Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

Direct photolysis of oxytetracycline: Influence of initial concentration, pH and temperature

Xin Jin^{a,b,e}, Huaizhou Xu^c, Shanshan Qiu^d, Mingyun Jia^a, Fang Wang^a, Aiqian Zhang^{b,f,*}, Xin Jiang^{a,e,**}

^a Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing, 210008, China

^b State Key Laboratory of Pollutant Control and Resource Reuse, School of Environment, Nanjing University, Nanjing, 210023, China

^c Key Laboratory of Pesticide Environmental Assessment and Pollution Control, Nanjing Institute of Environmental Science, Ministry of Environmental Protection, Nanjing, 210042, China

^d School of Chemical Engineering, Nanjing University of Science and Technology, Nanjing, 210094, China

^e University of Chinese Academy of Sciences, Beijing, 100049, China

^f Research Center for Eco-Environmental Science, Chinese Academy of Sciences, Beijing, 100085, China

ARTICLE INFO

Article history:

Received 22 February 2016

Received in revised form 1 August 2016

Accepted 30 August 2016

Available online 1 September 2016

Keywords:

Oxytetracycline
Direct photolysis
Quantum yield
pH
Temperature

ABSTRACT

Oxytetracycline (OTC) commonly undergoes direct photolysis as the main elimination pathway in surface waters. The photolysis of OTC was studied as a function of initial concentration, pH and temperature. The experiments were performed in the absence of oxygen to avoid oxygen induced indirect photolysis. The photolysis rate decreased when increasing the initial OTC concentration, and was accelerated as raising the temperature. pH was the key factor controlling the photolysis rate. By measuring the UV–vis absorption spectra of OTC under various pH conditions and fitting them with global analysis, four OTC species H_3L^+ , $H_2L^\pm$, HL^- , and L^{2-} (L represents OTC) were decomposed, meanwhile, the acid dissociation constant of OTC and the molar absorption coefficient of each species were resolved. Because apparent photolysis rate constants under varied light source conditions are often incomparable, therefore the direct photolysis quantum yield of OTC species were calculated by a mathematical approach which based on the molar absorption coefficients, the species composition and apparent kinetic rates of OTC under experimental pH conditions. The result shows the photolysis quantum yield of OTC followed the order $L^{2-} > H_2L^\pm > HL^- > H_3L^+$. The direct photolysis rates of OTC were tightly correlated to the energy dissipation processes at the excited states, e.g., the excited states inter-/intramolecular proton transfer (ESIPT), and the molecular conformation transfer induced internal energy transmission between ground state and excited state. Fluorescence emission was not an important energy dissipation process. The results of this study provide the useful parameters for predicting the environmental fate of OTC in surface waters. And the approach for calculating the photolysis quantum yield is also available for other antibiotics with multiple pKa values.

© 2016 Published by Elsevier B.V.

1. Introduction

Oxytetracycline (OTC) is a compound belonging to the class of tetracyclines (TCs). TCs are broad-spectrum antibiotics which are widely used for veterinary purposes in livestock and poultry industry [1,2]. Due to their poor absorption efficiency, more than

70% of the intake is excreted through feces into environment [3]. The non-point source discharge from urban waste and agricultural manure application further spread antibiotics into the environment. TCs find their way into the natural surface waters through municipal sewage [4] and field runoff [5]. TCs concentrations in surface waters are detected up to several tens of micro-gram per liter with a quite high detection frequency up to 90% [6–8].

The main elimination pathways for TCs in surface waters are adsorption [9,10], hydrolysis [11,12], and photochemical transformation [13–16], while biological processes seem not to be effective to degrade TCs [1,16]. Table S1 (Supplementary material, SM)

* Corresponding author at: 18 Shuangqing Road, Beijing, 100085, China.

** Corresponding author at: 71 East Beijing Road, Nanjing, 21008, China.

E-mail addresses: aqzhang@rcees.ac.cn (A. Zhang), jiangxin@issas.ac.cn (X. Jiang).

reviews hydrolysis and photolysis rates of OTC from previous studies. Compared to hydrolysis, the photolysis rate of OTC under sunlight exposure was about an order of magnitude faster [13–16]. It indicates photodegradation is a very important dissipation pathway of OTC in surface waters. Since, the photolysis kinetics typically depends on the light intensity, the photolysis rates from peers are incomparable, e.g., the kinetic rate under the exposure of artificial lights was one to three orders of magnitude higher than that under natural sunlight [14–19]. Therefore, the published kinetic data can hardly be used to predict the photolysis fate of OTC in the natural environment. It's necessary to provide the photolysis quantum yield which is independent of the illumination intensity, and represents the intrinsic photochemical reactivity of a compound. In previous studies, the quantum yields of tetracycline (TTC) species have already been provided by Werner et al. [21]. However, the physicochemical properties of TCs congeners could be significantly varied as was for instance observed in hydrolysis [11]. Therefore, it is worth to choose another compound belonging to TCs, e.g. OTC, for study.

Moreover, the photolysis quantum yields of TTC by Werner et al. [21] were obtained from an open air condition. It is known that TCs photodegradation in aerobic condition undergoes three pathways [22]: Direct photolysis, photosensitization, and catalytic degradation. Direct photolysis is the main decomposition process of TCs in natural surface waters [22]. The latter two are indirect pathways in the presence of oxygen [17,19,22,33–39]. In order to reveal the intrinsic photochemical reactivity of OTC, the direct photolysis was studied in the absence of oxygen. There are three reasons for getting rid of oxygen: (1) The concentration of reactive oxygen species (ROS) are temporally and spatially varied in laboratory conditions and in natural surface waters [23]. (2) Different light intensity will produce different concentrations of ROS. (3) Natural humus and NO_3^- play double roles of either enhancing TCs photolysis through photosensitization, or suppressing it as a result of competitive photo absorption and ROS scavenging [24,37,38].

In addition, previous studies revealed that antibiotic concentrations in surface waters or ground waters were seasonally variable. The concentration level was higher in winter than in summer [7,20]. Temperature could be a significant factor for hydrolysis and photolysis decomposition. The hydrolysis rate of TCs is closely temperature dependent that an increase of 10°C doubled the hydrolysis rate of OTC [11,13]. However, it is unknown whether and how significantly seasonal temperature variations will influence the direct photolysis rate of OTC.

Based on the above justifications, the main purpose of this study is to reveal the effect of pH and temperature on the direct photolysis of OTC, and to obtain the direct photolysis quantum yield of OTC species through experimental and mathematical approaches.

2. Materials and methods

2.1. Spectroscopy analysis

Oxytetracycline dihydrate (ACROS Organic, USA, chemical purity >95%) was initially dissolved in methanol, and diluted by Milli-Q water to the final concentration of $50\text{ }\mu\text{M}$. Methanol as a co-solvent accounted for 1% of the final volume. The OTC solutions were adjusted to a series of pH values from 2.0 to 12.0 by HCl or NaOH, and absorption spectra were measured by a UV-2450 spectrometer (SHIMADZU, Japan). The pH mediated fluorescence spectrum was measured by a Cary Eclipse Fluorescence Spectrophotometer (Agilent, USA) at an exciting wavelength of 365 nm. The fluorescence quantum yield was determined by taking Rhodamine B as a reference compound, $\phi_F = 0.70$ at 20°C [25].

2.2. Direct photolysis of OTC

The photolysis kinetics of OTC was studied as a function of initial concentration, pH, and temperature. The OTC solutions for photolysis were prepared in the same way as for the spectra study. In consideration of an appropriate degradation half-life and sampling interval, the initial concentration for photolysis was prepared at $80\text{ }\mu\text{M}$. HCl or NaOH was applied when the effect of pH was studied. The pH kept stable during the reaction, therefore no buffer was introduced to avoid any interference. Experiments were performed with a photochemical reactor XPA-1 (Xujiang Electromechanical Plant, Nanjing, China) equipped with a 100 W mercury lamp. The relative luminous intensity distribution of the mercury lamp was provided by the Xujiang Electromechanical Plant and shown in Fig. S1. The lamp was placed in the center of the instrument, and cooled down by a cooling water system. The quartz cuvettes as the reaction containers were placed in the holders surrounding the lamp. The actual radiation intensity of the light source in the position of the quartz cuvettes was measured to be $I_{(365\text{nm})} = 1500\text{ }\mu\text{W}/\text{cm}^2$, $I_{(297\text{nm})} = 840\text{ }\mu\text{W}/\text{cm}^2$, and $I_{(254\text{nm})} = 730\text{ }\mu\text{W}/\text{cm}^2$. Glass filters, ZWB2, were placed between the lamp and the quartz cuvettes to cut off the wavelength below 300 nm. The reaction solution can only be irradiated by the light passed through the glass filters. A 25 mL OTC solution was filled into the quartz cuvette ($\Phi 26\text{mm} \times 182\text{mm}$). During the reaction, the quartz cuvettes rotated around the lamp to get a homogeneous irradiation. In order to keep an oxygen free condition, the dissolved oxygen was purged by high pure N_2 (99.99%) at a consistent flow rate of 0.3 L/min 30 min before the reaction started, and during the reaction. The XPA-1 photochemical reactor was placed in a closed metal box to avoid sun light. A fan was in the wall of the metal box to exchange air from inside to outside. A thermometer was placed in the metal box to record the room temperature during the whole photolysis.

XPA-1 reactor cannot control the reaction temperature. In order to study the effect of temperature on the direct photolysis of OTC, another photochemical reactor XPA-7 (Xujiang Electromechanical Plant, Nanjing, China) equipped with a water bath controller was applied to control the reaction temperature at 15, 25 and 45°C .

2.3. Analytical method

OTC concentration was measured by HPLC (Agilent 1100, USA) with a ZORBAX SB-C18 column (Agilent, $4.6 \times 250\text{ mm}$, $5\text{ }\mu\text{m}$). Acetonitrile and water (with 10 mM oxalic, pH = 2.0) was used for the elution at a ratio of 30/70 (v/v), and at a flow rate of 0.5 mL/min. OTC was quantified by UV detector at 275 nm and 355 nm. The photolysis product was identified by LC–MS (Finnigan LCQ Advantage MAX, USA) with the electrospray ionization (ESI) source. The LC–MS method used the same ZORBAX SB-C18 column for separation, and the elution of acetonitrile/acetic solution (0.1%, pH 3.0), 40/60 (v/v).

3. Quantum yield calculation

OTC photolysis followed a pseudo-first order kinetics (show in the results part). The apparent degradation rate contributes by each component in the system:

$$e^{k_{app} \cdot t} = \alpha_1 \cdot e^{k_{comp,1} \cdot t} + \alpha_2 \cdot e^{k_{comp,2} \cdot t} + \dots + \alpha_n \cdot e^{k_{comp,n} \cdot t} \quad (1)$$

Where α is the fraction of the component. k_{comp} (s^{-1}) is the first-order rate constant of each individual component. k_{app} (s^{-1}) is the apparent first-order rate constant of OTC which was measured by the dissipation rate of OTC molecules. In this study, the quantum yield based on the initial photolysis rate, therefore t tends to be

Fig. 1. Molecular structure of oxytetracycline (OTC). The regions in dash frame were the three acid dissociation moieties.

zero. The Eq. (1) can be approximated to

$$k_{app} = \alpha_1 \cdot k_{comp.1} + \alpha_2 \cdot k_{comp.2} + \dots + \alpha_n \cdot k_{comp.n} \quad (2)$$

Set A , $K_{comp.}$, and $K_{app.}$ as the matrix of $\alpha_1 \sim \alpha_{nm}$, $k_{comp.}^1 \sim k_{comp.}^n$, and $k_{app.}^1 \sim k_{app.}^m$ of all the components in multi-conditions. n represents the total components. m represents the total pH conditions.

The independent rate constant of each component was then derived with

$$K_{comp.} = (A^{-1} \cdot A)^T \cdot A^{-1} \cdot K_{app.} \quad (3)$$

That is:

$$\begin{pmatrix} k_{comp.}^1 \\ \vdots \\ k_{comp.}^n \end{pmatrix} = \begin{pmatrix} \alpha_1 & \alpha_2 & \dots & \alpha_n \\ \alpha_{12} & \ddots & & \vdots \\ \vdots & & \ddots & \vdots \\ \alpha_{1n} & \dots & \dots & \alpha_{nm} \end{pmatrix}^{-1} \cdot \begin{pmatrix} \alpha_1 & \alpha_2 & \dots & \alpha_n \\ \alpha_{12} & \ddots & & \vdots \\ \vdots & & \ddots & \vdots \\ \alpha_{1n} & \dots & \dots & \alpha_{nm} \end{pmatrix}^T \cdot \begin{pmatrix} k_{app.}^1 \\ k_{app.}^2 \\ \vdots \\ k_{app.}^m \end{pmatrix}$$

The quantum yield of each OTC species was calculated from the individual rate constant and the molar absorption coefficient [26]:

$$\phi_d = \frac{-k_{comp.}}{2.303 \cdot \sum(I_{0\lambda} \cdot \varepsilon_{\lambda}) \cdot (S/V) \cdot l} \quad (4)$$

Where $I_{0\lambda}$ (Einstein $\text{cm}^{-2} \text{s}^{-1}$) is the incident light intensity at wavelength λ . $I(\text{photons} \cdot \text{cm}^{-2} \cdot \text{s}^{-1}) = I(W \cdot \text{cm}^{-2} \cdot \text{nm}^{-1}) \times \lambda(\text{nm}) \times 5.035 \times 10^{15}$ [27]. 1 Einstein = 1 mol of light = 6.02×10^{23} photons. ε_{λ} (L mole $^{-1} \text{cm}^{-1}$) is the molar absorption coefficient; S (cm^2) is the exposed area; V (mL) is the volume of solution; l (cm) is the light path length. In this case, the mercury lamp is a non-continue brand spectrum, the total light absorption efficiency, $\sum(I_{0\lambda} \cdot \varepsilon_{\lambda})$, was the sum of the photons absorbed under the multiple irradiation wavelengths.

4. Results

4.1. pH mediated absorption spectra

OTC generally presents four species, according to pH, they are H_3L^+ , $\text{H}_2\text{L}^{\pm}$, HL^- , and L^{2-} (L represents OTC). The acid dissociation constants have been studied to be 3.22 (pK_{a1}), 7.46 (pK_{a2}), 8.94 (pK_{a3}) by potentiometric titration [28]. The carbon frame of OTC is labeled in Fig. 1. The pK_{a1} occurs at OH_3 site of the tricarbonyl group ($\text{C}_1\text{--}\text{C}_3$), the pK_{a2} derives from the deprotonation of OH_{12} of the phenolic-diketone group ($\text{C}_{11}\text{--}\text{C}_{12}$), and the pK_{a3} is attributed to the deprotonation of the dimethyamino group NH_{4a} [29,30]. Duarte et al. [40] proposed a fourth pK_{a4} for TCs with the value up to 11.8 which should be assigned to the OH_{10} , because the hydrogen bond between OH_{10} and O_{11} stabilizes the hydroxyl. As shown in Fig. 2a, OTC has two absorption bands, namely the bathochromic band at 353–376 nm, and the hypsochromic band at around 270 nm. pH was the key factor of controlling the absorption spectrum. From pH 2.0–3.8, H_3L^+ transformed to $\text{H}_2\text{L}^{\pm}$ with the deprotonating of OH_3 . The hypsochromic band shifted to long-wavelength. From pH 5.5–12.0, $\text{H}_2\text{L}^{\pm}$ transformed into HL^- and L^{2-} , and the bathochromic band shifted widely to the long-wavelength while the hypsochromic band shifted in reverse. It was not possible to distinguish the transformation of $\text{H}_2\text{L}^{\pm} \rightarrow \text{HL}^-$ and $\text{HL}^- \rightarrow \text{L}^{2-}$ because the absorption spectrum changed smoothly from pH 5.5–12.0 without apparent segmentation. This is in agreement with Qing and Adam [28] according to the potentiometric titration curve where there was no significant boundary between pK_{a2} and pK_{a3} . It indicated proton dissociated continually and simultaneously from OH_{12} and NH_{4a} (Fig. 1).

The absorption spectra of OTC at multiple pH conditions (Fig. 2a) was fitted by the global analysis and derived the molar absorption coefficients of H_3L^+ , $\text{H}_2\text{L}^{\pm}$, HL^- , and L^{2-} (Fig. 2b), as well as the acid dissociation constants (pK_a) of OTC (Table 1). The process was performed by the software ReactLab EQUILIBRIA (Jplus Consulting). The computational pK_a values were very close to the values derived from experimental potentiometric titration [28]. Therefore, global analysis can be an economic and promising mathematical way to obtain the molar absorption coefficients and pK_a values of compounds with multiple deprotonation sites.

Fig. 2. (a) UV-vis absorption spectrum of OTC as a function of pH, [OTC] = 50 μM ; (b) Molar absorption constant of the four OTC species. L stands for the full deprotonated OTC molecule.

Table 1

Acid dissociation constants of OTC.

equilibrium	pKa		Ref. [28]
$\text{H}^+ + \text{L}^{2-} \rightleftharpoons \text{HL}^-$	8.71 ± 0.017^a	9.08 ± 0.004^b	8.94 ± 0.30
$\text{H}^+ + \text{HL}^- \rightleftharpoons \text{H}_2\text{L}^{\pm}$	7.54 ± 0.005^a	6.89 ± 0.047^b	7.46 ± 0.03
$\text{H}^+ + \text{H}_2\text{L}^{\pm} \rightleftharpoons \text{H}_3\text{L}^+$	3.26 ± 0.006^a	2.90 ± 0.301^b	3.22 ± 0.30

^a Parameters derived from global fitting of the UV–vis absorption spectra.^b Parameters derived from global fitting of the fluorescence spectra.

4.2. The static-state fluorescence spectra

As shown in Fig. 3, the fluorescence emission was greatly enhanced in alkaline condition. The independent fluorescence emission constants of H_3L^+ , $\text{H}_2\text{L}^{\pm}$, HL^- , and L^{2-} (Fig. 3b) and the acid dissociation constants (Table 1) were also resolved by global analysis in the software ReactLab EQUILIBRIA. The fluorescence intensity of L^{2-} was 10- to 30-fold stronger than the other protonated species. The fluorescence emission peaks of HL^- and L^{2-} appeared at around 500 nm, while those of H_3L^+ and $\text{H}_2\text{L}^{\pm}$ shifted to around 600 nm. The fluorescence quantum yield was determined by comparative method using Rhodamine B as the reference standard. The values were calculated to be 3.3×10^{-4} (H_3L^+), 4.5×10^{-4} ($\text{H}_2\text{L}^{\pm}$), 1.1×10^{-3} (HL^-), and 9.7×10^{-3} (L^{2-}), in the same order of magnitude to that reported by Carloti et al. [31 a], i.e. 6×10^{-4} (pH 2.3), 8.5×10^{-4} (pH 5.0), 1.8×10^{-3} (pH 9.0), and 3×10^{-3} (pH 11).

4.3. Photolytic kinetics

The photolysis of OTC followed a pseudo first-order kinetics. The apparent rate constants and the half-lives were fully provided in Table S2 (SM). According to the initial rate constant, the half-lives of OTC at pH 1.4, 3.9, 5.7, 7.6 and 9.2 were estimated to be 194, 41, 35, 47, and 22 min, respectively. The photochemical reactivity of OTC in alkaline conditions was much higher than that in acid and neutral conditions.

The effect of concentration on OTC photolysis was pH dependent. At pH 5.5, the photolysis rate constant decreased with increasing the initial OTC concentration from 20 μM to 80 μM (Fig. 4a). At pH 1.4, the photolysis rate after 30 min was higher than the initial rate (Fig. 4b). On the contrary, at pH 9.2, the rate constant greatly decreased as reaction proceeded (Fig. 4b). Since the aqueous pH kept stable during the whole reaction, it was supposed to attribute to the change of OTC concentration. The results were in coincidence with the previous reports of either acid conditions [17,18] or alkaline conditions [22].

Fig. 4. Direct photolysis kinetics of (a) OTC at pH 5.5 with different initial concentration; (b) OTC at different pH conditions, $[\text{OTC}]_0 = 80 \mu\text{M}$; (c) OTC at different temperature, $[\text{OTC}]_0 = 80 \mu\text{M}$.

Fig. 4c shows the effect of temperature on OTC photolysis. Increasing temperature accelerated the photolysis rate of OTC by about 1.4-fold (40 °C vs. 25 °C) and 1.2-fold (25 °C vs. 15 °C) at pH around 6.

Fig. 3. (a) The fluorescence spectra of OTC according to pH. (b) Independent fluorescence emission constants of the four species of OTC. $[\text{OTC}] = 50 \mu\text{M}$, L stands for the fully deprotonated OTC molecule.

Fig. 5. HPLC chromatograph of OTC and photolysis intermediates. OTC molecule and one intermediate were detected by LC–MS with the m/z = 461.3 and 416.5, respectively.

4.4. Products

The photolysis products appeared the same in different pH conditions. Four product peaks were detected in the HPLC chromatograph, and one product with m/z of 416.5 was recognized by LC–MS (Fig. 5). 4-dedimethylaminotetracycline (DTC) and lumitetracycline (LTC) were studied to be two primary photolysis products of tetracycline (TTC) in the oxygen free organic or aqueous solvents [44–46]. The dimethylamine was detected with the amount equivalent to the LTC [46]. Since OTC is analogous to TTC, the product with the m/z of 416.5 was assigned to be DOTC[−] whose dimethylamino group was dissected. LTC forms the bond between C₄ and C₁₁ but has the mass charge ratio 414.5. Liu et al. [37–39] presented detailed photodecomposition pathways of OTC by UV photolysis or UV/AOP (advanced oxidation process), but species with m/z of 416.5 was not detected. It probably suggested the decomposition pathway of OTC in the absence of oxygen is different to that in the presence of oxygen. The parent compound was detected to be H₃L⁺ with the m/z of 461.3. However, the other three product peaks were not detected by the mass spectra.

5. Discussion

5.1. The effect of concentration and pH on photolysis kinetics

It is speculated that photolysis of OTC will compete with the nonproductive energy loss pathways such as fluorescence emission and non-radiative vibrational relaxation in the excited states (OTC*). The bathochromic band of OTC appears at around 350 nm, while the maximum fluorescence emission was at 500 nm or 600 nm. Such a large Stokes shift suggested some non-radiative

dissipation processes occurred to OTC*. It is commonly recognized that aryl alcohols are considerably more acidic in their excited singlet states than in their ground states [41]. Take 2-Naphthol as an example, its pK_a in the ground state is 9.46, while the pK_a^* turns to 2.8 upon excitation to its lowest excited singlet state [42]. The photo induced molecular excitation promotes the proton dissociating in aqueous solutions. OTC follows the same law that the increased acidity of OTC* at the excited state facilitates the inter-/intramolecular proton transfer (ESIPT). It was evidenced by the time-resolved fluorescence spectra that the decay time of OTC* in methanol > deuterated water > normal water [32]. It was also evidenced by the Stokes shift and the fluorescence quantum yield that, from aqueous solution to organic solvent, the Stokes shift increased and the fluorescence quantum yield decreased [31a,b].

The effect of concentration and pH on the direct photolysis of OTC was possibly related to the ESIPT process. (1) When OTC was protonated at O₁₂ position, the ESIPT processes among OH₁₀ and OH₁₂ could cause significant energy dissipation of the excited OTC*. High concentrations of H₃L⁺ or H₂L[±] probably facilitated the inter-/intramolecular proton transfer. Therefore, the photolysis rate constant decreased as the concentration increased (Fig. 4a and Fig. 4b pH 1.4). (2) From the protonated species H₃L⁺ to the fully deprotonated species L^{2−}, the bathochromic absorption spectra of OTC shifted to red (Fig. 2b), while the fluorescence spectra shifted to blue (Fig. 3b). The Stokes shift reduced as pH increased. It suggested that highly protonated OTC molecule performs stronger energy dissipation processes like ESIPT. Therefore, the photolysis rate of OTC increased as pH increasing (Fig. 4b).

On the contrary, the photolysis rate constant decreased as the concentration decreased in alkaline conditions (Fig. 4b, pH 9.2). Werner et al. [21] observed the same phenomenon and ascribed it

Fig. 6. Second-order rate kinetic of OTC photolysis in alkaline condition. pH = 9.2. $[OTC]_0 = 80 \mu\text{M}$.

to the decrease of ROS generated by photosensitization. However, this photolysis of OTC was performed in the oxygen free condition where OTC molecule and its photolysis products could be the only photosensitizers. The fluorescence emission in alkaline conditions (500–600 nm) could not excite OTC molecules whose spectra absorption occurred below 400 nm. One possible hypothesis is that thermodynamic collision reaction occurred simultaneously with photo exciting under alkaline condition. It was assumed two OTC* molecules of excited states collided and induced the thermodynamic collision reaction. By this hypothesis, the degradation kinetics in alkaline condition should follow a second-order rate kinetics, $d[OTC^*]/dt = -k \cdot [OTC^*]^2$. OTC* population of the excited state was proportional to the concentration of OTC of the ground state. It was reformulated to $1/[OTC] = k \cdot t + c$, where k (min^{-1}) is the thermodynamic reaction rate constant, and c is the intercept. As shown in Fig. 6, $1/[OTC]$ vs. t at pH 9.2 perfectly followed the second-order rate kinetic model ($R^2 = 0.999$). In comparing, the degradation curve of pH 3.9, 5.7, 7.6 all followed the first-order rate kinetics (Fig. 4b). It indicated the thermodynamic collision process should be much more intensive in alkaline condition than that in acid/neutral condition.

5.2. The effect of temperature on photolysis kinetic

Photolysis of organic compounds is usually regarded as non-temperature dependent. However, a thermodynamic collision model was proposed to occur accompany with the photolysis of OTC* (Section 5.1). It strongly hints temperature would have an effect on the photolysis of OTC. In order to further verify the hypothesis, the effect of temperature on OTC photolysis was studied (Fig. 4c). According to the Arrhenius equation $-\ln k_{app} = \ln A + \frac{E_a}{R} \cdot \frac{1}{T}$, the experimental activation energy E_a of photolysis of $H_2L^{\pm*}$ was calculated to be 15.5 kJ/mol. The photolysis activation energy in neutral conditions was much smaller than that of OTC's hydrolysis which is 28.35 ~ 93.56 kJ/mol

[11,13]. In addition, the E_a was ignorable comparing to the electron transition energy from $H_2L^{\pm}$ to $H_2L^{\pm*}$ which was calculated to be 339 kJ/mol at 354 nm. Nevertheless, such activation energy indicated the direct photolysis of OTC will be accelerated in higher temperature conditions. And it can be speculated that the effect of temperature on OTC photolysis should be much more intensive in alkaline conditions than in acid/neutral condition.

5.3. Quantum yield of OTC species

Direct photolysis quantum yield (ϕ_d) represents the intrinsic photolysis efficiency independent of the light exposure. According to Eq. (4), in order to get the quantum yield of each OTC species, the molar absorption coefficient and the first-order rate constant of individual species should be obtained in advance. In this study, the independent molar absorption coefficients of H_3L^+ , $H_2L^{\pm}$, HL^- , and L^{2-} , as well as the pK_a values were successfully decomposed and resolved by global analysis (Fig. 2b). According to the pK_a values (Table 1), the species composition under certain pH conditions was calculated. The photolysis quantum yields (ϕ_d) of each OTC species were then calculated according to the mathematical approach in part 3 "Quantum yield calculation" and the result was provided in Table 2.

The quantum yield of L^{2-} was 2- to 10-fold higher than H_3L^+ , $H_2L^{\pm}$, and HL^- . The high photochemical reactivity in alkaline conditions was in agreement with the fluorescence activity, that fluorescence emission of L^{2-} was much stronger than the other species. Although fluorescence emission lost energy, it only covered less than 1.0% of the energy obtained according to the fluorescence quantum yield (9.7×10^{-3} of L^{2-}). In reverse, the intensive fluorescence emission of L^{2-} indicated L^{2-*} of the excited states are highly populated. This has been evidenced by the time-resolved fluorescence that the excited OTC* species held longer decay time in alkaline conditions [33]. On the other hand, it also could be contributed by the thermodynamic collision reaction which was proposed to occur to L^{2-*} accompanying with direct photolysis reaction.

The photolysis quantum yields of OTC species were in the same order of magnitude with TTC species reported by Werner et al. [21]. It is unexpected that the photolysis quantum yield of species HL^- (L stands for OTC) was lower than $H_2L^{\pm}$. The result was distinct from the previous reports [17,18,21,33]. However, the results are to some extent un-comparably because this study was performed in the oxygen-free condition additionally with 1% methanol. Methanol played a role of radical scavenger. It is already known the photolysis rate would be decreased in oxygen-free and ROS-free conditions [33,34]. Both $H_2L^{\pm}$ and HL^- showed relatively low fluorescence intensities, while the fluorescence shifted to blue from $H_2L^{\pm}$ to HL^- . The Stokes shift reduced from the width of 220 nm ($H_2L^{\pm}$) to that of 145 nm (HL^-). All suggested the energy dissipation processes probably changed when $H_2L^{\pm}$ was transferred into HL^- and L^{2-} . One possible explanation for this abnormal

Table 2
Quantum yield of OTC species^a.

Species	$ K_{app} /\text{min}^{-1}$	H_3L^+	$H_2L^{\pm}$	HL^-	L^{2-}
pH = 1.4	>0.00357	98.7%	1.3%		
pH = 3.9	0.0171	17.4%	82.6%		
pH = 5.7	0.0201	0.3%	98%	1.7%	
pH = 7.6	0.0148		40.8%	56.6%	2.6%
pH = 9.2	<0.0321		0.6%	34.4%	65.0%
$K_{comp}/10^{-4} \text{ s}^{-1}$		>0.6	3.3	1.7	<7.3
$\sum(I_{\lambda} \cdot \epsilon_{\lambda})/\text{s}^{-1}$		0.106	0.121	0.129	0.126
$\phi_d/10^{-4}$		>2.5	12	5.7	<25

phenomenon is that the molecular conformation transfer from the ground state to the excited state resulted in the internal energy transmission. Species $\text{H}_2\text{L}^\pm$ and HL^- holds the twisted conformation (A) of which the protonated dimethylamino group stands above the BCD plane as a result of steric hindrance. The species L^{2-} holds the extended conformation (B) where the deprotonated dimethylamino group lies under the BCD plane. The twisted conformation (A) has higher molecule internal energy than the extended conformation (B) [43]. As it is stated in Section 4.1, from $\text{H}_2\text{L}^\pm$ to HL^- and L^{2-} , proton dissociated simultaneously from OH_{12} and NH_{4a} sites. Therefore, OTC holds a small fraction of the extended conformation (B) after pK_{a2} , namely, OTC mainly exists as $\text{H}_2\text{L}^\pm$ and HL^- at pH 3.9 and 5.7, while partly exists as L^{2-} at pH 7.6. The acidic of OTC increases in the excited state, $\text{H}_2\text{L}^{\pm*}$ and HL^-* transferred to L^{2-*} flashily by UV excitation. During this process, the molecule geometry adjusted from the twisted conformation (A) to the extended conformation (B). The internal energy fall from conformation A to conformation B might contribute for OTC decomposition. As a result, less energy contributed for OTC decomposition at pH 7.6 comparing to pH 3.9 and 5.7. The direct photolysis rate at pH 7.6 was slower than that at pH 3.9 and 5.7.

6. Conclusion

The direct photolysis rates of OTC were tested in oxygen free conditions as a function of the initial concentration, pH and temperature. pH significantly affected the photolysis rate of OTC that the photolysis rate increased as pH increasing. The effect of pH and concentration was suggested to attribute to the energy dissipation process of the excited states intra-/intermolecular proton transfer (ESIPT), and the molecular conformation transfer induced internal energy transmission. The photolysis quantum yield was calculated as the order $\text{L}^{2-} > \text{H}_2\text{L}^\pm > \text{HL}^- > \text{H}_3\text{L}^+$. Temperature has an effect on OTC photolysis. It was implied that thermodynamic decomposition processes occurred along with photolysis reactions in neutral and alkaline conditions. Several parameters were derived from this study: The acid dissociation constants, the molar absorption coefficients, and the direct photolysis quantum yield of the four OTC species. These parameters can in reverse serve for modeling the photolysis fate of OTC. However, the quantum yield of OTC from this study only accounts for the direct photolysis. The whole photolysis decomposition of OTC is also contributed by photosensitization and catalytic degradation. To predict the natural photolysis fate of OTC in field conditions, the indirect photolysis pathways should be further investigated independently [35–39].

Acknowledgements

The authors wish to thank Dr. Reiner Schroll and Dr. Ulrike Dörfler of Helmholtz Zentrum München for their full support in the fluorescence measurement, and their helpful comments that improved the manuscript. This study was financially supported by the National Key Basic Research Program of China (No. 2014CB441105), the Outstanding Youth Fund of Natural Science Foundation of Jiangsu, China (BK20150050), and the National Natural Science Foundation of China (21507036, 21377138, 41271327).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jphotochem.2016.08.032>.

References

- [1] A.K. Sarmah, M.T. Meyer, A.B.A. Boxall, A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment, *Chemosphere* 65 (5) (2006) 725–759.
- [2] X. Pan, Z. Qiang, W. Ben, M. Chen, Residual veterinary antibiotics in swine manure from concentrated animal feeding operations in Shandong Province, China, *Chemosphere* 84 (5) (2011) 695–700.
- [3] R. Daghrir, P. Drogui, Tetracycline antibiotics in the environment: a review, *Environ. Chem. Lett.* 11 (3) (2013) 209–227.
- [4] H. Liu, G. Zhang, C.-Q. Liu, L. Li, M. Xiang, The occurrence of chloramphenicol and tetracyclines in municipal sewage and the Nanming River, Guiyang City, China, *J. Environ. Monit.* 11 (6) (2009) 1199–1205.
- [5] S.K. Frey, E. Topp, I.U.H. Khan, B.R. Ball, M. Edwards, N. Gottschall, M. Sunohara, D.R. Lapen, Quantitative *Campylobacter* spp., antibiotic resistance genes, and veterinary antibiotics in surface and ground water following manure application: influence of tile drainage control, *Sci. Total. Environ.* 532 (2015) 138–153.
- [6] R. Wei, F. Ge, S. Huang, M. Chen, R. Wang, Occurrence of veterinary antibiotics in animal wastewater and surface water around farms in Jiangsu Province, China, *Chemosphere* 82 (10) (2011) 1408–1414.
- [7] L. Jiang, X. Hu, D. Yin, H. Zhang, Z. Yu, Occurrence, distribution and seasonal variation of antibiotics in the Huangpu River, Shanghai, China, *Chemosphere* 82 (6) (2011) 822–828.
- [8] L. Yao, Y. Wang, L. Tong, Y. Li, Y. Deng, W. Guo, Y. Gan, Seasonal variation of antibiotics concentration in the aquatic environment: a case study at Jiangnan Plain, central China, *Sci. Total. Environ.* 527 (2015) 56–64.
- [9] D. Fernández-Calviño, A. Bermúdez-Couso, M. Arias-Estévez, J. Nóvoa-Muñoz, M. Fernández-Sanjurjo, E. Álvarez-Rodríguez, A. Núñez-Delgado, Kinetics of tetracycline, oxytetracycline, and chlortetracycline adsorption and desorption on two acid soils, *Environ. Sci. Pollut. Res.* 22 (1) (2015) 425–433.
- [10] K. Chen, J.L. Zhou, Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China, *Chemosphere* 95 (2014) 604–612.
- [11] K.A. Loftin, C.D. Adams, M.T. Meyer, R. Surampalli, Effects of ionic strength, temperature, and pH on degradation of selected antibiotics, *J. Environ. Qual.* 37 (2) (2008) 378–386.
- [12] H.J. Kang, M.Y. Lim, J.H. Kwon, Effects of adsorption onto silica sand particles on the hydrolysis of tetracycline antibiotics, *J. Environ. Monit.* 14 (7) (2012) 1853–1859.
- [13] X. Richeng, A. Lestley, W. Qiquan, S.R. Yates, K.C. Biswas, Hydrolysis and photolysis of oxytetracycline in aqueous solution, *J. Environ. Sci. Health* 45 (1) (2010) 73–81.
- [14] B. Halling-Sørensen, G. Sengeløv, F. Ingerslev, L.B. Jensen, Reduced antimicrobial potencies of oxytetracycline, tylosin, sulfadiazin, streptomycin, ciprofloxacin, and olaquinox due to environmental processes, *Arch. Environ. Contam. Toxicol.* 44 (1) (2003) 0007–0016.
- [15] H. Pouliquen, M. Larhantec-Verdier, M.L. Morvan, H.L. Bris, Comparative hydrolysis and photolysis of four antibacterial agents (oxytetracycline oxolinic acid, flumequine and florfenicol) in deionised water, freshwater and seawater under abiotic conditions, *Aquaculture* 262 (1) (2007) 23–28.
- [16] A. García-Rodríguez, V. Matamoros, C. Fontàs, V. Salvadó, The influence of light exposure, water quality and vegetation on the removal of sulfonamides and tetracyclines: a laboratory-scale study, *Chemosphere* 90 (8) (2013) 2297–2302.
- [17] J.J. López-Peñalver, M. Sánchez-Polo, C.V. Gómez-Pacheco, J. Rivera-Utrilla, Photodegradation of tetracyclines in aqueous solution by using UV and UV/ H_2O_2 oxidation processes, *J. Chem. Technol. Biotechnol.* 85 (10) (2010) 1325–1333.
- [18] J. Shaojun, S. Zheng, Y. Daqiang, W. Lianhong, C. Liangyan, Aqueous oxytetracycline degradation and the toxicity change of degradation compounds in photoirradiation process, *J. Environ. Sci.* 20 (7) (2008) 806–813.
- [19] Y. Chen, H. Li, Z. Wang, T. Tao, C. Hu, Photoproducts of tetracycline and oxytetracycline involving self-sensitized oxidation in aqueous solutions: effects of Ca^{2+} and Mg^{2+} , *J. Environ. Sci.* 23 (10) (2011) 1634–1639.
- [20] J. Tang, T. Shi, X. Wu, H. Cao, X. Li, R. Hua, F. Tang, Y. Yue, The occurrence and distribution of antibiotics in Lake Chaohu, China: seasonal variation, potential source and risk assessment, *Chemosphere* 122 (2015) 154–161.
- [21] J.J. Werner, W.A. Arnold, K. McNeill, Water hardness as a photochemical Parameter: Tetracycline photolysis as a function of calcium concentration, magnesium concentration, and pH, *Environ. Sci. Technol.* 40 (23) (2006) 7236–7241.
- [22] Y. Chen, C. Hu, J. Qu, M. Yang, Photodegradation of tetracycline and formation of reactive oxygen species in aqueous tetracycline solution under simulated sunlight irradiation, *J. Photochem. Photobiol. A* 197 (1) (2008) 81–87.
- [23] B.M. Peterson, A.M. McNally, R.M. Cory, J.D. Thoemke, J.B. Cotner, K. McNeill, Spatial and temporal distribution of singlet oxygen in Lake Superior, *Environ. Sci. Technol.* 46 (13) (2012) 7222–7229.
- [24] J. Niu, L. Yang, W. Wang, Light-source-dependent role of nitrate and humic acid in tetracycline photolysis: kinetics and mechanism, *Chemosphere* 92 (11) (2013) 1423–1429.
- [25] R.F. Kubin, A.N. Fletcher, Fluorescence quantum yields of some rhodamine dyes, *J. Lumin.* 27 (4) (1983) 455–462.
- [26] R.G. Zepp, Quantum yields for reaction of pollutants in dilute aqueous solution, *Environ. Sci. Technol.* 12 (3) (1978) 327–329.

- [27] R.G. Zepp, D.M. Cline, Rates of direct photolysis in aquatic environment, *Environ. Sci. Technol.* 11 (4) (1977) 359–366.
- [28] Z. Qiang, C. Adams, Potentiometric determination of acid dissociation constants (pKa) for human and veterinary antibiotics, *Water Res.* 38 (12) (2004) 2874–2890.
- [29] P. Kulshrestha, R.F. Giese, D.S. Aga, Investigating the molecular interactions of oxytetracycline in clay and organic matter: insights on factors affecting its mobility in soil, *Environ. Sci. Technol.* 38 (15) (2004) 4097–4105.
- [30] J. Wessels, W. Ford, W. Szymczak, S. Schneider, The complexation of tetracycline and anhydrotetracycline with Mg^{2+} and Ca^{2+} : a spectroscopic study, *J. Phys. Chem. B* 102 (46) (1998) 9323–9331.
- [31] (a) B. Carloti, A. Cesaretti, F. Elisei, Complexes of tetracyclines with divalent metal cations investigated by stationary and femtosecond-pulsed techniques, *Phys. Chem. Chem. Phys.* 14 (2) (2012) 823–834;
(b) B. Carloti, D. Fuoco, F. Elisei, Fast and ultrafast spectroscopic investigation of tetracycline derivatives in organic and aqueous media, *Phys. Chem. Chem. Phys.* 12 (48) (2010) 15580–15591.
- [32] S. Schneider, M.O. Schmitt, G. Brehm, M. Reiher, P. Matousek, M. Towrie, Fluorescence kinetics of aqueous solutions of tetracycline and its complexes with Mg^{2+} and Ca^{2+} , *Photochem. Photobiol. Sci.* 2 (11) (2003) 1107–1117.
- [33] C. Zhao, M. Pelaez, X. Duan, H. Deng, K. O'Shea, D. Fatta-Kassinos, D.D. Dionysiou, Role of pH on photolytic and photocatalytic degradation of antibiotic oxytetracycline in aqueous solution under visible/solar light: kinetics and mechanism studies, *Appl. Catal. B-Environ.* 134–135 (0) (2013) 83–92.
- [34] X.D. Zhu, Y.J. Wang, R.J. Sun, D.M. Zhou, Photocatalytic degradation of tetracycline in aqueous solution by nanosized TiO_2 , *Chemosphere* 92 (8) (2013) 925–932.
- [35] W.-R. Chen, C.-H. Huang, Transformation of tetracyclines mediated by Mn (II) and Cu (II) ions in the presence of oxygen, *Environ. Sci. Technol.* 43 (2) (2008) 401–407.
- [36] H. Wang, H. Yao, P. Sun, D. Li, C.-H. Huang, Transformation of tetracycline antibiotics and Fe(II) and Fe(III) species induced by their complexation, *Environ. Sci. Technol.* 50 (1) (2016) 145–153.
- [37] Y. Liu, X. He, Y. Fu, D.D. Dionysiou, Kinetics and mechanism investigation on the destruction of oxytetracycline by UV-254 nm activation of persulfate, *J. Hazard. Mater.* 305 (2016) 229–239.
- [38] Y. Liu, X. He, X. Duan, Y. Fu, D. Fatta-Kassinos, D.D. Dionysiou, Significant role of UV and carbonate radical on the degradation of 2 oxytetracycline in UV-AOPs: kinetics and mechanism, *Water Res.* 95 (2016) 195–204.
- [39] Y. Liu, X. He, Y. Fu, D.D. Dionysiou, Degradation kinetics and mechanism of oxytetracycline by hydroxyl radical-based advanced oxidation processes, *Chem. Eng. J.* 284 (2016) 1317–1327.
- [40] H.A. Duarte, S. Carvalho, E.B. Paniago, A.M. Simas, Importance of tautomers in the chemical behavior of tetracyclines, *J. Pharm. Sci.* 88 (1) (1999) 111–120.
- [41] M. Lawrence, C.J. Marzocco, C. Morton, C. Schwab, A.M. Halpern, Excited-state deprotonation of 2-naphthol by anions, *J. Phys. Chem.* 95 (25) (1991) 484–490.
- [42] A. Weller, Fast reactions of excited molecules, *Prog. React. Kinet. Mech.* 1 (1961) 187.
- [43] X. Jin, S. Qiu, K. Wu, M. Jia, F. Wang, C. Gu, A. Zhang, X. Jiang, The effect of Cu^{2+} chelation on the direct photolysis of oxytetracycline: a study assisted by spectroscopy analysis and DFT calculation, *Environ. Pollut.* 214 (2016) 821–839.
- [44] J.J. Hlavka, P. Bitha, Photochemistry IV. a photodeamination, *Tetrahedron Lett.* 32 (1966) 3843–3846.
- [45] R.E. Drexel, G. Olack, C. Jones, G.N. Chmurny, R. Santini, H. Morrison, Lumitetracycline: a novel new tetracycline photoproduct, *J. Org. Chem.* 55 (1990) 2471–2478.
- [46] H. Morrison, G. Olack, C. Xiao, Organic photochemistry. 93. Photochemical and photophysical studies of tetracycline, *J. Am. Chem. Soc.* 113 (21) (1991) 8110–8118.