Influence of pH and ozone dose on sulfaquinoxaline ozonation

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Sulfaquinoxaline is a sulfonamide routinely used in prophylactic veterinary treatment to prevent coccidiosis and bacterial infections [1]. Its presence in both surface (4.5 to 40.4 ng L$^{-1}$) [2] and groundwater (0.01 to 112.1 ng L$^{-1}$) [3] is an issue of great importance for ecosystems and human health. Issues such as acute and chronic effects of antibiotics on ecosystems and potential rise of antibiotic-resistant bacteria are not well understood and they are at the root of increasing public concern [4]. Ozonation is an effective alternative to conventional treatments when this type of pollutant is present. Two main oxidants (molecular O$_3$ and OH radical) may be acting in the ozonation reaction. Lower pH (pH < 4) favors the oxidation via molecular ozone. Increase of pH favors O$_3$ decomposition into hydroxyl radicals. In pH > 10, O$_3$ is instantaneously decomposed into hydroxyl radicals. In pH 7 both oxidants can be acting. Due the scarcity of scientific research in the literature about sulfaquinoxaline degradation by ozonation process, the objectives of this study were to evaluate the sulfaquinoxaline degradation and toxicity reduction in different ozone doses and pH values. Moreover, the intermediates formed during ozonation were evaluated.

**Methods**

Sulfaquinoxaline working solutions (500 µg L$^{-1}$ or 1.67 µmol L$^{-1}$) were prepared in 1 L of ultrapure water. A wide range of pH (3-11) was used to evaluate the sulfaquinoxaline degradation due to molecular ozone (O$_3$) and hydroxyl radical (•OH).

The experimental setup for the study consists of an ozone generator (O3R Philozon), a glass contact column (50 cm height x 7 cm diameter, volume of 1 L) and a reaction vessel for ozone gas measurement. One glass diffuser was used to sparge ozone gas into the solution at a constant flow rate of 4.0 L min$^{-1}$. The ozone generation was 5.52 ± 0.32 mg min$^{-1}$ L$^{-1}$. The experiments were conducted at room temperature (20 ± 1°C), varying reaction time from 0 to 15 minutes, i.e., ozone dose from 0 to 80 mg L$^{-1}$.

Gaseous ozone concentration was determined by a potassium iodide method (Method 2350 E) [5]. In the control experiment, oxygen gas was continuously bubbled into the solution at the same flow rate as used for the ozonation experiments and results indicated that sulfaquinoxaline was not stripped from the aqueous phase.

Solutions submitted to degradation process were concentrated by solid phase extraction using Varian C$_{18}$ (500 mg/6.0 mL) cartridges, conditioned with methanol (6.0 mL) and ultrapure water (6.0 mL). Analytes were eluted with methanol (4.0 mL) and the extract was filtered (0.22 µm porous membrane). The concentration of residual sulfaquinoxaline was quantified by HPLC with a photodiode array detector (Waters, USA). The limit of quantification was 0.04 µg L$^{-1}$.

Microtox® toxicity analyzer (Vibrio fischeri, 30 minutes of contact) was used to assess the toxicity of the solutions submitted to ozonation. Analyses were carried out according to Microtox® test procedures standard (81.9% screening test).
To identify the intermediates formed during ozonation, aqueous solutions (sulfaquinoxaline initial concentration of 500 µg L\(^{-1}\)) were evaluated in a UPLC/MS/MS (Waters, USA). After sampling, the samples were flushed with nitrogen for 20 min in order to remove residual ozone dissolved in the aqueous phase.

A design of experiments (DOE) was used under normalized conditions for modeling the influence of pH and ozone doses. These two variables were set following a central composite design. This study was conducted experimentally, varying reaction time from 0 to 2.0 minutes (ozone dose from 0 to 11.5 mg L\(^{-1}\)) and pH value from 3 to 11. Each variable assumes two levels (low and high), which correspond to the range of every variable specified in Table 1, including three central points for statistical validity and star points at \(-1.41 \times \sqrt{2}\) to \(+1.41 \times \sqrt{2}\).

### Table 1. Design of experiment and variable levels studied.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Codified Values</th>
<th>Variable levels</th>
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<tbody>
<tr>
<td></td>
<td>Ozone Doses</td>
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<tr>
<td>K</td>
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*Negative values have no sense and experiment was carried out at 0 mg L\(^{-1}\) O\(_3\).

### Results

The experimental results indicated that as the ozone gas doses increased, the sulfaquinoxaline concentration decreased (Graphical Illustration). This is expected because an increase in ozonation time results in an increase in aqueous ozone concentration which either directly reacts with sulfaquinoxaline or can decompose to produce \(^{1}\)OH, which reacts with the sulfonamides too [6].

Sulfaquinoxaline degradation by ozonation presented a pseudo-first order degradation rate: \(k = 6.36\) min\(^{-1}\) for pH 3, \(k = 2.21\) min\(^{-1}\) for pH 7 and \(k = 0.25\) min\(^{-1}\) for pH 11.

The reactivity of sulfonamides towards ozone is strongly related to their pKa values: in acidic conditions, protonated species are predominant, what decrease the reactivity with ozone [6]. However, in this study the effect of pH on degradation of sulfaquinoxaline by ozonation occurred under this decreasing order: pH 3 > pH 7 > pH 11. These results are in accordance with the results obtained by Lin et al [4] for sulfonamide and macrolide antibiotics. The authors concluded that degradation of contaminants containing unsaturated C-C bonds, which is sulfaquinoxaline’s case, occurred faster at low pH, consistent with O\(_3\) being the predominant oxidant and its aqueous concentration being higher at low pH.

Garoma et al. [6] observed that increasing the pH from 2.0 to 10.0 resulted in enhanced removal of the sulfadiazine, sulfamethizole, sulfamethoxazole and sulfathiazole. However, in this study, the opposite happened. Sulfaquinoxaline degradation increased in acidic conditions and decreased when pH was 11 (or higher). To obtain at least 50 % of contaminant degradation at pH 11, double amount of ozone load was required (12 mg L\(^{-1}\)) when compared with the experiment carried out at pH 7.

The Pareto chart reveals the weight of each variable in the response factor (Figure 1). The main effects are clearly influential variables, since they are higher than significant frontier 2.45. Contrary, the interaction of the two studied factories (ozone dosage and pH) may be neglected. Regarding the contaminant degradation, pH plays a less important role than O\(_3\) doses.

![Figure 1. Pareto chart of the standardized effects, response is C/C\(_0\), \(\alpha = 0.05\).](image)
Toxicity assays were carried out with samples submitted to ozonation process at pH 3, 7, and 11 (Figure 3). Toxicity for samples submitted to ozonation at pH 3 was almost neglected. At pH 7, inhibition of luminescence remained almost constant (around 30 %) throughout ozonation time. However, luminescence inhibition in pH 11 increased from 0 to 85% when ozonation time varied from 0 to 15 minutes, which corresponds to an ozone dose from 0 to 80 mg L⁻¹.

Intermediates formation was evaluated after samples have been submitted to ozonation process (Figure 4). The intermediates proposed during sulfaquinoxaline (m/z 301) degradation under pH 3 were m/z 213, m/z 215, and m/z 241. These intermediates did not increase toxicity of the samples (Figure 3).

**Conclusions**

Molecular ozone was more effective on sulfaquinoxaline degradation than hydroxyl radical. DOE showed that there was not significant interaction between the studied factors (ozone dose and pH). Moreover, solutions degraded in pH 3 presented the lowest toxicity for Vibrio fischeri bacteria. Several intermediates were detected by the mass spectra assay.

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**References**