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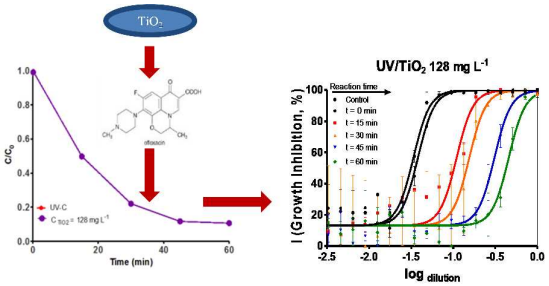
Graphical Abstract

Photocatalytic processes (UV/TiO_2 and $UV/TiO_2/ H_2O_2$) were used for ofloxacin degradation. The antimicrobial activity of ofloxacin solutions submitted to degradation processes was evaluated.



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ARTICLE

Photocatalytic Degradation of Ofloxacin and Evaluation of Residual Antimicrobial Activity

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Ofloxacin is an antimicrobial agent frequently found in significant concentrations in wastewater and surface water. Its continuous introduction into the environment is a potential risk to non-target organisms or to human health. In this study, ofloxacin degradation by UV/TiO_2 and UV/TiO_2/H_2O_2, antimicrobial activity (*E. coli*) of samples submitted to these processes, and by-products formed by the processes were evaluated. For UV/TiO_2, degradation efficiency was 89.3% in 60 min of reaction when 128 mg L^{-1} TiO_2 were used. The addition of 1.68 mmol L^{-1} hydrogen peroxide increased degradation to 97.8%. For UV/TiO_2, increasing catalyst concentration from 4 to 128 mg L^{-1} led to an increase in degradation efficiency. For both processes, antimicrobial activity was considerably reduced throughout the reaction time. The structures of two by-products were presented: *m/z* 291 (9-fluoro-3-methyl-10-(methyleneamino)-7-oxo-2,3-dihydro-7H-[1,4]oxazino[2,3,4-ij]quinoline-6-carboxylic acid); and *m/z* 157 ((Z)-2-formyl-3-((2-oxoethyl)imino)propanoic acid).

1. Introduction

The concentration of pharmaceuticals in the aquatic environment is low, ranging from ng L^{-1} to µg L^{-1}. However, in the long term continuous introduction of pharmaceuticals into the water may be a risk to aquatic and terrestrial organisms, so significant efforts must be made to develop effective means of minimizing exposure of environmental microbiota to antimicrobial compounds.

Ofloxacin (OFX) is an antimicrobial agent of the fluoroquinolone family. Fluoroquinolones are not completely metabolized by the human body. From 20 to 80% of fluoroquinolones that are ingested is excreted in pharmacologically active form.[1] For this reason, fluoroquinolones affect domestic sewage. Residual concentrations of fluoroquinolones and of other antimicrobials present in wastewater can be harmful to organisms in water that receives them.[2] Bacteria exposed to antimicrobial residues present in environmental matrices may undergo alterations in their genes, resulting in microorganisms that are resistant to the compound. More powerful drugs must be used in the treatment of diseases because of drug-resistant bacteria. So, it is very important to use treatment technologies that are capable of removing residual antimicrobial activity of these compounds.

Significant concentrations of ofloxacin have been detected in surface water (10-535 ng L^{-1}), municipal wastewater treatment plant effluent (53-1800 ng L^{-1}), and hospital wastewater (25,000-35,000 ng L^{-1}).[3-4] Studies show that advanced oxidation processes (AOPs) are effective at degrading ofloxacin in aqueous solutions. Michael *et al.*,[5] Hapeshi *et al.*,[4] Prieto-Rodriguez *et al.*,[6] and Vasquez *et al.*[7] used the photocatalytic process with UV/TiO_2. Despite the importance of minimizing bacterial resistance, most studies of antimicrobial degradation by AOPs evaluate only the removal of the parent compound. Biological effects that can be induced by partial oxidation and/or degradation by-products are rarely discussed.

Among the AOPs available, photocatalysis using titanium dioxide (TiO_2) as a catalyst is able to achieve complete oxidation of organic pollutants by generating hydroxyl radicals when the catalyst is exposed to ultraviolet radiation.[8] It should be noted that the adsorption of contaminants by TiO_2 is key to this AOP, since the compound needs to be adsorbed on the surface of the catalyst for the oxidation reaction to take place. The efficiency of the adsorption of the compound on the semiconductor varies. This depends on the pollutant, adsorbent concentration, pH, and matrix complexity.

The objectives of the present study were to assess the degradation of ofloxacin in aqueous solutions by photocatalysis with TiO_2 in suspension, to investigate the influence of adding hydrogen peroxide to the process, to evaluate the residual antimicrobial activity of solutions submitted to photocatalytic processes, and to identify by-products formed during the processes.

2. Materials and methods

2.1. Chemicals

The following chemicals were used in the assays: ofloxacin (99.8% w/w, $C_{18}H_{20}FN_3O_4$, 361.368 g mol^{-1}) and oxalic acid (99.5% w/w) from Sigma–Aldrich (St. Louis, USA); hydrogen peroxide (30% w/w), H_2SO_4 (97% v/v), and NaOH (97% w/w) from Synth (Diadema, Brazil); methanol (HPLC grade) and $BaCl_2.2H_2O$ (99% w/w) from J.T. Baker (Edo, Mexico); H_3PO_4 (85% v/v) and KH_2PO_4 (98% w/w) from Nuclear (São Paulo, Brazil); KOH (85% w/w) from Ecibra (São Paulo, Brazil); and NH_4VO_3 (99% w/w) from Honeywell Riedel-de Haën (Seelze, Germany). TiO_2 was obtained from Degussa (Frankfurt, Germany). Mueller-Hinton broth cultures and Mueller–Hinton Agar were purchased from Himidia (Mumbai, India). The ultrapure water used in the study was produced by a Milli-Q Academic water purification system (Millipore).

2.2 Stock solution

Ofloxacin stock solution (500 mg L^{-1}) was prepared in methanol and stored at 4 °C, protected from light. The ofloxacin working solutions (500 µg L^{-1}, pH 6) were prepared by diluting stock solution in 1 L of ultrapure water.

2.3. Experimental setup

The degradation assays were carried out in a 38.5 cm-long, 3.5 cm-inner-diameter borosilicate glass reactor (190 mL). A low pressure mercury lamp (15 W, λ_{max} = 254 nm and 2.4 cm internal diameter) was inserted in the centre of the tube. The lamp was in direct contact with the solution. The system was operated in batches with recirculation with a flow rate of 100 mL min^{-1}. The experimental system was made up of a magnetic stirrer, a 1000 mL reservoir, a peristaltic pump to re-circulate the solution, and a photochemical reactor. A similar experimental system was utilized by da Silva et al.[9] and Guadagnini et al.[10] Irradiance was 6.95 mW cm^{-2}, measured by a Cole Parmer (VLX 3W model) radiometer calibrated at 254 nm.

The reaction time varied from 0 to 60 min. The TiO_2 catalyst (Degussa P-25) was kept in suspension by stirring; the concentration varied from 4 to 128 mg L^{-1}. After the degradation assays, TiO_2 was removed from the solution by filtration with a GF 51-B glass membrane. In the UV/TiO_2/H_2O_2 experiments, 1.68 mmol L^{-1} H_2O_2 was added. Consumption of hydrogen peroxide was measured using the method described by Nogueira *et al.*[11]

Ofloxacin adsorption on the catalyst was evaluated by adding TiO_2 at concentrations ranging from 4 to 128 mg L^{-1} to 1 L of ofloxacin working solution at pH 3, 6, and 10. Contact time varied from 0 to 90 min. Equilibrium was reached after 30 minutes of testing, so the photocatalysis assays were carried out after ofloxacin solutions had been placed in contact with the catalyst for 30 minutes. TiO_2 was removed by filtration with a GF 51-B glass membrane. The aqueous solution with the remaining ofloxacin was then concentrated by solid phase extraction (SPE), and the extract was quantified by high performance liquid chromatography (HPLC) analysis.

2.4. Analytical methods

2.4.1. High performance liquid chromatography

Degradation of ofloxacin was assessed by HPLC with UV detection (298 nm). Before quantification, samples were concentrated using SPE with Waters OASIS HLB cartridges (500 mg/6 mL) conditioned with methanol (6.0 mL) and water (6.0 mL). One litre of sample was percolated through the cartridges at a flow rate of 10 mL min^{-1}. Analytes were eluted with methanol (4.0 mL). The extract was filtered (0.22 µm membrane filters) before HPLC analyses. Recovery ranged from 96.3 to 107% for samples containing 10–500 µg L^{-1} ofloxacin.

The HPLC consisted of a Waters Model 510 delivery system, a Rheodyne Model 7725 injector with a 20µL sample loop, and a tunable Waters Model 486 absorbance detector. Data was acquired by a Waters 746 Data Module integrator. Quantification was performed at 298 nm. The analytical column was a Waters X-Bridge® RP18 (250 mm x 4.6 mm I.D., 5 µm). Flow rate was 1.0 mL min^{-1}. The mobile phase consisted of methanol:0.01 mol L^{-1} oxalic acid (35:65 v/v). LOD was 0.04 µg L^{-1} and LOQ was 0.2 µg L^{-1}, considering a concentration factor of 250 times.

2.4.2. Mass spectrometry

To determine the by-products generated by the application of advanced oxidation processes, a Micro Four model (Waters, USA) triple quadrupole mass spectrometer was used with an electrospray ionization source (ESI) operated in positive mode. Ofloxacin solutions of 5 mg L^{-1} were used in order to avoid analyte loss during the SPE process. OFX solution aliquots (1 mL) were collected at 5, 10, 15, 30, and 60 min. Spectra were acquired between m/z 100 and 600. The typical operating conditions were as follows: Cone energy (25 V), capillary energy (3000 V), resolution (0.75 Da), cone gas flow (10 L h^{-1}), desolvation gas flow (1000 L h^{-1}), extraction cone energy (3 V), desolvation temperature (500 °C), infusion flow (20 µL min^{-1}). Data was acquired using MassLynx ® software.

2.5. Antimicrobial activity assay

Antimicrobial activity was evaluated quantitatively. It used an *E. coli* K12 culture with a population density of 1.0×10^6 CFU mL^{-1}. The assays followed the procedures in Rodrigues-Silva et al.,[12] with some modifications, using a 96-well microtiter plate. An aliquot of 350 µL of standard and degraded samples at pH 6 was added to the first well of each row. Then an 18-member serial dilution (1.4-fold) was performed by transferring 250 µL from the first well to the second well. This procedure was repeated until the penultimate well of each row. The last well (i.e., the 18th well) contained only the phosphate buffer solution. The antimicrobial activities of the ofloxacin standard, the initial samples (t = 0 min), and degraded samples (t = 15, 30, 45, 60 min) were quantified in duplicate. After the serial dilution, all of the wells of the 96-wells plate were inoculated with 100 µL of *E. coli* culture solution. Subsequently, the plate was covered, sealed and incubated on a shaker (Marconi model MA-420) for 8 h at 37 °C. After this period, the absorbance of each sample well was measured at 630 nm using a Multiskan MS microplate reader (Labsystems).Values for growth inhibition (%), I, were obtained using Equation 1.

$$I(\%) = \frac{A_0 - A}{A_0} x100\% \qquad \text{Equation 1}$$

In which A_0 is the absorbance of the *E. coli* culture incubated with phosphate buffer, equivalent to non-inhibited growth, and A is the absorbance recorded in each well at 630 nm.

The dose–response curves and the EC_{50} values of the degraded and untreated solutions were evaluated using Graph-Pad Prism 5.0 software (La Jolla, USA), as in Equation 2.

$$I(\%) = I_{\min,t} + \frac{I_{\max,t} - I_{\min,t}}{(1+10^{((\log EC - \log(1/m^n)\times H)})} \qquad \text{Equation 2}$$

In which logEC is equal to Log $EC_{50Control}$ plus Log $EC_{50Ratio}$; EC_{50} is the effective dose at which 50% growth inhibition is achieved; $I_{max,t}$ represents maximum growth inhibition; $I_{min,t}$ represents minimum growth inhibition for each sample; and H is the Hill slope.

The residual activity of the samples submitted to the degradation processes was compared to the antimicrobial activity of the untreated samples (t = 0 min). The effective dose capable of causing 50% inhibition of bacterial growth (EC_{50}) was determined by exposing the *E. coli* culture to ofloxacin concentrations varying from 250 µg L^{-1} to 152.4 ng L^{-1}.

Changes in the EC_{50} values of the samples during the degradation processes were evaluated by calculating the potency equivalent value (PEQ), as in Equation 3.

$$PEQ = EC_{50,0} / EC_{50,X} \qquad \text{Equation 3}$$

In which PEQ is the potency equivalent value, $EC_{50,0}$ is the effective dose at which 50% growth inhibition was observed in the untreated ofloxacin solution, and $EC_{50,X}$ is the EC_{50} value calculated for each sample submitted to the degradation process.

3. Results and Discussion

3.1. Adsorption of ofloxacin on TiO_2 surface

The adsorption of ofloxacin on the catalyst did not exceed 30% for any of the conditions evaluated. These results are consistent with those of Hapeshi et al.[4] who verified that 30 min was sufficient to ensure maximum adsorption of ofloxacin on the catalyst surface. This same period was used by Vasquez et al.[7] for photocatalysis assays.

3.2. pH effect

pH is an important parameter that can affect photocatalytic reactions. In this study the degradation of ofloxacin was evaluated at different pH values (3, 6, and 10), shown in Figure 1A. The efficiency of the photocatalytic process is related to the surface ionization state of the catalyst,[13] as shown in Equations 4 and 5.

$$TiOH + H^+ \leftrightarrow TiOH_2^+ \quad \text{Equation 4}$$

$$TiOH + OH^- \leftrightarrow TiO^- + H_2O \quad \text{Equation 5}$$

pH changes can influence ofloxacin molecule adsorption on the catalyst surface, which is decisive for the occurrence of photocatalytic oxidation reactions. The zero charge point of TiO_2 (Degussa P-25) is at pH 6. Thus, the catalyst surface is positively charged in acidic mediums (pH<6) and negatively charged in basic mediums (pH>6).

Ofloxacin is positively charged at pH values lower than the pKa_1 (6.05), negatively charged at pH values above the pKa_2 (8.11), and neutral at pH values between pKa_1 and pKa_2.[4] Therefore, pH 3 and 10 are not favorable for ofloxacin adsorption on the catalyst surface, since there is electronic repulsion between the charges of the compound and catalyst with the same sign. At pH 6, both the catalyst molecule and the drug are in their neutral forms, and no repulsion occurs between them.

When catalyst concentration was increased from 8 to 32 mg L^{-1}, the effect that pH has on drug degradation efficiency was more pronounced, that is, there was a more notable difference between the results obtained at pH 6 than for those at pH 3 and 10. Results from photocatalysis degradation at pH 3 and 10 were lower than those obtained for the same process at pH 6. This was probably due to the charge-repulsion phenomenon, which is not favorable to ofloxacin adsorption on TiO_2.

3.3. Ofloxacin degradation

Photocatalysis was effective for ofloxacin degradation (Figure 1B). For all concentrations tested, this process was more efficient than photolysis. An increase in degradation efficiency was observed when catalyst concentration was increased from 4 to 128 mg L^{-1}. This correlation between degradation rate and catalyst concentration is due to the large number of active TiO_2 sites available for the photocatalytic reaction.

Concentrations of TiO_2 higher than 128 mg L^{-1} were not used because they did not lead to significant gains in drug degradation efficiency. Very high catalyst concentrations cause turbidity in the solution, hinder the penetration of incident UV radiation, and impair the efficiency of the photocatalytic process.[14]

The maximum degradation of ofloxacin obtained by photolysis process was 51% after 60 min of testing. Using 4 mg L^{-1} TiO_2, drug degradation efficiency was slightly better than for photolysis, reaching 56.2% after 60 min. When 8 mg L^{-1} catalyst was used, degradation increased, reaching 57.4% after 60 min. The application of TiO_2 caused a significant OFX degradation gain for TiO_2 concentration varying from 16 to 64 mg L^{-1}. As can be seen in Figure 1B, after 60 min, the maximum degradation efficiency was 89.3%, when 128 mg L^{-1} catalyst was used.

TiO_2 photocatalysis was also used by Michael et al.[5] to degrade 10 mg L^{-1} ofloxacin (catalyst concentration of 3 g L^{-1}): 60% degradation was obtained after 120 min of irradiation. Vasquez et al.[7] achieved 100% degradation of a 10 mg L^{-1} OFX solution in 30 min using photocatalysis (1 g L^{-1} TiO_2). Hapeshi et al.[4] achieved 88% degradation of 20 mg L^{-1} ofloxacin solution in 240 min using UV/TiO_2 (250 mg L^{-1} TiO_2). In the study carried out by Prieto-Rodriguez et al.[6], a solution of 1.61 x10^{-3} mg L^{-1} OFX underwent a photocatalytic process (20 mg L^{-1} TiO_2) for 300 min, resulting in approximately 90% degradation. Ofloxacin degradation results obtained in present study were similar to those in the literature. This drug can be efficiently degraded by photocatalysis. However, the vast majority of existing studies use ofloxacin and TiO_2 concentrations much higher than those used in this study, that is, 500 µg L^{-1} ofloxacin and 128 mg L^{-1} TiO_2. This reduction in the levels of the drug and chemicals differentiates the present study from the others.

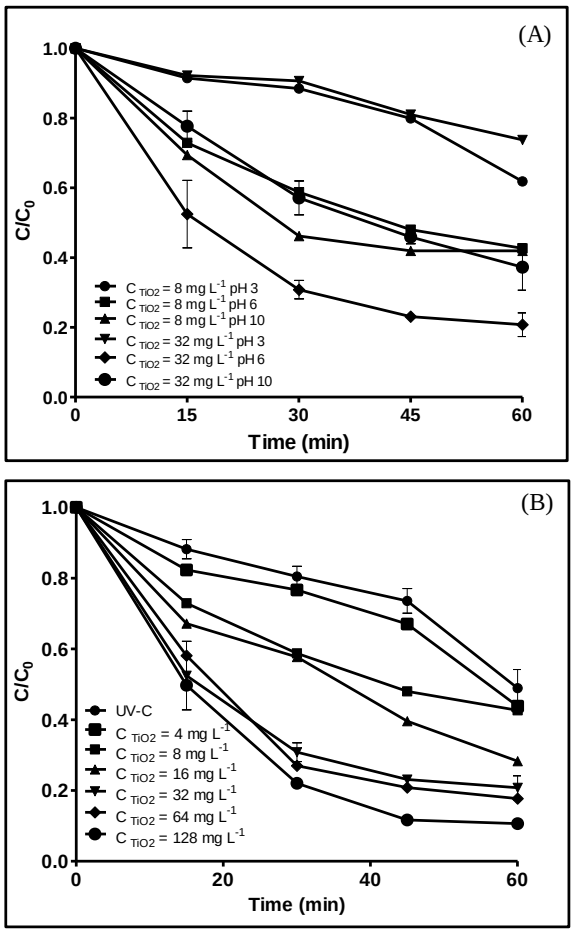

Figure 1. (A) Degradation of ofloxacin by UV/TiO_2 (pH 3, 6, and 10). (B) Degradation of ofloxacin by UV and UV/TiO_2 at pH 6; TiO_2 concentration ranging from 4 to 128 mg L^{-1}.

3.3.1. Influence of adding hydrogen peroxide

The addition of hydrogen peroxide to the photocatalytic process was assessed. According to Chu et al.,[15] when small amounts of hydrogen peroxide are introduced into the UV/TiO_2 process, the degradation efficiency increases because more hydroxyl radicals are generated by UV/H_2O_2. Moreover, Hapeshi et al.[4] reported that the use of hydrogen peroxide can result in higher degradation efficiency because there are more hydroxyl radical formation reactions, shown in Equations 6 and 7.

$H_2O_2 + O_2^{\bullet-} \rightarrow HO^{\bullet} + OH^- + O_2$ Equation 6

$H_2O_2 + e^- \rightarrow HO^{\bullet} + OH^-$ Equation 7

The addition of hydrogen peroxide led to an increase in the efficiency of the process, especially in the first 15 min of testing, as can be seen in Figure 2. Comparing the results obtained using 8 mg L^{-1} TiO_2, the addition of hydrogen peroxide resulted in a gain of about 30% in drug reduction efficiency, reaching 86.8% after 60 min. When 32 mg L^{-1} catalyst were used, after 60 minutes, the degradation efficiency increased from 75.8% to 94.6% when H_2O_2 was added.

Degradation of 95.6% was obtained by adding hydrogen peroxide to the UV/TiO_2 process, using 64 mg L^{-1} TiO_2. In the UV/H_2O_2 process (1.68 mmol L^{-1} H_2O_2), 25% of ofloxacin degradation was observed after 15 min. Maximum degradation was 96.8% after 60 min. The addition of catalyst (UV/TiO_2/H_2O_2 process, with 1.68 mmol L^{-1} H_2O_2 and 128 mg L^{-1} TiO_2) raised this result to 65.3% after 15 min of testing. The best result obtained from this process was 97.8% degradation after 60 min.

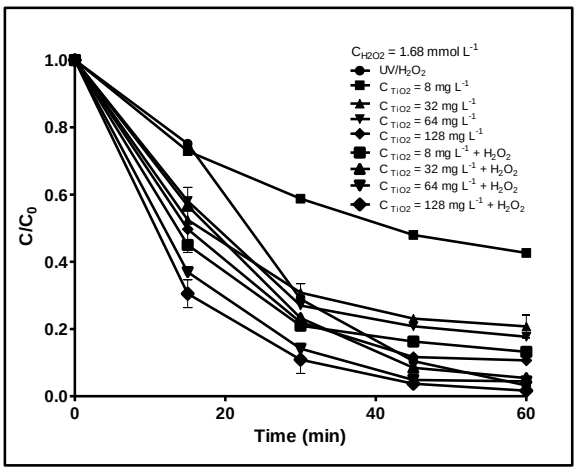

Figure 2. Degradation of ofloxacin by UV/H_2O_2, UV/TiO_2 and UV/TiO_2/H_2O_2 (1.68 mmol L^{-1} H_2O_2).

3.4. Antimicrobial activity

The EC_{50} values obtained ranged from 7.89 µg L^{-1} to 8.55 µg L^{-1}. The mean value was 8.22 µg L^{-1}, which was taken to be the EC_{50} of ofloxacin.

Dose-response curves illustrate the relationship between serial dilution and growth inhibition percentage, and they are shown in Figure 3. A shift of the dose-response curves to the right indicates that the antimicrobial activity of the sample was reduced. The application of photolysis (Figure 3A) for 60 min decreased antimicrobial activity by 42%. The UV/TiO_2 process was efficient at reducing biological activity. There was a 65% reduction when 8 mg L^{-1} TiO_2 was used (Figure 3C). Reduction was 77% when 32 mg L^{-1} TiO_2 was used (*figure not shown*). It reached 91% when 128 mg L^{-1} TiO_2 were used (Figure 3D). Reaction time was 60 min. UV/TiO_2/H_2O_2 (1.68 mmol L^{-1} H_2O_2) was able to decrease the activity by 67% when 8 mg L^{-1} TiO_2 was applied (Figure 3E), 93% when 32 mg L^{-1} TiO_2 was applied (*figure not shown*), and 96% when 128 mg L^{-1} TiO_2 was applied (Figure 3F).

Comparing the results for photolysis and UV/TiO_2, the addition of catalyst is seen to result in a greater reduction of antimicrobial activity. When used 128 mg L^{-1} TiO_2, UV/TiO_2/H_2O_2 (1.68 mmol L^{-1} H_2O_2) was more efficient than UV/H_2O_2 at reducing biological activity, even when four times as much oxidant was used (6.77 mmol L^{-1} H_2O_2) in the UV/H_2O_2 process (Figure 3B).

The antimicrobial activity of ofloxacin is linked to the presence of carboxylic and carbonyl groups in the molecule.[16] Thus, the reduction in antimicrobial activity could be related to the reactions that occurred in this domain.

A relationship between degradation efficiency and antimicrobial activity reduction was observed. Therefore, the degradation processes did not generate by-products with greater antimicrobial activity than the parent compound. Dodd et al.,[17] Paul et al.,[18] and da Silva et al.[9] verified that the antimicrobial activity of some fluoroquinolones decreased after drug degradation by advanced oxidation processes.

3.5. By-products formed during ofloxacin degradation

Mass spectrometry assays were performed to identify the products generated during the UV/TiO_2 process. Figure 4 shows mass spectra obtained from the best degradation condition (128 mg L^{-1} of TiO_2). A decrease in signal intensity of the original molecule (ofloxacin, *m/z* 362, t = 0 min) was observed when its degradation by-products appeared. Possible structures of some of the degradation by-products (*m/z* 291 and *m/z* 157) are shown in Figure 5.

The intermediate with *m/z* 291 is generated due to piperazine ring oxidation. According to Hapeshi et al.,[19] ofloxacin molecule cleavage ($C_{11}H_{15}FN_2$ loss) can form an intermediate with *m/z* 169. The subsequent loss of a methyl group gives rise to by-product *m/z* 157. The structures of all products generated couldn't be identified because hydroxyl radicals do not attack organic compounds selectively, i.e., the radical can interact with any region of the target molecule. The analyte's chemical structure can hinder the investigation of the intermediates formed.[20] It is worth mentioning that to prove the identity of the intermediates formed, analytical standards must be acquired. They are not always available,[20-21] so they must be synthesized.

There have been some studies showing that fluoroquinolones are susceptible to photochemical transformations by exposure to ultraviolet radiation.[22-23] They offer limited evidence that such processes may diminish the antimicrobial activity of solutions. According to Shen et al.,[24] the core of the molecular structure of fluoroquinolones is responsible for drug binding to bacteria DNA. Therefore, it is expected that the antimicrobial activity of the drug would be reduced only when this part of the structure is degraded. However, even if the degradation by-products have an intact quinolone core, their antimicrobial activity is lower;[18] this indicates that changes in auxiliary functional groups can also decrease antimicrobial activity. The piperazine ring of ofloxacin is responsible for providing recognition and binding the drug to the topoizomerase DNA enzymes of the bacteria. Therefore, the by-product with *m/z* 291, which has structural fragmentation, has lower affinity to the target bacteria. It has lower antimicrobial activity than the parent compound.[25]

According Shen et al.,[24] fluoroquinolones require the carboxyl group (-COOH) for activity; therefore, the proposed by-product with *m/z* 157 has no antimicrobial activity.

Paul et al.[18] showed that there was a significant decrease (over 90%) of antimicrobial activity of ciprofloxacin solutions undergoing peroxidation assisted by UV radiation (UV/H_2O_2) and photocatalysis.

Vasquez et al.[7] suggested that there are two main routes for the generation of ofloxacin by-products by photolytic and photocatalytic reactions: dealkylation of the piperazine ring and decarboxylation (removal of the carboxyl group, -COOH). Studies have shown the principal photolytic reactions that occur are that fluoroquinolones lose fluoride (F^-), followed by decarboxylation.[22,26]

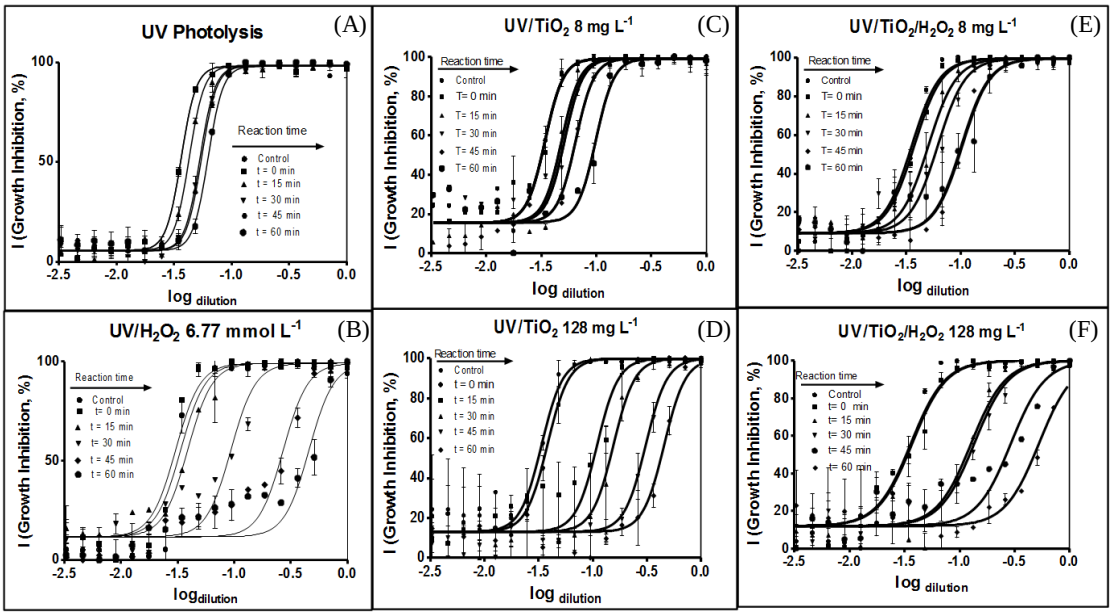

Figure 3. Antimicrobial activity of OFX solutions submitted to (A) photolysis, (B) UV/H_2O_2 (6.77 mmol L^{-1} H_2O_2), (C) UV/TiO_2 8 mg L^{-1}, (D) UV/TiO_2 128 mg L^{-1}, (E) UV/TiO_2/H_2O_2 8 mg L^{-1} TiO_2 and 1.68 mmol L^{-1} H_2O_2, and (F) UV/TiO_2/H_2O_2 128 mg L^{-1} TiO_2 and 1.68 mmol L^{-1} H_2O_2.

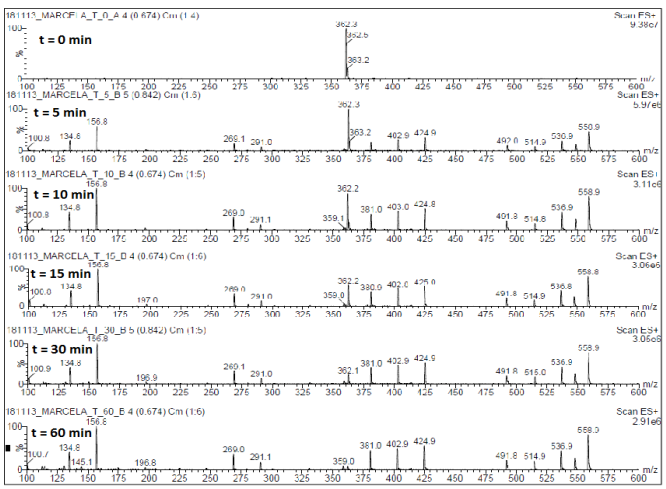

Figure 4. Mass spectra of ofloxacin solution submitted to UV/TiO_2 (128 mg L^{-1} of TiO_2) at t = 0, 5, 10, 15, 30, and 60 min.

The loss of fluoride in aqueous neutral solutions of fluoroquinolones does not occur in acidic conditions or for fluoroquinolones with electron donating groups, which is the case of ofloxacin. The insertion of an electron donor substituent at C-8 (alkoxy) stabilizes the molecule and makes the fluoride exit reaction inefficient.[27] In these conditions, the alkylamine side chain is partially degraded, leaving the fluorine attached to an intact heterocyclic chain.[22]

Small molecules such as water and carbon dioxide are eliminated by ofloxacin when this compound is submitted to photolysis and TiO_2 photocatalysis, as reported by Calza et al.[28] Subsequent attacks of hydroxyl radicals can lead to the formation of bi- or tri-hydroxylated products or dimerization of the ofloxacin molecule, resulting in by-products with *m/z* greater than the parent molecule.[19]

In this study, by-products with higher molecular mass than ofloxacin were also formed.

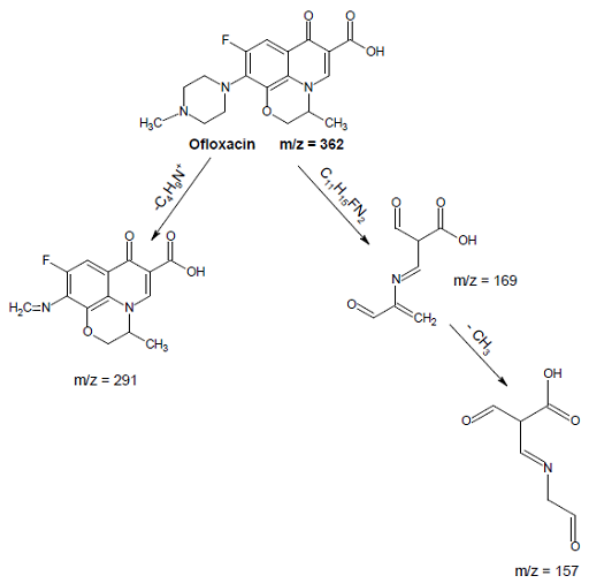

Figure 5. Proposed ofloxacin oxidation byproducts.

4. Conclusions

The photocatalytic process (UV/TiO_2) was highly effective at ofloxacin degradation: 89.3% was reduced after 60 min. The addition of hydrogen peroxide (UV/TiO_2/H_2O_2) resulted in more drug degradation (97.8%) and greater antimicrobial activity reduction. With the decrease of ofloxacin concentration, antimicrobial activity also decreased, showing that there is a relationship between degradation and antimicrobial activity reduction.

These photocatalytic processes can be applied in the treatment of

effluents containing ofloxacin, reducing the concentration of this compound and the solution antimicrobial activity. These advanced oxidation processes are new options for the treatment of effluents containing this class of compounds. They contribute to the reduction of environmental impacts when these compounds are disposed of in bodies of water.

5. Acknowledgements

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