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Chlorination of tramadol: Reaction kinetics, mechanism and genotoxicity evaluation

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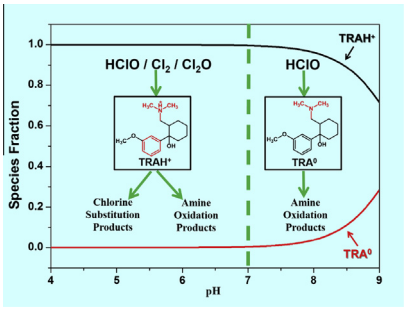
HIGHLIGHTS

- The reactivity between TRA and chlorine was found to increase with increasing pH.
- The active species Cl_2, Cl_2O and HOCl were considered during chlorine/TRA reaction.
- Eleven chlorination products were identified by UPLC–Q-TOF MS at various pH.
- Two possible reaction pathways were proposed under various pH conditions.
- The genotoxicity of TRA oxidation products increased with high dosage of chlorine.

GRAPHICAL ABSTRACT



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ABSTRACT

Tramadol (TRA) is one of the most detected analgesics in environmental matrices, and it is of high significance to study the reactivity of TRA during chlorination considering its potential toxicity to the environment. The chlorine/TRA reaction is first order with respect to the TRA concentration, and a combination of first-order and second-order with respect to chlorine concentration. The pH dependence of the observed rate constants (k_{obs}) showed that the TRA oxidation reactivity increased with increasing pH. k_{obs} can be quantitatively described by considering all active species including Cl_2, Cl_2O and HOCl, and the individual rate constants of $HOCl/TRA^0$, $HOCl/TRAH^+$, Cl_2/TRA and Cl_2O/TRA reactions were calculated to be $(2.61 \pm 0.29) \times 10^3\ M^{-1}\ s^{-1}$, $14.73 \pm 4.17\ M^{-1}\ s^{-1}$, $(3.93 \pm 0.34) \times 10^5\ M^{-1}\ s^{-1}$ and $(5.66 \pm 1.83) \times 10^6\ M^{-1}\ s^{-1}$, respectively. Eleven degradation products were detected with UPLC–Q-TOF-MS, and the corresponding structures of eight products found under various pH conditions were proposed. The amine group was proposed to be the initial attack site under alkaline pH conditions, where reaction of the deprotonated amine group with HOCl is favorable. Under acidic and neutral pH conditions, however, two possible reaction pathways were proposed. One is an electrophilic substitution on the aromatic ring, and another is an electrophilic substitution on the nitrogen, leading to an N-chlorinated intermediate, which can be further oxidized. Finally, the SOS/umu test showed that the genotoxicity of TRA chlorination products increased with increasing dosage of chlorine, which was mostly attributed to the formation of some chlorine substitution products.

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1. Introduction

The widespread occurrence of pharmaceuticals and personal care products (PPCPs) in water has gained much attention in recent years (Daughton and Ternes, 1999). Although PPCPs detected in the environment are at trace concentration levels (Daughton and Ternes, 1999), their potential risk to human healthy cannot be ignored considering their constant accumulation and high resistance to biodegradation (Joss et al., 2006). Tramadol (TRA, with chemical structure shown in Fig. 1) is a synthetic, centrally acting analgesic agent used for the relief of acute and chronic pain, which has been used in both human and veterinary medicine (Matthiesen et al., 1998; De Leo et al., 2009). In 2004, the total consumption of TRA was as much as 25.3 tons in Germany, and it is reported that 15–35% of TRA is excreted unchanged via urine (Kasprzyk-Hordern et al., 2007). The concentrations of TRA detected in secondary effluent and surface water were up to 97 μg L^{-1} and 6 μg L^{-1}, respectively (Kasprzyk-Hordern et al., 2009). Additionally, according to subacute and chronic toxicity studies on TRA, exposure to TRA may lead to the clinical signs of intoxication, like behavioral disorders and convulsions (Matthiesen et al., 1998). Therefore, it is of high significance to understand the fate of TRA considering its potential toxicity to the environment.

Owing to its low cost, chlorine is one of the most frequently used disinfectants for drinking water disinfection (Deborde and von Gunten, 2008). Prior studies reported that chlorine can easily react with some PPCPs, like sulfamethoxazole, ciprofloxacin, enrofloxacin, diclofenac, carbamazepine and tetracycline (Dodd and Huang, 2004; Wang et al., 2011; Soufan et al., 2012, 2013). Generally, the former studies considered hypochlorous acid (HOCl) and hypochlorite (ClO^-) as the main chlorine species, and the degradation reactions followed second-order kinetics, first order in the concentration of target compound, and first order in the chlorine concentration (Dodd and Huang, 2004; Soufan et al., 2012). Recently, some researchers pointed out that some other active species such as chlorine monoxide (Cl_2O) and molecular chlorine (Cl_2) take part in the chlorination disinfection process as well, in addition to HOCl/ClO^- (Sivey et al., 2010; Sivey and Roberts, 2012). Despite their relatively lower concentrations, such species (Cl_2, Cl_2O) possess much higher reactive activity with organic products compared with HOCl/ClO^- (Sivey et al., 2010; Sivey and Roberts, 2012; Cai et al., 2013). Therefore, the contributions of HOCl/ClO^-, Cl_2 and Cl_2O should all be considered during the PPCPs chlorination process. Additionally, chlorine attack generally leads to the generation of halogenated organic compounds, which typically possess relatively higher toxicity compared with the original organic products (Richardson and Ternes, 2011). Hence, evaluation of the toxicity of chlorination products should not be overlooked.

Recently, the kinetics and mechanism of the oxidation of TRA by ferrate and ozone have been investigated, where the apparent second-order rate constants were calculated to be $7.4 \pm 0.4\ M^{-1}\ s^{-1}$ and $(4.2 \pm 0.3) \times 10^4\ M^{-1}\ s^{-1}$ at pH 8. The lone electron pair of the amine-N has been identified as the predominant site of oxidant attack (Zimmermann et al., 2012). However, the fate of TRA during the chlorine disinfection process is unclear. Accordingly, detailed kinetics experiments were conducted from pH 4 to 9 during this work. Then the basic reactions considering all active species (including HOCl/ClO^-, Cl_2 and Cl_2O) were employed to calculate the individual kinetic constants. Additionally, the degradation products under various pH conditions were detected and analyzed with Ultra Performance Liquid Chromatography–Quadrupole-Time of Flight-Mass Spectrometry (UPLC–Q-TOF-MS). Then, possible reaction pathways and mechanisms were proposed. Finally, the potential genotoxicity of the TRA chlorination products was evaluated.

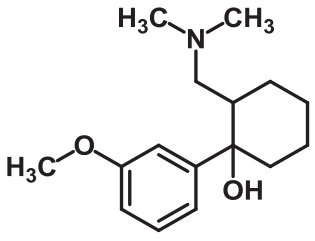

Fig. 1. Chemical structure of tramadol (TRA).

2. Materials and methods

2.1. Chemicals

Tramadol was obtained from Sigma–Aldrich (Saint Louis, USA) with purity more than 99%. Sodium hypochlorite solution with 10% available chlorine concentration was purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). The solvent acetonitrile was HPLC grade (Fisher Scientific, USA), and other reagents (CH_3COOH, CH_3COONa, $Na_2HPO_4{\cdot}12H_2O$, $NaH_2PO_4{\cdot}2H_2O$, H_3BO_3, $Na_2B_4O_7{\cdot}10H_2O$, NaOH, H_2SO_4, NH_4Cl), all obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China), were at least analytical grade. Reaction solutions were prepared using ultrapure water produced by a Millipore Water Purification System (Advantage A10, Millipore, USA). Free-chlorine stock solution was prepared by diluting initial sodium hypochlorite solution to yield about 100 mg/L free available chlorine and standardized by the DPD (N,N-diethyl-p-phenylenediamine, Sigma–Aldrich, Saint Louis, USA) colorimetric method (APHA, 1998).

2.2. Experimental procedures

To investigate the chlorination kinetics of TRA, batch experiments were conducted following pseudo-first-order conditions, with at least 10-fold excess of chlorine. Reactions were initiated by injecting an aliquot of chlorine stock solution into the prepared TRA solution (0.03 mM) containing 10 mM buffer solution. The previous studies reported that thiosulfate may convert the N-chlorinated intermediate back to the parent compound (Dodd et al., 2005; El Najjar et al., 2013). In order to avoid the potential interference of thiosulfate, NH_4Cl as a "soft" quenching reductant (1 mM), was used during this experiment (Dodd et al., 2005). At predetermined time intervals, samples (1 mL) were withdrawn and quenched. The resulting solutions were filtered (0.22 μm) and then analyzed by HPLC to measure the residual concentration of TRA as soon as possible. The reaction pH ranged from 4 to 9, which was maintained by 10 mM acetate buffer for pH 4–5, phosphate buffer for pH 6–8 and borate buffer for pH 9, respectively. A small quantity of H_2SO_4 or NaOH was used to control the pH if necessary. Unless otherwise specified, all experiments were conducted at 20 ± 2 °C. It should be noted that although the environmental concentration of TRA has been reported to be at the ng L^{-1}–μg L^{-1} level, a relatively high concentration was chosen in this work to facilitate the detection of TRA loss and the identification of degradation products. Since rate constants are independent of the initial TRA concentration, this discrepancy would not affect the kinetic results.

In order to identify reaction intermediates and products, a series of higher concentration TRA solutions (0.05 mM) were treated with 10-fold dosages of chlorine from pH 4 to pH 9. These experiments were conducted for sufficient time (24 h) to reach