Spectroscopic characterization of degradation of antibiotics in wastewater treated by ozonation

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Abstract

Ozonation of a municipal wastewater effluent containing thirteen fluoroquinolone, macrolide and lincosamide antibiotic species was conducted with aims to examine the removal of these trace-level contaminants and the effects of ozonation on the evolution of effluent organic matter (EfOM) present in wastewater. The absorbance of ozonated wastewater was found to decrease consistently with increased ozone dose or treatment time due to the destruction of chromophores in EfOM molecules by ozone, thus the relative changes of absorbance ($\Delta A/A_0$) measured at a typical wavelength of 254 nm and an alternative wavelength of 330 nm were used to indicate the evolution of wastewater EfOM during the oxidation process. The degradation of antibiotics was efficient and strongly correlated with the changes of EfOM absorbance. Application of a formal model for the concurrent degradation of trace level contaminants and EfOM reactive sites allowed achieving a very good fitting between the experimentally observed and modeled $\Delta C/C_0$ vs. $\Delta A/A_0$ correlations.

Key words: ozonation; antibiotics; EfOM; absorbance; $\Delta C/C_0$ vs. $\Delta A/A_0$.

Introduction

Antibiotics, including groups of fluoroquinolone, sulfonamide, tetracycline, ß-lactam and macrolide, etc., comprise a significant amount of pharmaceuticals that are used as human and veterinary treatments. The environmental concentrations of these antibiotics are very low but they are already defined as detrimental emerging pollutants because of their continuous input into the environment, persistence and adverse effects associated with the proliferation of antibiotic-resistant pathogens (Kuemmerer, 2009; Fick et al., 2009). The real treatment of municipal or hospital wastewater treatment facilities usually fails to remove these drugs completely (Batt et al., 2007; Oulton et al., 2010); whereas, ozonation, as one notable advanced oxidation
process (AOP) in this regard, is efficient for their removals from wastewater (Huber et al., 2005; Snyder et al., 2006; Sirtori et al., 2009). For instance, Ternes et al. (2003) have reported around 90% depletion of antibiotic species in a pilot test at relatively low O$_3$ doses ($\leq$ 5mg/L) to wastewaters containing DOC as high as 23 mg/L.

The degradation of these and other trace-level contaminants by AOPs takes place simultaneously with the engagement of effluent organic matter (EfOM) present in all wastewaters or natural organic matter (NOM) in surface waters (Rosario-Ortiz et al., 2008; Shon, 2006; Wert et al., 2007). Interactions of EfOM or NOM with ozone or other oxidants have been shown to result in consistent changes of their absorbance (Ben Abdelmelek et al. 2011). Relationships between the degradation of contaminants such as EDC/PPCP and the evolution of spectroscopic parameters of AOP-treated wastewater have been addressed in considerable detail in recent publications (Nanaboina and Korshin, 2010; Rosario-Ortiz et al., 2010; Wert et al., 2009). These studies established the existence of strong correlations between the degradation of a wide variety of organic contaminants and the relative changes of EfOM absorbance at 254 nm ($\Delta A_{254}/A^0_{254}$). This was theoretically interpreted assuming the presence of kinetically different chromophore groups in EfOM. In principal, real-time $\Delta A_{254}/A^0_{254}$ measurements can be utilized to indicate AOP process efficiency; however, this approach has not been applied to examine the degradation of antibiotics by ozonation.

In this study, thirteen fluoroquinolone, macrolide and lincosamide antibiotics were chosen as target contaminants present in a municipal secondary wastewater effluent matrix. Ozonation was carried out in laboratory-scale experiments by applying a series of ozone doses or treatment times. Absorbance spectroscopy was detected to indicate transformations of wastewater EfOM caused by ozonation. Correlations between the removal of the parent antibiotic species and, on the other hand, changes of wastewater optical properties were examined in detail.

**Materials and Methods**

**Reagents and wastewater**

LC/MS-grade water, methanol and acetonitrile were purchased from EMD chemicals (Gibbstown, NJ, USA). LC/MS-grade formic acid, mass spectrometric grade ammonium acetate, pCBA, selected antibiotics (norfloxacin, ciprofloxacin, levofloxacin, enrofloxacin, sparfloxacin, danofloxacin, sarafloxacin, lomefloxacin, pefloxacin, roxithromycin, azithromycin, tylosin, lincomycin) were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Na$_2$SO$_3$ and NaOH were purchased from JT Baker (Phillipsburg, NJ, USA).

Stock solutions of antibiotics and pCBA were prepared by weighing and dissolving requisite amounts of each compound in MilliQ water and acetonitrile mixture (50:50 v/v) to yield a 1000 mg/L concentration. A few drops of 1 M NaOH were added to the
stock solutions to facilitate the dissolution of fluoroquinolones. A mixed solution of all antibiotics and pCBA was prepared by weighing each individual stock solution to yield a 1 mg/L concentration.

A grab sample of wastewater secondary effluent before disinfection was collected in Seattle area and transported to the laboratory within hours of sampling. The sample was filtered with a 0.45μm filter and stored at 4 °C. During the experiments, antibiotics were spiked into wastewater to make a 1 μg/L concentration for each compound. The samples were also injected with 75 µg/L pCBA with an aim to evaluate the OH· exposure.

**Ozonation experiment**

An AC 2000 series ozone generator with Mini Hicon ozone analyzer was used to produce an approx. 50 mg/L O₃ stock solution. O₃ concentration was determined by the standard 4500-O₃ indigo colorimetric method (Eaton et al., 1995). Ozonation was conducted by injecting predefined volumes of O₃ stock solution into each wastewater sample flask (initial pH ~7.50) to achieve desired O₃ concentrations. Additional experiments were carried out by exposing 1000 mL wastewater to a 1.5 mg/L continuous steady-state ozone flow. In this case, samples were collected at different time intervals. Sodium sulfite was dosed into flasks in advance to immediately quench residual O₃.

**Analytical Methods**

Concentrations of antibiotics were determined by a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system that included a Shimadzu Prominence HPLC (Kyoto, Japan) coupled with a 4000 Q Trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) using electrospray ionization (ESI) in positive mode. An Inertsil ODS-3 C₁₈ column (150 × 2.0 mm ID, 5 μm particle size) was used as stationary phase with a mobile phase containing (A) water (0.1% formic acid) and (B) 1:1 methanol-acetonitrile. The gradient started with 2% B for 2 min followed by a linear rise to 90% B within 17 min, kept isocratic up to 22 min, then dropped to initial conditions followed by a re-equilibration step up to 30 min. Antibiotics were quantified by injecting 50 μL sample into the column at a 0.2 mL/min flow rate in the multiple reaction monitoring (MRM) mode under ambient temperature. Quantification of pCBA was performed on the same column in negative ionization mode using its precursor and product ions as m/z 155.0 and 111.0 respectively. UV absorbance was measured from 200 to 600 nm at 1 nm increment, using a dual-beam Lambda-18 spectrophotometer (Perkin-Elmer GmbH., Überlingen Germany) with 5 cm quartz cell at 240 nm/min scan rate.
Experimental data and interpretation

Degradation of antibiotics by ozonation

Figure 1 shows the concentrations of all the examined antibiotics (norfloxacin, ciprofloxacin, levofloxacin, enrofloxacin, sparfloxacin, danofloxacin, sarafloxacin, lomefloxacin, pefloxacin, roxithromycin, azithromycin, tylosin, lincomycin) and pCBA in the wastewater decreased over a range of ozone doses (data of varying ozone exposure times not shown in this paper). The applications of a 1 mg/L ozone dose resulted in approximately 50% removals of all these species, except lomefloxacin for which ca. 40% removal was observed. When the ozone dose was increased to 3 mg/L or within 5 minutes of treatment at a constant steady-state ozone concentration, all these antibiotics were degraded virtually completely. Given that little removal of the hydroxyl radical probe pCBA (Park et al., 2004) took place at ozone doses < 3.0 mg/L, the oxidation of these antibiotics in these experimental conditions appeared to be driven primarily by molecular O₃.

**FIGURE 1.** Degradation of selected antibiotics and pCBA in wastewater treated over a range of ozone doses.

Oxidation of levofloxacin, enrofloxacin, sparfloxacin, danofloxacin and pefloxacin was faster than that of the rest fluoroquinolones. The differences in the removal of these antibiotics appear to reflect their varying intrinsic rates of interaction with the dominant oxidant ozone (k"_{O₃,app}) while the reported oxidation rates of these species
by hydroxyl radical \( (k''_{\text{OH,app}}) \) are relatively close (Dodd et al., 2006; An et al., 2010; Qiang et al., 2004; Park et al., 2004). Tylosin was oxidized faster than the other macrolides. This is likely to be due to the presence of the conjugated diene moiety in the macrolactone ring of this species. This functional group typically reacts with ozone with rates of \( 10^5 - 10^6 \text{ M}^{-1}\text{s}^{-1} \) (Dowideit and Sonntag, 1998).

**Effects of ozonation on absorbance spectra of wastewater**

![Graph](image)

**FIGURE 2.** Evolution of (a) absorbance and (b) relative changes of absorbance spectra of wastewater treated with varying ozone doses.

Because the change of TOC concentration during ozonation was less than 5%, only
absorbance was considered in this paper instead of the specific UV absorbance (SUVA). Most of the reduction of absorbance is due to the loss of aromatic and conjugated double boned carbon structures in EfOM as has been observed in previous studies, thus changes of absorbance can be used as an indication of changes in the equivalent amount of EfOM chromophores. Figure 2(a) illustrates the absorbance spectra of wastewater before and after treatment with varying ozone doses. In all cases, no prominent peaks can be found except a weak shoulder in the range of 250 nm to 280 nm, and the spectra gradually merged into a tailing when \( \lambda > 400 \) nm. With increasing ozone dose, an overall decrease in absorbance is clearly observed. Similar profiles were obtained from experiment with varying ozonation times. By quantifying the degree of absorbance deduction based on differential absorbance spectra approach, more details on the response of EfOM chromophores to ozonation can be acquired. Figure 2(b) clearly presents that the detection at bigger wavelength gives greater absorbance removal efficiency. At ozone dose of 5.0 mg/L, for instance, around 75% and 55% of absorbance were reduced for detection at 330 nm and 260 nm, respectively. It is also interesting to find a subtle decease in the absorbance at high wavelength observation when zone dose increased up to 3.0 mg/L which is likely due to the presence of oxidation products that have UV absorptivity thus compromise the reduction of overall absorbance of wastewater EfOM.

Correlations between degradation of antibiotics and changes of EfOM absorbance

Prior research has demonstrated the existence of strong correlations between relative changes of EfOM absorbance measured at 254 nm (\( \Delta A_{254}/A_{254}^0 \)) and concurrent degradation of a wide variety of organic contaminants other than antibiotics (e.g., Wert et al., 2009; Rosario-Ortiz et al., 2010). In this study, similar relationships were found to exist between \( \Delta A_{254}/A_{254}^0 \) values and relative changes of concentrations of all tested antibiotics (Figure 3). For enrofloxacin, levofloxacin, sparfloxacin, danofloxacin, pefloxacin, lincomycin and the macrolides, ca. 50% of their initial concentrations was removed at \( \Delta A_{254}/A_{254}^0 \) values around 0.10 (also shown in Table 1). Ciprofloxacin, norfloxacin, lomefloxacin, sarafloxacin along with pCBA were less reactive with their 50% removals observed at \( \Delta A_{254}/A_{254}^0 \) values close to 0.20. Increase of \( \Delta A_{254}/A_{254}^0 \) values from 0.20 to 0.25 was accompanied by >95% degradation of these contaminants, and when \( A_{254}/A_{254}^0 \) values were > 0.30, complete removals were achieved.

Despite the prevalent application of absorbance observation at 254 nm, in this paper, an alternative of 330 nm located in the higher wavelength range with more prominent relative changes of EfOM absorbance was chosen to examine the \( \Delta C/C_0 \) vs. \( \Delta A/A_0 \) relationships, because we argue that the wavelength of 254 nm may not be necessarily optimal for monitoring changes of EfOM chromophores caused by ozonation.
FIGURE 3. Correlations between the degradation of representative antibiotics and relative changes of absorbance at 254 nm and 330 nm of wastewater treated with different ozone doses and ozonation times.
Main features of the $\Delta C/C_0$ vs. $\Delta A_{330}/A_{0}^{330}$ correlations are shown in Figure 3. These correlations were parameterized using two parameters, the slope of the initial leg of the observed correlation corresponding to $\Delta A_{330}/A_{0}^{330}$ values < 0.10, and the value of $\Delta A_{330}/A_{0}^{330}$ corresponding to 50% removal of target contaminants ($S_{\text{initial}}$-$A_{330}$ and ($\Delta A_{330}/A_{0}^{330}$)$_{50\%}$ as compiled in Table 1). Prior research has shown that the $S_{\text{initial}}$ and ($\Delta A/A_{0}^{0}$)$_{50\%}$ values are correlated with the apparent kinetic rates of the degradation of many PPCP compounds by ozone and that higher $S_{\text{initial}}$ and/or lower ($\Delta A/A_{0}^{0}$)$_{50\%}$ values correspond to increasing reactivities of PPCPs towards the oxidant (Nanaboina and Korshin 2010). All the macrolides and fluoroquinolones, except norfloxacin, ciprofloxacin, sarafloxacin and lomefloxacin had $S_{\text{initial}}$-$A_{330}$ values as high as 4.34 and ($\Delta A_{330}/A_{0}^{330}$)$_{50\%}$ values < 0.20 indicative of their high degradability. On the other hand, ciprofloxacin, lomefloxacin, norfloxacin and sarafloxacin with corresponding $S_{\text{initial}}$-$A_{330}$ values < 1.50 and ($\Delta A_{330}/A_{0}^{330}$)$_{50\%}$ values > 0.30 showed relatively less reactivity toward ozone.

Table 1. Parameters of $\Delta C/C_0$ vs. $\Delta A/A_0$ correlations for the studied antibiotics and $p$CBA in ozonated wastewater with values of $S_{\text{initial}}$ and ($\Delta A/A_{0}^{0}$)$_{50\%}$ obtained from visual observation of experimental $\Delta C/C_0$ vs. $\Delta A/A_0$ correlations and values of $r_{C/S2}$ calculated based on a model assumption described in the text.

<table>
<thead>
<tr>
<th>Compound</th>
<th>absorbance at 254 nm</th>
<th>absorbance at 330 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S_{\text{initial}}$</td>
<td>($\Delta A_{254}/A_{254}^{0}$)$_{50%}$</td>
</tr>
<tr>
<td></td>
<td>$A_{254}$</td>
<td>$A_{254}^{0}$</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>1.69</td>
<td>0.17</td>
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<td>Danofloxacin</td>
<td>5.23</td>
<td>0.10</td>
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<td>Enrofloxacin</td>
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<td>0.12</td>
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<tr>
<td>Levofloxacin</td>
<td>2.85</td>
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</tr>
<tr>
<td>Lomefloxacin</td>
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<td>Norfloxacin</td>
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<td>Pefloxacin</td>
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<td>0.10</td>
</tr>
<tr>
<td>Sarafloxacin</td>
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<td>0.16</td>
</tr>
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<td>Sparfloxacin</td>
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<td>Azithromycin</td>
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<td>Roxithromycin</td>
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<tr>
<td>Tylosin</td>
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</tr>
<tr>
<td>Lincomycin</td>
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<td>0.11</td>
</tr>
<tr>
<td>pCBA</td>
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<td>0.33</td>
</tr>
</tbody>
</table>

It is interesting to notice that the functional shape of these relationships at 330 nm detection were similar to those observed at 254 nm (Figure 3), and the formal estimation of $S_{\text{initial}}$ and ($\Delta A/A_{0}^{0}$)$_{50\%}$ values at both wavelengths were also very close (Table 1). These results demonstrate the reliability and applicability of the measurement of absorbance changes of wastewater EfOM as an indicator of the treatment efficacy for target contaminants regardless of observation wavelength.
chosen in real time monitoring operation.

Modeling of correlations between changes of EfOM absorbance and the degradation of antibiotics

A mathematical model describing the $\Delta C/C_0$ vs. $\Delta A/A_0$ relationships for PPCP contaminants was developed by Nanaboina and Korshin (2010). That model was based on the hypothesis that EfOM chromophores can be subdivided into at least two kinetically distinct functionalities (denoted as $S_1$ and $S_2$). It was also postulated that in the range of ozone concentrations and/or treatment times that are similar as employed in this study, the apparent oxidation rates of $S_1$ and $S_2$ can be estimated as $k_{S_1} = k_{S_1}^{OH} \Gamma_{OH/O_3} + k_{S_1}^{O_3}$ and $k_{S_2} = k_{S_2}^{OH} \Gamma_{OH/O_3} + k_{S_2}^{O_3}$ (Eqs. 1 and 2), where $\Gamma_{OH/O_3}$ is the average apparent ratio of molar concentrations of hydroxyl radical and molecular ozone participating in the oxidation of any specific trace-level contaminants and EfOM.

This approach was applied here to simulate the relationships between the absorbance changes at both 254 nm and 330 nm and the degradation of antibiotic contaminants in ozonated wastewater. Briefly, the intensity of EfOM absorbance at any conditions of treatment was described as below:

$$ A = \delta_1[S_1^0][S_2^0]^{r_{S_1/S_2}} + \delta_2[S_2] + A_f $$

(Eq. 3)

where $\delta_1$ and $\delta_2$ are molar extinction factors of substrates $S_1$ and $S_2$, and we suppose

$$ \delta_1 = \delta_2 = (A_{initial} - A_f) / (S_1^0 + S_2^0); \quad r_{S_1/S_2} = (k_{S_1} / k_{S_2}) $$

is the ratio of apparent rates of oxidation of these two EfOM groups, $A_f$ represents absorbance of EfOM species that do not involve in ozonation.

Eq.3 can be transformed to Eq.4 as below:

$$ \int_{C_0}^{C} d \ln [C] = \int_{A_f}^{A} \left[ r_{C/S_2} dA \right] $$

(Eq. 4)

where $r_{C/S_2} = (k_C / k_{S_2})$ is the ratio of apparent rates of oxidation of contaminant C and EfOM substrate $S_2$.

In this case, modeling of all $\Delta C/C_0$ vs. $\Delta A/A_0$ correlations assumed the existence of the same initial concentrations of $S_1$ and $S_2$ in wastewater (1.0 and 2.0 µM,
respectively), with $S_1$ being oxidized 40 times faster than $S_2$ by ozone ($r_{S_1/S_2} = 40$). Values of $A_F$ were 0.0122 and 0.0024, and correspondingly values of $\delta_1$ and $\delta_2$ were $7.5 \times 10^3$ and $3.7 \times 10^3$ for absorbance detection at 254 nm and 330 nm, respectively.

Adjusting the compound-specific dimensionless $r_{C/S_2}$ parameter allowed achieving very good fitting between experimentally observed and model predicted correlations between the degradation of the studied antibiotics and changes of EfOM absorbance (Figure 4). The compound-specific $r_{C/S_2}$ values determined via the fitting of these datasets are compiled in Table 1. It demonstrates that in most cases the use of absorbance at alternative observation wavelengths could result in very close $r_{C/S_2}$ values for each antibiotic compound which are also strongly correlated with the parameters ($S_{\text{initial}}$ and $(\Delta A/A_0)_{50\%}$) shaping the $\Delta C/C_0$ vs. $\Delta A/A_0$ relationships. In principle, the determination of $r_{C/S_2} = (k_c/k_s_2)$ values allows estimating apparent second-order degradation constants for any trace-level compound if the absolute value of $k_s_2$ is known which was not addressed in this study but worth to pursue in future research.

**FIGURE 4.** Fitting plots of experimentally observed and modeled parameters of $\Delta C/C_0$ vs. $\Delta A/A_0$ relationships for all studied antibiotics in ozonated wastewater.
Conclusions

This study examined effects of ozonation of wastewater on concentrations of fluoroquinolone, macrolide and, in a limited extent, lincosamide antibiotics. These compounds were observed to exhibit different reactivities, with the macrolides generally more degradable than the fluoroquinolones and lincosamide. Ozonation caused decrease of the absorbance of the treated wastewater due to the oxidation of EfOM chromophores. The removal of antibiotics was strongly correlated with the changes in EfOM absorbance via ozonation in terms of either varying ozone doses or ozone exposure times. Applications of a formal model describing the concurrent degradation of trace level contaminants and relative changes of EfOM absorbance obtained good fittings between the experimentally observed and model predicted $\Delta C/C_0$ vs. $\Delta A/A_0$ data. These results have demonstrated the validity of detection of EfOM optical changes as an alternative indication of the removal efficacy during oxidation treatment for target contaminants present in trace level magnitude.

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Reference


