



Photoproducts of tetracycline and oxytetracycline involving self-sensitized oxidation in aqueous solutions: Effects of Ca^{2+} and Mg^{2+}

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Abstract

Tetracyclines constitute one of the most important antibiotic families and represent a classic example of phototoxicity. The photoproducts of tetracyclines and their parent compounds have potentially adverse effects on natural ecosystem. In this study, the self-sensitized oxidation products of tetracycline (TC) and oxytetracycline (OTC) were determined and the effects of Ca^{2+} and Mg^{2+} on self-sensitized degradation were investigated. The Ca^{2+} and Mg^{2+} in the natural water sample accounted for enhancement (pH 7.3) and inhibition (pH 9.0) of photodegradation of TC and OTC due to the formation of metal-ions complexes. The formation of Mg^{2+} complexes was unfavorable for the photodegradation of the tetracyclines at both pH values. In contrast, the Ca^{2+} complexes facilitated the attack of singlet oxygen ($^1\text{O}_2$) arising from self-sensitization at pH 7.3 and enhanced TC photodegradation. For the first time, self-sensitized oxidation products of TC and OTC were verified by quenching experiments and detected by LC/ESI-DAD-MS. The products had a nominal mass 14 Da higher than the parent drugs (designated M+14), which resulted from the $^1\text{O}_2$ attack of the dimethylamino group on the C-4 atom of the tetracyclines. The presence of Ca^{2+} and Mg^{2+} also affected the generation of M+14 due to the formation of metal-ions complexes with TC and OTC. The findings suggest that the metal-ion complexation has significant impact on the self-sensitized oxidation processes and the photoproducts of tetracyclines.

Key words: tetracyclines; complexation; self-sensitized oxidation; photoproducts

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Introduction

As an emerging class of environmental pollutants, pharmaceuticals and personal care products (PPCPs) have been the subject of increasing concern and scientific interest (Daughton and Ternes, 1999; Halling-Sørensen et al., 1998). Vast arrays of pharmaceuticals have been detected in aquatic environments throughout the world every year. Of particular concern is the detection of antibiotics due to increasing bacterial resistance and detrimental impact on the ecosystems (Witte, 1998; Sarmah et al., 2006; Pruden et al., 2006). Tetracyclines are one of the most frequently prescribed antibiotics used as therapeutics and growth promoters in cattle, swine, poultry and fish husbandry (Sanderson et al., 2005; Fritz and Zuo, 2007). They are poorly absorbed after intake by human and animals, with more than 70% of tetracyclines leaving the organism unmetabolized via urine or feces (Hirsch et al., 1999). Tetracyclines have been widely detected in soils, surface

waters, groundwater and swine wastewater (Jacobsen et al., 2004; Lindsey et al., 2001; Kolpin et al., 2002; Batt and Aga, 2005; Ben et al., 2008).

Due to the antibacterial activity of tetracyclines, biodegradation can be anticipated to play a negligible role in the natural elimination processes (Eichhorn and Aga, 2004). Tetracyclines absorb light in the UV-A region and they are photolabile. Tetracyclines are also phototoxic antibiotics (Dürckheimer, 1975) and their photoproducts may exhibit a potentially higher adverse risk on microorganisms compared to the parent drugs (Jiao et al., 2008a, 2008b). Knowledge of the photochemical processes and detection of the photoproducts are therefore important to assess the environmental impact of tetracyclines. A number of studies have identified the photoproducts of TC in organic or aqueous media under different light source irradiation (Eichhorn and Aga, 2004; Jiao et al., 2008a, 2008b; Oka et al., 1989; Drexel et al., 1990; Morrison et al., 1991; Davies et al., 1979; Moore et al., 1983; Hasan et al., 1985). However, very few studies have been carried out under environmentally relevant conditions. A

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quinone derivative was reported in the presence of oxygen in aqueous TC solution, even though the structure of this compound has been less well characterized (Davies et al., 1979). Additionally, Oka et al. (1989) isolated seven photoproducts, which had lost their TC structure under conditions similar to a culture pond. While our previous work demonstrated that TC photodegradation was associated with self-sensitized oxidation processes (Chen et al., 2008), little is known about self-sensitized oxidation products.

The self-sensitization process is related to the deprotonation of TC. Higher pH is more favorable for the deprotonation and the self-sensitized oxidation (Chen et al., 2008). At natural pH, deprotonation is generally attributed to the β diketone moiety (C11–C12) of the BCD rings and the C4 dimethylammonium of the A ring, respectively (Fig. 1). Both groups can be implicated by direct interaction with Ca²⁺ and Mg²⁺ ions (Wessels et al., 1998; Lambs et al., 1988), which are ubiquitous in natural water. Accordingly, Ca²⁺ and Mg²⁺ ions are likely to influence the deprotonation and self-sensitized oxidation of TC. Although Ca²⁺ and Mg²⁺ ions affect TC photodegradation (Werner et al., 2006), the effect of the metal ions on self-sensitized oxidation processes and photoproducts has not been well understood.

In the present study, TC and OTC were chosen as the model compounds of tetracyclines. The kinetics of TC and OTC were examined in a natural and a deionized water sample to screen the effect factors on photodegradation. The effect of Ca²⁺ and Mg²⁺ on the photodegradation of TC and OTC was investigated. The self-sensitized oxidation processes were assessed via a series of ¹O₂ quenching experiments in aqueous solutions. The self-sensitized oxidation products of TC and OTC were identified by liquid chromatography/electrospray-mass spectrometry (LC/ESI-MS). Based on the ¹O₂ quenching experiments

and the photoproducts identified, a self-sensitized oxidation process was proposed.

1 Materials and methods

1.1 Chemicals

Both TC (98%) and OTC (95%) were purchased from the Sigma Chemical Corporation (USA) and used as received. The CaCl₂, MgCl₂·6H₂O, and sodium azide were supplied by Wuhan Chemicals Corporation, China. Stock solutions of TC and OTC were prepared fresh daily from the solid in deionized water for photolysis experiments. All chemicals used were of at least analytical-reagent grade.

1.2 Photolysis experiments

Tetracyclines solutions (20 μ mol/L) in deionized water (DI H₂O) were photolyzed alongside tetracyclines solutions (20 μ mol/L) in a natural water sample (DJW, from Dongjiang river in southern China; 0.22 μ m filtered; Ca²⁺ and Mg²⁺ concentrations of 2.11 and 1.87 mg/L, respectively; and pH = 7.3) (Table S1). The photodegradation experiments were conducted under 150-W xenon short arc lamp in a 60 mL cylindrical Pyrex vessel (40 mm i.d., containing 50 mL of solution). Light of wavelengths of less than 300 nm were filtered with the Pyrex glass to simulate sunlight. Inhibition experiments were carried out with addition of ¹O₂ quencher sodium azide (10 mmol/L). All experiments were conducted in Tris-HCl (10 mmol/L) buffered solutions. Aliquots of samples (300 μ L) were withdrawn at various intervals and substrate decay was measured by high performance liquid chromatography (HPLC).

1.3 HPLC analysis

The concentrations of TC and OTC were analyzed by an Alliance 2695 HPLC system with 2996 Diode Array Detector (Waters, USA) and XTerra MS column (5 μ m, 250 mm \times 2.1 mm). The optimized mobile phase was 15% methanol-25% acetonitrile-60% 0.01 mol/L oxalic acid aqueous solution. The flow rate was 1.0 mL/min and the photodiode array detector was set at 355 nm.

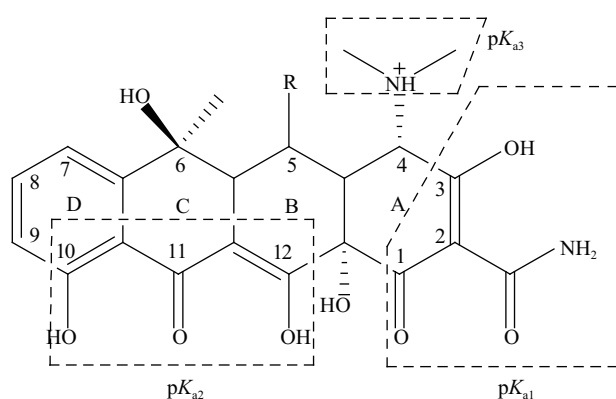
1.4 Mass spectrometry

Photoproducts were analyzed by an Alliance 2690 HPLC system equipped with a single-quadrupole mass spectrometer ZQ 4000 (Micromass, Manchester, UK). Full-scans of product ions were obtained in positive ionization mode. Mass spectrums were obtained by scanning the quadrupole from 200 to 500 *m/z* with a 1-sec scan and setting the cone voltage at 30 V.

2 Results and discussion

2.1 Natural water photolysis

Photodegradation of TC and OTC in DJW was about twice as rapid as that in DI H₂O at pH 7.3 (Table 1). However, the inhibition effect was observed when the pH of DJW was raised to 9.0. According to the water



Compound	R ₁	R ₂	pK _{a1}	pK _{a2}	pK _{a3}
TC	H	H	3.30	7.68	9.69
OTC	HO	H	3.27	7.32	9.11

Fig. 1 Molecular structure of TC (R=H) and OTC (R=OH). The corresponding pK_a values in aqueous solutions were from Stephens et al. (1956).

Table 1 Observed rate constants (k_{obs}) for photodegradation of TC and OTC in the DI H₂O and DJW at different pH values

Compound	System	k_{obs} (min ⁻¹)	
		pH 7.3	pH 9.0
TC	DI H ₂ O	0.0083	0.037
	DJW	0.0192	0.021
OTC	DI H ₂ O	0.0084	0.033
	DJW	0.0156	0.016

Results are the means of triplicate measurements; initial concentration of TC or OTC was 20 $\mu\text{mol/L}$.

DI H₂O: deionized water; DJW: a nature water sample.

characterization data (Table S1), the concentrations of photoreactive species such as iron ion (0.0062 mmol/L), nitrate (0.012 mmol/L), and dissolve organic matter (TOC 1.96 mg/L) were low. In view of the rapid direct photodegradation of the tetracyclines, their effect on the degradation of TC and OTC was less important in this study. Although the concentration of Na⁺ (0.41 mmol/L) was high enough, the effect of Na⁺ on photodegradation was negligible (data not shown). It is known that TC and OTC can form complexes with Ca²⁺ and Mg²⁺ (Wessels et al., 1998; Lambs et al., 1988; Schmitt and Schneider, 2000), which are rich in DJW. The complexation behavior was pH dependent, thereby giving rise to a distinct effect on the photodegradation of TC and OTC at different pH values. Accordingly, the formation of metal-ion complexes of tetracyclines was likely responsible for the enhancement or inhibition of photodegradation.

2.2 Effect of Ca²⁺ and Mg²⁺ on photolysis

As shown in Fig. 2a, the addition of Mg²⁺ slightly inhibited the photolysis of TC and OTC at pH 7.3, while an obvious enhancement was found in the presence of Ca²⁺. Therefore, Ca²⁺ was responsible for the enhancement effect of photodegradation of the tetracyclines in DJW at pH 7.3. For both Ca²⁺ and Mg²⁺, the pronounced inhibition effect on photodegradation was observed at pH 9.0 (Fig. 2b), which accounted for the slower degradation of TC and OTC in DJW compared to the DI H₂O. The complexation of tetracyclines Ca²⁺ and Mg²⁺ also results in a bathochromic shift of the absorption spectra (Schmitt and Schneider, 2000), which contributes to the light absorption. The inhibition effect of Ca²⁺ and Mg²⁺ at pH 9.0 suggested that spectra change played a minor role in the different photodegradation between pH 7.3 and 9.0. The enhancement or inhibition effect at different pH values was attributed to the metal ion complexation with TC and OTC, which may affect the underlying degradation mechanism.

2.3 Self-sensitized oxidation

It has been established that ¹O₂ can be generated in irradiated TC and OTC solutions (Chen et al., 2008; Seto et al., 2010). In our previous work, we demonstrated that self-sensitized oxidation occurred concurrently with direct photodegradation of TC (Chen et al., 2008). In this experiment, the self-sensitization effect was examined in the presence of Ca²⁺ and Mg²⁺ in the tetracyclines solutions. As shown in Fig. 3, the addition of the ¹O₂

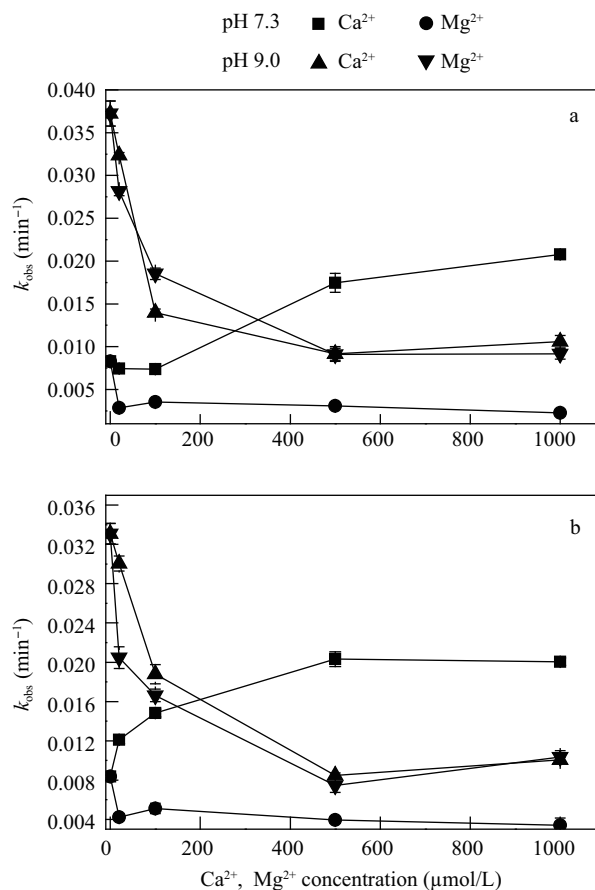


Fig. 2 Photodegradation of TC (a) and OTC (b) in the presence of Ca²⁺, Mg²⁺ at pH 7.3 and 9.0. Error bars indicate 95% confidence intervals ($n = 3$).

quencher sodium azide did not affect the photodegradation of tetracyclines in the presence of Mg²⁺ at pH 7.3 and 9.0, suggesting self-sensitized oxidation was negligible due to the complexation of Mg²⁺ with TC and OTC at both pH values. A similar result was obtained for Ca²⁺ at pH 9.0 (Fig. 3). However, the addition of sodium azide markedly inhibited the photodegradation of TC and OTC in the presence of Ca²⁺ at pH 7.3, indicating that self-sensitized oxidation occurred in the Ca²⁺-tetracyclines solutions. Accordingly, the ¹O₂ quenching experiments explained the enhancement effect of Ca²⁺ in the natural water sample at pH 7.3.

The distinct role of Ca²⁺ at pH 7.3 can be attributed to the different metal-ion binding sites at the complexes of tetracyclines. According to our previous results, the higher solution pH was more favorable for the self-sensitized oxidation of TC (Chen et al., 2008), suggesting that the deprotonation of the dimethylamino group facilitated the molecule attack of electrophilic ¹O₂. In addition, previous reports have indicated that the electron transfer reaction occurs between some amines and ¹O₂ (Baciocchi et al., 2006a, 2006b). Therefore, ¹O₂ formed in the tetracyclines attacks the dimethylamino group. This attack ability depends on the availability of the lone-pair electrons on the N atom (N-electrons). Deprotonation of N4 of the dimethylamino group increases the availability of N-electrons. The coordinating to the dimethylamino-O3 will decrease the availability of N-electrons, thereby inhibiting

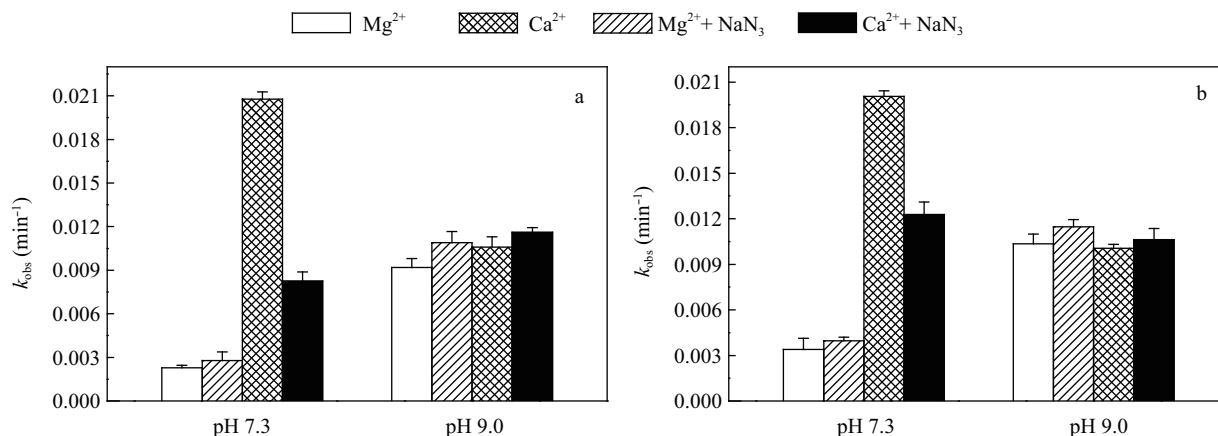


Fig. 3 Inhibition effect of sodium azide on the photodegradation of TC (a) and OTC (b) in the presence of Ca²⁺ and Mg²⁺. Initial conditions: 10 mmol/L sodium azide, 20.0 μmol/L TC and OTC, 10 mmol/L Ca²⁺ and Mg²⁺, respectively. Error bars indicate 95% confidence intervals for $n = 3$.

the self-sensitized oxidation.

Different binding sites have been assigned for the two metal ions at pH 8, i.e., Mg²⁺ coordinating to the N4-O3 site and Ca²⁺ coordinating to O10-O11 and O12-O1 sites of TC (Wessels et al., 1998). Assuming similar coordinating occurred at pH 7.3, the Mg²⁺ complexation will lead to a decrease in the availability of N-electrons and thus disfavor the attack of ¹O₂ on the dimethylamino group of tetracyclines. For Ca²⁺, the coordinating to O10-O11 and O12-O1 sites gave rise to the deprotonation of N4 (Wessels et al., 1998), and thus facilitated the self-sensitized oxidation. When the pH was raised, the N4 atoms compete easily with the BCD ring system for the binding of metal ions (Lamb et al., 1988). Accordingly, the inhibition effect of Ca²⁺ and Mg²⁺ was likely associated with the competitive metal ions coordinating to the N4 atoms of the tetracyclines at pH 9.0.

2.4 Identification of photoproducts

The experiments described above clearly showed that TC and OTC photodegradation was related to self-sensitized oxidation. To further ascertain the attack site of ¹O₂ on the dimethylamino group and clarify the underlying mechanism for direct photolysis, the self-sensitized oxidation products of TC and OTC were monitored by LC/ESI-DAD-MS in (+)-full-scan mode from m/z 100 to 550. Identical photoproducts were formed under solar irradiation as obtained in the laboratory (Fig. S1), indicating that photochemical processes of tetracyclines were identical in both light sources. As shown in Fig. 4, the main photoproducts were m/z 413.5 and 459.5 for TC (m/z 445.5). The photoproducts of OTC (m/z 461.5) were m/z 475.5 and 413.5 (Fig. S2).

As seen from the main photoproducts, the products m/z 459.5 of TC and m/z 475.5 of OTC were both 14 Da higher than the parent compounds (designated M+14). The kinetics experiments indicated that both photoproducts were diminished after the addition of the ¹O₂ quencher sodium azide in the tetracyclines solutions (Fig. S3). Therefore, the photoproducts M+14 were attributed to the self-sensitized oxidation of TC and OTC. Figure 5a shows the ESI (+)-MS of the initial solution of TC (1). An intense

and predominant ion of m/z 445.5 was observed, which corresponded to [1+H]⁺. The two fragment ions were identified as [1+H-NH₃]⁺ and [1+H-NH₃-H₂O]⁺ (Oka et al., 1998). As shown in Fig. 5b, the photoproduct m/z 459.5 (2) displayed similar fragment ions to TC, but an important fragment ion m/z 430.5, corresponding to [2+H-CHO]⁺, was observed. This indicated an aldehyde group (HC=O) was formed after irradiation of TC in aqueous solution. Likewise, the OTC photoproduct m/z 475.5 showed a fragment ion of m/z 446.5 [M+H-CHO]⁺ (Fig. S2). Based on the self-sensitized oxidation mechanism and the molecular fragments of the photoproducts [M+14], the aldehyde group (HC=O) resulted from the attack of ¹O₂ to the dimethylamino moiety on C-4 atom of the tetracyclines (Fig. 6).

2.5 Mechanism and proposed photolysis processes

There is a general agreement on the hypothesis that the physical and chemical quenching of ¹O₂ by amines involves the formation of an exciplex with partial charge transfer (CT) as the reaction intermediate (Schweitzer and Schmidt, 2003). In addition, a close analogy exists between the properties of ¹O₂ (an electronically excited state) and those of ketone triplets (Gorman et al., 1984). Therefore, it is reasonable that an electron-transfer interaction occurred

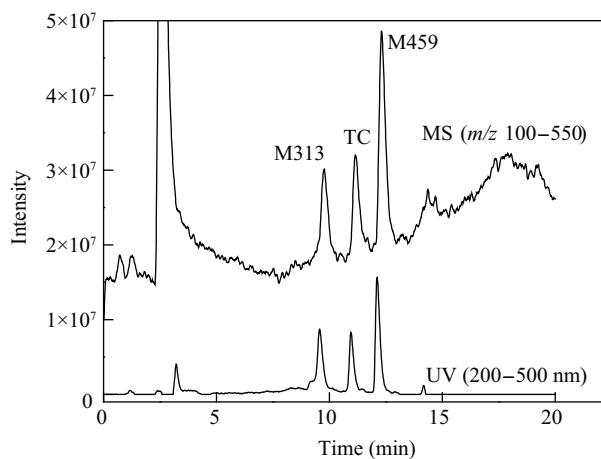


Fig. 4 (+)-LC/ESI-DAD-MS chromatogram of the photoproducts from the irradiation experiment of TC.

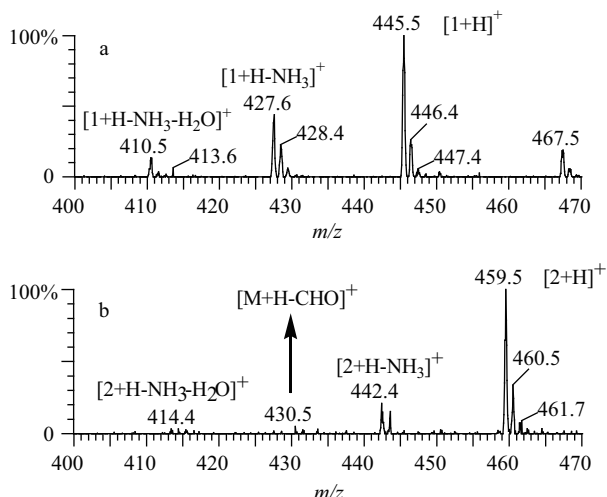


Fig. 5 (+)-ESI mass spectra of TC solution before (a) and after (b) irradiation.

between amines and $^1\text{O}_2$ similar to the reactions of triplet ketones with amines (Chen et al., 2009; Canonica et al., 1995). The effect of Ca^{2+} and Mg^{2+} in this study showed that the formation of metal-ions complexes of the tetracyclines affected the availability of the N-electrons, thus enhancing or inhibiting self-sensitized photolysis (Fig. 2). Accordingly, an electron-transfer interaction between $^1\text{O}_2$ arising from self-sensitization and the tetracyclines (TC and OTC) was proposed. As illustrated in Fig. 6, the electron-transfer interaction between $^1\text{O}_2$ and TC (OTC) led to the exciplex (process a). The exciplex was transient intermediate which can undergo intersystem crossing (isc) to give the tetracyclines and $^3\text{O}_2$ (physical quenching, process b), or be converted into products (chemical quenching, process c and d). For chemical quenching, the first step was the electron-transfer interaction between the N atom

of the tetracyclines and $^1\text{O}_2$, followed by a rapid transfer of the hydrogen from α -carbon (α -hydrogen) on the dimethylamino group (process c) or the A ring (process d) to form the carbon radical intermediates. The carbon radical intermediate from process d reacted with O_2 and led to m/z 459.5 or 475.5 (process e). Another carbon radical intermediate from process d may lose the dimethylamino group and be further oxidized to quinone (process f), which has been detected in irradiated TC solution by Davies et al. (1979).

3 Conclusions

The self-sensitized degradation of TC and OTC occurred in the aqueous solutions under simulated sunlight. The $^1\text{O}_2$ arising from the self-sensitization process oxidized the dimethylamino moiety on the C4 atom of the tetracyclines into N-methylformamide group. The presence of Ca^{2+} in the solution facilitated the generation of self-sensitized oxidation products at pH 7.3, but a marked inhibition was observed at pH 9.0. In contrast, Mg^{2+} was unfavorable for the generation of the photoproducts at both pH values. The metal-ion complexes of tetracyclines may have different ecological impacts from the metal-free tetracyclines due to the effect of the complexes on the degradation rate and the self-sensitized photoproducts.

Acknowledgments

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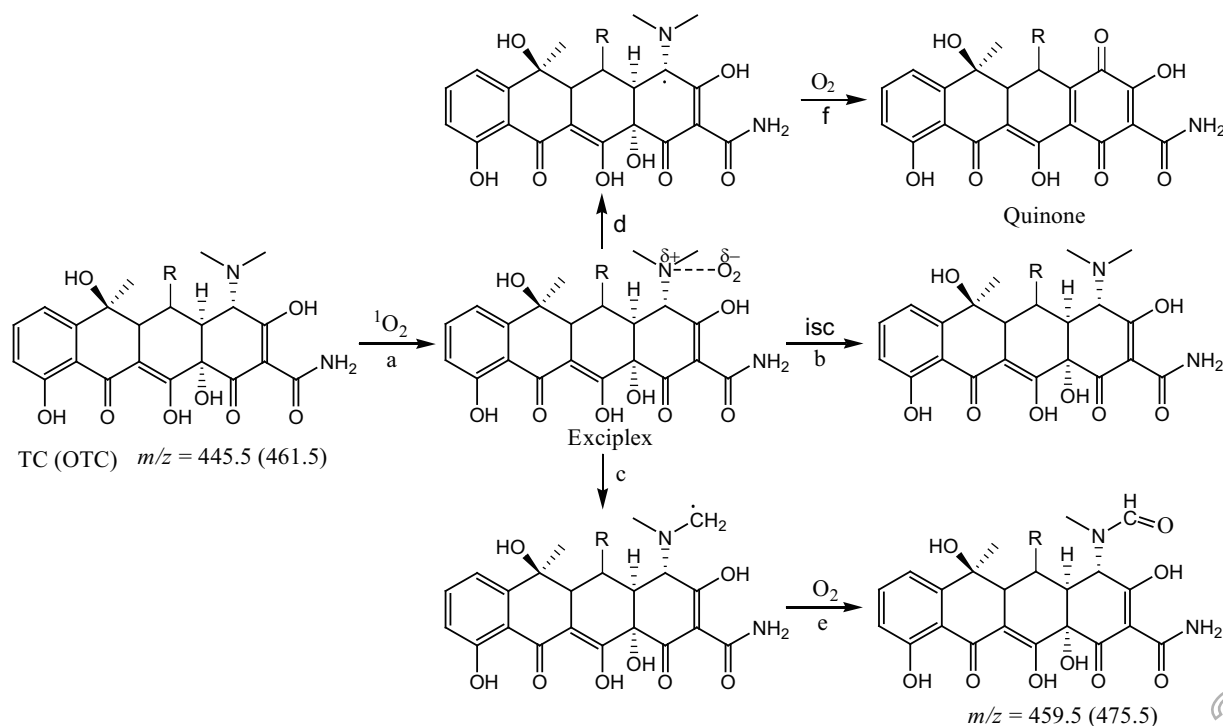


Fig. 6 Photoproducts and proposed pathway of TC (R=H) and OTC (R=OH) in aqueous solutions.

al Water Environmental Special Program of China (No. 2008ZX07211-10-02).

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Supporting materials

Supplementary data associated with this article can be found in the online version.

Table S1 Characteristics of Dongjiang River natural water sample used in this work

Parameter	Parameter		
Na	9.34 mg/L	SO ₄	15.22 mg/L
Mg	1.87 mg/L	Cl	4.97 mg/L
Al	0	NO ₃	0.73 mg/L
K	1.36 mg/L	pH	7.3
Ca	2.11 mg/L	TOC	1.96 mg/L
Fe	0.35 mg/L		
Mn	0.53 mg/L		

Total organic carbon (TOC) of the water sample was analyzed with a Phoenix 8000 TOC analyzer. The anions were analyzed with a DX-120 Ion Chromatograph Dionex ion chromatography system. The cations were analyzed by ICP mass spectrometry from PerkinElmer, USA.

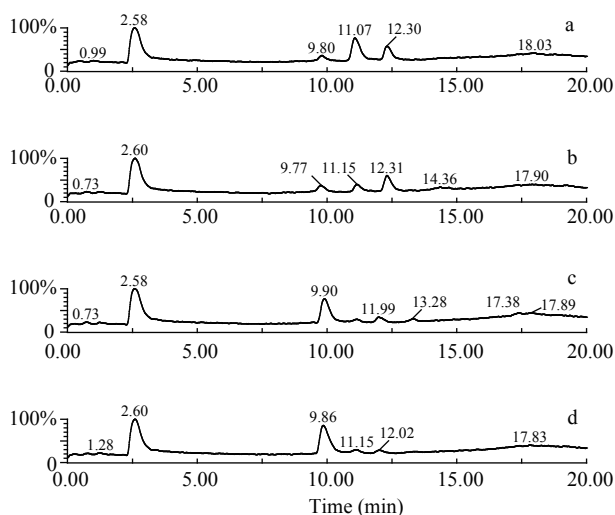


Fig. S1 (+)-LC/ESI-MS total ion chromatograms of the photoproducts from the irradiation experiments of (a) TC under sunlight, (b) TC under simulated sunlight, (c) OTC under sunlight, and (d) OTC under simulated sunlight.

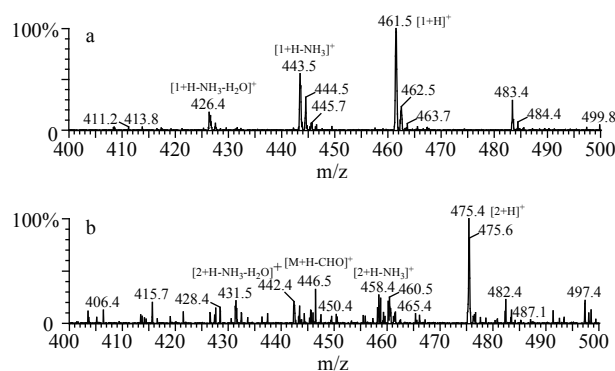


Fig. S2 ESI mass spectra of OTC (a) and its sensitized oxidation photo-products (b).

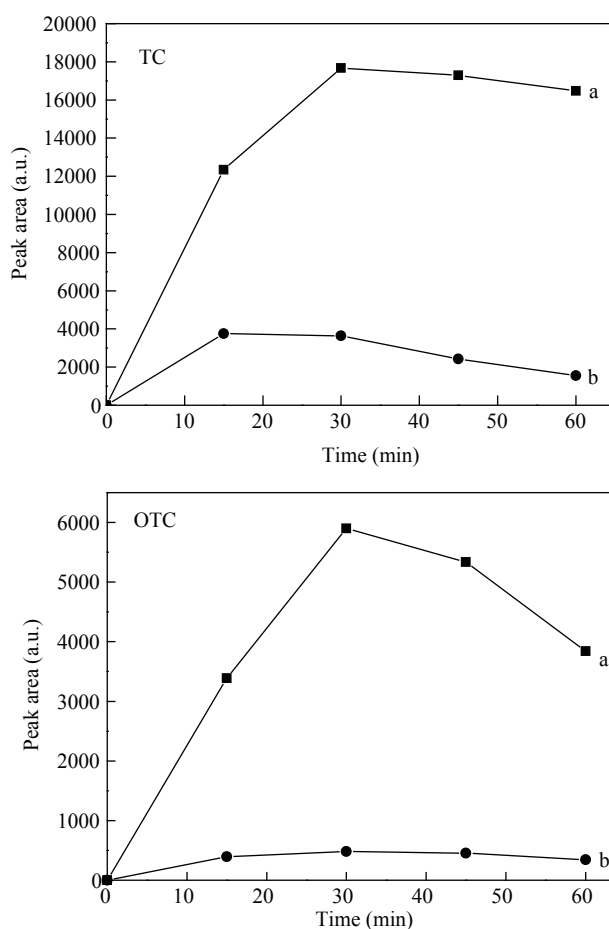


Fig. S3 Comparison of the peak area of the photoproducts M+14 in the TC and OTC solutions in the absence (line a) and presence (line b) of the ¹O₂ quencher sodium azide (10 mmol/L).