Redox Reactions of Chloramphenicol and Some Aryl Peroxyl Radicals in Aqueous Solutions: A Pulse Radiolytic Study

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Redox reactions of aqueous chloramphenicol (CPL) solutions with $H^•$, e_{aq}^-, $OH^•$, $(CH_3)_2(OH)C^•$, and $CO_2^{•-}$ were studied using pulse radiolysis techniques. Rate constants for the reaction of these radicals with CPL were determined. Using $MV^{2+}/MV^{•+}$ as the standard couple, the one-electron redox potential of the CPL was determined. High-performance liquid chromatography was employed for the investigation of the degradation products. The possible formation of aryl radicals from CPL and nitrosobenzoic acid (one of the several products formed in irradiated CPL aqueous solution) and its subsequent reaction with O_2 can lead to the formation of aryl peroxyl radicals. The reactivity of various *p*-substituted aryl peroxyl radicals with 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) was studied. Such a reaction product could be responsible for observed side effects of CPL.

Introduction

Ionizing radiation is increasingly being used for sterilizing medical products.[1−3] Drugs for parenteral applications need to be sterilized before use. Sterilizing by ionizing radiation has its own desirable advantages and therefore is preferred over other available techniques of sterilizations. Applications of ionizing radiation for sterilizing drugs are however limited, possibly due to the formation of undesirable chemical degradation products.[1−3]

Chloramphenicol (CPL) has been used extensively in the treatment of typhoid and meningitis. It is a broad spectrum antibiotic and is also used in the treatment of microbial infections of the eye and ear. Various side effects of CPL are known. The most serious is a non-dose-related aplastic anemia that often appears after the drug therapy has been discontinued. It has been suggested that in the metabolism of CPL, nitroso compounds can be formed and this could be the cause of non-dose-related aplastic anemia.[4] However, no basic mechanisms have been suggested for the formation of metabolite in vivo, although the formation of p-nitrobenzaldehyde, *p*-nitrobenzoic acid, and *p*-nitrosobenzoic acid is reported on exposure of CPL to sunlight and ionizing radiations.[4]

Oxygen is also present during metabolism of CPL in the blood stream. Some of the degradation products may be formed on reaction with O_2 due to various enzymatic reactions, which could be responsible for observed side effects.

It is known that living cells are more sensitive to ionizing radiation in the presence of oxygen than in its absence. Many drugs have been developed which can mimic this oxygen effect. Most of these radiation sensitizers are nitro compounds.[5] CPL is often used for comparing the properties of these compounds.

Effects of ionizing radiation on CPL have been studied in detail.[1−3] It degrades less than 1% in solid state and more than 25% in aqueous solution at sterilizing dose (25 kGy).

Degradation of CPL is attributed to various redox reactions. Considering the importance of such reactions as mentioned above to various free radical/enzymatic reactions, we have tried to investigate transient behavior and various redox reactions occuring in CPL solution using pulse radiolysis techniques. In addition, the effect of pH, both in the presence and in the absence of dissolved oxygen, and the possible toxic reactions of aryl peroxyl radicals with various compounds of biological interest are presented. The one-electron redox potential for the couple $CPL/CPL^{•-}$ at neutral pH is also mentioned. The various degradation products formed on irradiation were separated using HPLC and separation profiles used for comparison and analogies made in the present study.

Experimental Section

CPL obtained from Alltech Associates, Inc., Applied Sciences Labs, IL, was used as received. All other chemicals and reagents were HPLC, AR, or GR grade. Methyl viologen (MV^{2+}) (Aldrich), *tert*-butanol, 2-propanol, bromobenzene (Aldrich), dibromobenzene (Sigma), bromobiphenyl (BDH), dibromobiphenyl (BDH) riboflavin (Sigma), bilirubin (Sigma), and 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) (ICN) were also used as received. IOLAR grade (purity > 99.9%) gases (N_2 or N_2O) used for purging the solutions were obtained from Indian Oxygen Limited.

Normal-phase and reverse-phase chromatographic techniques were employed for separation of degradation products. The following configuration of HPLC was used: pump, Constametric III, LDC; flow rate, 1.5 mL/min; injection port, Rheodyne Model 7125, LDC; injection loop, 50 uL; detector, Spectromonitor II, LDC, 273 nm, 0.16 AUFS; data logger PDP 11/23 computer system DEC; column (NP), Partisil PXS 4.6 mm × 25 cm, analytical column; mobile phase (NP), chloroform, methanol, formic acid, 90:2:0.2 (v/v); column (RP), ODS C-18, 25 cm, 4 mm o.d., NOVAPAK analytical column; mobile phase (RP), 0.1 N sodium acetate and acetonitrile (85:15 v/v).

All solutions were prepared in 0.001 mol dm^{-3} phosphate buffer (equimolar mixtures of $Na_2HPO_4 \cdot H_2O$ and KH_2PO_4) unless otherwise stated. Acidic and alkaline pH were adjusted by KH_2PO_4, $HClO_4$, and NaOH to the required value. Nanopure water (conductivity 0.1 $\mu S\ cm^{-1}$) was used for making the solutions.

UV−vis ground-state absorption spectra were measured on a Hitachi (Model 330) spectrophotometer. CPL shows a prominent absorption peak at 275 nm. With the change of pH (1.8−9) no effect on the absorption band of CPL was observed.

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This shows that the CPL exists in only one form in the entire studied pH range, as expected from its structure.

Pulse radiolytic studies were carried out by irradiating solutions in rectangular quartz cells of 1 cm optical path length. Pulses of 50 ns, 500 ns, and 2 μs of 7 MeV electrons from a linear accelerator were employed. The details of the LINAC are given elsewhere.[6] An aerated 0.05 mol dm^{-3} KSCN solution was used for dosimetry, and the $(SCN)_2^{\bullet-}$ radical was monitored at 500 nm. The absorbed dose per pulse was calculated assuming $G\epsilon$ for the $(SCN)_2^{\bullet-}$ radical to be 21 520 dm^3 mol^{-1} cm^{-1} per 100 eV,[7] where G is the radiation chemical yield expressed as the number of molecules formed or destroyed per 100 eV of energy absorbed and ϵ is the molar absorptivity. The dose employed in the present study, unless otherwise stated, was typically 16 Gy/pulse. However, lower doses of about 9 Gy/pulse were used for the determination of formation rate constants, molar absorptivity, and equilibrium constant.

One-Electron Reduction. To study the reaction of e_{aq}^- with CPL at pH 7, 0.1 mol dm^{-3} *tert*-butanol in N_2-saturated solution was used as the scavenger for $OH^\bullet$ radicals according to reaction 1.

$$OH^\bullet + (CH_3)_3COH \rightarrow H_2O + (CH_3)_2C(CH_2^\bullet)OH \quad (1)$$

The $G(e_{aq}^-)$ value at pH 7 was taken as 2.7 from the literature.[9,10] The CPL concentration was varied from 0.5×10^{-4} mol dm^{-3} to 2.0×10^{-4} mol dm^{-3} to determine the rate of reaction.

To study reactions of the isopropyl alcohol radical and of the carboxyl radical with CPL, N_2O-saturated solution containing 1.0 mol dm^{-3} 2-propanol or 0.01 mol dm^{-3} formate was used. In these cases e_{aq}^- was converted into $OH^\bullet$ radicals according to eq 2,

$$N_2O + e_{aq}^- + H_2O \rightarrow N_2 + OH^- + OH^\bullet \quad (2)$$

and in the presence of 2-propanol or formate the following reactions take place:

$$H^\bullet/OH^\bullet + (CH_3)_2CHOH \rightarrow (CH_3)_2C^\bullet OH + H_2/H_2O \quad (3)$$

$$H^\bullet/OH^\bullet + HCO_2^- \rightarrow H_2/H_2O + CO_2^{\bullet-} \quad (4)$$

Hence all the primary radicals of water radiolysis were quantitatively converted to the isopropyl radical or $CO_2^{\bullet-}$ radical.

$H^\bullet$ radical reactions at pH 1.8 were studied in the presence of 0.1 mol dm^{-3} *tert*-butanol in the N_2-saturated solutions. The concentration of CPL was varied from 1.0×10^{-4} to 4.0×10^{-4} mol dm^{-3}.

Although the primary reducing radicals are the major reductants, they can get converted to $HO_2^\bullet$ and $O_2^{\bullet-}$ in the presence of oxygen according to reactions 5−7:

$$H^\bullet + O_2 \rightarrow HO_2^\bullet \quad (5)$$

$$e_{aq}^{\bullet-} + O_2 \rightarrow O_2^{\bullet-} \quad (6)$$

$$HO_2^\bullet \Leftrightarrow O_2^{\bullet-} + H^+ \quad (7)$$

Reactions of $OH^\bullet$ and Peroxyl Radicals. Reactions of CPL with $OH^\bullet$ radicals were studied in N_2O-saturated solutions at pH 7 or in oxygen-saturated solutions at pH 1.8. In acidic pH, solutions were saturated with O_2 to scavenge both $H^\bullet$ and e_{aq}^- as explained above.

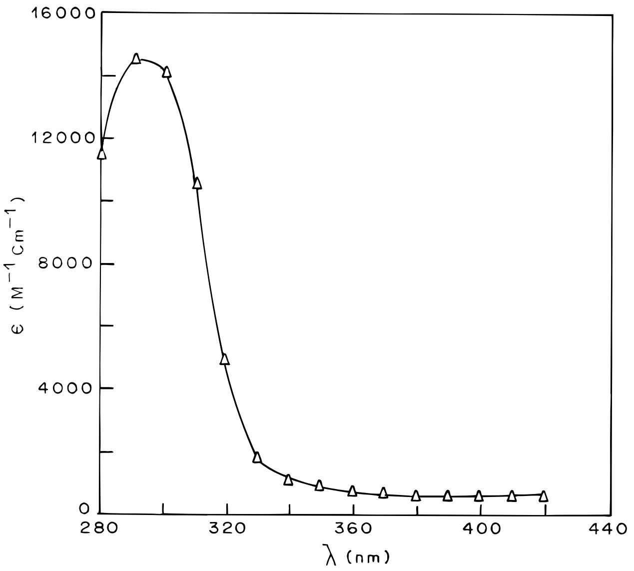

Figure 1. Absorption spectrum of the transient obtained in N_2-saturated solution of 1.0×10^{-4} mol dm^{-3} CPL in water at pH 7 containing 0.1 mol dm^{-3} *tert*-butanol after 11 μs. Dose = 16 Gy.

Peroxyl radical reactions were studied in aerated alcohol or alcohol−water solutions. They were produced in solution by dehalogenation from the respective molecule as explained in the literature.[10,11]

The rates of reactions were determined by carrying out the experiments with at least three different concentrations of CPL, varying by at least a factor of 4. Bimolecular rate constants were derived from plots of the first-order rates vs concentration. The rate constants reported in the table are generally accurate to ±15%. Second-order constants were checked at different doses (16−80 Gy).

Results and Discussion

Pulse Radiolytic Studies. The absorption spectrum of the transient formed after 11 μs of irradiation by 50 ns electron pulses in nitrogen-saturated aqueous solution containing 1.0×10^{-4} mol dm^{-3} CPL and 0.1 mol dm^{-3} *tert*-butanol at pH 7 is shown in Figure 1. Nitrobenzene anion shows an absorption maximum at 285 nm,[12] which is similar to the absorption observed in the present study. Therefore, it seems that the solvated electron reacts with the nitro group of CPL. It indicates that the presence of *p*-substituent with respect to the nitro group in CPL has a negligible effect on the spectral profile. This assignment can be confirmed by examining the protonation properties of the observed radical anion. We find that on increasing the pH from 7 to 9, there was no effect up to 4000 μs either on the spectral properties or on the decay kinetics of the transient absorbing at 290 nm. However, at pH 4.3 the decay of the transient increases. This is evident from the spectral changes shown in Figure 2. The transient spectrum was taken at 10 and 4000 μs after the 50 ns electron pulse. The nature of the spectrum obtained at 10 μs is similar to that obtained in nitrogen-saturated aqueous solution at pH 7. This shows that the spectrum observed at 10 μs is of the CPL radical anion. As can be seen from Figure 2, the transient spectrum at 4000 μs is slightly broader than that at 10 μs with a slight red shift in the absorption maximum. This could be due to the formation of some product which absorbs at λ's > 320 nm. An attempt to provide a satisfactory explanation for the same, however, failed due to the reason that the transient with λ_{max} at 290 nm does not decay completely within the time window of our experimental conditions. No effect of pH (between 4.3 and 9.0) on

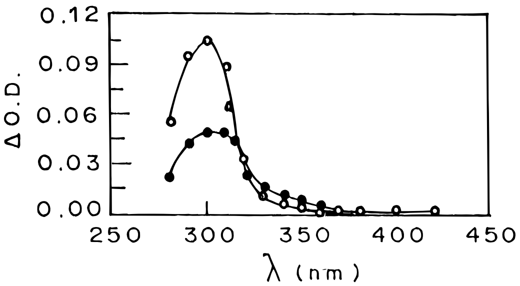

Figure 2. Time-resolved absorption spectrum of the transient obtained in N_2-saturated solution of 5.0×10^{-5} mol dm^{-3} CPL and 0.2 mol dm^{-3} 2-propanol at pH 4.3 (○) after 10 μs and (●) after 4000 μs. Dose = 16 Gy.

the absorption yields of the transient was observed. This indicates that if the pK_a exists, it must be less than 4.3.[12] Measurement of the formation kinetics of the transient was carried out at 300 nm to avoid ground-state absorption of CPL. In aqueous solutions at pH 7, the decay rate of e_{aq}^- at 700 nm matches the formation rate kinetics of the 300 nm band. This band was observed to grow by pseudo-first-order kinetics, with k_{obs} increasing linearly with CPL concentration $(0.5-2.0) \times 10^{-4}$ mol dm^{-3}. The bimolecular rate constant for the reaction of e_{aq}^- with CPL, determined from the slope of the linear plot of k_{obs} vs CPL concentration, was 2.3×10^{10} dm^3 mol^{-1} s^{-1}, as shown in Table 1. The observed bimolecular rate constant is in reasonably good agreement with the literature value.[13] The electron adduct was observed to decay very slowly, and under our experimental conditions it was not possible to measure it. The intensity of the 300 nm absorption band remained constant with the change of CPL concentration from 5.0×10^{-5} to 2.0×10^{-4} mol dm^{-3}. It indicates that all the e_{aq}^- produced reacted with CPL and the concentration of e_{aq}^- can be taken equal to the concentration of the transient produced at 300 nm. The molar absorptivity at 300 nm was calculated to be 14 600 ± 1500 dm^3 mol^{-1} cm^{-1}.

Reaction of $CO_2^{\bullet -}$ and Isopropyl Radical with CPL at Neutral pH. $CO_2^{\bullet -}$ and isopropyl radical were produced in N_2O-saturated aqueous solution containing solute and 1.0×10^{-2} mol dm^{-3} formate or 1.0 mol dm^{-3} 2-propanol. The nature of the transient spectrum obtained on reaction of CPL with $CO_2^{\bullet -}$ or $(CH_3)_2C{\cdot}OH$ is comparable with that observed in the case of e_{aq}^-. Therefore, it seems that both $CO_2^{\bullet -}$ or $(CH_3)_2C{\cdot}OH$ reduce the CPL via

$$\mathrm{CPL} + \mathrm{CO_2}^{\bullet -} \rightarrow \mathrm{CPL}^{\bullet -} + \mathrm{CO_2} \tag{8}$$

$$\mathrm{CPL} + \mathrm{(CH_3)_2C{\cdot}OH} \rightarrow \mathrm{CPL}^{\bullet -} + \mathrm{(CH_3)_2CO} + \mathrm{H}^+ \tag{9}$$

The bimolecular rate constants for the reaction of isopropyl radical, and $CO_2^{\bullet -}$ with CPL, determined from the slope of the linear plot of k_{obs} vs [CPL], were 1.0×10^9 dm^3 mol^{-1} s^{-1} and 6.0×10^8 dm^3 mol^{-1} s^{-1}, respectively.

Reaction of CPL with $H^\bullet$. The transient absorption spectrum observed in the pulse radiolysis of N_2-saturated CPL solution containing 0.1 mol dm^{-3} *tert*-butanol at pH 1.8 exhibits an absorption maximum at 420 nm (Figure 3). This spectrum was formed over 7 μs after the 50 ns electron pulse and decayed within about 0.5 ms. This spectrum is not due to the anion radical of CPL since the observed spectrum is different from that observed on reaction of CPL with e_{aq}^-. Its molar absorptivity at λ_{max} is given in Table 1.

The observed transient band at 420 nm (pH 1.8) grows with the concentration of CPL. As shown in Table 1, the measured rate constant for the $H^\bullet$ radical addition to CPL has a value of 1.0×10^9 dm^3 mol^{-1} s^{-1}. The CPL concentration was varied from 1.0×10^{-4} to 4.0×10^{-4} mol dm^{-3}. A comparison of the $H^\bullet$ radical reaction rate constant of CPL with nitrobenzene[14,15] suggests that the presence of the alkyl chain does not have any significant effect on the kinetics of this reaction. Since the $H^\bullet$ radical can undergo addition reactions with aromatic compounds,[14,15] the observed transient could be due to the formation of a CPL-H adduct. The transient decayed by first-order kinetics, as shown in Table 1. This indicates that the transient does not undergo any dimerization or addition reaction with the CPL molecule.

Reaction of CPL with $HO_2^\bullet$ and $O_2^{\bullet -}$ Radicals. In O_2-saturated solutions the reactions of CPL with $HO_2^\bullet$ and $O_2^{\bullet -}$ radicals were studied at pH 1.8 and 7. The solution contained 0.1 mol dm^{-3} *tert*-butanol and 2.0×10^{-5} mol dm^{-3} CPL. Under our experimental conditions we could not see any transient formation in the accessible range (280−750 nm) due to the reaction of HO_2 or $O_2^{\bullet -}$ with CPL. The reactivity of oxygen radicals with biologically important compounds is a matter of concern.[10,11,16] The above observations indicate that both these O_2 radicals are unreactive toward CPL.

Reaction of CPL with $OH^\bullet$ Radicals. Reactions of CPL with $OH^\bullet$ radicals were studied at pH 7 (N_2O-saturated) and at pH 1.8 (oxygen-saturated) aqueous solutions. The transient absorption spectrum observed in the pulse radiolysis of N_2O-saturated CPL solution at pH 7 is shown in Figure 3. The transient absorption spectrum exhibits absorption maxima at 350 and 420 nm. The spectrum was formed over 13 μs and decayed within 1.0 ms. It is known that H and OH radicals on reaction with aromatic compounds give a cyclohexadienyl type of radical with similar absorption characteristics.[14,15] The formation of two bands in the absorption spectrum shows that $OH^\bullet$ radicals undergo addition reaction with CPL at more than one site. The attempts to resolve this, however, failed. The molar absorptivity and the measured rate constant are given in Table 1. It can be seen from Figure 3 that the spectral profiles of the transients obtained on reaction of CPL with $OH^\bullet$ radical at pH 7 and 1.8 are similar.

Assignment of End Products. Some of the major degradation products formed on γ-irradiation of aqueous solution of CPL at natural pH were identified using HPLC as *p*-nitrobenzoic acid, *p*-nitrobenzaldehyde, *p*-nitrosobenzoic acid, and HCl. The degradation products could have formed by the reaction of primary species with CPL and their subsequent reactions/rearrangement. A mechanism for the degradation of aqueous solutions of CPL using visible light has been suggested earlier.[4,17] In that study also the formation of *p*-nitrobenzoic acid, *p*-nitrobenzaldehyde, and *p*-nitrosobenzoic acid is reported. The mechanism proposed is based on the formation of a carbon-centered radical on absorbing light. Therefore it seems that whether the degradation of CPL takes place via primary species of water radiolysis or via carbon-centered radicals,[4] the mentioned end products are found to be identical. This shows that the effect of light and ionizing radiation on degradation of CPL is similar.

Possible Role of Aryl Radical Reactions in the Radiolysis of CPL. In recent years one-electron reduction of nitro compounds has received considerable attention in view of the application of nitro aromatics as radiosensitizers. The reactivities of various *p*-substituted nitrobenzene compounds have been studied with carbon-centered radicals.[18−20] Very recently, it has been shown that aryl radicals may be formed by the thermal decomposition of nitrosobenzene,[21] and the reactivity of aryl radicals toward various substrates has been a subject of considerable interest.[22,23] As discussed above, nitrosobenzoic

TABLE 1: Spectral and Kinetic Parameters of the Transient Formed by Reaction of CPL with Various Radicals

pH	radical	λ_{max} (nm)	ϵ ($dm^3\ mol^{-1}\ cm^{-1}$)	observed species	k_2 ($dm^3\ mol^{-1}\ s^{-1}$)	k_1 or $2k/\epsilon l$ (s^{-1})
7	e_{aq}^-	290	14 600 ± 1500	radical anion	2.3×10^{10}	
7	$(CH_3)_2C{\cdot}OH$	290		radical anion	1.0×10^9	
natural	$CO_2^{\bullet -}$	290		radical anion	6.0×10^8	
1.8	$H^\bullet$	420	3150 ± 300	adduct	1.0×10^9	1.2×10^{6a}
7	$OH^\bullet$	350,420	3130 ± 300 (420 nm)	adduct	1.8×10^9	2.1×10^5
1.8	$OH^\bullet$	420		adduct	2.5×10^9	1.8×10^6
7	CCl_3O_2	295		adduct	2.3×10^9	1.7×10^5

[a] First-order decay.

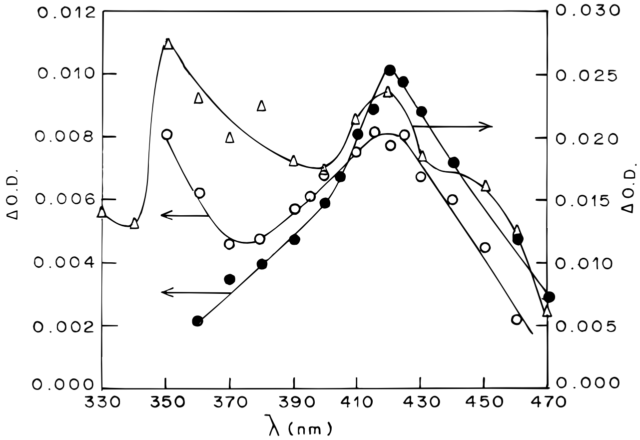

Figure 3. Transient absorption spectrum obtained from aqueous solution containing 1.0×10^{-4} mol dm^{-3} CPL: (●) H-adduct spectrum at pH 1.8, N_2-saturated; (△) OH-adduct spectrum at pH 7, N_2O-saturated; (○) OH-adduct spectrum at pH 1.8, O_2-saturated. Dose = 16 Gy.

acid is one of the radiation degradation products of CPL. Nitroso compounds on thermal decomposition can form aryl radicals,[21] and in the presence of O_2 the aryl radicals can also form aryl peroxyl radicals which have intense absorption at wavelengths < 350 nm. However, they also show absorption maxima at wavelengths > 400 nm depending on the substituents.[24,25] The possibility of formation of aryl radicals from nitrosobenzoic acid cannot be ruled out. Therefore, we have studied the reactivity of various aryl peroxyl radicals with biologically important compounds such as riboflavin and bilirubin because oxygen is present in the blood stream during metabolism of CPL. ABTS is generally used to study such reactions because of the high molar absorptivity of its radical cation. We have generated aryl peroxyl radicals from various *p*-substituted compounds, viz., dibromobenzene, 4-bromobiphenyl, and 4,4′-dibromobiphenyl. On pulse irradiation peroxyl radicals formed from dibromobenzene (DBB) and 4,4′-dibromobiphenyl (DBP) show absorption maxima in aerated solutions at 480 and 560 nm, respectively (Figure 4). Due to the low solubility of these compounds, the transient absorption spectra of DBB and DBP were taken in 60:40 (v/v) methanol−water and in neat methanol, respectively. It was observed that peroxyl radicals of DBB and DBP oxidize ABTS with a rate 0.5×10^9 $dm^3\ mol^{-1}\ s^{-1}$ and 0.8×10^8 $dm^3\ mol^{-1}\ s^{-1}$, respectively. However, no reaction was observed with bilirubin and riboflavin under our experimental conditions. Since the aryl peroxyl radicals show intense absorption at wavelengths < 350 nm, therefore it was not possible to study the reactivity of aryl peroxyl radicals with CPL. For completeness, however, the reactivity of halogenated alkyl peroxyl radicals was studied with CPL. The transient spectrum formed, just after the pulse at pH 7 in an aerated alcohol−water solution containing 1.0×10^{-4} mol dm^{-3} CPL, 5 mol dm^{-3} 2-propanol, and 1.0×10^{-2} mol dm^{-3} CCl_4, on reaction of CCl_3O_2 with solute is shown in Figure 5. The 290 nm transient band was observed to grow by pseudo-first-order kinetics with respect to CPL concentration. The bimolecular rate for the reaction of CCl_3O_2 with CPL in an aerated alcohol−water solution determined from the slope of the linear plot of k_{obs} vs solute concentration was 2.3×10^9 $dm^3\ mol^{-1}\ s^{-1}$. The transient decays by second-order kinetics, with $2k/\epsilon l = 1.7 \times 10^5\ s^{-1}$. It is known that the reactivity of aryl peroxyl radicals is more than that of alkyl peroxyl radicals.[24,25] Therefore, the above results show that if the formation of aryl radicals takes place during metabolism of CPL (or later), they can react with the CPL in the presence of oxygen. This may have important biological consequences.

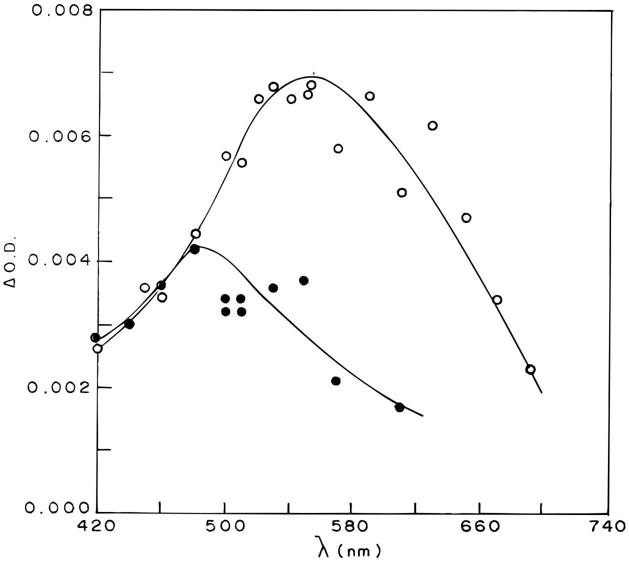

Figure 4. Transient absorption spectrum of brominated phenyl peroxyl radicals: (●) (4-bromophenyl) peroxyl in 60:40 methanol−water; (○) (4-bromobiphenyl) peroxyl in methanol. (See text for details.) Dose = 80 Gy.

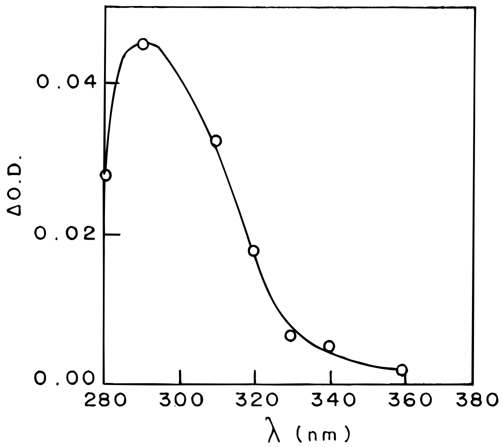

Figure 5. Transient absorption spectrum obtained in aerated aqueous solution containing 1.0×10^{-4} mol dm^{-3} CPL, 1.0×10^{-2} mol dm^{-3} CCl_4, and 5.0 mol dm^{-3} 2-propanol at pH 7.

Redox Studies. The redox potential for the nitrobenzene, NB/NB^-, couple has been reported as −0.486 V (vs NHE).[26]

TABLE 2: Effect of pH on the Absorbance of $MV^{\bullet+}$ at Constant [CPL] and $[MV^{2+}]$

pH	CPL, mol dm^{-3}	MV^{2+}, mol dm^{-3}	OD_{578} nm
4.9	5.0×10^{-5}	2.0×10^{-4}	0.0894
7.0	5.0×10^{-5}	2.0×10^{-4}	0.0918
9.2	5.0×10^{-5}	2.0×10^{-4}	0.0927

It has been suggested that the nitrobenzene and methyl viologen (MV^{2+}) redox system undergoes very fast equilibrium.[27] The redox potential for MV^{2+} has been reported as −0.446 V for the MV^{2+}/MV^{+} pair (vs NHE). In N_2-saturated aqueous solution containing CPL and MV^{2+} we have observed that the MV^{2+} and CPL redox system forms an equilibrium:

$$\mathrm{CPL}^{\bullet-} + \mathrm{MV}^{2+} \rightleftharpoons \mathrm{MV}^{\bullet+} + \mathrm{CPL} \tag{10}$$

It was observed that for a fixed concentration of CPL and MV^{2+} in N_2-saturated aqueous solution in pulse radiolysis there was no change in the absorbance yield of $MV^{\bullet+}$ at 578 nm. The pH of the solution was varied between 4.9 and 9.2. The results are given in Table 2.

Under the equilibrium conditions, the equilibrium constant K for eq 10 can be related to the concentrations of the species by

$$K = \{[\mathrm{MV}^{\bullet+}][\mathrm{CPL}]\}/\{[\mathrm{CPL}^{\bullet-}][\mathrm{MV}^{2+}]\} \tag{11}$$

The validity of the above equation is based on the assumption that when either [CPL] or $[MV^{2+}]$ was varied, the total radiolytic yield of radicals ($CPL^{\bullet-} + MV^{\bullet+}$) remained constant under our experimental conditions at a dose of 9 Gy used.

On pulse radiolysis of N_2-saturated neutral aqueous solution containing 0.1 mol dm^{-3} *tert*-butanol and 1.0×10^{-5} mol dm^{-3} MV^{2+} and at various concentration of CPL, the formation of the transient band at 578 nm of MV^{2+} was observed. The interference of $CPL^{\bullet-}$ at the wavelength of measurement for $MV^{\bullet+}$ (578 nm) was negligible. Equation 11 can be rearranged to

$$[\mathrm{CPL}^{\bullet-}]/[\mathrm{MV}^{\bullet+}] = (1/K)\{[\mathrm{CPL}]/[\mathrm{MV}^{2+}]\} \tag{12}$$

By calculating the equilibrium concentrations of each in eq 12, the equilibrium constant K can be determined by plotting $[CPL^{\bullet-}]_{eq}/[MV^{\bullet+}]_{eq}$ vs $[CPL]_{eq}/[MV^{2+}]_{eq}$. The plot is shown in Figure 6.

The redox potential of the couple $CPL/CPL^{\bullet-}$ was determined by the following relation:[28]

$$E^\circ = E^\circ_{\mathrm{MV}^{2+}/\mathrm{MV}^{+}} - E^\circ_{\mathrm{CPL}/\mathrm{CPL}^{-}} = 0.059 \log K \tag{13}$$

From the value of $K = 16.6$ the redox potential value for the couple CPL/CPL^{-} is determined to be −0.543 ± 0.03 V vs NHE.

Conclusion

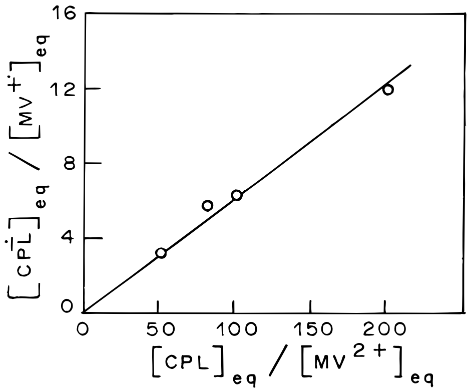

Figure 6. Plot of the ratio of the equilibrium concentration of $CPL^{\bullet-}$ and $MV^{\bullet+}$ against the ratio of the concentration of CPL and MV^{2+} at equilibrium.

The present study indicates the mechanism of degradation of chloramphenicol (CPL) in aqueous solution. Formation and reactivity of nitroso compounds could lead to the formation of degradation products responsible for observed toxic side effects. Considering the various reaction rate constants determined in the present study, it could be possible to stabilize the aqueous solution of CPL for radiation sterilization. Reactions of CPL and its degradation products with globular proteins could give more insight for reactions occurring in living systems.

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