1 and S_2 , photoionization is biphotonic (Motten *et al.*, 1985). We attributed the discrepancy between our results and those of Navaratnam *et al.* (1978) to the high light levels required for the laser flash photolysis experiments. Indeed, Lachish *et al.* (1976) have reported that the use of high light levels in laser flash experiments often confounds the analyses of monophotonic and biphotonic photoionization. Nevertheless, the incongruity between our results and those of Navaratnam *et al.* (1978) called for further study of the photoionization processes of PZ and CPZ.

We now present direct evidence that, at light levels typical of laser flash photolysis, CPZ can undergo stepwise biphotonic photoionization during S_1 excitation ($\lambda > 300$ nm); and we use computer simulation of the intramolecular kinetics to show that at the light levels that have been used in laser flash photolysis to investigate CPZ and PZ photoionization, stepwise biphotonic photoionization can appear to be monophotonic.

MATERIALS AND METHODS

Chlorpromazine (Smith, Kline and French Labs, Philadelphia, PA) and promazine (Wyeth Labs, Philadelphia, PA) were used as received. For the experiments described here, CPZ and PZ were dissolved in nitrogen-bubbled 10 mM pH 6.5 citrate buffer, unless otherwise noted. Changing the buffer concentration to 2.5 mM did not affect the results.

Laser flash photolysis experiments were performed at the Center for Fast Kinetic Research, Austin, TX, using a Nd:YAG Q switched laser with a 12 ns pulse. Excitation was accomplished with either the 266 nm output, 13 mJ pulse energy, or the 355 nm output, 22 mJ pulse energy. A mode-locked Nd:YAG laser was used to produce a 30 ps 10 mJ pulse at 355 nm. (Energies quoted represent energy to the sample in a volume of $3 \times 5 \times 10$ mm path length.) In the experiments comparing e_{aq}^- with light intensity, the transient decay curves used for the analysis were a 4–6 shot average. The laser flash intensity was varied by placing wire-screen neutral density filters in the

beam immediately before the sample. Conventional flash photolysis was performed with a PRA FP-1000 flash photolysis system.

Computer simulation of photoionization rates were performed using the TUTSIM program (Applied I, Palo Alto, CA) developed at the Technische Hogeschool Twente, Netherlands. The program was run on a Kaypro IV personal computer using the mathematical model described in the Results and Discussion section.

Fluorescence lifetimes for PZ and CPZ in aqueous buffer at pH 6.5 were measured with an SLM 4800 Subnanosecond Lifetime Spectrofluorimeter (Urbana, IL). 1,4 bis-2-(4-Methyl-5-phenyloxazolyl)-benzene in aerated absolute ethanol was used as a lifetime standard (Lakowicz *et al.*, 1981). The lifetime of the standard was taken to be 1.45 ns.

The absorption coefficients for PZ ($\epsilon_{302 \text{ nm}} = 3880 \text{ M}^{-1} \text{ cm}^{-1}$) and CPZ ($\epsilon_{308 \text{ nm}} = 4030 \text{ M}^{-1} \text{ cm}^{-1}$) at their absorption maxima were determined as the slopes of linear plots of absorbance vs concentration, and were calculated at other wavelengths by reference to the complete absorption spectra.

RESULTS AND DISCUSSION

CPZ and PZ transient absorption spectra

The transient absorption spectra of CPZ and PZ following excitation at 266 or 355 nm are presented in Figs. 1 and 2. These spectra are similar to those presented by Navaratnam *et al.* (1978). The slowly decaying transients at 510 nm for PZ and 530 nm for CPZ are assigned to the well known cation radicals, $\text{PZ}^{\cdot+}$ and $\text{CPZ}^{\cdot+}$ (Borg and Cotzias, 1962; Piette and Forrest, 1962; Iwaoka and Kondo, 1974; Navaratnam *et al.*, 1978; Davies *et al.*, 1979).

The triplet–triplet absorptions for PZ and CPZ have been previously assigned to the transients with a maximum at 460 nm (Davies *et al.*, 1976; Navaratnam *et al.*, 1978; Alkaitis *et al.*, 1975). Very little absorption was observed at 460 nm for CPZ, while for PZ a large triplet–triplet absorption peak was visible (Figs. 1 and 2). The triplet state lifetime of PZ determined from the 12 ns pulse system was 3100 ns‡ in nitrogen-bubbled solution, 400 ns in air-saturated solution, and 86 ns in oxygen-saturated solution. These lifetimes were determined using a double first-order decay analysis for the 460 nm transient; the slower decaying transient corresponded to the decay of $\text{PZ}^{\cdot+}$. The lifetime of the 460 nm transient of CPZ was estimated using the fast data acquisition capabilities of the 30 ps laser flash photolysis system at the CFKR. Our value of 23 ns in nitrogen-bubbled solution is comparable to the value of approximately 100 ns obtained by Navaratnam *et al.* (1978). The much shorter triplet state lifetime of CPZ, compared to PZ, is undoubtedly related to the chlorine present and possibly the dechlorination reaction.

We attribute the broad, very fast-decaying transient absorption *ca.* 700 nm to e_{aq}^- [$\lambda_{\text{max}} = 720$ nm,

†We have chosen to use the term 'stepwise biphotonic' to clearly distinguish consecutive two-photon processes from processes that result from simultaneous two-photon absorption. See Scaiano *et al.* (1988).

‡In general, the standard errors of the fitted rate constants were 5–10% of the analysed value.

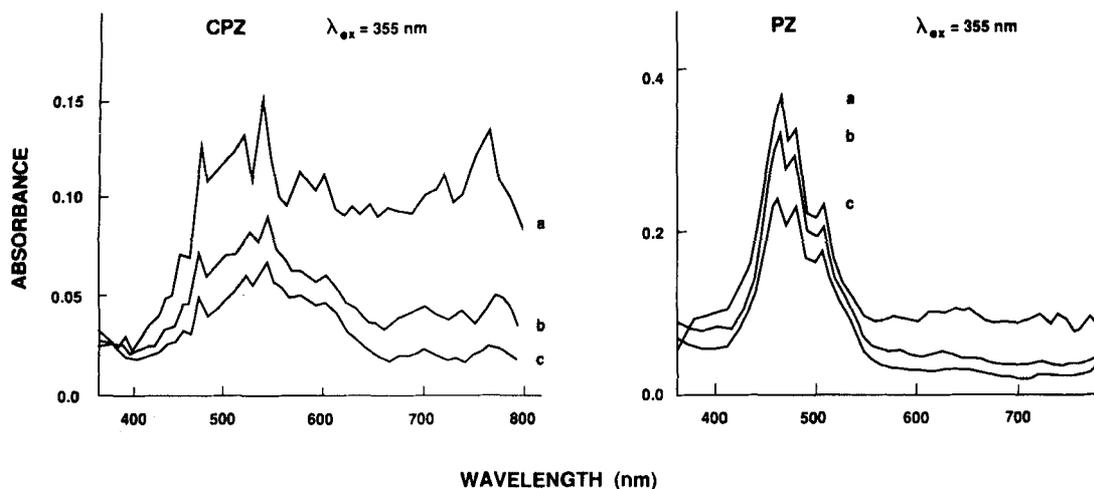


Figure 1. Transient absorption spectra of CPZ and PZ: 12 ns, 355 nm excitation. Left: nitrogen-bubbled 0.54 mM CPZ; absorbance at 355 nm was 0.18. Spectra were taken at (a) 0.12 μ s, (b) 0.44 μ s, and (c) 1.42 μ s after the laser flash. Right: nitrogen-bubbled 0.55 mM PZ; absorbance at 355 nm was 0.09. Spectra were taken at (a) 0.10 μ s, (b) 0.70 μ s, and (c) 2.2 μ s after the laser flash. Absorption data were taken at 10 nm intervals. Pulse energy was *ca.* 22 mJ.

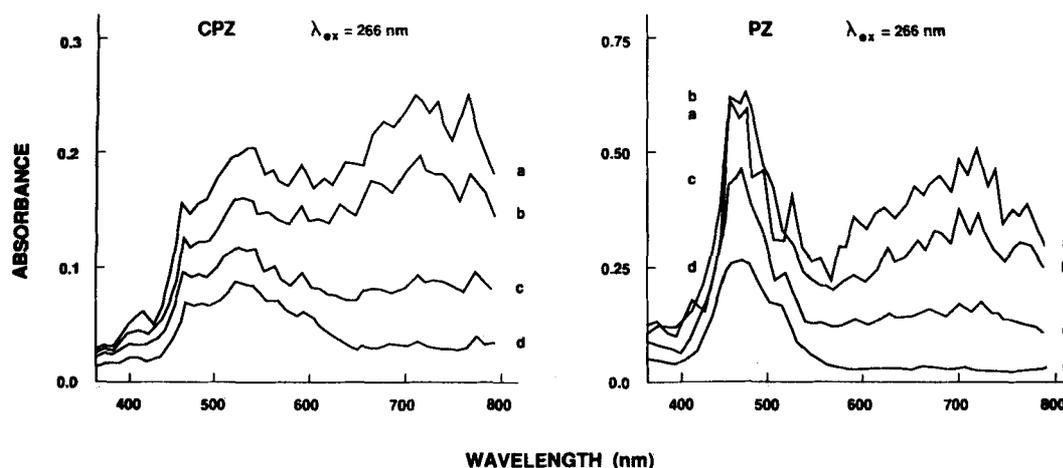


Figure 2. Transient absorption spectra of CPZ and PZ: 12 ns, 266 nm excitation. Left: nitrogen-bubbled 0.09 mM CPZ; absorbance at 266 nm is 1.1. Spectra were taken at (a) 0.14 μ s, (b) 0.26 μ s, (c) 0.64 μ s, and (d) 1.29 μ s after the flash. Right: nitrogen-bubbled 0.09 mM PZ; absorbance at 266 nm was 1.1. Spectra were taken at (a) 0.040 μ s, (b) 0.10 μ s, (c) 0.27 μ s and (d) 1.35 μ s after the laser flash. Absorption data were taken at 10 nm intervals. Pulse energy was *ca.* 22 mJ.

$\epsilon_{720} = 1.9 \times 10^4 \text{ mol}^{-1} \text{ cm}^{-1}$, width at half maximum *ca.* 350 nm (Jou and Freeman, 1977)]. To confirm our assignment, we tested for quenching by N_2O . At an N_2O concentration of *ca.* 22 mM (saturated solution) the transient was effectively quenched as expected; N_2O reacts with e_{aq}^- at $8.9 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (Anbar *et al.*, 1973). As a further check, we also measured the reaction rates of this transient with CPZ and with the spin trapping agent DMPO. The reaction of e_{aq}^- with CPZ was found to be first-order in CPZ by Navaratnam *et al.* (1978) with a rate constant of $1.9 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$. From our observed pseudo-first-order rate constants we determined that the rate constant for the reaction

of our *ca.* 700 nm transient with CPZ was $1.7 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$. (For promazine, this rate constant was $8.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$.) The reaction of our *ca.* 700 nm transient with the spin trap DMPO had a second-order rate constant of $6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, similar to that reported by Hoebcke *et al.* (1986), $1.6 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ for the reaction of hydrated electron with DMPO. Taken together, these observations are consistent with the production of e_{aq}^- from the photoionization of PZ and CPZ.

$[e_{\text{aq}}^-]$ vs. light intensity

In an effort to determine whether the observed photoionization process is monophotonic or step-

wise biphotonic, we measured $[e_{aq}^-]$ as a function of light intensity. Solutions were flashed with the 266 nm or 355 nm laser output with a series of intensities, and the absorbance of e_{aq}^- at 690 nm was monitored. The transient decay curve of the electron absorbance was analyzed using first-order kinetics, and an extrapolated absorbance at time zero, D_0 , was calculated and plotted against flash intensity (Figs. 3 and 4). In such a plot the slope is related to the mechanism of photoionization as discussed below.

For moderate flash intensities, the initial absorbances of a transient chemical species can be described as a function of intensity by Eq. 7:

$$D_0 = k I_p^n \quad (7)$$

where n is an integer and k is a constant encompassing the specified experimental conditions (Pillof and Albrecht, 1968). The value of n can be conveniently

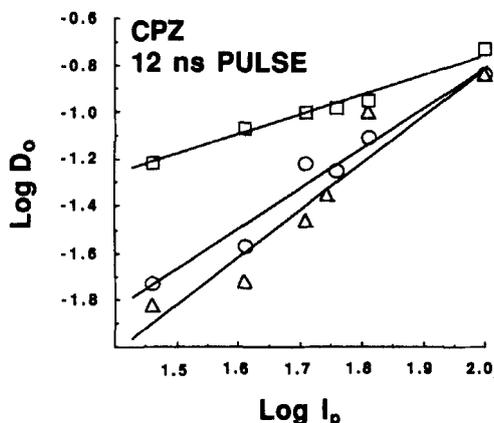


Figure 3. $\text{Log}(D_0)$ at 690 nm vs $\text{Log } I_p$ for CPZ. Δ — 12 ns, 355 nm excitation of 0.5 mM CPZ; absorbance at 355 nm is 0.18. \circ is a repeat of Δ . \square — 12 ns, 266 nm excitation of 90 μM CPZ, absorbance at 266 nm = 1.08.

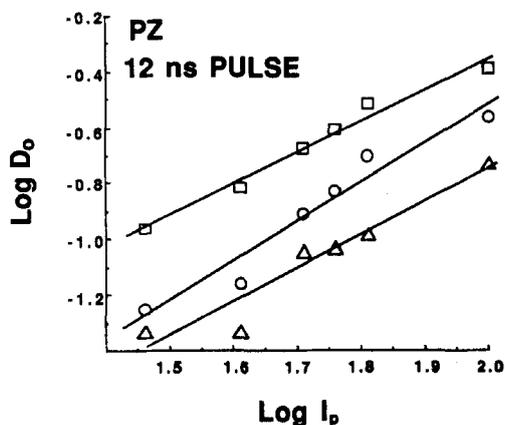


Figure 4. $\text{Log}(D_0)$ at 690 nm vs $\log(I_p)$ for PZ. Δ — 12 ns, 355 nm excitation of 0.5 mM PZ, absorbance at 355 nm = 0.10; \circ — 12 ns, 355 nm excitation of 0.5 mM PZ, absorbance at 355 nm = 0.09; \square — 12 ns, 266 nm excitation of 90 μM PZ; absorbance at 266 nm is 1.08.

determined by plotting the logarithms of the terms in Eq. 7:

$$\log(D_0) = \log k + n \log(I_p) \quad (8)$$

A plot of $\log(D_0)$ vs $\log(I_p)$ then yields a straight line, the slope of which gives n . Note that the absolute values of $[e_{aq}^-]$ and light intensity are not necessary to obtain n .

Lachish *et al.* (1976) and Pillof and Albrecht (1968) have analyzed the conditions under which it is possible to use the value of n to determine mechanisms of photoionization. Using the Beer Lambert law, Lachish *et al.* (1976) have derived a qualitative criterion for determining when the value of n obtained from equation 8 can be used to distinguish single- from multi-photon processes. This criterion can be expressed as

$$\sigma Y < 1 \quad (9)$$

where σ is the absorption cross-section of any absorbing species in the photoionization process and Y is the photon fluence (density) of the flash in photons/cm². Subject to this condition, $n=1$ for a monophotonic process and $n=2$ for a biphotonic process. However, when the photon fluence Y is high compared with the absorption cross-sections of the initial substrate or its excited-state intermediates, i.e. where σY is greater than one at the exciting wavelength over the range of intensities used in an experiment, saturation of excited states can occur and stepwise biphotonic processes can mimic monophotonic ones. In the present study, it was found that laser excitation of both PZ and CPZ at 355 nm (12 ns pulse) gave $\sigma Y = 0.06$ at highest flash intensity, while at 266 nm σY was 0.5 at the highest flash intensities. These results suggest that laser flash intensities were low enough in the present experiments to exclude trivial saturation effects during the initial $S_0 \rightarrow S_1$ (355 nm) and $S_0 \rightarrow S_2$ (266 nm) excitation. Effects arising from further excitation of transient species are discussed in the next section.

Figure 3 presents the plots of D_0 vs laser light intensity for the experiments using the 12 ns pulsed laser to excite CPZ. Using the laser's 355 nm output, the slopes of two separate experiments are 1.7 and 2.0. Excitation at 266 nm yields a slope of 0.9. Thus, photoionization of CPZ clearly occurs through a stepwise biphotonic process during S_1 excitation, while excitation at 266 nm (S_2) appears to produce monophotonic photoionization.

The same experiments with PZ produced slopes of 1.1 and 1.4 with 355 nm excitation and a slope of 1.0 with 266 nm excitation (Fig. 4). The slopes at 355 nm are greater than one but not near two as with CPZ. The results at 266 nm also suggest a monophotonic ionization.

Since our experimental results were not entirely consistent with previous laser flash photolysis work (Navaratnam *et al.*, 1978) or with our ESR data (Motten *et al.*, 1985), we turned to a computer

model that could take ground state depletion and/or triplet state saturation into account.

Computer simulation

The TUTSIM computer simulation algorithm is based on a mathematical model of electrical current flow through resistors and capacitors and can be used for any system whose evolution in time can be described by a small number of differential equations. Our model was a five-level system consisting of the ground state, two excited singlet states, one triplet state, and the ionized state, with each pair of states connected by rate constants. (See Fig. 5 and Table 1.) Monitoring the absorbance of the hydrated electron allows us to ignore the dechlorination reaction (Eq. 4). We have also omitted processes such as triplet-triplet annihilation and the decay of the photoionization products because we presumed them to be slow or rare compared to photoionization itself. The model is described by the following differential equations:

$$\frac{dS_0}{dt} = -(k_{A1} + k_{A2})[S_0] + k_T[T_1] + k_F[S_1] \quad (10)$$

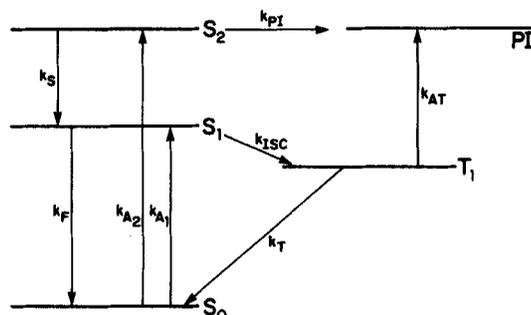


Figure 5. Energy levels scheme for computer simulations of photoionization. Only excitation into S_1 was simulated in the present work.

Table 1. Rate constants used to simulate photoionization in the five-level system of Figure 5

Rate constant	Chlorpromazine	Promazine
k_F	$1.8 \times 10^9 \text{ s}^{-1}$	$4.29 \times 10^8 \text{ s}^{-1}$
τ_F	0.5 ns*	2.1 ns*
k_{ISC}	$2.0 \times 10^8 \text{ s}^{-1}$	$4.76 \times 10^7 \text{ s}^{-1}$
ϕ_T	0.1†	0.1†
k_T	$4.3 \times 10^7 \text{ s}^{-1}$	$3.2 \times 10^5 \text{ s}^{-1}$
τ_T	23 ns*‡	3100 ns*‡
k_S	10^{12} s^{-1}	10^{12} s^{-1}
k_{PI}	10^{11} s^{-1}	10^{11} s^{-1}
k_{A2}	0	0
$k_{A1}\S$	$10^6\text{--}10^9 \text{ s}^{-1}$	$10^6\text{--}10^9 \text{ s}^{-1}$
$k_{AT}\S$	$10^6\text{--}10^9 \text{ s}^{-1}$	$10^6\text{--}10^9 \text{ s}^{-1}$

*Measured in this study.

†From Navaratnam *et al.* (1978).

‡Nitrogen-bubbled solution.

§In curve b of Fig. 6.

$$\frac{dS_1}{dt} = -(k_F + k_{ISC})[S_1] + k_{A1}[S_0] + k_S[S_2] \quad (11)$$

$$\frac{dS_2}{dt} = -(k_S + k_{PI})[S_2] + k_{A2}[S_0] \quad (12)$$

$$\frac{dT_1}{dt} = -(k_{AT} + k_T)[T_1] + k_{ISC}[S_1] \quad (13)$$

$$\frac{dPI}{dt} = k_{PI}[S_2] + k_{AT}[T_1] \quad (14)$$

We assigned rate constants that were consistent with measured rates where possible, and where measurement was not feasible we assigned reasonable values. We calculated k_T from the triplet lifetimes; it represents all triplet deactivation processes except photoionization. k_F , the rate constant for deactivation of the singlet state by all routes other than intersystem crossing, and k_{ISC} , the intersystem crossing rate from S_1 to T_1 , were assigned values consistent with both the measured triplet quantum yield of 0.1 as determined for CPZ by Navaratnam *et al.* (1978) and our measured fluorescence lifetimes of 0.5 ns for CPZ, 2.1 ns for PZ. For the present work only stepwise biphotonic photoionization *via* the triplet state ($S_0 \rightarrow S_1 \rightarrow T_1 \rightarrow PI$) was modeled. The second excited singlet state was included in the model for completeness and for our planned extension to S_2 excitation. In the present study the rate constant k_{A2} was set to zero. The decay from the second excited singlet state, S_2 , to either S_1 (k_S) or to PI (k_{PI}) cannot affect our results in this limited calculation because the S_2 state was not populated.

The excitation rate constants k_{A1} and k_{AT} depend on the photon fluence rate (flux) of the beam, the number of molecules in the beam, and the extinction coefficients $\epsilon_{S_0 \rightarrow S_1}$ and $\epsilon_{T_1 \rightarrow PI}$, respectively; these absorption rate constants were varied over wide ranges as we investigated the behavior of the model system. For a single concentration and a single excitation wavelength, both the number of molecules in the beam and the extinction coefficients are constant, so changing k_{AT} and k_{A1} in concert is equivalent to changing the fluence rate, Γ . Changing k_{AT} but not k_{A1} is equivalent to changing the ratio of extinction coefficients, $\epsilon_{T_1 \rightarrow PI}/\epsilon_{S_0 \rightarrow S_1}$ (which we will henceforth call R_e) without changing Γ . Therefore, all simulations were interpreted in terms of these two variables, Γ and R_e , and the rate constants for each simulation were chosen so that only one of these two factors would be changed at a time.

The parameters in Table 1 were used as input for the model, and the population of the photoionized state was calculated at 40 ps intervals for 20 ns total time. The population of the PI state was taken as the population at the end of 20 ns. After a simulated 20 ns excitation flash into S_1 , $\log[PI]$ is plotted in Fig. 6a–c over a range of fluence rates for three different R_e values. In Fig. 6a, R_e is 100, in 6b R_e is 0.1, and in 6c it is 0.01. Curve 6d was generated

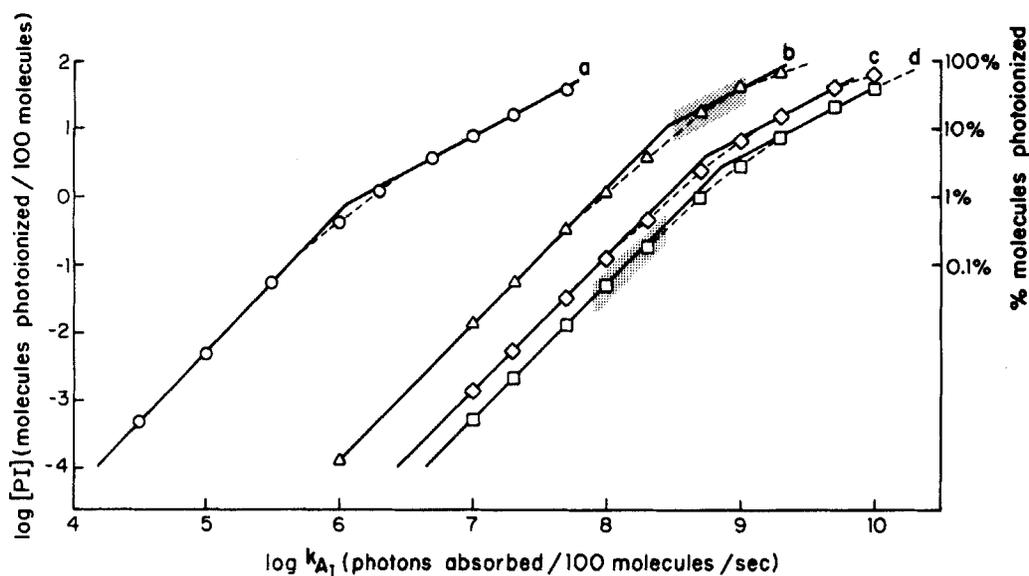


Figure 6. Log-log plot of computer simulated biphotonic photoionization. The solid lines represent the closest extrapolation of tangential fit of lines with slope 1.0 and slope 2.0 to the relevant portions of the calculated curves (plotted points). When simulated over a wide range of photon fluences, all our calculated curves contain a biphotonic (slope two) section and an apparently monophotonic (slope one) section, even though all the data were generated using a stepwise biphotonic model. Curves (a) through (c) represent different values of R_e with a flash time of 20 ns: (a) $R_e = 100$; (b) $R_e = 0.1$; (c) $R_e = 0.01$. Curve (d) represents a 12 ns flash time with all other parameters the same as in curve (c). The shaded areas are located on the x-axis so that they correspond to Navaratnam's light conditions (upper area) and our light conditions from Fig. 3 (lower area). Their placement on curves (b) and (d) demonstrate that a reasonable difference in R_e at 347 nm and 355 nm can account for both our clearly biphotonic measurements and the apparently monophotonic results of Navaratnam *et al.* (1978).

using the same parameters as 6c, including the same fluence rate and the same R_e , but with only a 12 ns flash time. All four curves contain a linear region with a slope of two at lower fluence rates and at higher fluence rates a continuously varying region whose slope decreases monotonically from two to zero. A portion of the latter region (roughly 10% to 80% conversion to the photoionized state) has the form of a monophotonic or quasimonophotonic line with an average slope of *ca.* one.

The slope n of the $\log [PI]$ vs $\log k_{A1}$ curve at any given point is related to the way the triplet state population changes during the flash. If the triplet state population increases throughout the duration of the flash (Fig. 7a,b), n will be 2 for a biphotonic process. If, however, the triplet state population reaches and maintains a steady state during the flash (Fig. 7d), information about steps involved in reaching the triplet state will be lost, the photoionization will *look* monophotonic, and n will be 1. Intermediate values of n , between 1 and 2, will result when the triplet population follows an intermediate course, approaching but not actually reaching a steady state, as in Fig. 7c.

The same fluence rate can give different values of n for different molecular species, especially if R_e varies from one species to another. At $\log k_{A1} =$

7, for example, $n = 2$ for molecules with $R_e = 0.01$ or 0.1, but $n = 1$ for a molecule with $R_e = 100$. In Fig. 7a-d we have plotted the triplet state population during the flash for a series of hypothetical molecules with R_e varying from 0.01 to 1000.

On the other hand, the entire range of values for the slope can also be obtained for a single molecule if the fluence rate can be sufficiently varied. For example, in Fig. 6c ($R_e = 0.01$), the slope is two, and a plot of the triplet state population during the flash would give a shape similar to Fig. 7a-b when $\log k_{A1}$ is in the range of 7 to 8. At $\log k_{A1} = 8.7$, a plot of the triplet state population during the flash would look similar to Fig. 7c, with n intermediate between 1 and 2. When $\log k_{A1} = 9.3$, the triplet state population reaches a steady state during the flash (similar to Fig. 7d), and $n = 1$.

Note that it is the change in, rather than the magnitude of, the triplet population that determines the slope of the $\log [PI]$ vs $\log k_{A1}$ curve. As long as a steady state is reached during the flash, $n = 1$ whether the level of steady state triplet population is 1% or 50%. For any given value of R_e , a fluence rate for the laser flash (expressed as k_{A1}) can be found that will give a slope of approximately one, but the magnitude of the triplet population at the end of the flash can vary (Fig. 7e-h). R_e and Γ

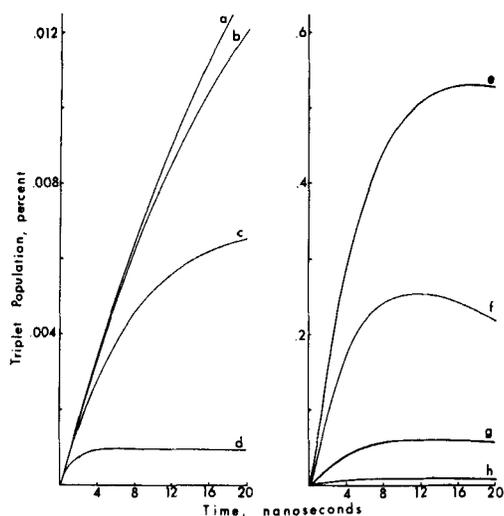


Figure 7. Simulated triplet state populations for a stepwise biphotonic process during a 20 ns flash. 'Triplet population, percent' is $100 \times$ (number of triplet state molecules)/(total number of molecules in all states). Rate constants were the same as for chlorpromazine in Table 1. In (a)–(d) k_{A1} is constant (corresponding to constant photon fluence rate) while R_e is varied from 0.01 (a) to 10 (b) to 100 (c) to 1000 (d). These rate constants correspond to points on $\log [PI]$ vs $\log k_{A1}$ plots in the slope two region (a–b), in the intermediate region (c), and in the pseudomonophotonic, slope one region (d). We chose to hold k_{A1} constant rather than R_e because with R_e constant the change in shapes is not readily visible on a single scale graph. In (e)–(h) k_{A1} is adjusted to give a point in the slope one region for various values of R_e : (e) 0.01; (f) 0.1; (g) 1; (h) 10. Note that it is not the total triplet state population that determines whether pseudomonophotonic behavior will occur in the $\log [PI]$ vs $\log k_{A1}$ plot, but rather the extent to which the triplet population has established a reasonably steady state. Triplet state populations during a 12-ns flash can be seen in the first 12 ns of each curve.

interact to give the final value of n , with smaller R_e requiring higher fluence rates for pseudomonophotonic behavior.

We varied several parameters other than R_e and Γ to probe the sensitivity of our model. Shortening the flash lowers the percentage of photoionized molecules after the flash without changing the general shape of the $\log [PI]$ vs $\log k_{A1}$ curve (compare Fig. 6c and d). Increasing the triplet state quantum yield (not shown) slightly increases the photoionized product concentration. Varying the fluorescence lifetime $[\tau_F = 1/(k_{ISC} + k_F)]$ has virtually no effect on this time scale, nor does lengthening the triplet lifetime $(\tau_T = 1/k_T)$. Thus, the single most important parameter in terms of effect on the results of the simulation is R_e .

Comparison of computer simulations with experiments

We can account for both our slope two curve and the slope one curve of Navaratnam *et al.* (1978) for

CPZ $S_0 \rightarrow S_1$ excitation using measured parameters if we adjust R_e in the model (Table 1). However, if we could find a single set of parameters that could simultaneously account for our data and those of Navaratnam *et al.* (1978), we might be able to infer the range for R_e . The maximum photon fluence† used in our work and that of Navaratnam *et al.* are separated by only *ca.* 50%, so it is unlikely that our different slopes can wholly be accounted for by the different photon fluences alone. We can speculate that because two different excitation wavelengths were used, it is possible that $\epsilon_{T_1 \rightarrow PI}$ is greater at 347 nm than at 355 nm. (Note: $\epsilon_{T_1 \rightarrow PI}$ is not necessarily a triplet–triplet absorption; it could be an absorption from T_1 directly into the continuum of photoionized states. Our model cannot address the question of possible involvement of intermediate higher triplet states.) The slope of a $\log [PI]$ vs $\log k_{A1}$ plot could be changed from one at the 347 nm excitation to two at 355 nm (see shaded regions in Fig. 6) if $\epsilon_{T_1 \rightarrow PI}$, and thus R_e , differs sufficiently at 347 nm from its 355 nm value. Even if $\epsilon_{T_1 \rightarrow PI}$ (347 nm) is equal to $\epsilon_{T_1 \rightarrow PI}$ (355 nm), there will be a difference in R_e of a factor of two due to the two-fold difference in $\epsilon_{S_0 \rightarrow S_1}$ between these two wavelengths. Thus, an additional factor of only two to four in $\epsilon_{T_1 \rightarrow PI}$ would allow our model of the biphotonic mechanism to account for both our data and those of Navaratnam and coworkers (1978).

For promazine excited at 355 nm, our slopes of 1.4 and 1.1 are consistent with stepwise biphotonic photoionization measured in the region of changing slope. An example of this type of intermediate case is presented in Fig. 7c.

In our spin-trapping experiments with PZ (Motten *et al.*, 1985), we were not able to trap e_{aq}^- with steady state photolysis into either S_1 or S_2 , while the laser flash photolysis results appear consistent with monophotonic photoionization from S_2 .

†Laser light intensity (I_p , units = mJ/pulse) was converted to the rate constant k_{A1} (units = photons absorbed/100 molecules solute/s) as follows:

$$k_{A1} = \frac{100 [1 - \exp(-\epsilon C)] I_p}{C N v t_p h \nu}$$

where ϵ is the extinction coefficient at the appropriate wavelength (CPZ, $\epsilon_{355} = 342$, $\epsilon_{347} = 693$; PZ, $\epsilon_{355} = 50.5$, $\epsilon_{347} = 115$, in units of $M^{-1} \text{ cm}^{-1}$); C is the concentration (0.5 mM in all cases); N is Avogadro's number; v is the volume illuminated (0.15 cm^3 in all cases); and t_p is the pulse width in seconds. At 355 nm the laser light intensity at 100% power ($I_p = 22 \text{ mJ/pulse}$) corresponds to a photon fluence of 7.9×10^{16} photons/cm² in our 12 ns pulse experiments. In the 20 ns pulse experiments of Navaratnam *et al.* (1978), we estimate the photon fluences to have been 1.1×10^{17} photons/cm² at 100% power ($I_p = 30 \text{ mJ/pulse}$). Although the photon fluence in the two sets of experiments are somewhat different, the actual photon fluence rates (photon $\text{cm}^{-2} \text{ s}^{-1}$) are nearly the same due to the different laser pulse widths.

The discrepancy remains unresolved as yet, but almost certainly relates to the different light conditions used. However, as the discussion above illustrates, it may be extremely difficult to distinguish between monophotonic and biphotonic mechanisms in all but the most ideal cases. In the difficult cases, as Lachish *et al.* (1976) point out, the steady-state experiment might be a closer approximation to photochemical reactions of biological interest than flash experiments.

CONCLUSIONS

We have demonstrated that photoionization of CPZ during $S_0 \rightarrow S_1$ excitation is stepwise biphotonic. Our laser flash photolysis results with PZ are less clearcut but are consistent with a stepwise biphotonic mechanism. We have used computer simulation of the intramolecular kinetics to account for the apparent monophotonic ionization that has been observed previously. The stepwise biphotonic mechanism for CPZ photoionization through the S_1 excited state illustrates the difficulty of interpreting experimental results that are obtained with very high light levels.

It should be kept in mind that these experiments were done in aqueous solution. Since the photoionization efficiency may change as the polarity of the solvent changes (Navaratnam *et al.*, 1978), it will be important to extend these experiments to other solvents.

The stepwise biphotonic photoionization of CPZ and (probably) PZ when excited into S_1 ($\lambda > 300$ nm) suggests that photoionization is not an important mechanism for the observed phototoxic and photoallergic effects of these drugs in sunlight.

Acknowledgements—The laser flash photolysis experiments and analyses of the data produced were performed at the Center for Fast Kinetic Research at The University of Texas at Austin. The CFKR is supported jointly by the Biomedical Research Technology Program of the Division of Research Resources at NIH (RR 00886) and by the University of Texas at Austin. We thank Dr. Steve Atherton for his efforts while we were at the CFKR.

REFERENCES

- Alkaitis, S. A., G. Beck and M. Grätzel (1975) Laser photoionization of phenothiazines in alcoholic and aqueous micellar solution. Electron transfer from triplet states to metal ion acceptors. *J. Am. Chem. Soc.* **97**, 5723–5729.
- Anbar, M., M. Bambenek and A. B. Ross (1973) Selected specific rates of reactions of transients from water in aqueous solution. I. Hydrated electron. *Nat. Stand. Ref. Data Ser., Nat. Bur. Standards* (NSRDS-NBS 43).
- Borg, D. C. and G. C. Cotzias (1962) Interaction of trace metals with phenothiazine drug derivatives. II. Formation of free radicals. *Proc. Natl. Acad. Sci.* **48**, 623–642.
- Chignell, C. F., A. G. Motten and G. R. Buettner (1985) Photoinduced free radicals from chlorpromazine and related phenothiazines. Relationship to phenothiazine-induced photosensitization. *Environ. Hlth Perspec.* **64**, 103–110.
- Davies, A. K., E. J. Land, S. Navaratnam, B. J. Parsons and G. O. Phillips (1979) Pulse radiolysis study of chlorpromazine and promazine free radicals in aqueous solution. *J. Chem. Soc. Faraday Trans. I*, **75**, 22–35.
- Davies, A. K., S. Navaratnam and G. O. Phillips (1976) Photochemistry of chlorpromazine [2-chloro-N-(3-dimethyl-aminopropyl) phenothiazine] in propan-2-ol solution. *J. Chem. Soc. Perkin II*, 25–29.
- Harber, W. C. and R. L. Baer (1969) In *The Biologic Effects of UV Radiation* (Edited by F. Urbach), pp. 519–529. Pergamon Press, Oxford.
- Hoebeke, M., E. Gandin and Y. Lion (1986) Photoionization of tryptophan: An electron spin resonance investigation. *Photochem. Photobiol.* **44**, 543–546.
- Iwaoka, T. and M. Kondo (1974) Mechanistic studies on the photooxidation of chlorpromazine in water and ethanol. *Bull. Chem. Soc. Japan* **47**, 980–986.
- Jou, F. Y. and G. R. Freeman (1977) Shapes of optical spectra of solvated electrons. Effect of pressure. *J. Phys. Chem.* **81**, 909–915.
- Lachish, U., A. Shafferman and G. Stein (1976) Intensity dependence in laser flash photolysis experiments: Hydrated electron formation from ferrocyanide, tyrosine, and tryptophan. *J. Chem. Phys.* **64**, 4205–4211.
- Lagercrantz, C. (1962) Light-induced free radicals in solutions of some phenothiazine derivatives. *Psychopharmacol. Serv. Center Bull.* **2**, 53–54.
- Lakowicz, J., H. Cherek and A. Balter (1981) Correction of timing errors in photomultiplier tubes used in phase-modulation fluorometry. *J. Biochem. Biophys. Meth.* **5**, 131–146.
- Motten, A. G., G. R. Buettner and C. F. Chignell (1985) Spectroscopic studies of cutaneous photosensitizing agents. VIII. A spin trapping study of light induced free radicals from chlorpromazine and promazine. *Photochem. Photobiol.* **42**, 9–15.
- Navaratnam, S., B. J. Parsons, G. O. Phillips and A. K. Davies (1978) Laser flash photolysis study of the photoionisation of chlorpromazine and promazine in solution. *J. Chem. Soc. Faraday Trans. I*, **74**, 1811–1819.
- Piette, L. H. and I. S. Forrest (1962) Electron paramagnetic resonance studies of free radicals in the oxidation of drugs derived from phenothiazine *in vitro*. *Biochim. Biophys. Acta* **57**, 419–420.
- Pileni, M. P., D. Lavalette and B. Muel (1975) Wavelength dependence of the photoionization of phenolic compounds. *J. Am. Chem. Soc.* **97**, 2283–2284.
- Pillof, H. S. and A. C. Albrecht (1968) Biphotonic photoionization: a flash photoconductivity study of TMPD in 3-methylpentane solutions. *J. Chem. Phys.* **49**, 4891–4901.
- Scaiano, J. C., L. J. Johnston, W. G. McGimpsey and D. Weir (1988) Photochemistry of organic reaction intermediates: Novel reaction paths induced by two-photon laser excitation. *Acc. Chem. Res.* **21**, 22–29.
- Zelickson, A. S. and H. C. Zeller (1964) A new and unusual reaction to chlorpromazine. *J. Am. Med. Assoc.* **188**, 394–396.