E-beam degradation of thiamphenicol and florfenicol

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Abstract Thiamphenicol (THA) and florfenicol (FLO) aqueous solutions were irradiated to different doses by 1 MeV electron beams, and redox reactions of THA/FLO and radicals of OH, e_{aq}^{-} and 'H were investigated under various conditions. The results showed that at 8 kGy, the decomposition rate of THA and FLO was 97.2% and 98.5%, respectively. Quantitative and qualitative analysis indicated that OH played a key role in E-beam degradation of the THA and FLO in aqueous solutions, and e_{aq}^{-} and 'H radicals could initiate the degradation, too. Seven primary products of the THA and FLO degradation were identified. These indicate that E-beam radiation is an effective method to reduce THA and FLO in aqueous solutions.

Key words Thiamphenicol, Florfenicol, E-beam irradiation, Mechanism.

1 Introduction

Antibiotic, such as THA (thiamphenicol) and FLO (florfenicol), which are common phenicol antibiotics widely used in aquaculture^[1–2], had been over used for a very long period of time. This caused environment contamination, affecting human through the food-chain to such an extent that many antibiotics are no longer as effective in combating microbial infections as they used to be. Therefore, policies to limit the use of antibiotics have been implemented in many countries. The United States, Canada and many European countries have regulated the use of THA and FLO in aquaculture^[3–6]. And efforts were made in China to limit the maximum residue levels of the antibiotics in various tissues^[7].

THA and FLO are derivatives of chloramphenicol (Fig.1)^[8,9]. They are of broad-spectrum antibiotics. THA was established over half a century ago as an antibiotic against *Gram positive* and *Gram-negative* bacteria, and thousands of metric tons were produced over the years as human and veterinary antibiotic. FLO has been used for decades to prevent

and treat infections in pig, fish, and other animals. Antibiotics cannot be completely eliminated by mechanical-biological methods^[10] and biodegradation needs a long period of time to achieve complete biodegradation of veterinary antibiotics in surface water^[11]. It was reported that chloramphenicol was degraded after 4 h exposure with TiO₂ P-25 and ZnO as photocatalysts^[12], and the FLO in deionized water, freshwater and seawater under abiotic condition resisted hydrolysis and or photolysis in 14 days of exposure^[13]. Much of the THA and FLO went into surface water through wastewater that was not treated properly, and efforts should be made to seek better ways of controlling the contamination.



Fig.1 Thiamphenicol and Florfenicol.

Electron beam irradiation has shown a great promise in the treatment of industrial wastewater and groundwater^[14]. The E-beam induced radicals can

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react with a wide range of pollutants in wastewater ^[15–17]. In this paper, we report E-beam degradation of THA and FLO in aqueous solutions in different conditions. The reaction products are characterized by chromatography-mass spectrometry (GC/MS) and high performance liquid chromatography (HPLC). The inorganic anions of Cl⁻, SO₄²⁻ and F⁻ are analyzed by ion chromatography(IC). The reaction process and mechanisms are discussed.

2 Experimental

2.1 Materials

THA and FLO (>98%), ethyl acetate and *t*-butyl alcohol (Sigma, HPLC grade) were used without further purification. Purity of the N₂ or N₂O was > 99.9%. Ultrapure water (18.2 M Ω) was used. The pH of THA and FLO solutions was adjusted with KOH or HClO₄ aqueous solution.

2.2 Irradiation

THA or FLO solution (10 mL, 2-mm thickness) in an open polyethylene container was irradiated to 1, 3, 5, 8, 10, 15, 20, 25 or 30 kGy by 1 MeV 1 mA E-beams at room temperature. Before irradiation, the solutions were saturated by N_2 or N_2O for 20 min. Ultra-pure water and non-irradiated THA and FLO solutions were used as blank and control, respectively.

2.3 Analysis

Prior to the GC/MS (Shimadzu GCMS/QP2010, Japan), the primary products from THA and FLO degradation were extracted with ethyl acetate. The GC separation was accomplished by a Rtx-5MS fused silica capillary column (Restek; 30-m length, 0.25 mm ID, 0.25 μ m thickness). The samples were injected in seamless mode. The column oven temperature was programmed from 50°C (kept for 1 min) to 150°C at rate of 10°C/min and at 15°C/min to 280°C (kept to 5 min). The MS was operated at 280°C of the source temperature, and one scan per second from 40 to 600 amu. The carrier gas was helium at a column flow-rate of 1.0 mL/min.

THA and FLO were quantitatively analyzed using HPLC paired with an UV detector (Agilent 1200). All the products were conducted with sterile 0.2- μ m syringe micro-filters (Acrodisk-13, Gelman Sciences, Ann Arbor, MI), and the 10- μ L extract was injected into the C₁₈ column (150 mm×4.6 mm, Agilent). The UV ray at 225 nm was detected. The mobile phase was a mixed of methanol and water (40/60, v/v) at flow rate of 1.0 mL·min⁻¹.

The Cl⁻, SO₄^{2–}, and F⁻ anions in the irradiated THA and FLO solutions were determined by IC (Metrosep Asupp 5-250, Metrohm) at the flow rate of 0.7 mL·min⁻¹. The mobile phase with $5-\mu$ L injection volume was 3.2-mM Na₂CO₃/1.0-mM NaHCO₃ (50/50 (v/v).

3 Results and discussion

3.1 Reactive species

In interactions between E-beam and water molecules in the solution, hydroxyl radicals (OH), hydrated electrons (e_{aq} ⁻), hydrogen ions (H) and small amounts of hydrogen (H₂) and hydrogen peroxide (H₂O₂) are formed ^[18]:

H₂O→ OH (2.7),
$$e_{aq}^{-}$$
(2.6), H^{*}(0.55), H₂ (0.45), H₂O₂
(0.71), H₃O⁺ (2.6) (1)

The H and e_{aq}^{-} can be converted into HO₂ and O₂ - under O₂ atmospheres via the following reactions:

$$H' + O_2 \rightarrow HO_2'$$
 (2)

$$\mathbf{e}_{\mathrm{aq}}^{-} + \mathbf{O}_2 \to \mathbf{O}_2^{-} \tag{3}$$

$$\mathrm{HO}_2^{-} \to \mathrm{O}_2^{-+} \mathrm{H}^+ \tag{4}$$

In studying the e_{aq}^{-} reactions with THA and FLO, we used 15 mM *t*-BuOH in N₂-saturated solution at pH7. The OH radicals were scavenged according to reaction (5):

$$OH + t-BuOH \rightarrow t-BuO' + H_2O$$
 (5)

In studying the OH reactions with THA and FLO, we used N₂O-saturated solution at pH7. The e_{aq} radicals were converted into OH radicals according to reaction (6):

$$e_{aq} + N_2O \rightarrow OH + OH + N_2$$
 (6)

H and OH radicals react with aromatic compounds to form a radical of the cyclohexadienyl type with similar absorption characteristics. The H radical reactions were studied with N_2 -saturated solution at pH 2 with 15 mM *t*-BuOH.

Fig.2 shows the removal of THA and FLO in initial concentrations of 500 mg \cdot L⁻¹ irradiated by the E-beams. THA and FLO were decomposed completely

after E-beam irradiation of relatively low doses. At 8 kGy, the degradation rates of THA and FLO was 97.2% and 98.5%, respectively. At \leq 5 kGy doses, the OH, e_{aq}^{-} , and H could accelerate the THA and FLO degradation, with the THA degradation rates being 100%, 98.1% and 96.0% for OH, e_{aq}^{-} , and H, respectively (Fig.2a), and FLO being 100%, 97.9% and 96.4% for OH, e_{aq}^{-} , and H, respectively (Fig.2b), hence the formation of more radical fraction and primary products. It may be concluded that the higher OH-adduct and H-abstract reactions occur, and the H forms H adduct with aromatic rings^[17,19].



Fig.2 Degradation of THA (a) and FLO (b) at different conditions.

3.2 Analysis of Cl⁻, SO₄^{2–}, and F⁻

The inorganic anions removed from THA and FLO were detected (Fig.3). At 5 kGy, the major anions in the THA aqueous solution were Cl⁻ and SO₄²⁻, and F⁻ was found in the FLO aqueous solutions; whereas Cl⁻, SO₄²⁻ and F⁻ were not detected in the blank and control.

As shown in Fig.4, concentrations of CI^- , SO_4^{2-} , and F^- in the aqueous solutions increase with the doses up to 15 kGy, where the CI^- concentrations were 57.9% and 53.1% in THA and FLO aqueous solutions,

respectively, i.e. the covalent chlorine atoms can be converted by oxidizing primary products formed in the E-beam irradiation^[20]. But a substantial increase of inorganic Cl⁻ concentration was not recorded by increasing the dose from 15 kGy to 30 kGy. This is due to the primary oxidation products of polar chloroorganic compounds, as will be seen in the GC/MS analysis in Section 3.3. OH-adduct reaction occurred during the irradiation can be deduced as reaction (7).



Fig.3 IC of aqueous THA (a), FLO (b) solutions at 5-kGy absorbed dose.

The $SO_4^{2^-}$ concentrations in the aqueous THA and FLO solutions increased with the dose, too, reaching 16.0% and 15.1% of the $SO_4^{2^-}$ concentration in the THA and FLO aqueous solutions, respectively, at 20 kGy, where the trend of increase stopped. The $SO_4^{2^-}$ may be generated by hydrolyzing $HSO_3^{2^-}$, $SO_3^{2^-}$ and $S_2O_5^{2^-}$ under moderate pH after the OH⁻ and H⁻ adduct^[21], as shown in reaction (8). The HF of FLO can be eliminated easily, as shown in reaction (9), and with its boiling point at 19.54°C, HF released from the FLO solution, hence the low F⁻ concentration in Fig.4 (b).



Fig.4 Concentrations of Cl^- , SO_4^{2-} , and F^- from aqueous THA (a) and FLO (b) vary with doses of E-beam irradiation.

3.3 Primary products

The degradation of THA and FLO, like all the other organic pollutants, is a companied with a number of primary products. They should be scrutinized, so as to completely mineralize the toxic products, if any, by improved irradiation processes. For this reason, organic products from the THA and FLO solutions irradiated to 5 kGy were extracted using a liquid-liquid extraction and analyzed by GC/MS as shown in Fig.5 (Refer to Fig.6 for correlating their structures with the m/z).

Seven primary products of radiolysis degradation of THA and FLO were identified by GC/MS, at the peaks of m/z = 127, 185, 199, 200, 212, 231, and 337. The primary products may be generated by the OH abstracting hydrogen atom in aliphatic chains. Fig.6 shows that the OH and H first attack γ - or α -

C-H bonds, to form reactive carbon radical intermediates, which can be fragmented from the C-H, N-H, O-H, and C-C bonds. Further, the OH can react with C=C bonds or phenyl rings at more than one sites^[22], thus fragmenting and rearranging into these products, which is similar to the reaction of chloramphenicol with OH and $H^{[23]}$.



Fig.5 GC/MS of THA (a) and FLR (b) at 5 kGy.



Fig.6 Radiolytic primary products vs m/z in aqueous THA and FLO solutions at 5 kGy.

4 Conclusions

The aqueous THA and FLO solutions can effectively be degraded by the reaction of OH-adduct and H-abstract to result in the reactive radical fractions, and ultimately mineralized. The concentrations of Cl⁻ or SO_4^{2-} increases with the absorbed dose up to 15 or 20 kGy, and the F⁻ concentration is low due to releasing HF. The OH may have a dominant effect on degrading the THA and FLO.

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