HYDROXYL RADICAL BASED DEGRADATION OF CIPROFLOXACIN IN AQUEOUS SOLUTION

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ABSTRACT

This study reports the degradation of ciprofloxacin (CIP) by means of ionizing radiations. Kinetic studies with aqueous concentrations of 4.6, 10, 15, 17.9 mg/L reveals that degradation of CIP follows pseudo-first order kinetics and the decay constant increased with decrease in initial concentration of CIP. The removal efficiency, represented by G-value, decreased with increasing absorbed dose and increased with higher [CIP]o concentration at a given absorbed dose. The effects of bubbling CIP solution with N2, NO or air on CIP degradation were also studied. The influence of various radical scavengers like tert-butanol, isopropanol, HCOO−, CO32−, NO−3 and NO2− as radical scavengers in N2-saturated solutions of CIP indicated that OH were more closely associated with the radiolytic decomposition of CIP than other radicals, such as eaq− or H. The pH value of aqueous media played a crucial role in the degradation of CIP. It was observed that degradation efficiency was higher under acidic condition compared to degradation in natural or alkaline media. F−, CH3COO− and HCOO− were formed as a result of CIP degradation that were analyzed by ion-chromatography.

Keywords: Hydroxyl radical, ciprofloxacin, degradation, gamma-irradiation, kinetics

1. INTRODUCTION

The presence of antibiotics in the aquatic bodies provides a new challenge to drinking water, wastewater, and water reuse system [1-4]. Ciprofloxacin, a member from fluoroquinolone group is widely used as humans and veterinary medicine [5]. The presence of these broad spectrum antibiotics in aquatic environments, may pose serious threats to the ecosystem and human health by causing proliferation of bacterial drug resistance [6, 7]. Most fluoroquinolones are not fully metabolized in the body and thus are excreted and introduced to the environment through wastewater effluents [6, 8]. Advanced oxidation processes (AOPs), with highly reactive hydroxyl radical (•OH) as the main oxidative species, provide an alternate technique to transform and decontaminate these soluble human antibiotics [1, 9]. Radiation-induced decontamination of CIP and other toxic chemicals in water is a new and more promising technique [10]. This process has been found to be more economical and efficient than other wastewater treatment processes [11]. The underlying principles behind gamma ray or electron beam treatment processes involve radiation chemistry of water, thoroughly studied and accepted technique. [12]. Irradiation of water using gamma ray can be explained by following equation, where the number in parenthesis represents the radiation yield (G-value) of each species per 100 eV of absorbed energy [12].

\[ \text{H}_2\text{O} \rightarrow \cdot \text{OH}^+ + \text{H}_2 \]  

(1)

The most reactive species in Equation (1) are the oxidizing hydroxyl radicals (•OH) and the reducing aqueous electrons (•eaq−) and hydrogen atoms (H). High concentrations of these species can be produced in a very short interval of time and can react with target contaminant. Table 1 shows an estimate of the concentration of these reactive species at different absorbed doses applied in the present study.

Table 1: Concentration of different species at various doses of gamma radiation.

<table>
<thead>
<tr>
<th>Dose (Gy)</th>
<th>OH (mM)</th>
<th>H (mM)</th>
<th>eaq− (mM)</th>
<th>H2O2 (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>145</td>
<td>0.0406</td>
<td>0.0087</td>
<td>0.03915</td>
<td>0.01015</td>
</tr>
<tr>
<td>290</td>
<td>0.0812</td>
<td>0.0174</td>
<td>0.0783</td>
<td>0.0203</td>
</tr>
<tr>
<td>435</td>
<td>0.1218</td>
<td>0.0261</td>
<td>0.11745</td>
<td>0.03045</td>
</tr>
<tr>
<td>580</td>
<td>0.1624</td>
<td>0.0348</td>
<td>0.1566</td>
<td>0.0406</td>
</tr>
<tr>
<td>870</td>
<td>0.2436</td>
<td>0.0522</td>
<td>0.2349</td>
<td>0.0609</td>
</tr>
</tbody>
</table>

The present study focused on the use of gamma radiations as an AOP to degrade CIP and to determine the role of •OH in the degradation of CIP by means of radical scavengers experiments. The main objectives of the present study were: (1) to study the decomposition of ciprofloxacin (CIP) in water using gamma radiations; (2) to investigate the decomposition kinetics of CIP; and (3) to evaluate the efficiency of •OH in the degradation of CIP removal using radical promoters and scavengers, often present in water.

2. EXPERIMENTAL

2.1 Chemicals and reagents

All of the chemical used (tert-butanol, isopropanol, sodium carbonate, sodium bicarbonate, sodium nitrate, sodium nitrite, perchloric acid, sodium hydroxide) were of high-purity analytical grade reagents supplied by Sigma-Aldrich. The ultrapure water was obtained from a Milli-Q® system (Millipore). Nitrogen and nitrous oxide gases used in the present study were of at least 99.99% purity. Ciprofloxacin (100%) used in this study was supplied by Sigma-Aldrich. The chemical structure of this compound is shown in Fig.1.

Fig. 1: Chemical Structure of Ciprofloxacin.

1.2 Experimental procedures

1.2.1 Sample preparation

CIP solution at initial concentration of 4.6 mg/L was prepared by dissolving weighted amount of solid CIP in ultrapure water. The different additives (isopropanol, tert-butanol, CO32−, HCOO−, NO−3, and NO2−) of different concentrations were added into CIP aqueous solution to examine their effects on CIP degradation. HClO4 (0.05 mol/L) or NaOH (0.05 mol/L) were added into 4.6 mg/L CIP solution to make solution of desired pH and to test the effect of pH value on degradation efficiency. CIP solutions at different concentrations were also prepared to test the effect of initial concentration of CIP on degradation at the given radiation dose.

The present study focused on the use of gamma radiations as an AOP to
2.2.2 Irradiation procedures and bubbling of CIP solution by N$_2$ or N$_2$O gases

The irradiation source used for irradiation studies was a Co-60 gamma-ray source (Issledovatol, former USSR) available at NIFA, Tarnab, Peshawar, Pakistan. The source was calibrated by using aqueous ferrous sulfate (Fricke dosimetry) solution [12]. The dose rate was found to be 296 Gy/hr.

N$_2$-saturated experiments were conducted in 250 mL glass vessel, where N2-gas was passed through CIP solution for at least 25 minutes. For N2O saturation of CIP solution, same procedure was applied, replacing N2 by N2O gas. The purity of N$_2$ and N$_2$O gases used in this study was 99.999%.

2.3 Analytical methods

Analysis of CIP was performed using high performance liquid chromatography (Agilent 1200 series HPLC, Agilent Technologies, USA), equipped with UV detector. The separation was achieved using reversed phase C-18 column, with water/methanol/acetonitrile/1% acetic acid (15/15/20/50, V/V) as mobile phase in isocratic mode at flow rate of 1ml min$^{-1}$. The evaluation of UV-Vis absorption spectra of CIP in aqueous solution before and after irradiation was recorded by Lambda-800 UV-Visible Spectrophotometer (Perkin Elmer Instruments, USA). For the analysis of by-products, like F$^-$, CH$_3$COO$^-$ and HCOO$^-$, Metrohm Ion-Chromatograph (IC) was used. Triplicate samples were prepared and analyzed for each sample.

2.4 Calculation of G-Value and dose constants

The G-value is defined as the number of molecules of products formed or of reactant destroyed, by absorbing 100 eV of energy, it can be calculated using the following equation [13].

$$G = \frac{R \times (6.02 \times 10^{23})}{D \times (6.24 \times 10^{21})}$$  \hspace{1cm} (2)

Where R is the change in the concentration of reactant, CIP (M), D is the absorbed dose (Gy), 6.02 x 10$^{23}$ is the Avogadro’s number and 6.24 x 10$^{21}$ is the conversion factor from Gy to 100 eV/L.

The dose constant, k, was calculated from the slope of the natural logarithm (ln) of the organic pollutant concentration in M versus dose (Gy). Dose constants were used to calculate dose required for 50% and 90% CIP degradation (D$_{50}$ and D$_{90}$ values) by using Eqs. (3) and (4):

$$D_{50} = \frac{\ln 2}{k} \hspace{1cm} (3)$$

$$D_{90} = \frac{\ln 10}{k} \hspace{1cm} (4)$$

D$_{50}$ and D$_{90}$ were used for the analysis of solute removal [14].

3. RESULTS AND DISCUSSION

3.1 Degradation of CIP by ionizing radiations

During irradiation, the reactions between reactive radicals (OH, $e^-_a$, H) and any additive occur in aqueous solution as follows: In aerated media, the reducing species 'H and $e^-_a$ are converted into HO$^-$ and O$_2^-$ because of their fast reactions given by Eqs. (5) and (6); under acidic conditions, the H$^+$ ion reacts with $e^-_a$ to produce H', as by Eq. (7); HO$^-$ and its conjugate base O$_2^-$ exist in a pH-dependent equilibrium as given by Eq. (8);

$$H + O_2 \rightarrow HO_2^- \hspace{1cm} k = 2.1 \times 10^{-9} \text{ L/mol.s} \hspace{1cm} (5)$$

$$e^- + O_2 \rightarrow O_2^- \hspace{1cm} k = 1.9 \times 10^{-10} \text{ L/mol.s} \hspace{1cm} (6)$$

$$e^- + H^+ \rightarrow H \hspace{1cm} k = 2.3 \times 10^{10} \text{ mol/L/s} \hspace{1cm} (7)$$

$$HO_2^- + H^+ \leftarrow \text{pK}_a = 4.88 \hspace{1cm} (8)$$

Eqs. (9)-(11) may also occur depending on experimental conditions.

$$HO_2^- + O_2 \rightarrow H_2O_2 + O_2 \hspace{1cm} (9)$$

$$HO_2^- + HO_2 \rightarrow H_2O_2 + O_2 \hspace{1cm} (10)$$

$$H + OH \rightarrow e^- + H_2O \hspace{1cm} (11)$$

Aqueous solution of 4.6 mg/L CIP was irradiated with radiation doses of 0, 145, 290, 435, 870 Gy. Fig. 2 shows the UV-absorbance spectra of CIP solution before and after irradiation at 200-400nm (Fig. 2a) and decrease in concentration of CIP as a result of after gamma ray irradiation (Fig. 2b). The results showed that the UV-absorbance and thus the conc. of CIP in the 200 - 400 nm region decreased with increase of radiation dose. At radiation dose of 870 Gy, 4.6 mg/L of CIP was almost completely degraded as shown in Fig. 2b.

According to Eq. (2), the G-values of CIP (4.6 mg/L) at different radiation doses were calculated as shown in Table 2. The G-values decreased with increasing radiation dose, a trend that has been reported in several other studies [15-17]. Firstly, this trend can be explained by competition for solute molecules (CIP) between the reactive species produced during radiolysis of water. With increasing radiation dose, greater competition occurs between the reactive radicals, resulting in decrease of G-values. Secondly, there is possibility of competition for reactive radicals between the parent compound (CIP) and the reaction by-products [17]. The third possibility is the radical-radical recombination reactions, including ‘OH, $e^-_a$, H also increases with increasing radiation dose [15] as shown by reactions such as given in Eqs. (12-15), so the radical concentration for interaction with CIP reduced.

<table>
<thead>
<tr>
<th>G value (molecules/100 ev)</th>
<th>0.5635</th>
<th>0.3582</th>
<th>0.2671</th>
<th>0.1402</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absorbed dose (Gy)</td>
<td>145</td>
<td>290</td>
<td>435</td>
<td>870</td>
</tr>
</tbody>
</table>

The efficiency of irradiation process for the decomposition of CIP was compared between the percentage removal (%) and G-values as shown in Fig. 3.
3.2 Effect of dissolved oxygen, \(N_2\)-saturated and \(N_2\text{O}\)-saturated gases on CIP degradation.

Fig. 4 shows the effect of dissolved oxygen, \(N_2\)-saturated and \(N_2\text{O}\)-saturated gases on CIP degradation by gamma irradiation. In this figure the fractions of CIP at a given radiation dose (\(C/C_0\)) have been plotted against radiation dose (where \(C\) is the conc. of CIP after irradiation and \(C_0\) is the initial conc. of CIP). It is clear from Fig. 4 that CIP was degraded in all the three conditions and degradation efficiency increased with the increase in radiation dose. However, the degradation efficiency in the solution saturated with nitrous oxide was relatively higher than that in the solution saturated with air, and the latter was relatively lower than that in the absence of them at the same radiation dose. The results showed that at the same absorbed dose the degradation efficiency was lower in the presence of \(N_2\text{O}\) and \(N_2\text{O}\text{h}\) than that in the absence of them. The reason was that in the presence of \(N_2\text{O}\) the \(\cdot\text{OH}\) radical still can react with CIP. Whereas in the aqueous solution saturated with nitrous oxide, the \(\cdot\text{OH}\) radical can react with CIP. This shows that \(\cdot\text{OH}\) radicals are more effective in degradation of CIP.

Under this condition the concentration of H is only 1/10 of that of \(\cdot\text{OH}\) and \(\cdot\text{OH}\) is the main radical that reacts with CIP.

In aerated solution, oxygen reacts with \(e^-\text{aq}\) and \(\cdot\text{H}\) by fast reactions (Eqs. (5, 6)), forming \(\cdot\text{O}_2\text{H}\) and \(\cdot\text{HO}_2\). However, \(\cdot\text{OH}\) radical still can react with CIP. These radicals can then react with CIP to increase the degradation efficiency of CIP. Therefore, it appears that among the three radicals, the \(\cdot\text{OH}\) radicals are more effective in degradation of CIP.

3.3 CIP degradation in the presence of \(\text{OH}\) scavengers (\(2\text{-PrOH, tert-BUOH, CO}_2\text{O}, \text{NO}_2\), \(\text{HCO}_3\), and \(\text{NO}_3\))

Iso-propional (\(2\text{-PrOH}\)) can scavenge \(\text{OH}\) and \(\cdot\text{H}\) by the reactions shown in Eqs. (17) and (18) [18], whereas tert-BUOH can scavenge \(\cdot\text{OH}\) by the reactions shown in Eq. (20) [18]:

\[
\begin{align*}
\cdot\text{OH} + 2\text{-PrOH} & \rightarrow (\text{CH}_3)_2\text{COH} + \text{H}_2\text{O} & (17) \\
\text{H}^+ + 2\text{-PrOH} & \rightarrow (\text{CH}_3)_2\text{COH} + \text{H}_2 & (18) \\
\cdot\text{OH} + \text{t-BUOH} & \rightarrow \text{CH}_3\text{C} (\text{CH}_3)\text{CH} \cdot\text{OH} + \text{H}_2\text{O} & (19)
\end{align*}
\]

Since both \((\text{CH}_3)_2\text{COH}\) and \(\text{CH}_3\text{C} (\text{CH}_3)\text{CH}\) are relatively slow reacting species, \(H^+\) and \(e^-\) play the predominant role in the presence of tert-butanol while \(e^-\) plays the crucial role in the presence of iso-propanol. Table 4 shows the degradation efficiency at different doses for these three conditions i.e., without scavenger, with the presence of 80 mM iso-propanol, or 80 mM of tert-butanol. This shows that \(\cdot\text{OH}\) played the most important role in the degradation process while \(\cdot\text{H}\) and hydrated electron only played minor role.

Table 3: Effect of Irradiation on dose constant, \(D_{ka}\) and \(D_{ks}\) at different concentrations of CIP solution.

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>Dose constant (k)</th>
<th>(D_{ka}) (Gy)</th>
<th>(D_{ks}) (Gy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.6</td>
<td>4.9 x 10^-1</td>
<td>138.62</td>
<td>460.51</td>
</tr>
<tr>
<td>10</td>
<td>2.9 x 10^-1</td>
<td>231.04</td>
<td>767.52</td>
</tr>
<tr>
<td>15</td>
<td>1.9 x 10^-1</td>
<td>346.57</td>
<td>1151.29</td>
</tr>
<tr>
<td>17.9</td>
<td>1.6 x 10^-4</td>
<td>433.21</td>
<td>1439.11</td>
</tr>
</tbody>
</table>

Fig. 4: Degradation of aqueous CIP solution in air, \(N_2\), or \(N_2\text{O}\) saturated solution by gamma irradiation.

In aqueous solution of CIP saturated with nitrogen, all of \(e^-\text{aq}\), \(\cdot\text{H}\) and \(\cdot\text{OH}\) may react with CIP. Whereas in the aqueous solution saturated with nitrous oxide, the conc. of \(\cdot\text{OH}\) is increased due to conversion of \(e^-\text{aq}\) to \(\cdot\text{OH}\) as shown in Eq. (16).

\[e^- + \text{N}_2\text{O} + \text{H}_2\text{O} \rightarrow \text{OH} + \text{N}_2 + \text{OH}^- \quad k = 9.1 \times 10^9 \text{L/mol.s} \]  

(16)

\[\text{NO}_3^- + e^- = \text{NO}_2^- \quad k = 4.1 \times 10^8 \text{L/mol.s} \]  

(24)

\[\text{CO}_2^- + e^- = \text{products} \quad k = 3.9 \times 10^9 \text{L/mol.s} \]  

(25)

3.4 Kinetic study for the radiolytic degradation of CIP

As shown in Fig. 2b the concentration of CIP decreases exponentially with the absorbed doses, which can be represented by Eq.26 [20, 21].

\[C = C_0 e^{-kD} \]  

(26)

Where \(C_0\) and \(C\) represents initial and final concentrations of CIP before and after irradiation, respectively. \(k\) is the dose constant and \(D\) the absorbed dose. This Eq. (26) can be re-arranged to pseudo-first-order reaction as:

\[-\ln (C/C_0) = kD \]  

(27)

Eq. (27) was used to calculate the dose constant from a linear least-squares fit of the experimental data.

Batch kinetic experiments were performed to study the dependency of Initial concentrations of 4.6, 10, 15 and 17.9 mg L^-1, and absorbed doses ranging from 0 to 870 Gy were used for these experiments. The aqueous CIP solutions were prepared at room temperature (ca. 25°C) and initial pH of solution was 6.2.
Table 4: % degradation of CIP in the presence of radical scavengers (2-PrOH, tert-BuOH, CO$_3^{2-}$, NO$_2^-$, HCO$_3^-$ and NO$_3^-$)

<table>
<thead>
<tr>
<th>Absorbed dose (Gy)</th>
<th>Blank</th>
<th>% degradation in the presence of tert-BuOH</th>
<th>% degradation in the presence of iso-PrOH</th>
<th>% degradation in the presence of CO$_3^{2-}$</th>
<th>% degradation in the presence of NO$_2^-$</th>
<th>% degradation in the presence of HCO$_3^-$</th>
<th>% degradation in the presence of NO$_3^-$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>145</td>
<td>65.41</td>
<td>11.44</td>
<td>3.89</td>
<td>25.14</td>
<td>4.55</td>
<td>31.43</td>
<td>42.95</td>
</tr>
<tr>
<td>290</td>
<td>86</td>
<td>22.57</td>
<td>11.8</td>
<td>50.89</td>
<td>9.85</td>
<td>52.56</td>
<td>60.47</td>
</tr>
<tr>
<td>435</td>
<td>93.9</td>
<td>32.6</td>
<td>23.32</td>
<td>73.79</td>
<td>12.09</td>
<td>67.02</td>
<td>69.75</td>
</tr>
<tr>
<td>870</td>
<td>96.3</td>
<td>-----</td>
<td>-----</td>
<td>90.11</td>
<td>14.04</td>
<td>80.87</td>
<td>81.95</td>
</tr>
</tbody>
</table>

Fig. 5: Effect of 2-PrOH and tert-BuOH additives on CIP degradation.

Fig. 6: Kinetic results for the radiolytic degradation of CIP at different initial concentrations. (A) Removal of CIP at different initial conc. vs. irradiation doses and (B) the G-value at different initial CIP concentrations.

The G-values calculated for four different initial CIP concentrations of CIP versus absorbed dose are presented in Fig. 6B. As shown in Fig. 3, the G-values decreased with increasing radiation dose. However, for a given radiation dose, the G-values increased with the higher initial CIP concentration, which is similar to previously reported results with chloroform and MTBE [17]. This can be explained by the fact that at higher CIP concentrations, the reactive radicals have a greater chance of reacting with CIP molecules, leading to higher removal efficiency, represented by higher G-values.

3.5 Effect of solution pH

Fig. 7 shows the influence of pH of aqueous medium on CIP degradation. The degradation was enhanced in acidic (pH 3.56) conditions. The reason is that, under acidic condition, hydrated electron is converted to $\cdot$H and the relative concentration of $\cdot$H was much higher (Eq. (7)). On the other hand in alkaline media, $\cdot$H reacts with OH to generate $e_{aq}^-$ (Eq. (12)), so increasing the concentration of $e_{aq}^-$ and thus enhancing the probability of recombination between $e_{aq}^-$ and $\cdot$OH (Eq. (16)). This results in decrease of $\cdot$OH concentration, and thus the degradation efficiency of CIP decreased at higher pH values [23].

4. By-Product Analysis

The gamma irradiation can degrade CIP effectively producing, fluoride (F$^-$), acetate (CH$_3$COO$^-$) and formate (HCOO$^-$) ions. Concentration of F$^-$, CH$_3$COO$^-$, HCOO$^-$ were determined using Metrohm 800 series ion chromatograph, equipped with electrical conductivity detector. The column used was Assup-5 (250/4.0mm), with 3.2 mM Na$_2$CO$_3$/1 mM NaHCO$_3$/50 mM H$_2$SO$_4$/water at flow rate of 1mL/min. As shown in Fig. 8, with the increase in the absorb dose the CIP concentration decreased while the concentration of F$^-$, CH$_3$COO$^-$ and HCOO$^-$ ions increased. Acetate ion was the major product of CIP degradation while the concentration of formate ion and fluoride was relatively small. After dose of 600Gy the concentration of acetate ions are also starting to decrease, probably because of degradation of acetate ions as a result of irradiation.
5. CONCLUSIONS

The results of this study showed that CIP was degraded more in N2-saturated solution as compared to N2-saturated and aerated solutions. The radiolytic decomposition of CIP. The results of kinetic study showed that radiolytic oxidation of CIP followed pseudo first-order reaction. With respect to G-value, two patterns were observed in our study. The removal efficiencies as represented by G-values decreased with higher irradiation doses. At a given dose, the G-values increased with increasing aqueous CIP concentration. The pH value also affect the CIP degradation efficiency, the degradation yield was higher in acidic conditions as compared to neutral and alkaline media. In conclusion the application of radiation technology shows promising results in the treatment of industrial and municipal effluents containing various antibiotic compounds.

REFERENCES