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Gamma Radiolysis of Cu(II) Complex of Metronidazole

P. C. Mandal, D. K. Bardhan, and S. N. Bhattacharyya*
Nuclear Chemistry Division, Saha Institute of Nuclear Physics, 1/AF, Bidhannagar, Calcutta-700064, India

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Aqueous solutions of Cu(II)Metronidazole ($Cu^{II}M$) at neutral pH were irradiated with ^{60}Co γ-rays under different conditions of radiolysis. The radiolytic formation of HNO_2 and Cu^I was followed. The radiolytic yields of chromophore loss of $Cu^{II}M$ were also determined under different conditions. The OH radicals attack the metal complex to give the OH adducts of the ligand at C_2, C_4, and C_5 either directly or through the formation of Cu(III) species. The C_5-OH adduct, however, undergoes oxidative denitration and as a result the metal complex is decomposed. The OH adducts also undergo electron transfer to Cu^{II} ion to give reduced complex. No denitration was observed due to the reaction of e_{eq}^- with the metal complex. On the other hand, the nitro group of the ligand in the complex undergoes successive 4-electron reduction to give hydroxylamino derivative. From the competition kinetics using *t*-butyl alcohol as the scavenger of OH in N_2O saturated solution of the metal complex, the rate constant for the reaction of OH with complex was evaluated to be ca. 2.1×10^9 dm^3 mol^{-1} s^{-1} which is of the same order as that observed in the case of free metronidazole.

Metronidazole or flagyl, a nitroimidazole derivative is used for a long time as an antibacterial drug.[1] Several years back it was observed[2-4] that these nitroimidazole derivatives could be fruitfully utilized in the treatment of cancer cells by radiotherapy. But due to their toxicity in the body tissues,[5-7] their use in the clinics is becoming limited. Because of the photo toxicity, after prolonged treatment with these drugs the patients show an increased susceptibility to skin cancer.[8] At present, an alternative approach is to find a radiosensitizer which contains a transition metal ion having a high binding capability with the cellular DNA.[9,10] Several groups have studied sensitization by complexes of Pt and related metals with various radiosensitizers. Because of the interaction with radiation, and DNA-binding properties of the metals, the relative concentration of the complex at the DNA target will be increased.[9] Also due to complexation with the metals the photo-toxicity of these nitroheterocyclic drugs would be decreased.[11]

Several Cu^{II} and Cu^I complexes are known to bind with DNA.[12-17] Hence complexation of metronidazole with Cu(II) would give a compound which might be a better sensitizer in comparison with both the free metal ion or metronidazole. However, before studying its radiosensitizing behavior in vivo or in vitro, it is necessary to study its radiation chemical behavior in dilute aqueous solution. Here some aspects of its radiation chemistry in dilute aqueous solution have been described.

Experimental

Materials: Metronidazole (M) or flagyl (2-methyl-5-nitro-1H-imidazole-1-ethanol) was procured from Sigma. All other chemicals were of analytical reagent grade. 1:1 Complex of Cu(II) and metronidazole ($Cu^{II}M$) (molecular formula: $CuC_{10}H_{15}N_3O_7$) was prepared[18] by mixing equimolar amounts of methanolic solution of Cu^{II} acetate and metronidazole at room temperature. The solution was evaporated to a smaller volume in a current of nitrogen and left at 0 °C for overnight when bluish crystals of $Cu^{II}M$ separated out. The precipitate was collected by filtration and washed with little methanol and dried. Formation of the complex was further checked by comparing its IR and UV spectra with that reported in the literature.[18] Triply distilled water was always used for the preparation of solutions for irradiation. Deaeration of solutions prior to irradiation was carried out by saturating the solution with extra pure argon. Pure N_2O was used in the experiments.

Irradiation: Irradiation was carried out with ^{60}Co γ-rays. The dose rate (12 Gy min^{-1}) was determined with the help of the Fricke dosimeter, taking $G(Fe^{3+})$ as 15.6.

Analysis: $Cu^{II}M$ contents in the experimental samples before and after irradiation were ascertained by measuring the absorbances near the absorption maximum, 315 nm, ($\varepsilon\approx8714$ dm^3 mol^{-1} cm^{-1}) without further treatment except appropriate dilution with doubly distilled water. The amount of nitrous acid (HNO_2) produced was determined by spectrophotometry with sulphanilic acid and 1-naphthylamine reagents.[19] Cu^I was determined spectrophotometrically by measuring[20] the absorbance of its neocuproin complex at 457 nm. The presence of H_2O_2, if any, in the irradiated solutions was tested with titanium(III)sulphate[21] reagent.

Results

Figure 1 shows the change in absorption spectrum of $Cu^{II}M$ solution on irradiation. It is evident that in the range 260 nm to 380 nm the nature of spectrum remains unaltered on irradiation, while only the absorbances decrease with the increase in the absorbed dose. Further, the decrease in absorption is more pronounced in N_2O saturated solution when compared with that in argon saturated solution. From the molar extinction coefficient of the $Cu^{II}M$ the change in its concentration (for the chromophore loss) was determined.

Figure 2 shows the the amount of $Cu^{II}M$ loss as determined from the loss of chromophoric group as a function of absorbed dose in N_2O saturated solutions. Apart from the loss of the parent compound, the radiolytic products comprise HNO_2 and Cu^I. The yields of HNO_2 and Cu^I against dose are also shown in

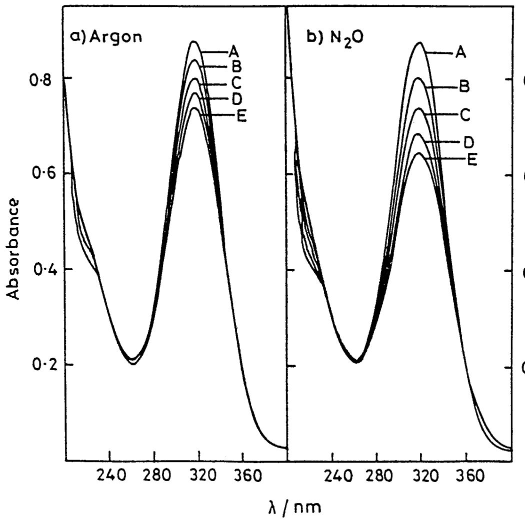


Fig. 1. Change in absorption spectrum on irradiation of (a) argon saturated and (b) N_2O saturated $Cu^{II}M$ (10^{-4} mol dm^{-3}) solution at neutral pH. A: Unirradiated; B,C,D, and E: irradiated to doses of 24 Gy, 48 Gy, 72 Gy, and 96 Gy respectively.

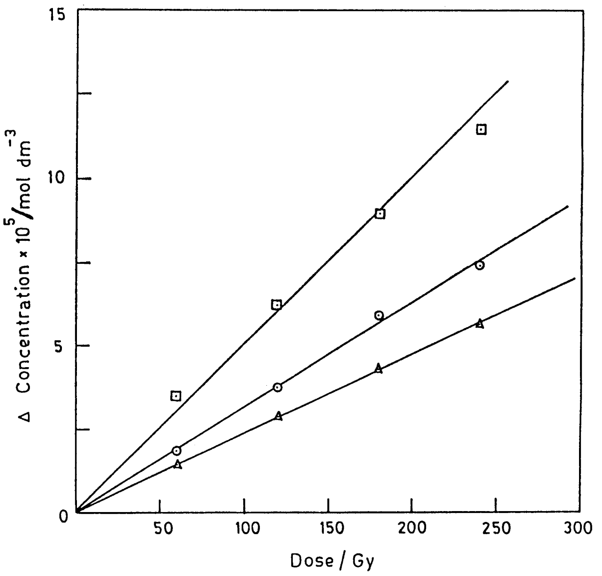


Fig. 2. Yield vs. dose plot for the decomposition of $Cu^{II}M$ and for the formation of HNO_2 and Cu^I in the γ-radiolysis of 5×10^{-4} mol dm^{-3} $Cu^{II}M$ in N_2O saturated solution at neutral pH. (○), loss of $Cu^{II}M$; (Δ), formation of HNO_2 and (⊡), formation of Cu^I.

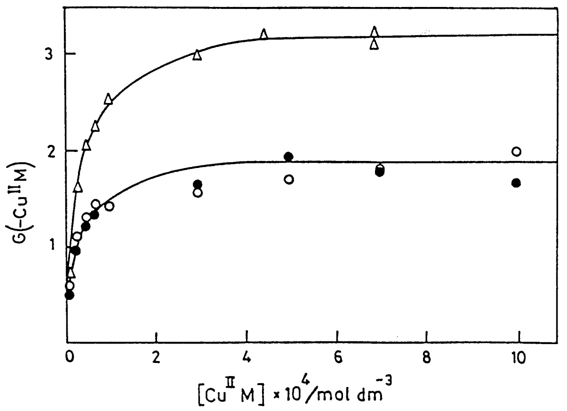


Fig. 3. Change of $G(\text{-}Cu^{II}M)$ values with increase in the initial concentration of the complex in the γ-radiolysis of $Cu^{II}M$ under aerated (○); argon saturated (●), and N_2O saturated (Δ) solutions.

Table 1. G-Values of the Products Formed in the Radiolysis of $Cu^{II}M$ (5×10^{-4} mol dm^{-3}) under Different Conditions

Condition	$G(\text{-}Cu^{II}M)$	$G(Cu^I)$	$G(HNO_2)$
Argon satd	2.0±0.1	2.7±0.05	0.9±0.05
N_2O satd	3.2±0.1	4.8±0.1	2.4±0.1
Aerated	2.0±0.1	2.4±0.1	1.2±0.1
0.4 mol dm^{-3} *t*-Butyl alcohol+Argon satd	0.4±0.05	0.2±0.01	0.0

the same figure. It is evident from the figure that the loss of chromophoric group in $Cu^{II}M$ as well as the formation of HNO_2 and Cu^I are linear in the dose range 60 to 300 Gy. Hence the G-values for the loss of chromophore of $Cu^{II}M$, $G(\text{-}Cu^{II}M)$ and those for the formation of HNO_2 and Cu^I were determined from the slopes of the respective straight lines. The G-values for the loss of chromophore as determined for various initial concentration of $Cu^{II}M$ under different conditions of radiolysis are shown in Fig.3. From the Fig. 3 it is observed that under these conditions the $G(\text{-}Cu^{II}M)$ at first increases with increase in the concentration of $Cu^{II}M$ and then attains a steady state when the solute concentration reaches about 5×10^{-4} mol dm^{-3}. The limiting G-values for the chromophore loss in aerated and argon saturated solution is, $G(\text{-}Cu^{II}M)=2.0\pm0.1$ while that in N_2O saturated solution is 3.2±0.1. The G-values for the formation of HNO_2 and Cu^I were also determined at the concentration, e.g., 5×10^{-4} mol dm^{-3} where the steady state is reached and the results are tabulated in the Table 1. It is evident from the table that under all the conditions except in the presence of 0.4 mol dm^{-3} *t*-BuOH, appreciable amounts of Cu^I and HNO_2 are formed as the product of radiolysis. In the presence of *t*-BuOH, the $G(\text{-}Cu^{II}M)$ is greatly decreased. Apart from this, no HNO_2 is detected under this condition, indicating that no denitration of $Cu^{II}M$ takes place in the presence of *t*-butyl alcohol.

Discussion

In γ-irradiated aqueous solutions of $Cu^{II}M$ water-borne free radicals[22]

$$H_2O \longrightarrow H,\ OH,\ e_{aq}^- \qquad (1)$$

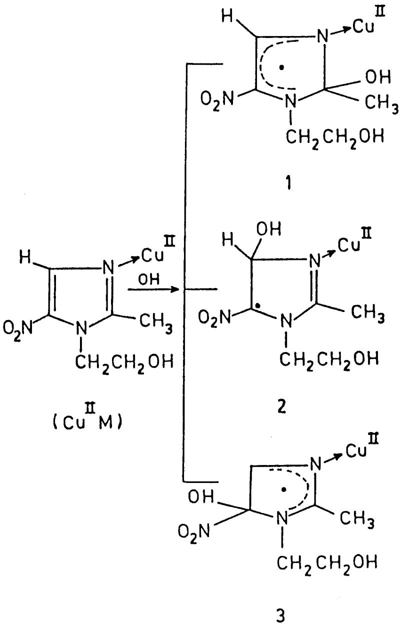
react with the solute giving rise to certain transient species which undergo subsequent transformation to give rise to different products and as a result the compound is decomposed. From Table 1, it is evident that in argon saturated solution, $G(\text{-Cu}^{\text{II}}\text{M}) \approx 2.0$, which is much less in comparison to the total yield of the active species ($G\text{e}_{\text{aq}}^{-} + G_{\text{OH}} + G_{\text{H}} = 6.0$). This indicates that all the transient species do not undergo reactions which lead to the degradation of the compound. Further, when the concentration of $\text{Cu}^{\text{II}}\text{M}$ is below 5×10^{-4} mol dm^{-3}, the decomposition yield is lower than the steady-state value (Fig 3). This is due to the fact that at lower solute concentrations radical-radical reactions predominate over the reactions of the active species with the solute. The radiolytic behavior of $\text{Cu}^{\text{II}}\text{M}$ in absence of any additive appears to be quite complex. The role of individual active species in the degradation of the molecule could be studied in presence of suitable scavengers and hence attempts have been made to study the radiolysis of $\text{Cu}^{\text{II}}\text{M}$ in presence of the scavengers for e_{aq}^{-} and OH radical.

Effect of Dinitrogen Monoxide: When the radiolysis is carried out in dinitrogen monoxide saturated solution, the e_{aq}^{-}, is converted to equivalent amount of OH radicals[23] [$G(\text{OH})=5.4$].

$$\text{e}_{\text{aq}}^{-} + \text{N}_2\text{O} \longrightarrow \text{OH} + \text{OH}^{-} + \text{N}_2 \qquad (2)$$

A small amount of H atoms ($G_{\text{H}}=0.6$) also remains unscavenged in the system. Hence the loss of $\text{Cu}^{\text{II}}\text{M}$ as well as the formation of Cu^{I} and HNO_2 might have arisen from the reaction of the H atom and OH with $\text{Cu}^{\text{II}}\text{M}$. However, when N_2O saturated solution of 5×10^{-4} mol dm^{-3} $\text{Cu}^{\text{II}}\text{M}$ is radiolysed in the presence of 0.4 mol dm^{-3} *t*-BuOH, a good scavenger of OH radicals,[24] neither appreciable loss of $\text{Cu}^{\text{II}}\text{M}$ nor the formation of HNO_2 was detected. Also, the yield of Cu^{I} was negligible.

Hence, the products arising from the radiolysis of 5×10^{-4} mol dm^{-3} in N_2O saturated solution is mainly due to the reactions of OH with $\text{Cu}^{\text{II}}\text{M}$. Under this condition, apart from the loss of $\text{Cu}^{\text{II}}\text{M}$, the products comprise HNO_2 and Cu^{I}. No hydrogen peroxide, however, could be detected in the system. The reaction of OH radicals with aromatic and heterocyclic compounds generally proceeds by the addition of OH to the unsaturated carbon atoms.[25–28] In a similar way, the OH radical might add to the unsaturated carbon atoms of the metronidazole molecule in $\text{Cu}^{\text{II}}\text{M}$ producing radicals **1**, **2**, and **3**.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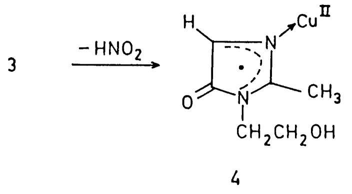
The C_5-OH adduct **3** bearing the hydroxyl and nitro groups at the same ring carbon undergoes HNO_2 elimination[27,29,30] to give radical **4**. In the case of $\text{Cu}^{\text{II}}\text{M}$, the OH radical also might react with the metal ion giving Cu^{III} species **5**, which undergoes electron transfer from π-electron system of imidazole ring to Cu^{III} producing a cation radical, **6**. This cation radical, however, would undergo immediate solvolysis to give radicals **1**, **2**, and **3**.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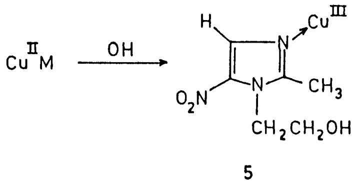
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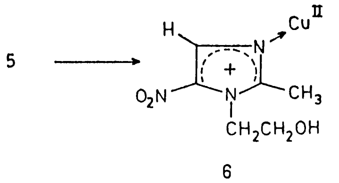
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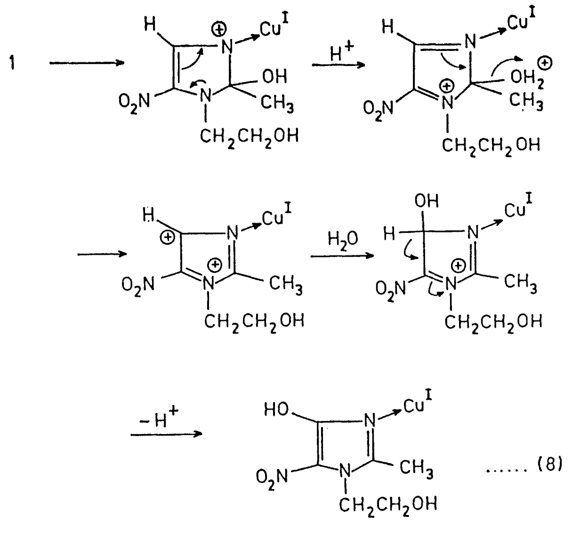
$$6 \xrightarrow{\text{H}_2\text{O}} (1) + (2) + (3) \ldots\ldots (7)$$

From a comparison of the relative yields of HNO_2 in the radiolysis of $\text{Cu}^{\text{II}}\text{M}$ with that formed in free metronidazole under similar condition one might have an idea about the site of attack of OH radicals in the complex. When free metronidazole (5×10^{-4} mol dm^{-3}) is radiolysed in N_2O saturated solution, $G(\text{HNO}_2)$ was found to be only ~1.6. But in the case of $\text{Cu}^{\text{II}}\text{M}$, the same quantity is increased to 2.4 (Table 1). Now, due to the presence of nitro group at C_5 position as well as due to the co-ordination of N_3 with Cu^{II}, the C_5 position of the imidazole ring will be more electron deficient compared to that in uncomplexed metronidazole. Hence addition of OH radical to this position leading to the formation of **3** would be less favored and as a consequence the yield of HNO_2 due to denitration of **3** would be less in comparison with that in free ligand. But the results show a reverse trend. The increased yield of HNO_2 in the presence of Cu(II) complex may, however, be explained by assuming that at least a part of the OH radicals attack the metal ion to give Cu^{III} species which gives cation radical **6**. Due to the electron deficient nature of the C_5 position, attack by H_2O (or OH^-) will be favorable in **6**, and hence the relative yield of **3** as well as that of HNO_2 would be increased.

The reduction of the metal center in the complex has also been determined under this condition and G-value for the reduction of the metal, $G(Cu^I)$ was found to be ≈ 4.8, which is slightly less than the total yield of OH radical [$G(OH) \approx 5.4$]. Pulse radiolysis studies with the imidazoles showed that the decay of the OH adduct of free imidazoles was found to be second order.[25,28] But when they are complexed with metal ions, the decay of the OH adduct follows first order rate law.[28] This has been ascribed to the intramolecular electron transfer from the transient OH adducts to the metal center of the complexes.[28,31,32] By analogy, it can be said that the radicals **1**, and **4** would reduce the metal center and give rise to products (reactions 8 and 9).



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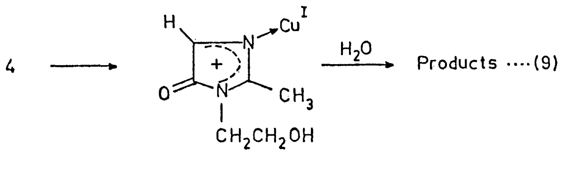
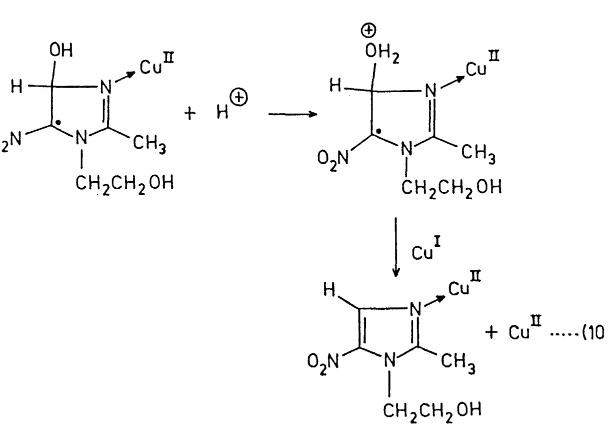


However, the 4-hydroxy adduct **2**, where the unpaired electron is *β* to the hydroxyl group,[25] does not reduce Cu(II) due to the presence of electron withdrawing NO_2 group at C_5-position of the imidazole ring. On the contrary, it undergoes H^+ catalysed OH^- elimination and consequently gets reduced by Cu^I formed in reactions (8) and (9) (cf. Ref. 25).

As the yield of $G(Cu^I)$ is not reduced much in comparison with the total yield of G(OH), the contribution of radical **2** should be very small. It should be mentioned here that the decomposition of $Cu^{II}M$ is measured by the loss of chromophore at 315 nm. The product **7** should have similar absorption behavior as that of parent compound and so could not be accounted by the method. The chromophore loss would be only due to denitrated product formed in reaction (9).



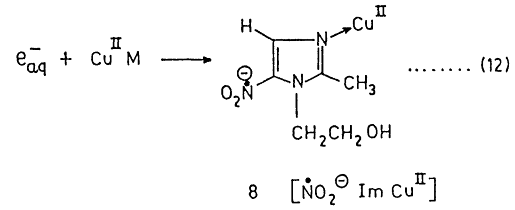
Hence from the aforementioned discussion, the $G(-Cu^{II}M)$ should be $\approx G(HNO_2)$. However, the yield of HNO_2 is less than $G(-Cu^{II}M)$. It is to be noted here that no hydrogen peroxide could be detected in the system. It is well known[33] that the highly reducing HNO_2 can decompose H_2O_2. Then considering the reaction of HNO_2 and H_2O_2, the $G(HNO_2)$ should correspond to $\sim G(-Cu^{II}M) - G(H_2O_2) = 3.2 - 0.7 = 2.5$ and the observed yield of HNO_2, $G(HNO_2) \approx 2.4$ is in good agreement with that expected.

Effect of *t*-Butyl Alcohol: In order to asses the role of e_{eq}^- in the radiolysis of $Cu^{II}M$, the system was studied in the presence of 0.4 mol dm^{-3} *t*-butyl alcohol, where OH radicals are expected to be scavenged by it.

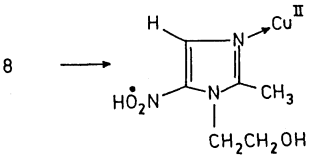
$$OH + (CH_3)_3COH \longrightarrow H_2O + CH_2(CH_3)_2COH \qquad (11)$$

The yields of chromophore loss as well as that of Cu^I formation were shown in Table 1. No HNO_2 and H_2O_2 were, however, detected under this condition. Very small amount of Cu^I has been observed (Table 1) indicating that e_{aq}^- does not react with the central metal ion directly.

It has been reported earlier[28] that metal complexes containing nitro benzoate ligand can trap hydrated electron (e_{aq}^-) through the electron-deficient nitro group in preference to the metal ion. Like wise it may be argued that the nitroimidazole ligand is reduced by e_{aq}^- forming nitro anion radical **8** which protonates to give **9** (reactions 12 and 13).



8 $[\dot{N}O_2^{\ominus}\ Im\ Cu^{II}]$



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9 $[Cu^{II}\ Im\ NO_2H]$

The co-ordinated ligand radical of the complex **9** would undergo intramolecular electron transfer[34] to metal ion.

$$[Cu^{II}ImNO_2H] \longrightarrow [Cu^{I}ImNO_2] + H^+ \qquad (14)$$

But the yield of Cu^I=0.2 only. Attention should also be drawn to that no H_2O_2 or HNO_2 is detected in the system. The molecular H_2O_2 might react with HNO_2 and/or Cu^I. From a comparison of the yield of HNO_2 observed in dearated solution, it appears that no HNO_2 is formed when the radiolysis is carried out in the presence of *t*-BuOH. If it is formed due to the reactions of e_{aq}^- with the ligand, the yield of HNO_2, in fact, would have been larger than that which is observed in dearated solution. Hence it might be argued that H_2O_2 has disappeared through reaction with Cu^I.

$$H_2O_2 + Cu^I \longrightarrow OH + Cu^{II} + OH^- \qquad (15).$$

Hence the yield of Cu^I arising from the electron transfer in **9** should correspond to $G(Cu^I)_{obsd} + G(H_2O_2) = 0.2 + 0.7 = 0.9$. The rest of the radicals **9** (equivalent to a *G*-value $\approx 2.7 - 0.9 = 1.6$), may have disappeared through bimolecular disproportionation.[34,35]

$$2\ Cu^{II}ImNO_2H \longrightarrow Cu^{II}ImNO_2 + Cu^{II}ImNO + H_2O \quad (16)$$

The Cu(II)ImNO undergoes further reduction to Cu(II)ImNHOH (discussed latter).

It has been reported earlier[35] that reduction of N_1-substituted nitroimidazoles takes place by successive 4-electron processes to hydroxylamino derivatives or by successive 6-electron processes to amino derivative. Here the ratio of the *G*(**9**) undergoing disproportionation to $G(-Cu^{II}M)$ is 1.6/0.4=4. It thus appears that similar to that observed in free metronidazole, the reduction of its Cu^{II} complex also takes place through successive 4-electron processes to give hydroxylamino derivative.

$$\begin{tikzcd} Cu^{II}ImNO_2 \arrow[r, "2e^-{,}2H^+", "-2H_2O"'] \arrow[rr, bend right, "4e^-"'] & Cu^{II}ImNO \arrow[r, "2e^-{,}2H^+"] & Cu^{II}ImNHOH \end{tikzcd} \qquad (17)$$

In the light of above discussion, the results obtained in argon saturated solution could be explained satisfactorily. Neglecting the small contribution of the H atoms the reactive species present under this condition are e_{aq}^- (G=2.7) and OH (G=2.7) radicals. Considering that the OH and e_{aq}^- would react in a similar manner as that observed in N_2O saturated solution and in the presence of *t*-butyl alcohol respectively, the $G(-Cu^{II}M)$ under this condition, should correspond to $G(-Cu^{II}M)_{Argon} = 1/2G(-Cu^{II}M)_{N_2O} + G(-Cu^{II}M)_{t\text{-}BuOH} = 1.6 + 0.4 = 2.0$ and the observed value agrees quite well. Similarly, the $G(Cu^I)_{Argon}$ should be equal to 1/2 $G(Cu^I)_{N_2O} + G(Cu^I)_{t\text{-}BuOH} + G_{H_2O_2} = 2.4 + 0.2 + 0.7 = 3.3$ and $G(HNO_2)_{Argon}$ should correspond to 1/2 $G(HNO_2)_{N_2O} = 1.2$ and the observed results are not far from these.

Effect of Dissolved Oxygen: The *G*-values observed in aerated solution (Table 1) is found to be almost identical with those observed in argon saturated solution. In presence of dissolved oxygen, the e_{aq}^- and H atoms would react[22] with molecular oxygen to give O_2^- and HO_2.

$$O_2 + e_{aq}^- \longrightarrow O_2^- \qquad (18)$$

$$H + O_2 \longrightarrow HO_2 \qquad (19).$$

The O_2^- and HO_2 would give rise to H_2O_2 according to equations (20) and (21).

$$2HO_2 \longrightarrow H_2O_2 + O_2 \qquad (20)$$

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2 \qquad (21)$$

The OH radicals react with $Cu^{II}M$ in a similar manner as that described earlier. However, the $G(-Cu^{II}M)$ is somewhat higher than that is expected from the reactions of OH only. Here also no H_2O_2 was detected. The yield of HNO_2 is well accounted by the reactions of OH with $Cu^{II}M$ i.e., $G(HNO_2)_{air} = 1/2G(HNO_2)_{N_2O}$. The disappearance of H_2O_2 could not be accounted by the reactions of H_2O_2 with Cu^I. However, the results could be explained if one assumes that the O_2^- and/or HO_2 do not give rise to H_2O_2 but react with the ligand through electron transfer at the nitro group forming nitro anion radical **8** which subsequently undergoes similar reactions as was observed in deaerated solutions.

Competition Kinetics with *t*-Butyl Alcohol in Dinitrogen Monoxide Saturated Solution: It has already been shown that in N_2O saturated solution the loss of $Cu^{II}M$ is due to the reactions of OH with the complex. If an OH scavenger, *t*-butyl alcohol is present in the system, then a competition ensues between the scavenger and $Cu^{II}M$ for the OH radicals.

$$Cu^{II}M + OH \longrightarrow \mathbf{1} + \mathbf{2} + \mathbf{3} \qquad (3)$$

$$(CH_3)_3COH + OH \longrightarrow CH_2(CH_3)_2COH + H_2O \qquad (11)$$

Then considering the reactions (3) and (11), it can be shown that

$$\frac{G(-Cu^{II}M)_0}{G(-Cu^{II}M)} = 1 + \frac{k_{11}}{k_3}\,\frac{[(CH_3)_3COH]}{[Cu^{II}M]} \qquad (22)$$

where $G(-Cu^{II}M)_0$ is the yield of chromophore loss in the absence of *t*-butyl alcohol and $G(-Cu^{II}M)$ is the yield in the presence of alcohol, $[(CH_3)_3COH]$ and $[Cu^{II}M]$ represents the concentration of *t*-butyl alcohol and complex respectively, k_3 and k_{11} are the reaction rate constants for the reactions (3) and (11) respectively. What transpires from equation (22) is that the

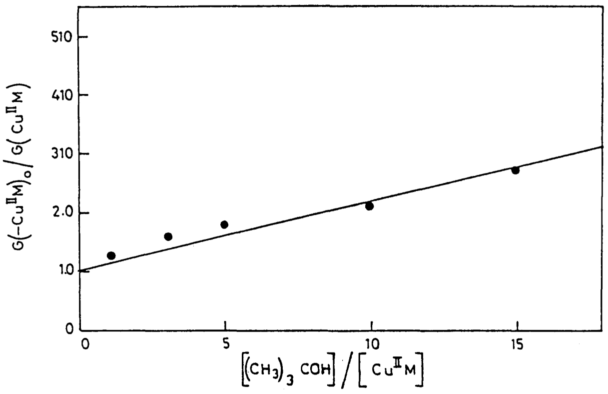

Fig. 4. Kinetic plot for the reaction of OH with $(CH_3)_3COH$ and $Cu^{II}M$ in N_2O saturated solution. $(Cu^{II}M)=5\times10^{-4}$ mol dm^{-3}).

plot of $G(-Cu^{II}M)_0/G(-Cu^{II}M)$ against $[(CH_3)_3COH]/[Cu^{II}M]$ should yield a straight line. The observed results have been shown (Fig. 4) to yield a straight line as expected from which the k_{11}/k_3 value is found to be 0.1208. Taking the value[24] of $k_{11}=2.5\times10^6$ dm^3 mol^{-1} s^{-1}, the rate constant for the reaction of OH with the Cu^{II}-Metronidazole, k_3, was evaluated to be $\approx2.1\times10^9$ dm^3 mol^{-1} s^{-1}. This rate constant value for the reaction of OH with the complex is, however, of the same order as that observed[2] in the case of free metronidazole.

Conclusions

From these experimental results it was observed that like free metronidazole, the Cu(II) complex of the compound also reacts with OH very rapidly and oxidative denitration takes place followed by intramolecular electron transfer from the ligand radical to central metal ion. No denitration is observed when e_{aq}^- reacts with the complex compound. On the other hand, successive 4-electron reduction takes place due to the reactions of e_{aq}^-.

Further work on the isolation and analysis of the ligand degradation products will be the subject matter of future studies.

References

1) F. J. C. Roe, *J. Antimicrob. Chemother.*, **3**, 205 (1977).
2) D. W. Whillans and G. E. Adams, *Radiat. Res.*, **62**, 407 (1975).
3) P. Wardman, *Curr. Top. Radiat. Res. Q.*, **11**, 347 (1977).
4) J. Cadet and R. Teoule, *J. Radiat. Res.*, **18**, 93 (1977).
5) B. A. Moore, B. Palcic, and L. D. Skarsgard, *Radiat. Res.*, **67**, 459 (1976).
6) A. J. Varghese and G. F. Whitmore, *Cancer Res.*, **40**, 2615 (1980).
7) R. J. Knox, R. C. Knight, and D. I. Edwards, *Biochem. Pharmacol.*, **30**, 1925 (1981).
8) G. E. Kelly, W. D. Meikle, and D. E. Moore, *Photochem. Photobiol.*, **49**, 59 (1989).
9) K. A. Skov, *Radiat. Res.*, **112**, 217 (1987).
10) K. A. Skov, N. P. Farrell, and H. Adomat, *Radiat. Res.*, **112**, 273 (1987).
11) A. Garnier-Suillerot, "Anticancer Agents," ed by J. W. Lown, Elsvier (London), (preprint kindly supplied by the author).
12) M. Howe-Grant and S. J. Lippard, *Biochemistry*, **18**, 5762 (1979).
13) L. E. Marshall, D. R. Graham, K. A. Reich, and D. S. Sigman, *Biochemistry*, **20**, 244 (1981).
14) D. R. Graham and D. S. Sigman, *Inorg. Chem.*, **23**, 4188 (1984).
15) S. Goldstein and G. Czapski, *J. Am. Chem. Soc.*, **108**, 2244 (1986).
16) T. B. Thederahn, M. D. Kuwabara, T. A. Larson, and D. S. Sigman, *J. Am. Chem. Soc.*, **111**, 4941 (1989).
17) G. Czapski and S. Goldstein, "Radiation Research," Vol-II ed by E. M. Fielden, J. F. Fowler, J. H. Hendry, and D. Scot, Taylor & Francis, London (1987), Vol. II, p. 167.
18) B. J. Wilkins and D. E. Moore, *Photochem. Photobiol.*, **47**, 481 (1988).
19) A. I. Vogel, "A Text Book of Quantitative Inorganic Analysis," ELBS, London (1962), p. 784.
20) A. I. Vogel, "A Text Book of Quantitative Inorganic Analysis," ELBS, London (1962), p. 902.
21) G. M. Eisenberg, *Ind. Eng. Chem. Anal.*, **15**, 327 (1943).
22) I. G. Draganic and Z. D. Draganic, "The Radiation Chemistry of Water," Academic Press, New York (1971).
23) J. P. Keeni, *Radiat. Res.*, **22**, 1 (1964).
24) M. Anbar and P. Neta, *Int. J. Apply. Radiat. Isot.*, **18**, 493 (1967).
25) K. M. Bansal and R. M. Sellers, *J. Phys. Chem.*, **79**, 1775 (1975).
26) A. Samuni and P. Neta, *J. Phys. Chem.*, **77**, 1629 (1973).
27) H. Ide, O. Ohkubo, A. Sakamoto, S. Nishimoto, T. Wada, and T. Kagia, *J. Radiat. Res.*, **25**, 246 (1984).
28) H. A. Boucher, G. A. Lawrance, A. M. Sargeson, and D. E. Sangster, *Inorg. Chem.*, **22**, 3482 (1983).
29) C. L. Greenstock, I. Dunlop, and P. Neta, *J. Phys. Chem.*, **77**, 1187 (1973).
30) P. Neta and C. L. Greenstock, *J. Chem. Soc., Chem. Commun.*, **1973**, 309.
31) M. Z. Hoffman and M. Simic, *J. Am. Chem. Soc.*, **94**, 1757 (1972).
32) M. Simic, M. Z. Hoffman, and N. V. Brezniak, *J. Am. Chem. Soc.*, **99**, 2166 (1977).
33) F. A. Cotton and G. Wilkinson, "Advanced Inorganic Chemistry," Wiley Eastern Ltd, New Delhi (1972).
34) K. D. Whitburn, M. Z. Hoffman, N. V. Brezniak, and M. G. Simic, *Inorg. Chem.*, **25**, 3037 (1986).
35) T. Kagiya, H. Ide, S. Nishimoto, and T. Wada, *Int. J. Radiat. Biol.*, **44**, 505 (1983).