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# Degradation of flumequine by photocatalysis and evaluation of antimicrobial activity

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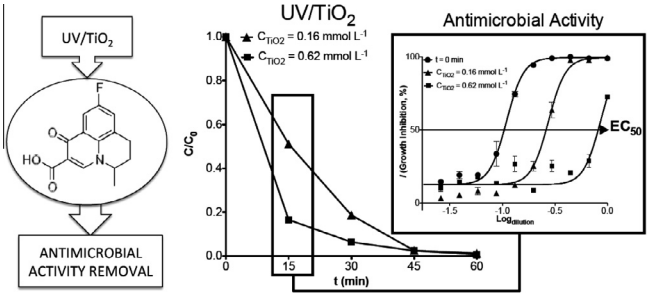
## HIGHLIGHTS

- $UV/TiO_2$ was able to degrade 98% of flumequine within 45 min.
- A total loss of antimicrobial activity was observed in 30 min.
- $H_2O_2$ addition improved the degradation efficiency and biological activity reduction.
- Byproducts with *m/z* of 244, 234, 232, and 202 were identified.

## GRAPHICAL ABSTRACT

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## ABSTRACT

This study evaluated the efficiencies of advanced oxidation processes ($UV/TiO_2$ and $UV/TiO_2/H_2O_2$) for the flumequine degradation, an antimicrobial and emergent pollutant. The photocatalytic process was capable of degrading approximately 55% of the drug after 15 min of reaction using 0.31 mmol $L^{-1}$ $TiO_2$ in suspension; the addition of $H_2O_2$ (0.5 mmol $L^{-1}$) increased flumequine degradation to 81.6%. For $UV/TiO_2$, increasing catalyst concentration (0.08–0.62 mmol $L^{-1}$) corresponded to an increase in the degradation efficiency. Using mass spectrometry, it was possible to identify five putative byproducts formed during the advanced oxidation processes. Due to the risks of bacterial resistance, the antimicrobial activity of the treated solutions was evaluated and compared to an untreated solution. Residual antimicrobial activity was detected after 15 min of reaction in all of the evaluated conditions. The antimicrobial activity was considerably reduced by the photocatalytic processes ($UV/TiO_2$ and $UV/TiO_2/H_2O_2$) throughout the reaction.



## 1. Introduction

Veterinary drugs are widely used in animals for infection control, prophylaxis and therapeutic uses [1]. The majority of these drugs are not fully metabolized by animals due to their low absorption in the digestive tract. Therefore, these drugs can be excreted *in natura* via feces and urine in their non-metabolized form or as active metabolites, thereby contaminating soils and surface waters [2].

Flumequine, an antimicrobial agent of the fluoroquinolone family, is commonly used in veterinary medicine. Due to its continuous introduction into the environment and resistance to degradation, flumequine residues have been detected in aquatic environments (2.5–50 ng $L^{-1}$) [3–5], and in soil (6.9 μg $g^{-1}$) [6]. When present in the environment, flumequine may cause alterations in microbial communities (e.g., the development of resistant bacteria) and can affect organisms. The presence of this drug in water and soil has attracted the attention and concern of the scientific community [3,7,8]. Veterinary drugs are also frequently released into the environment as a result of manufacturing processes, illegal disposal, metabolic excretion and accidental discharges during drug applications [9–11]. Several studies have reported the low efficiency of the

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removal of these compounds by water and sewage treatments plants [12–14].

Advanced oxidation processes (AOPs) present efficient mechanisms for the reduction of persistent substances. Among the commonly used AOPs, photocatalytic processes have been successfully used for the degradation of antimicrobial drugs in aqueous solutions [15–24]. $TiO_2$ is the most widely used photocatalyst due to its high stability, low cost, and low environmental impact [25].

In our previous study [26], flumequine in aqueous solutions was degraded by UV/$H_2O_2$, whereas Miranda-García et al. [15], Paul et al. [17], Nieto et al. [20], Palominos et al. [22] and Mansilla et al. [24] used photocatalysis to degrade this fluoroquinolone. Despite the available literature regarding flumequine degradation by photocatalysis, reports of residual antimicrobial activity are rare. This evaluation is needed because even after the extensive degradation of the original compound, the generated byproducts may present antimicrobial activity. In the reports of Palominos et al. [22] and Mansilla et al. [24], irradiated solutions were examined in terms of their antimicrobial activity against *Escherichia coli* based on measurements of the inhibition halo formed around the micro drops seeded in bacteria-inoculated agar plates. However, these studies aimed to qualitatively or semi-quantitatively evaluate the antimicrobial activity.

The quantitative analysis of antimicrobial activity in degraded drug samples is seldom reported in the scientific literature. Paul et al. [18] studied modifications in the antibacterial potency of aqueous ciprofloxacin solutions during photocatalytic treatment processes. In our previous work [26], a reduction in antimicrobial activity was evaluated during flumequine degradation by photolysis, peroxidation and UV/$H_2O_2$. This study is the first dedicated to the quantitative evaluation of residual antimicrobial activity of flumequine solutions submitted to UV/$TiO_2$ and UV/$TiO_2$/$H_2O_2$ processes.

The objective of the present study was to evaluate the reduction in antimicrobial activity during flumequine degradation by photocatalysis with $TiO_2$ in suspension. In addition, the influence of hydrogen peroxide additions was assessed, and the resulting degradation byproducts were investigated.

## 2. Experimental

### 2.1. Chemicals

Flumequine (99%) was purchased from Sigma–Aldrich (St. Louis, USA). The molecular formula and molecular weight of flumequine are $C_{14}H_{12}FNO_3$ and 261.25 g mol$^{-1}$, respectively. Methanol (HPLC grade) and $BaCl_2{\cdot}2H_2O$ (99%) were purchased from J.T. Baker (Edo. de México, Mexico); oxalic acid (99.5%) was purchased from Merck (Darmstadt, Germany); hydrogen peroxide (30%, v/v), $NaHSO_3$ (58.5% of $SO_2$), concentrated $H_2SO_4$ and NaOH (97%) were purchased from Synth (Diadema, Brazil); $H_3PO_4$ (85%) and $KH_2PO_4$ (98%) were purchased from Nuclear (São Paulo, Brazil); KOH (85%) was purchased from Ecibra (São Paulo, Brazil); $NH_4VO_3$ (99%) was purchased from Honeywell Riedel-de Haën (Seelze, Germany); and $TiO_2$ was obtained from Degussa (Frankfurt, Germany). Mueller–Hinton broth cultures and Mueller–Hinton:Agar were purchased from Himidia (Mumbai, India). Ultrapure water used throughout the study was obtained using a Milli-Q Academic water purification system (Millipore).

### 2.2. Stock solution

A flumequine stock solution (250 mg L$^{-1}$) was prepared in methanol and stored at 4 °C while protected from light. Flumequine working solutions (500 μg L$^{-1}$) were prepared daily by appropriate dilutions of the stock solution in 1000 mL of ultrapure water.

### 2.3. Experimental setup

AOP studies were performed using a previously described system [26], which was composed of a reactor (made of borosilicate glass with a 3.5 cm inner diameter and a 38.5 cm length containing a concentric low-pressure mercury lamp operating at 15 W with $\lambda_{max}$ = 254 nm and a 2.4 cm inner diameter; 190 mL volume), a magnetic stirrer, a 1000 mL reservoir, and a peristaltic pump, to maintain the solution in constant recirculation. In this experimental system, the lamp was in direct contact with the circulating solution. The irradiance was 8.3 mW cm$^{-2}$, as measured by a Cole Parmer (VLX 3 W model) radiometer previously calibrated at 254 nm.

Photocatalysis experiments were performed in an aqueous solution at pH 7. The concentration of the catalyst ($TiO_2$, Degussa P-25), which was held in suspension by agitating the solution, varied from 0.08 to 1.88 mmol L$^{-1}$. Following the degradation assays, titanium dioxide was removed by filtration with a GF 51-B glass membrane. In the UV/$TiO_2$/$H_2O_2$ experiments, 0.5 mmol L$^{-1}$ $H_2O_2$ was added, and the reactions were stopped by the addition of sodium bisulfite with $H_2O_2$ at a 1:1 M ratio.

### 2.4. $TiO_2$ adsorption test

The adsorption of flumequine was obtained in the dark. A total volume of 1 L of flumequine working solution (500 μg L$^{-1}$) was maintained in the presence of different concentrations of $TiO_2$ (0.08, 0.62, and 1.88 mmol L$^{-1}$) at pH 7 under agitation. The temperature was maintained at 25 °C. After the assays, $TiO_2$ was removed by filtration with a GF 51-B glass membrane. The aqueous solution with the remaining flumequine was then concentrated by solid phase extraction (SPE), and the extract was quantified by high performance liquid chromatography (HPLC) analysis.

### 2.5. Analytical methods

Flumequine degradation was evaluated by HPLC with UV detection. Sample preparation prior to quantification was performed by SPE. SPE was performed using a $C_{18}$ solid phase cartridge (Varian 500 mg/6 mL) conditioned with methanol (6 mL) and water (6 mL). One liter of sample was percolated through the cartridges at a flow rate of 10 mL min$^{-1}$. Subsequently, the sorbent was dried under vacuum, and the analyte was eluted with methanol (4.0 mL). The eluate was filtered (0.22 μm membrane filters) prior to HPLC analyses. The recovery ranged from 89% to 104% for samples containing flumequine in the concentration range of 50–500 μg L$^{-1}$.

The chromatographic system consisted of a Waters solvent delivery system (model 510), a tunable absorbance detector (Waters model 486) and a Rheodyne 7725 injector with a 20 μL sample loop. Quantitation was performed at 236 nm. An XBridge® RP18 column from Waters (250 mm × 4.6 mm I.D., 5 μm) was used as the analytical column. The flow rate was 1.0 mL min$^{-1}$. Methanol:0.01 mol L$^{-1}$ oxalic acid (60:40 v/v) was used as the mobile phase. The LOD and LOQ were 0.24 μg L$^{-1}$ and 1.0 μg L$^{-1}$, respectively.

### 2.6. Identification of byproducts by mass spectrometry

To identify the byproducts formed during the degradation processes, aqueous solutions containing 5 mg L$^{-1}$ of flumequine were used to avoid the loss of byproducts during the concentration step. The photocatalytic processes were evaluated from 0 to 30 min using 0.16 mmol L$^{-1}$ $TiO_2$ and 5.0 mmol L$^{-1}$ $H_2O_2$.