Application of Fe$^{III}$-TAML/H$_2$O$_2$ system for treatment of fluoroquinolone antibiotics

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A B S T R A C T

Over the recent past, fluoroquinolone antibiotics (FQs) have raised extensive attention due to their potential to induce the formation of resistance genes and “superbugs”, thus various advanced oxidation techniques have been developed to eliminate their release into the environment. In the present study, the prototype tetraamido macrocyclic ligand (Fe$^{III}$-TAML)/hydrogen peroxide (H$_2$O$_2$) system is employed to degrade FQs (i.e., norfloxacin and ciprofloxacin) over a wide pH range (i.e., pH 6-10), and the reaction rate increases with the increase in pH level. The effect of dosage of Fe$^{III}$-TAML and H$_2$O$_2$ on the degradation of FQs is evaluated, and the reaction rate is linearly correlated with the added amount of chemicals. Moreover, the impact of natural organic matters (NOM) on the removal of FQs is investigated, and the degradation kinetics show that both NOM type and experimental concentration exhibit negligible influence on the oxidative degradation of selected antibiotics. Based on the results of liquid chromatography-high resolution mass spectrometry and theoretical calculations, the reaction sites and pathways of FQs by Fe$^{III}$-TAML/H$_2$O$_2$ system are further predicted and elucidated.

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Introduction

Fluoroquinolone antibiotics (FQs) have been widely employed in medical treatment and animal husbandry as a series of broad-spectrum pharmaceutical (Riaz et al., 2018; Van Doorslaer et al., 2014). However, due to the incapability of biodegradation, most of FQs were excreted into the environment in the form of parent molecule or bioactive metabolites, thus leading to their frequent detection in various matrices (e.g., surface water, soil and sediment), especially in wastewater treatment plants (WWTPs) (Verlicchi and Zambellos, 2015). FQ pharmaceuticals such as ciprofloxacin (CIP), have been frequently detected in both influent and effluent of WWTPs, in which the concentration of FQs could even reach mg/L level (Carvalho and Santos, 2016; Tran et al., 2018; Van Doorslaer et al., 2014). In addition to inducing the generation of antibiotic resistance genes, FQ antibiotics have raised great concerns of human health, such as cardiovascular diseases or renal calculi (Kang et al., 2001; Rubinstein, 2001). Therefore, it is necessary to develop efficient methods to eliminate FQ chemicals.

Over the past decades, various techniques have been developed to degrade FQs, such as treatments by ozone (Ling et al., 2018), chlorine (Dodd et al., 2005; He et al., 2019; Zhang et al., 2019), sulfate radical (Feng et al., 2017, 2018b; Jiang et al., 2016;
Zhou et al., 2018) and Fenton reagents (Giri and Golder, 2014; Salari et al., 2018). As a traditional treatment process, chlorine has been applied for the removal of typical FQs, whereas more toxic disinfection byproducts would be generated during chlorination process (Zhang et al., 2019). Although FQs could be eliminated rapidly by sulfate radical (SO$_4^-$·) generated from peroxymonosulfate (PMS), substrates could not be completely degraded at low concentration of PMS (Feng et al., 2017). Moreover, the oxidation of FQs by Fenton reagents has also been adequately surveyed and fast removal could be achieved for ciprofloxacin (Giri and Golder, 2014). However, strong acidic condition, quick consumption of ferrous ions and production of ferric hydroxide inevitably inhibit the application of Fenton technique in treatment of FQs (Giri and Golder, 2014; Gupta and Garg, 2018).

Recently, high-valent iron-oxo unit (e.g., Fe$^{IV}$=O, Fe$^{V}$=O, and Fe$^{VI}$=O) based oxidative treatment system has received increasing attention due to its high efficiency in detoxification of various organic pollutants, including pesticides, estrogens and pharmaceuticals (Feng et al., 2018a; Sharma et al., 2015; Zhao et al., 2011). Feng et al. (2017) investigated the degradation of FQs by high-valent iron generating from ferrate (Fe$^{VI}$), finding that rapid elimination could be obtained via Fe$^{VI}$ oxidation. It has been reported that the reactivities of Fe$^{IV}$ and Fe$^{V}$ are 2-5 orders higher than that of Fe$^{VI}$, following the order Fe$^{IV}$ > Fe$^{V}$ > Fe$^{VI}$ (Sharma, 2002; Teryn et al., 2017). Therefore, Fe$^{IV}$ and Fe$^{V}$ exhibit great potential to remove FQs.

The commonly used prototype tetraamido macrocyclic ligand (Fe$^{III}$-TAML), its molecular structure is illustrated in Appendix A Table S1 has been widely applied to treat wastewater containing various types of organic contaminants, especially for selective degradation of aromatic compounds (e.g., estrogenic chemicals (Chen et al., 2012; Onundi et al., 2017; Shappell et al., 2008), triphosphate pesticides (Chanda et al., 2006), and persistent chloro- (Gupta et al., 2002), bromo- (Wang et al., 2017a) and nitro- (Kundu et al., 2015a) phenols). Fe$^{III}$-TAML must be activated by an oxidant (commonly hydroxyl peroxide, H$_2$O$_2$) to form oxidative Fe-TAML (i.e., Fe$^{IV}$-TAML (Kundu et al., 2012) or Fe$^{V}$-TAML (Kundu et al., 2015b)), which subsequently undergo redox reaction with pollutants and being oxidized back to Fe$^{III}$-TAML. In addition, due to the low toxicity and trace usage dose of Fe$^{III}$-TAML, the iron-based chemical has been classified as a “green” catalyst (Banerjee et al., 2006; Gupta et al., 2002; Wang et al., 2017a). However, few researches have been conducted to investigate the performance of Fe$^{III}$-TAML/H$_2$O$_2$ system for the elimination of antibiotic pharmaceuticals. Therefore, it is greatly needed to explore Fe$^{III}$-TAML based techniques for purifying wastewater containing FQs antibiotics. In this work, norfloxacin (NOR) and CIP, which were the most frequently reported FQs in WWTPs even detected up to mg/L level, were selected as target pollutants (Van Doorslaer et al., 2014).

As common components in natural water, natural organic matters (NOMs) play a dual role in many reactions in aqueous phase. Li et al. (2018) found that the presence of NOMs slightly inhibited the degradation of p-chlorophenol (4-CP) using PMS/Fe$^{III}$-TAML, while the suppression effect was more obvious with regard to the traditional PMS system via hydroxyl radicals (·OH) and SO$_4^-$·. However, few studies mentioned about the influence of NOMs on the degradation efficiency of Fe$^{III}$-TAML/H$_2$O$_2$ system. This study aims to investigate the degradation efficiency and mechanism of selected FQs antibiotics (NOR and CIP) by Fe$^{III}$-TAML/H$_2$O$_2$ system. The effects of common water constitutes (i.e., pH (ranging from 6.0 to 10.0) and natural organic matter (NOM)) as well as the amount of Fe$^{III}$-TAML and H$_2$O$_2$ were also systematically examined to evaluate the factual treatment performance of FQs by Fe$^{III}$-TAML/H$_2$O$_2$. Moreover, the degradation pathways of NOR and CIP were elucidated, respectively, based on the identified intermediates and theoretically calculated reaction sites.

1. Materials and methods

1.1. Reagents

NOR, CIP and H$_2$O$_2$ were obtained from J&K Chemical Ltd. (Shanghai, China). The prototype Fe$^{III}$-TAML activator was purchased from GreenOx Catalysts, Inc. (Mellon Institute, Pittsburgh, PA, USA), and the concentration of Fe$^{III}$-TAML stock solution was quantified by spectrophotometric method measured at 366 nm with the absorption coefficient of 6600 mol/1·cm). Five NOM, i.e., SRHA (Suwannee River humic acid), SRFA (Suwannee River fulvic acid), PPFA (Pahokee Peat humic acid), PPFA (Pahokee Peat fulvic acid) and SRNOM (Suwannee River NOM) were obtained from the International Humic Substances Society. Other chemicals for experiments were of at least analytical grade and used as received. Milli-Q water was used for preparation of all the aqueous solutions related to the experiments.

1.2. Experimental methods

1.2.1. Degradation of FQs by Fe$^{III}$-TAML/H$_2$O$_2$ system

Degradation experiments were carried out in 100 mL glass bottles at 25°C under vigorous magnetic stirring. The reaction was initiated by adding 30 vol.% H$_2$O$_2$ into the mixed solution containing 20.0 μmol/L FQs (NOR or CIP), 1.0 μmol/L Fe$^{III}$-TAML and 10.0 mmol/L buffer salts (i.e., carbonate and phosphate). The molar ratio of Fe$^{III}$-TAML/H$_2$O$_2$ was 1:100. An aliquot of 0.5 mL sample was withdrawn and mixed with 1 mL methanol and 10 μL concentrated perchloric acid (3 mol/L) to quench the reaction at predetermined time intervals. To investigate the effect of pH, the initial pH of the solution was adjusted to 6.0, 7.0, 8.0 and 10.0 by carbonate or phosphate buffer. The change of pH was within ±0.1 during the reaction. When studying the effect of H$_2$O$_2$ and Fe$^{III}$-TAML concentration, dosage of chemicals was varied in the range of 0.25-5.0 mmol/L and 0.25-5.0 μmol/L for H$_2$O$_2$ and Fe$^{III}$-TAML, respectively. Moreover, SRNOM was pre-added to the solution at concentrations ranging from 0 to 20 mg/L to investigate the influence of NOM on the degradation of selected fluoroquinolone antibiotics by Fe$^{III}$-TAML/H$_2$O$_2$ system. Furthermore, to explore the impact of NOM properties on the removal of FQ pharmaceuticals, the degradation of NOR and CIP was also conducted in the presence of SRHA, SRFA, PPFA and PPFA, respectively. The initial concentration of FQs in the experiments above was fixed to 20.0 μmol/L.

1.2.2. Analytical methods

The samples of FQs were determined using high performance liquid chromatography (HPLC, Waters Alliance 2695, Milford, MA, USA) equipped with an Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm, Agilent Technologies, USA) and a UV detector monitoring at 277 nm. The analysis was conducted with a flow rate of 1.0 mL/min. For NOR and CIP, the mobile phase was 85% 50 mmol/L citric acids and 15% acetonitrile, the pH of which was adjusted by 3.5 mL triethylamine.

Meanwhile, the release of F$^{−}$ during the reactions under pH 10.0 was measured by fluoride selective electrode (FSE, Thermo ORION 9609BNWP, USA). Detailed information was shown in Appendix A Section S1.

Prior to identification of the reaction products, the sample was concentrated by solid phase extraction method using Waters Oasis HLB cartridge after reaction for a specific period. The HLB cartridge was activated by adding 5 mL methanol
followed by 5 mL Milli-Q water at a flow rate of 5 mL/min. Subsequently, 10 mL reaction solution passed through the cartridge and the extract remained by HLB cartridge was eluted with 1 mL methanol (Chen et al., 2018; Shi et al., 2019). The degradation products were identified by liquid chromatography/mass spectrometry (LC-MS) (Agilent 1260, Agilent Technologies, USA; Triple TOF 5600, AB Sciex, USA) equipped with a Atlantis T3 C18 column (2.1 mm × 100 mm, 3 μm) under the positive electrospray ionization mode. The mobile phase was consisted of 0.2% formic acid (V/V) and methanol. Detailed information on product analysis was listed in Appendix A Section S2.

1.2.3. Theoretical calculations
Theoretical calculations based on Gaussian 09W program with density functional theory (DFT) were used to investigate the molecular geometry, frontier electron density (FED) and contour surfaces of Fukui function for FQs. All calculations were performed under B3LYP method 6-311G (d,p) basis set, which was applied to all atoms of CIP and NOR, i.e., C, H, O, N and F. Moreover, FEDs of the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) were calculated, and the values of 2FEDHOMO and FEDLUMO were acquired in order to predict the reaction sites of electron extraction and hydroxyl addition, respectively. Furthermore, Fukui function of FQs was obtained according to the results of Milliken and Hirshfeld algorithms (Yang and Mortier, 1986), and the visualized contour surfaces were constructed to reflect Fukui indices. Detailed information about Fukui function was provided in Appendix A Section S3.

2. Results and discussion

2.1. Degradation of FQs by FeIII-TAML/H2O2 system at different pHs

Fig. 1 shows the degradation kinetics of CIP and NOR by FeIII-TAML/H2O2 system at pH 6.0-10.0, and the strong pH-dependent reactivity was observed. Negligible removal of FQs was observed at pH 6.0, whereas dramatic increase was obtained with the increase in pH level. The degradation of NOR and CIP followed the pseudo-first-order kinetics over the pH range from 7.0 to 10.0, and the fitted pseudo-first-order reaction rate constants (kobs) were listed in Table 1 and Appendix A Fig. S1. The fitted kobs are 0.0013, 0.0308 and 0.0493 min\(^{-1}\) for CIP degradation and 0.0068, 0.0304 and 0.0678 min\(^{-1}\) for NOR degradation by FeIII-TAML/H2O2 system at pH 7.0, 8.0 and 10.0, respectively. For comparison, the control groups of bare FeIII-TAML or H2O2 exhibited no effects on the degradation of CIP and NOR (Fig. 1).

The release of F\(^{-}\) was also investigated with FSE during the reactions at pH 10.0, as shown in Appendix A Fig. S2. In the early period of the reactions (0-30 min), the release of F\(^{-}\) was quite slow, although more than 80% FQs had been removed. Subsequently, with the complete elimination of parent FQs and enhanced degradation of transformation products, defluorination process was accelerated after 30 min. Finally, the defluorination rate for both FQs reached about 20% of the total fluoride in NOR or CIP, indicating that defluorination was not the main pathway for FQ degradation by FeIII-TAML/H2O2 system.

Table 1 – Fitted pseudo-first-order reaction rate constants (kobs) of NOR and CIP at different pH.

<table>
<thead>
<tr>
<th>pH</th>
<th>NOR</th>
<th>CIP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kobs (min(^{-1}))</td>
<td>R(^2)</td>
</tr>
<tr>
<td>6.0</td>
<td>1.27 × 10(^{-4})</td>
<td>0.2614</td>
</tr>
<tr>
<td>7.0</td>
<td>1.29 × 10(^{-3})</td>
<td>0.9852</td>
</tr>
<tr>
<td>8.0</td>
<td>3.08 × 10(^{-2})</td>
<td>0.9977</td>
</tr>
<tr>
<td>10.0</td>
<td>4.93 × 10(^{-2})</td>
<td>0.9829</td>
</tr>
</tbody>
</table>

Fig. 1 – Degradation kinetics of (a) norfloxacin (NOR) and (b) ciprofloxacin (CIP) at different pHs. Experimental conditions: initial concentration of FQs [\([\text{FQ}]\)] = 20 μmol/L, [FeIII-TAML] = 1 μmol/L, [H2O2] = 1 mmol/L, temperature (T) = 25°C. Control experiments were conducted at pH 10.0. FQs: the fluoroquinolone antibiotics, NOR and CIP in our experiment; [X]: the concentration of species X; TAML: the prototype tetraamido macrocyclic ligand; C: the initial concentration of FQ; C\(_{0}\): the concentration of FQ at the sampling time.
2.2. Kinetic modelling

According to the stoichiometric mechanism of catalysis by FeIII-TAML (Eqs. (1)–(4)) proposed in previous studies (Banerjee et al., 2015; Ghosh et al., 2008; Li et al., 2018), the kinetic modelling is conducted. All of the reactive intermediates formed from FeIII-TAML are described as oxidized Fe-TAML in the equations below.

$$\text{Fe}^{III} - \text{TAML} + \text{H}_2\text{O}_2 \xrightarrow{k_1} \text{Oxidized Fe} - \text{TAML}$$  \hspace{1cm} (1)

$$\text{Oxidized Fe} - \text{TAML} + \text{FQ} \xrightarrow{k_2} P_{primary} + \text{Fe}^{III} - \text{TAML}$$  \hspace{1cm} (2)

$$\text{Oxidized Fe} - \text{TAML} + P_{primary} \xrightarrow{k_3} P_{final} + \text{Fe}^{III} - \text{TAML}$$  \hspace{1cm} (3)

$$\text{Oxidized Fe} - \text{TAML} + \text{H}_2\text{O}_2 \xrightarrow{k_4} \text{O}_2 + \text{H}_2\text{O} + \text{Fe}^{III} - \text{TAML}$$  \hspace{1cm} (4)

where $k_1$ and $k_3$ represent the reversible reaction rates for the activation of FeIII-TAML to generate oxidized Fe-TAML, $k_2$ is the reaction rate for the oxidation of FQs by oxidized Fe-TAML, $k_3$ is the reaction rate for further oxidation of the primary product in Eq. (2), $k_4$ represents the rate of H$_2$O$_2$ decomposition by oxidized Fe-TAML to elute O$_2$. $P_{primary}$ is the primary product directly generated from FQ through Eq. (2), and $P_{final}$ is the final degradation product of FQ.

Subsequently, the steady state approximation was applied to oxidized Fe-TAML, in which the amount of oxidized Fe-TAML generated ([Oxidized Fe-TAML] from Eq. (1)) equals the amount of oxidized Fe-TAML consumed by Eqs. (1)–(4). And the mass balance of FeIII-TAML was also applied as the equation $[\text{Fe}^{III} - \text{TAML}]_0 = [\text{Fe}^{III} - \text{TAML}] + [\text{Oxidized Fe-TAML}]$, where $[\text{Fe}^{III} - \text{TAML}]_0$ represents the initial concentration of FeIII-TAML. As reported before (Ghosh et al., 2008), the reverse reaction of Eq. (1) is negligible when adding an appropriate electron donor (FQs in this study). Thus, the concentration of oxidized Fe-TAML in a steady state can be calculated through Eq. (5), where $k_3$ is not taken into consideration. Since the reaction rate of FQs can be respectively determined through Eqs. (2) and (6), the pseudo-first-order reaction rate constants $k_{obs}$ can be calculated based on Eq. (7).

$$[\text{Oxidized Fe} - \text{TAML}] = \frac{k_1[\text{Fe}^{III} - \text{TAML}]_0[H_2O_2]}{k_2[FQ] + \Sigma k_i P_{primary} + (k_1 + k_3)[H_2O_2]}$$  \hspace{1cm} (5)

$$\frac{d[FQ]}{dt} = k_{obs}[FQ] = k_2[\text{Oxidized Fe} - \text{TAML}][FQ]$$  \hspace{1cm} (6)

$$k_{obs} = \frac{k_3[k_2[\text{Fe}^{III} - \text{TAML}]_0[H_2O_2]}{k_2[FQ] + \Sigma k_i P_{primary} + (k_1 + k_3)[H_2O_2]}$$  \hspace{1cm} (7)

$$\frac{1}{k_{obs}} = \frac{k_3[k_2[\text{Fe}^{III} - \text{TAML}]_0}{[H_2O_2]} + \frac{1}{k_1 k_2[\text{Fe}^{III} - \text{TAML}]_0}$$  \hspace{1cm} (8)

where $X$ is the concentration of the species $X$.

2.3. pH-dependence of FeIII-TAML/H2O2 system

The strong pH-dependent reactivity correlates with the nature of FeIII-TAML/H$_2$O$_2$ system. Generally, reactive intermediates (e.g., FeIV-TAML and FeV-TAML) can be produced from the reaction of FeIII-TAML with H$_2$O$_2$, which can effectively remove substrates from the solution. Actually, diaqua [FeIII-TAML(H$_2$O)$_2$]$^{2-}$ and aqua/hydroxo [FeIII-TAML(OH)(H$_2$O)]$^{2-}$ are the main reactive species of FeIII-TAML in aqueous solution (Ghosh et al., 2008; Wang et al., 2017a). In addition, H$_2$O$_2$ itself undergoes ionization process with the generation of HO$_2^-$. Thus, four stoichiometric formula could be established for the formation of oxidized Fe-TAML (Appendix A Scheme S1), in which $k_a$, $k_b$, $k_c$, and $k_d$ represent the formation rates of the oxidized Fe-TAML via the reactions between [FeIII-TAML(H$_2$O)$_2$]$^{2-}$ and H$_2$O$_2$, [FeIII-TAML(H$_2$O)(OH)]$^{2-}$ and H$_2$O$_2$, [FeIII-TAML(H$_2$O)$_2$]$^{2-}$ and HO$_2^-$, and [FeIII-TAML(H$_2$O)(OH)]$^{2-}$ and HO$_2^-$, respectively (Ghosh et al., 2008). The deprotonated species [FeIII-TAML(OH)(H$_2$O)]$^{2-}$ with higher electron density are more likely to donate electrons to H$_2$O$_2$ than [FeIII-TAML(H$_2$O)$_2$]$^{2-}$. As a result, reaction rate $k_b$ is much faster than $k_b$. Similarly, it is easier for H$_2$O$_2$ to receive electrons ($k_b > k_c$, $k_d > k_4$) since it has lower electron density and weaker electrostatic repulsion than the latter one. Considering the dissociation constants ($pK_a$) of FeIII-TAML (between 9 and 10) (Ghosh et al., 2008) and H$_2$O$_2$ (approximately 11) (Popescu et al., 2008), the reaction rate will increase first and then decrease with the increasing pH levels. The maxi-
mum is predicted to occur between 10 and 11, which has been widely reported for the degradation of various organic pollutants (Chanda et al., 2006; Kundra et al., 2015a; Li et al., 2018; Wang et al., 2017a).

Additionally, on the basis of the pK_a values of CIP and NOR (Appendix A Table S1), the fraction of monoprotonated and deprotonated species of NOR and CIP increased when pH varied from 7.0 to 10.0, which was confirmed by the results simulated by Visual MINTEQ software. As shown in Appendix A Fig. S3, >95% of NOR and CIP existed in the deprotonated species at pH 10.0. Deprotonation would enhance the electron density of target FQs, thus making them more sensitive to the attack of electrophiles or oxidants (Ding et al., 2017; Dodd et al., 2005; Ling et al., 2018).

In terms of the proposed model, discussion about Fe^{III}-TAML and H_2O_2 speciation corresponded to the pH dependence of k_2, while influence of FQ speciation corresponded to k_2. Considering the previous researches (Ghosh et al., 2008; Li et al., 2018), the rate constants could be determined by the slope and intercept of Eq. (9) as below:

\[
\frac{[\text{Fe}^{III} - \text{TAML}]}{r_{ini}} = \frac{1}{k_1 k_2} + \frac{k_1 + k_2}{k_1 [\text{H}_2\text{O}_2]}[\text{FQ}]_0 \tag{9}
\]

where [FQ]_0 was the initial concentration of FQs, \( r_{ini} \) represented the initial reaction rate of FQs, which was determined by the slope of FQ concentration change at the beginning of the reaction. By investigating the initial rates under different concentration of H_2O_2 (0.25-5.0 mmol/L), \( k_3 \) for NOR and CIP at pH 10.0 were calculated to be 2.46 \times 10^2 and 2.80 \times 10^2 L/(mol·sec), respectively. For the determination of \( k_3 \), it was usually conducted through the intercept, neglecting \( k_3 \). Herein, \( k_3 \) would approximately be 1.39 \times 10^1 and 1.42 \times 10^3 L/(mol·sec) for NOR and CIP applying the method.

2.4. Effects of H_2O_2 and Fe^{III}-TAML dosage

As illustrated in Fig. 2a, the degradation rate was enhanced as the H_2O_2 concentration increased from 0.25 to 5 mmol/L, and the fitted \( k_{obs} \) increased from 0.09 to 0.20 min^{-1} for NOR (Appendix A Table S2). Similar trend was also observed for CIP degradation by Fe^{III}-TAML/H_2O_2 system with the \( k_{obs} \) value ranging from 0.10 to 0.22 min^{-1} (Appendix A Table S2). The correlation between H_2O_2 concentration and \( k_{obs} \) was observed to be non-linear. According to Eq. (7), the equation can be transformed into Eq. (8) to investigate the correlation. It can be seen that there should be a linear relationship between \( 1/k_{obs} \) and \( 1/[\text{H}_2\text{O}_2] \). As expected, \( 1/[\text{H}_2\text{O}_2] \) and \( 1/k_{obs} \) obtained in the experiment are plotted in Appendix A Fig. S4a, indicating an excellent agreement with Eq. (8). It has been reported that the degradation rates of ruthenium dye ([Ru(bpy)_{2}Cl_{2}]) (Ghosh et al., 2008) and p-chlorophenol (Li et al., 2018) increased linearly with the concentration of H_2O_2 and PMS. However, different from the results of previous studies, activation of Fe^{III}-TAML (Eq. (4), k_1[\text{H}_2\text{O}_2] in Eq. (7)) and decomposition of H_2O_2 (Eq. (6), k_2[\text{H}_2\text{O}_2] in Eq. (7)) should not be neglected under our experimental conditions. Therefore, the non-linear correlation between \([\text{H}_2\text{O}_2]\) and \( k_{obs} \) could be explained.

Moreover, a positive linear correlation was obtained for \( k_{obs} \) and Fe^{III}-TAML dosage (Appendix A Table S3 and Fig. S4b). As the dosage of Fe^{III}-TAML increased from 0.25 to 5 μmol/L, the reaction was promoted (Fig. 3). The fitted \( k_{obs} \) linearly increased from 0.0625 to 0.3664 min^{-1} and from 0.0648 to 0.4138 min^{-1} for NOR and CIP, respectively. These results are in line with the kinetic model, which is consistent with the results reported in previous study that various organic contaminants were degraded by Fe^{III}-TAML/H_2O_2 system (Ghosh et al., 2008; Li et al., 2018).

According to the stoichiometric mechanism, the addition of Fe^{III}-TAML or H_2O_2 both contributed to the enhanced generation of oxidized Fe-TAML. And the fitted experimental results match the kinetic model well. Therefore, the concentration of added Fe^{III}-TAML and H_2O_2 would be an important factor affecting the degradation efficiency of FQ antibiotics.

2.5. Effect of NOM on the degradation of FQs

NOM has been reported to influence the elimination of various organic contaminants, such as inhibition due to the effect of competition and promotion resulted from activated NOM species (Lado Ribeiro et al., 2019; Wang et al., 2017b; Yang et al., 2020). Therefore, the effect of NOM on the degradation of CIP and NOR was studied in the presence of different types and dosages of NOM. As shown in Fig. 4a and b, slight inhibition could only be obtained when the concentration of NOM reached 20 mg/L. Moreover, different NOMs were employed to investigate their effect on the degradation of FQ antibiotics,
and no significant differences were obtained for the degradation rates of CIP and NOR in the presence of selected NOMs (Fig. 4c and d). Therefore, NOMs exhibited negligible effects for the removal of CIP and NOR by Fe}^{III}\text{-TAML/H}_2\text{O}_2 system under our experimental concentrations, which might be attributed to the selectivity of Fe}^{III}\text{-TAML (Su et al., 2019). It was reported that the presence of NOM showed no effect on the enzyme or enzyme-like reactions, such as lignin peroxidase system and laccase system (Auriol et al., 2007; Georgi et al., 2007; Lu and Huang, 2009; Mao et al., 2009). Fe}^{III}\text{-TAML was synthesized as a mimic enzyme of cytochrome P450, indicating that the degradation of FQs by Fe}^{III}\text{-TAML was an enzyme-like catalytic reaction. Moreover, Lu and Huang (2009) found that the coupling interaction between NOM and substrates could enhance the removal of contaminants. As the concentration of NOM increased, slight inhibition effect might appear due to the competition between NOM and substrate (Li et al., 2018). Therefore, both of the two mechanism might contribute to the negligible effects of NOM on the degradation of FQs by Fe}^{III}\text{-TAML/H}_2\text{O}_2 system. Comparing with most advanced oxidation processes, reactive species, e.g., \text{.OH and SO}_4^{2-}, were produced to achieve superior elimination of organic contaminants and NOM via indiscriminate attack (Lee et al., 2007; Lei et al., 2020; Luo et al., 2016; Ma et al., 2018). Due to the competition from NOMs, however, the decomposition efficiency of target pollutants would be weakened (Lei et al., 2020; Luo et al., 2016; Shah et al., 2019).

2.6. Degradation pathways

The samples at different reaction time were analyzed by LC-MS and the structures of transformation products were deduced based on the MS spectra and previous studies. Finally, eight and seven transformation products were verified for NOR and CIP, respectively. Most of them could also be found in previous studies using different degradation methods. In these researches, the piperazone ring, quinolone group and fluorine atom were main reaction sites in the oxidation process of FQs and related to their efficacy of sterilization (Ding et al., 2017; Jiang et al., 2016; Ling et al., 2018). Based on the transformation products detected and theoretical calculations, the degradation pathways of NOR and CIP were proposed in Fig. 5a and b. The transformation products of NOR and CIP were named as TP-N1 to TP-N8 and TP-C1 to TP-C7, respectively. For both NOR and CIP, the reactive site was mainly located at the piperazone ring, despite that several defluorinated products were also observed via the cleavage of C-F bond. For NOR, the transformation product TP-N2 was detected first during the oxidation process, which could be ascribed

![Fig. 4](image-url)
Fig. 5 – Proposed degradation pathways of (a) NOR and (b) CIP with Fe$^{III}$-TAML/H$_2$O$_2$ system. m/z: the mass to charge ratio. TP-N$_x$ and TP-C$_x$ ($x = 1, 2, 3, 4, 5, 6, 7$ and $8$) refer to the transformation products of NOR and CIP, respectively.

to the hydroxylation and carbonylation process of NOR. It was reported that the Fe$^{III}$-TAML-induced oxidation was initiated from an electron-deprival reaction (Banerjee et al., 2015; Wang et al., 2017a), suggesting that the sites with high electron density (e.g., 38-N atom and 40-N atom) would be more facile to be attacked (Fig. 6). Subsequently, the unpaired electron will relocate to the $\alpha$-carbon atoms, thus leading to the nucleophilic substitution by H$_2$O and the formation of hydroxyl and carbonyl groups. Then TP-N$_2$ underwent C-C bond cleavage and piperazine opening, generating TP-N$_3$. Similarly, TP-N$_3$ went through demethylation and decarbonylation, forming TP-N$_4$ and TP-N$_5$, respectively. After elimination of aminomethyl-group, TP-N$_5$ was transformed into TP-N$_6$. Then carbonylation occurred on TP-N$_6$ with the production of TP-N$_7$, in which the piperazine ring had been substituted with amino-group thoroughly. Interestingly, the oxidized Fe-TAML could also break C-F bond in TP-N$_7$, producing a defluorinated product TP-N$_8$, which could also be confirmed by the partial release of F$^-$ during the reaction (Appendix A Fig. S2a).

Similarly, two degradation pathways of CIP are also summarized from the intermediates detected by MS. CIP could go through substitution of fluorine atom by hydroxyl group, resulting in the formation of TP-C$_1$. On the other hand, the successive oxidation of CIP piperazine ring by the oxidized Fe-TAML was also obtained with the products from TP-C$_2$ to TP-C$_7$ step by step.
3. Conclusions

In this study, Fe$^{III}$-TAML activated by H$_2$O$_2$, an environmentally-friendly system, was introduced to the efficient removal of typical fluoroquinolone antibiotics. Complete degradation of 20 μmol/L NOR and CIP was achieved in 60 min under pH 10.0. With the increase of pH from 6.0 to 10.0, the fitted pseudo-first-order reaction rate constants significantly increased. The kinetic model was proposed based on the degradation mechanism, and with the increasing dosage of H$_2$O$_2$ and Fe$^{III}$, TAML increase in reaction rates agreed well with the proposed model. The presence of different NOMs exhibited no significant impact on the degradation of FQ antibiotics. Moreover, degradation products were verified, and the respective reaction pathways were elucidated for NOR and CIP, which involve the piperazine opening and defluorination resulting from the gradual cleavage of C-C bond, C-N bond and C-F bond.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.jes.2020.06.006.

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