Water Research 102 (2016) 52–62

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Electrochemical oxidation of fluoroquinolone antibiotics: Mechanism, residual antibacterial activity and toxicity change

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ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form
4 May 2016
Accepted 2 June 2016
Available online 8 June 2016

Keywords:
Fluoroquinolone antibiotics
Electrochemistry-mass spectrometry
Antibacterial activity
Toxicity change

ABSTRACT

In this paper, we studied the electrochemical oxidation mechanisms of three typical fluoroquinolone antibiotics (FQs), and investigated residual antibacterial activity and toxicity changes after oxidation processes. Electrochemistry coupled to mass spectrometry (EC-MS) was used to study the oxidation processes of ciprofloxacin (CIP), norfloxacin (NOR) and ofloxacin (OFL). Eight oxidation products for each parent compound were identified and their chemical structures were elucidated. The transformation trend of each product, with the continuous increase of voltage from 0 to 3000 mV, was recorded by online EC-MS. The oxidation pathways were proposed based on the structural information and transformation trends of oxidation products. We found the oxidation mechanisms of FQs consisted of the hydroxylation and cleavage of piperazinyl ring via reactions with hydroxyl radicals, while the fluoroquinolone core remained intact. The antibacterial activity of the parent compounds and their oxidation mixtures was estimated using zone inhibition tests for gram-negative bacteria *Salmonella typhimurium*. It was found that the oxidation mixtures of CIP and NOR retained the antibacterial properties with lower activity compared to their parent compounds, while the antibacterial activity of OFL was almost eliminated after oxidation. Furthermore, the toxicity of the three FQs and their oxidation mixtures were evaluated using algal growth inhibition test (*Desmodesmus subspicatus*). The median effective concentration (EC_{50}) values for the algal inhibition tests were calculated for the end point of growth rate. The toxicity of CIP and NOR to green algae after electrochemical oxidation, remained unchanged, while that of OFL significantly increased. The results presented in this paper contribute to an understanding of the electrochemical oxidation mechanisms of FQs, and highlight the potential environmental risks of FQs after electrochemical oxidation processes.

1. Introduction

Fluoroquinolone antibiotics (FQs) are the most widely used group of antibiotics in the treatment of respiratory and bacterial infections, due to their board-spectrum activity against bacteria. FQs have been detected in wastewater treatment plant (WWTP) effluents (Kostich et al., 2014), surface water (Zhang et al., 2014) and in various environment matrixes (Kümmerer, 2009a) with concentrations from ng/L to μg/L level.

The presence of FQs in the environment can induce adverse effects on organisms and human beings in the long term, even at trace concentrations (Isidori et al., 2005; Johansson et al., 2014). First, continuous release of FQs into the aquatic environment may induce antibiotic resistance in native bacterial population (Bos et al., 2015). Resistance has the potential to adversely affect the health of aquatic and terrestrial organisms including humans (Bengtsson-Palme and Larsson, 2015; Kümmerer, 2009b). Secondly, the presence of FQs raises great concern about their toxicity in the

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http://dx.doi.org/10.1016/j.watres.2016.06.005
0043-1354/

environment. Robinson et al. (2005) conducted a study which found seven FQs exhibiting selective toxicity to five aquatic organisms. The combined toxicity of FQs and other antibiotics was investigated by González-Pleiter et al. (2013) on cyanobacterium and green alga, finding that strong synergism between these compounds observable in both organisms. Therefore, it is of great importance to effectively eliminate FQs from wastewater.

Advanced oxidation processes (AOPs) include a large variety of methods, such as ozone based processes, photolysis and photocatalysis processes and Fenton reaction based processes, which can effectively combine with conventional processes to remove resistant pharmaceuticals. Among them, electrochemical advanced oxidation processes (EAOPs) have recently received increasing attention due to their high-energy efficiency, versatility, and safety (Sirés and Brillas, 2012). The simplest and most popular EAOP is anodic oxidation with electrogenerated hydroxyl radicals (·OH) on boron-doped diamond (BDD) electrodes (Eq. (1)). BDD electrodes are preferred for water remediation since they can generate high amounts of weakly physisorbed hydroxyl radicals (Marselli et al., 2003; Moreira et al., 2014), which enhance the removal of organic chemicals.

$$BDD + H_2O \rightarrow BDD(\bullet OH)_{ads} + H^+ + e^- \quad (1)$$

Numerous investigations have been conducted on the removal of FQs by EAOPs, most of which were focused on the optimization of reaction conditions, oxidation kinetics, and efficiency (Carlesi Jara et al., 2007; Guinea et al., 2009, 2010; Yahya et al., 2014). However, little attention has been paid to the understanding of oxidation mechanisms of FQs during EAOPs.

Online or offline coupling electrochemistry with mass spectrometry (EC-MS) was first used to study the redox reactions of biomolecules (Hambitzer and Heitbaum, 1986; Volk et al., 1989) and simulate drug metabolism (Karst, 2004). In our previous studies (Chen et al., 2012, 2014; Hoffmann et al., 2011), EC-MS has been shown to be a reliable and rapid laboratory tool to investigate the oxidative mechanisms of organic pollutants in the environment and water treatment processes. In particular, the online monitoring of electrochemical oxidation processes has the advantage of directly detecting highly reactive and short-lived intermediates without a time delay. Therefore, we applied this approach to investigate oxidative mechanisms and identify oxidation products of FQs by electrochemical oxidation.

However, the abatement of the FQs during electrochemical oxidation can lead to the formation of various oxidation intermediates and products. There is a great possibility that electrochemical oxidation products also retain the biological effects of their parent compounds, and even develop new undesired bioeffects. In earlier studies (De Bel et al., 2009; Michael et al., 2010; Vasquez et al., 2013), residual antibacterial activity and toxicity changes in FQs after other oxidation processes, such as ozonation, UV treatment, and photocatalysis have been reported. Therefore, the issue cannot merely be addressed by elucidating the structures of oxidation products. Moreover, the toxicological effects of treated solutions arising from a mixture of residual parent compounds and their oxidation products should be evaluated.

The objective of the present paper is to study electrochemical oxidation mechanisms of FQs and evaluate the antibacterial activity and toxicity of the FQs and their reaction mixtures. Three typical FQs (Fig. 1), ciprofloxacin (CIP), norfloxacin (NOR) and ofloxacin (OFL), which are the most frequently detected in WWTPs and natural water (Kostich et al., 2014; Zhang et al., 2014; Van Doorslaer et al., 2014), were selected to conduct this study. We identified oxidation products and proposed oxidation pathways of the three FQs using EC-MS. A bulk EC cell for rapid electro-synthesis,

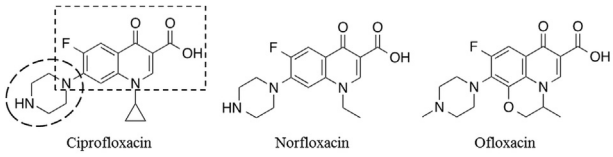

Fig. 1. Structures of ciprofloxacin, norfloxacin and ofloxacin. Dashed box contains fluoroquinolone core structure. Dashed oval contains piperazinyl moiety.

introduced in our latest work (Zhu et al., 2015), was used to prepare mg quantities of the reaction mixtures for biological tests. The antibacterial activity of the FQs and their oxidation mixtures was assessed using a typical zone inhibition test with gram-negative bacteria, *Salmonella typhimurium,* as a reference bacterium. The toxicity change of the FQs during electrochemical oxidation processes was investigated using algal growth inhibition tests with green algae (*Desmodesmus subspicatus*).

2. Materials and methods

2.1. Chemicals and regents

All solvents (chromatographic grade) and chemicals (analytical grade) were used as received from the commercial suppliers. Ciprofloxacin (99%, CAS 85721-33-1), norfloxacin (99%, CAS 70458-96-7), and ofloxacin (99%, CAS 82419-36-1) were purchased from Sigma-Aldrich. Ammonium acetate (p.a.), methanol (LiChrosolv purity), and dimethyl sulfoxide (DMSO) were purchased from Merck KGaA (Darmstadt, Germany). Formic acid was obtained from ROMIL (Cambridge, UK). High purity water (18.2 MΩ cm) was produced by a MilliQ plus 185 (Millipore, Molsheim, France).

2.2. Online EC-MS setup

2.2.1. Electrochemical reactions

A commercial EC reactor (Antec Leyden, The Netherlands) with a built-in platinum counter electrode and Roxy potentiosat was set up as reported in previous investigations (Chen et al., 2012; Hoffmann et al., 2011).

The electrochemical reactions were conducted in a flow-through "ReactorCell" (Antec Leyden, The Netherlands) containing a working electrode and a pH-dependent HyREF electrode for reference. In this study, a BDD working electrode was used for oxidation. Each reaction solution was composed of 50 μM of the parent compound with a 10 mM ammonium acetate buffer, in a mixture of methanol and water (2:3) containing 0.1% formic acid. The reaction solution was pumped through the EC cell at a constant flow rate of 10 μL/min. The residence time of the solution at the working electrode was approximately 3 s. A potential ramp at a scan rate of 10 mV/s was applied to record the dynamic transformation processes of target ions. The mass spectra of the FQs at different reaction voltages were recorded by applying constant voltages to the EC cell. All reactions were conducted at a constant temperature (25 °C) and repeated in triplicate to ensure the stability of the system and minimize bias and random errors.

2.2.2. Mass spectrometry conditions

ESI-MS experiments were carried out on a QTRAP 2000 (ABSciex, Darmstadt, Germany) and a high-resolution Fourier Transform Ion Cyclotron Resonance mass spectrometer (ESI-FTICR-MS) Ultra (ThermoFisher Scientific, San Jose, CA, USA), respectively. The settings of the method were performed as given in detail by Chen et al. (2012) and Hoffmann et al. (2011).