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Evaluation of the oxidation of enrofloxacin by permanganate and the antimicrobial activity of the products

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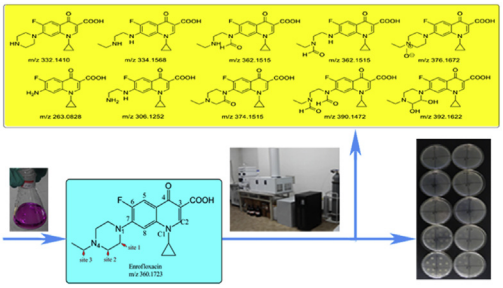
HIGHLIGHTS

- The oxidation mechanism of ENR by Mn(VII) is investigated.
- LC–Q-TOF is first used to determine the degradation products of ENR.
- The oxidation by Mn(VII) occurred on the piperazine ring.
- An identified new product by amine oxide and a new pathway is first reported.
- The fragmentation of the piperazine ring decreased antibacterial potency.

GRAPHICAL ABSTRACT



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ABSTRACT

Permanganate [Mn(VII)] oxidation of the fluoroquinolone (FQ) antibiotic enrofloxacin (ENR) was investigated with respect to kinetics and mechanisms, and the products were evaluated for residual antibacterial activity. The degradation of ENR by Mn(VII) obeyed second-order kinetics. A modern liquid chromatography coupled to a hybrid quadrupole time-of-flight mass spectrometer (LC–Q-TOF) was used to determine the accurate mass of the measured degradation products. The structures of nine oxidation products were identified at a neutral pH, one of which was an N-oxide product formed from the oxidation of tertiary amines. One proposed plausible reaction pathway was that the oxidation occurred on the piperazine ring; the C–H adjacent to the amine group was attacked by Mn(VII). The identified products from ENR arose through four pathways involving two mechanisms of N-dealkylation, C-hydroxylation and the reactions of amine oxides. The quinolone core remained intact for all of the products. The residual antibacterial activity of the oxidative reaction byproducts against the nonresistant *Escherichia coli* (G^-) reference strain DH5α was evaluated by quantifying the bacterial colonies. The oxidation products exhibited reduced antibacterial activity compared with their parent compound.

1. Introduction

Enrofloxacin (ENR), a broad-spectrum antibacterial agent from the class of fluoroquinolones (FQs), is the antibiotic most frequently used for the treatment of domestic animals. Because of its incomplete metabolic transformation in mammals, residual ENR is

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excreted into sewage (Kuemmerer, 2009; Speltini et al., 2010; Fatta-Kassinos et al., 2011). One of ENR's metabolic products, ciprofloxacin (CIP), still has antibacterial activity. Conventional water and wastewater treatment practices are inadequately designed for the reduction of antibiotic concentrations (Amorim et al., 2014), leading to a continuous input of ENR byproducts into the aquatic environment. In China, FQs have been detected in surface water at concentrations from 10 ng L^{-1} to 300 ng L^{-1}, and the highest concentration of ENR was measured at 210 ng L^{-1} (Wei et al., 2012). Thus, FQs are considered ubiquitous contaminants of emerging importance (Sturini et al., 2012b). This potential risk can be observed in the emergence of antibiotic-resistant bacteria (Dodd et al., 2009). Antibiotic-resistant bacteria pose a serious threat to humans (Dodd, 2012). The existence of FQs and other antibiotics in the environment may lead to adaptation by their target infectious organisms, rendering these antibiotics less effective. Antibiotic-resistant bacteria and their antibiotic-resistant genes can be transported to enhance the spread of antibiotic-resistant bacteria among non-resistant bacteria communities by means of both vertical and horizontal gene transfer processes, even after being fully inactivated in water treatment processes (Dodd, 2012). New treatment processes must be developed to treat contaminated water sources and to eliminate the biological activity of these antibacterial compounds.

Numerous previous studies have shown that FQs are susceptible to water treatment oxidants (e.g., chlorine (Dodd et al., 2005), chlorine dioxide (Wang et al., 2010), ozone (Dodd et al., 2006), manganese oxide (Zhang and Huang, 2005), and permanganate (Hu et al., 2010)). Studies of the photodegradation pathway in water have been performed for selected FQs (Knapp et al., 2005; Paul et al., 2010; Sturini et al., 2010; Li et al., 2011). These studies have described potential processes that could effectively cleave the piperazine ring of FQs and thus eliminate the biological activity of the parent antibacterial compounds (Dodd et al., 2009; Paul et al., 2010; Hu et al., 2011). Studies have indicated that the piperazine moiety of fluoroquinolone (FQ) was the predominant reactive site for these oxidants. Within the piperazine moiety, the inner aromatic N_1 atom and the outer aliphatic N_4 atom were considered to be the primary reactive centers. Minor substitutions on the piperazine ring affected not only the degradation rate with different oxidants but also the degradation products of the same oxidant. In ENR, there is an additional ethyl substituent on the outer aliphatic N_4 atom compared with ciprofloxacin (CIP). Research investigating the reaction of FQs with chlorine has indicated that the piperazine moiety in secondary-amine-containing FQs such as CIP should be more readily transformed than that in tertiary-amine-containing FQs such as ENR (Dodd et al., 2005). In contrast, ENR reacted faster than CIP with chlorine dioxide (Wang et al., 2010). Similarly, during ozone oxidation, ENR reacted at higher rates than CIP at neutral pH (Dodd et al., 2006). However, ENR and CIP had comparable reaction rates with manganese oxide (Zhang and Huang, 2005). ENR and CIP also had similar rate constants with the permanganate [Mn(VII)] at a pH of 5 and a pH of 9 (Hu et al., 2010). After examining the oxidation pathway of CIP, Hu et al. also stated that the oxidation target sites were the tertiary aromatic and secondary aliphatic amine groups on the piperazine ring and cyclopropyl group. However, in ENR, there is additional ethyl substituent in the N_4 atom on the piperazine ring, which contains the tertiary aromatic group and tertiary aliphatic amine groups. A previous investigation has shown that minor substitutions on the piperazine ring might affect the degradation products with chlorine (Dodd et al., 2005); ENR might illustrate a different degradation pathway with CIP with the reaction of Mn(VII). Indeed, there is limited information regarding the treatment of ENR with Mn(VII) in the overall pH value, and the oxidation products and mechanism of Mn(VII) are still not clear.

Although FQs can be decomposed by chemical oxidants and photolysis, recent studies have revealed that FQs are transformed without complete mineralization. The main degradation products probably retain most of the structure moiety, which might be more active than their parents (Li et al., 2011). A few studies have demonstrated that the mixture of the products of antibiotics retain less antibacterial potency than their parents. (Hu et al., 2011) found that a mixture of CIP products resulting from Mn(VII) reactions retained negligible antibacterial potency compared with the parent antibiotics. The reactions of ozone and hydroxyl radicals with ENR led to stoichiometric deactivation, indicating that the oxidation products retained significantly less antibacterial potency (Dodd et al., 2009). However, (Sturini et al., 2012c) and (Li et al., 2011) reported that the photolysis of ENR involves three main pathways and the generation of intermediates more active than ENR. The residual antibacterial activity of the oxidation products must be studied to evaluate the overall efficacy of oxidants. Studies investigating the risk associated with the formation of byproducts with unknown toxicological properties are thus urgently required (Sturini et al., 2012a).

In this study, the reactions of the important anthropogenic contaminant ENR with aqueous Mn(VII) were studied. The reaction sites were also determined to be the two tertiary amines on the piperazine ring. First, the study addresses (i) the examination of the kinetics in synthetic buffered waters over a pH range of 5–10, (ii) the identification of the main degradation products of ENR, and (iii) the elucidation of the possible oxidation degradation pathway. Second, the study examines the residual antibacterial activity of the reaction solutions at a neutral pH for a representative species of bacteria. Finally, the interaction between the substituent core FQ structure and bacterial enzyme was estimated.

2. Experimental section

2.1. Reagents and materials

Enrofloxacin was purchased from Sigma–Aldrich at greater than 98% purity. All other reagents employed were of the highest available quality and purchased from Tianjin Bodi or J&K Chemical Technology. All chemicals were used directly without further purification. Solutions were prepared in ultrapure water purified to 18.2 MΩ cm by a Milli-Q Academic (Millipore) water purification system. The glassware was washed by soaking the glass in diluted HNO_3 for >24 h and repeatedly rinsing with deionized water prior to use. The autosampler vials were washed by soaking the vials in 5% methanol for >24 h and repeatedly rinsing with deionized water. ENR stock solutions were prepared in a methanol/H_2O mixture (50/50 v/v) at a concentration of 1 mM, stored in a 4 °C freezer protected from light, and used within one month of preparation. Sodium thiosulfate is an oxidant scavenger; a 50 mM stock solution was prepared by dissolving $Na_2S_2O_3$ crystals in deionized water. The 0.02 M Mn(VII) stock solution was prepared by dissolving potassium permanganate standard into deionized water and calibrating with sodium oxalate. The required concentrations of potassium permanganate solutions were prepared by diluting the Mn(VII) stock solution with deionized water.

2.2. Oxidation experiments

Batch reactors were used to measure the reaction kinetics of ENR with Mn(VII). Reaction solutions were maintained at a constant pH with a 20 mM buffer (an acetate buffer for a pH of 4–5, a phosphate buffer for a pH of 6–8, and a borate buffer for a pH of 9–10). The tests showed that a slight inhibition was observed for ENR in the phosphate buffer. The reaction was initiated by adding