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Technical Note

# A novel approach to modeling the reaction kinetics of tetracycline antibiotics with aqueous ozone

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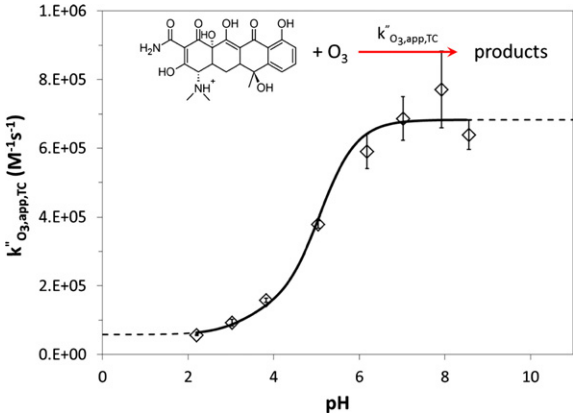
## HIGHLIGHTS

- The apparent second–order rate constants for transformation of five tetracyclines by ozone were determined.
- The reactivity with ozone at pH 7 was chlortetracycline < tetracycline < rolitetracycline < doxycycline < oxytetracycline.
- Disinfection–level ozone doses are sufficient to achieve complete transformation of tetracyclines.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Tetracycline antibiotics represent one of the most successful classes of pharmaceuticals and are extensively used around the world for human and veterinary health. Ozone-based processes have emerged as a selective water treatment process for many pharmaceuticals. The primary objective of this study was to determine the reaction kinetics for transformation of five tetracycline antibiotics (*i.e.*, chlortetracycline, doxycycline, oxytetracycline, rolitetracycline, and tetracycline) by ozone across the pH 2 to 9 range. The apparent second-order rate constant for tetracycline was on the order of 1–6 × $10^4$ $M^{-1}$ $s^{-1}$ at low pH, and 0.6–2.0 × $10^6$ $M^{-1}$ $s^{-1}$ at near neutral pH. The apparent second-order rate constants did not fit a conventional p$K_a$-based model, presumably due to the complex acid/base speciation of tetracycline antibiotics. A model that considers the net charge on tetracycline molecules in solution provided a nice fit to experimental data for all five tetracyclines. The five tetracycline antibiotics demonstrated similar reaction kinetics with ozone, and a cumulative analysis of all kinetics data provides a baseline model for other tetracycline compounds. The ozone exposure required for complete transformation of tetracycline antibiotics ($10^{-5}$ M — s) is well below that achieved during ozone disinfection processes ($10^{-3}$ M — s), indicating that ozone is an effective treatment for tetracycline antibiotics.



*Abbreviations:* CA, cinnamic acid; CTC, chlortetracycline; DAD, diode array detector; DC, doxycycline; DI, deionized water; ESI, electrospray ionization; HPLC, high performance liquid chromatography; MS/MS, tandem mass spectrometry; OTC, oxytetracycline; RTC, rolitetracycline; *t*-BuOH, *tert*-butanol; TC, tetracycline; TET, tetracycline antibiotics; WWTP, wastewater treatment plant.

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## 1. Introduction

Tetracyclines are one of the most popular classes of antibiotics, having produced global sales of $1.6 billion in 2009 (Hamad, 2010). Since the 1950s, tetracycline antibiotics have been widely used in human and veterinary medicine to treat bacterial infections and promote animal growth. This widespread use has resulted in the development of tetracycline resistance in almost all bacteria genera; however, tetracyclines are still employed for specific purposes, including treatment of urinary tract infections, chlamydia, acne, rosacea, and as a malaria prophylaxis, among other uses (Chopra et al., 1992; Roberts, 1996). In addition, antibiotic use in animal husbandry is at an all-time high. The US used over 29.8 million pounds of antibiotics in 2009 (Sperry, 2012), which is significantly greater than the 24.4 million pounds used in 2005 (AHI, 2008). Tetracycline antibiotics were a key component of that increase (AHI, 2008). Recently, a federal judge ordered the Federal Drug Administration to review antibiotic use in livestock (Sperry, 2012) given evidence that excessive use may increase development of antibiotic resistance in humans.

As tetracycline antibiotics are widely used in human and veterinary medicine, detection of these compounds in wastewater (Batt et al., 2007; Gao et al., 2012; Karthikeyan and Meyer, 2006; Leung et al., 2012; Li and Zhang, 2011; Miao et al., 2004; Thomas et al., 2007) and surface water (Kolpin et al., 2002; Li et al., 2008; Lin and Tsai, 2009; Spongberg et al., 2011; Wei et al., 2011) is not surprising. Batt et al. (2007) measured tetracycline concentrations up to 1.1 μg/L through four different wastewater treatment plants (WWTPs). Influent wastewater streams in Wisconsin were reported to contain doxycycline, oxytetracycline, and tetracycline concentrations of 10, 47, and 48 μg/L, respectively (Karthikeyan and Meyer, 2006). Others have detected tetracycline antibiotics in municipal, hospital, and drug manufacturer wastewater streams (Lin and Tsai, 2009; Miao et al., 2004; Thomas et al., 2007). In a landmark study, Kolpin et al. (2002) reported maximum concentrations of chlortetracycline, oxytetracycline, and tetracycline in US surface waters of 690 ng/L, 340 ng/L, and 110 ng/L, respectively. Even higher concentrations (*i.e.*, 810–2400 ng/L) were recorded by Wei et al. (2011) in Chinese surface waters. Tetracycline antibiotics have also been detected in biosolids and manure (Gottschall et al., 2012; Motoyama et al., 2011; Munir and Xagoraraki, 2011; Shafrir and Avisar, 2012); furthermore, tetracyclines have been shown to leach from solids and have been detected in food crops (Grote et al., 2007; Hamscher et al., 2002; Kumar et al., 2005).

A primary concern associated with the presence of antibiotics in WWTPs and the release of antibiotics into surface waters is the development of antibiotic resistance. Szczepanowski et al. (2009) measured 23 tetracycline resistance genes in activated sludge and final effluent from a wastewater treatment plant. Tetracycline resistance genes are routinely detected in small- and large-scale WWTPs (Auerbach et al., 2007; Mispagel and Gray, 2005). The number concentration of antibiotic resistant genes in wastewater effluent and treated biosolids from wastewater treatment plants in Michigan was quantified by Munir et al. (2011). Given, the widespread efflux of antibiotic resistance genes from WWTPs, advanced processes aimed at treatment of tetracycline antibiotics are merited.

Various treatment processes have been studied to remove tetracycline antibiotics from wastewater. Individual studies have focused on adsorption of tetracycline onto activated carbon (Chen et al., 2011; Choi et al., 2008a, 2008b; Fu et al., 2011; Sithole and Guy, 1987b; Torres-Perez et al., 2012), metal oxides (Gu and Karthikeyan, 2005), metal oxide-activated carbon composites (Shao et al., 2012), carbon nanotubes (Ji et al., 2009; Oleszczuk et al., 2009), and clays (Sithole and Guy, 1987a; Tolls, 2001), among others. For the most part, these technologies have shown relatively good potential to sorb tetracycline antibiotics. Regardless, adsorption processes transfer tetracyclines into the solid phase. As indicated above, tetracyclines have been shown to leach from solids. For this reason, oxidation-based processes, which chemically transform tetracyclines, may represent a more sustainable solution.

Previous researchers (Ben et al., 2012; Gomez-Pacheco et al., 2011; Khan et al., 2010; Kim et al., 2012; Uslu and Balcioglu, 2008; Wang et al., 2012) have investigated ozonation of tetracycline antibiotics in various water and wastewater matrices. Transformation products generated from ozonation of solutions containing tetracycline have been identified (Dalmazio et al., 2007; Khan et al., 2010); ozone mainly attacked the double bonds, aromatic ring, and tertiary amine moieties, as expected. Gomez-Pacheco et al. (2011) found that transformation of tetracycline is enhanced at higher pH, greater $H_2O_2$ concentrations, and in the presence of activated carbon due to increased hydroxyl radical exposure associated with faster ozone decay. Background electrolytes can impact transformation of oxytetracycline (Yalap and Balcioglu, 2009); however, those impacts are probably associated with hydroxyl radical scavenging. Ozone treatment of tetracycline antibiotics in agricultural wastewater has been shown to be effective (Lee et al., 2011; Uslu and Balcioglu, 2008), indicating fast reaction kinetics.

Previous research efforts into the transformation kinetics of tetracycline antibiotics by ozone have yielded varied results. Dodd et al. (2006) measured the apparent second-order rate constant ($k''_{O3,app,TC} = 9.6 \times 10^4$–$4.7 \times 10^6\ M^{-1}\ s^{-1}$) for transformation of tetracycline by ozone over the pH range of 3–9. Higher apparent second-order rate constants for tetracycline ($k''_{O3,app,TC} = 9.8 \times 10^6\ M^{-1}\ s^{-1}$), oxytetracycline ($k''_{O3,app,OTC} = 6.9 \times 10^6\ M^{-1}\ s^{-1}$), and chlortetracycline ($k''_{O3,app,TC} = 1.7 \times 10^7\ M^{-1}\ s^{-1}$) at pH 7.0 were reported by Ben et al. (2012). Rivas et al. (2011a) measured the apparent second-order rate constant for doxycycline at pH 2.5 to be $4.8 \times 10^4$–$3.6 \times 10^5\ M^{-1}\ s^{-1}$. While these results give an indication of the general reactivity of tetracycline antibiotics with ozone, disagreement between published rate constants exists and no model for the apparent rate constant over the pH range of environmental interest has been developed.

For that reason, the primary objectives of this research were (1) to determine the specific second-order rate constants for five tetracycline antibiotics (*i.e.*, tetracycline, chlortetracycline, oxytetracycline, doxycycline, and rolitetracycline) with ozone, (2) to model the apparent rate constants using a novel method of accounting for the complex speciation chemistry of tetracycline antibiotics, and (3) to contextualize the transformation of tetracycline antibiotics during ozone-based disinfection processes.

## 2. Experimental materials and methods

### 2.1. Chemicals

Chlortetracycline (CTC) hydrochloride (>80%), doxycycline (DC) hyclate (>98%), oxytetracycline (OTC) dihydrate (>99%), rolitetracycline (RTC; >91%), and tetracycline (TC; >98%) were purchased from Sigma-Aldrich (St. Louis, MO). Stock solutions of 1 g/L were prepared by dissolving the appropriate mass of individual tetracycline antibiotics in deionized water (DI) water; these stock solutions were stored in amber bottles at 4 °C. Salient information for the five tetracyclines of concern is shown in Table 1.

Cinnamic acid (CA) and *tert*-butanol (*t*-BuOH) were employed during reaction kinetics experiments. Cinnamic acid served as an ozone probe and was used to calculate second-order rate constants for transformation of tetracycline antibiotics with ozone in competition kinetics experiments. On the other hand, *t*-BuOH was employed as a hydroxyl radical scavenger to ensure that reaction kinetics with ozone were effectively isolated. LC-MS grade methanol, acetonitrile, and oxalic acid were obtained from Fisher Scientific (Houston, TX) for use as eluents in the LC-MS analysis of tetracycline antibiotics.