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Hydrolysis and photolysis of oxytetracycline in aqueous solution

RICHENG XUAN[1], LESTLEY ARISI[1], QIQUAN WANG[1], SCOTT R. YATES[2] and KEKA C. BISWAS[3]

[1]*Chemistry Department, Delaware State University, Dover, Delaware*
[2]*US Department of Agriculture-Agricultural Research Science, U.S. Salinity Laboratory, Riverside, California*
[3]*Department of Science, Wesley College, Dover, Delaware*

Oxytetracycline ((2Z,4S,4aR,5S,5aR,6S,12aS)-2-(amino-hydroxy-methylidene)-4-dimethylamino-5,6,10,11,12a-pentahydroxy-6-methyl-4,4a,5,5a-tetrahydrotetracene-1,3,12-trione) is a member of tetracycline antibiotics family and is widely administered to farm animals for the purpose of therapeutical treatment and health protection. Increasing attention has been paid to the environmental fate of oxytetracycline and other veterinary antibiotics with the occurrence of these antibiotics in the environment. The hydrolysis and photolysis degradation of oxytetracycline was investigated in this study. Oxytetracycline hydrolysis was found to obey the first-order model and similar rate constant values ranging from 0.094 ± 0.001 to 0.106 ± 0.003 day^{-1} were obtained at different initial concentration ranging from 10 to 230 μM. Solution pH and temperature were shown to have remarked effects on oxytetracycline hydrolysis. The hydrolysis in pH neutral solution appeared to be much faster than in both acidic and alkaline solutions. Oxytetracycline half-life decreased from 1.2×10^2 to 0.15 day with the increasing temperature from 4 ± 0.8 to 60 ± 1°C. The presence of Ca^{2+} made oxytetracycline hydrolytic degradation kinetics deviate from the simple first-order model to the availability-adjusted first-order model and greatly slowed down the hydrolysis. Oxytetracycline photolysis was found to be very fast with a degradation rate constant at 3.61 ± 0.06 day^{-1}, which is comparable to that of hydrolysis at 60°C. The presence of Ca^{2+} accelerated oxytetracycline photolysis, implying that oxytetracycline become more vulnerable to sunlight irradiation after chelating with Ca^{2+}. The photolysis may be the dominant degradation pathway of oxytetracycline in shallow transparent water environment.

Keywords: Oxytetracycline; hydrolysis; photolysis; degradation; calcium cation; kinetics.

Introduction

Veterinary antibiotics are widely administrated to animals in modern confined animal feeding operations to treat infection and disease, protect animal health, and improve feed efficiency.[1] Based on the sales data released by Animal Health Institute, 21.8 million pounds of antibiotics were applied to farm and companion animals in the United State in 2004, of which 30% are tetracyclines.[2] The administered veterinary antibiotics may eventually enter the environment and become environmental contaminants. In fish farming, antibiotics are given as feed additives, resulting in direct releases of antibiotics into the water environment.[3,4] In livestock and poultry farming, antibiotics are excreted in urine and feces by animals shortly after medication.[5] With the disposal of animal waste into the agricultural lands as fertilizers, antibiotics enter the soil environment.[6] Through leaching and runoff, antibiotics may contaminate water bodies.[7,8]

The contamination of veterinary antibiotics in the water environment has been reported. Based on a nationwide reconnaissance of stream water conducted 1999-2000 by the U.S. Geological Survey, at least one antibiotic was detected in approximately 50% samples with a maximum concentration of 1.9 μg L^{-1}.[9] Another investigation of antibiotic contamination in the water of 13 U.S. fish hatcheries. revealed that the detection frequency of tetracycline, oxytetracycline, and sulfadimethoxine were 1, 4, and 12%, respectively.[4] Besides surface water, ground water contamination by antibiotics has also been reported. The concentrations of sulfamethazine in ground water samples from an agricultural area in Germany were detected to be 0.08–0.16 μg L^{-1}.[10]

The occurrence of antibiotics in the environment may lead the development of antibiotic resistance genes in microorganisms, which can be transferred to human beings and animals through food chains and drinking water,[11,12] resulting in failure of antibiotic treatment of infections. In addition, the occurrence of antibiotics in

Address correspondence to Qiquan Wang, Chemistry Department, Delaware State University, Dover, DE 19901; E-mail: qwang@desu.edu
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water environment exposes human beings and animals to constant low concentrations of antibiotics through drinking water contamination. Though the effects of such kind of long-term exposure are not clear, the potential danger resulting from veterinary antibiotic contamination to human and animal health can not be neglected.[6]

Tetracyclines are a major veterinary antibiotic family with good activities against acute diseases caused by gram-positive and gram-negative bacteria. Tetracyclines have been widely administered to fish,[4] pigs,[13] poultry animals,[14] and cattle[5] and some of their environmental fate and transport have been investigated. A soil sorption study indicated that oxytetracycline, a major member of tetracyclines, is strongly adsorbed in soil regardless of soil type and thus only weakly mobile.[15] In an overland flow study, oxytetracycline concentration in runoff from a manure-applied field was detected to be 71.1 μg L^{-1}, illustrating overland flow as a route by which oxytetracycline may be transported to surface water.[7] The half-life of oxytetracycline in calf manure was determined to be 30 days, which is much longer than that of tylosin, a macrolide antibiotic.[5] Microorganisms were found to be responsible for the major degradation of oxytetracycline in animal manure.[16]

In the water environment, hydrolysis and photolysis may be major degradation routes for antibiotics. It has been reported that acidic conditions favor oxytetracycline stability and alkaline conditions favor oxytetracycline degradation.[17] Oxytetracycline was found to be much more vulnerable to both hydrolysis and photolysis than the other three investigated antibiotics, including oxolinic acid, flumequine, and florfenicol.[18] Temperature and pH were found to significantly affect the hydrolytic degradation of oxytetracycline.[19] Nearly complete dissipation of oxytetracycline was found in seawater in quartz tubes after 21 days under the illumination of sunlight with and without 1 m-depth seawater sealing.[3] However, the effect of various factors, especially the multi-covalent cation, on the hydrolytic and photolytic degradation kinetics of oxytetracycline has not yet been well-documented. The hydrolytic and photolytic degradation rates of oxytetracycline in aqueous solution have not been compared.

In this study, the hydrolysis of oxytetracycline in aqueous solutions with different initial concentrations, solution pHs, temperatures, and Ca^{2+} concentrations was investigated. The photolytic degradation kinetics with and without the presence of Ca^{2+} was compared. The dominant degradation pathway of oxytetracycline in water environment was discussed.

Materials and methods

Chemicals

Oxytetracycline dehydrate (≥98%) was purchased from Sigma (St. Louis, MO). Sodium monobasic phosphate monohydrate (American Chemical Society [ACS] certified), sodium dibasic phosphate hexahydrate (ACS certified), sodium hydroxide (ACS certified), calcium chloride (ACS certified), *o*-phosphoric acid (85%, ACS certified), water high performance liquid chromatography (HPLC grade), methanol (HPLC grade), and acetonitrile (HPLC grade) were purchased from Fisher Scientific (Suwanee, GA). 4-Epioxytetarcycline (97%), α- and β-apo-oxytetracycline were purchased from ACROS (Morris Plains, NJ).

Hydrolysis experiments

The hydrolysis of oxytetracycline was investigated in pH buffer solutions unless otherwise stated. All experiments were conducted in triplicates. Solutions were sealed in 125-mL glass serum bottles and incubated at darkness in incubators at different temperatures. At different incubation durations, 1.00 mL sample was taken out from each bottle and delivered into a 2-mL sample vial containing 0.20 mL methanol-85% H_3PO_4 (1:1 in volume) solution. Samples were stored at −21°C until sample analysis.

To investigate the effect of pH on oxytetracycline hydrolysis, 5 buffer solutions were prepared and oxytetracycline was added into each solution to achieve an initial concentration at 50 μM. The buffer solution at pH 3.09 was prepared by adding H_3PO_4 into water. The buffer solution at pH 5.07 was obtained by dissolving NaH_2PO_4 in water (at 10 mM). The buffer solution at pH 6.91 was prepared by dissolving NaH_2PO_4 and Na_2HPO_4 in water (each at 10 mM). The buffer solution at pH 9.06 was obtained by dissolving Na_2HPO_4 in water (at 10 mM) and then adjusting pH to 9.06 using H_3PO_4. The buffer solution at pH 10.54 was prepared by dissolving Na_2HPO_4 in water (at 10 mM) and then adjusting pH to 10.54 using 1 M NaOH solution. Solutions were incubated at 25 ± 0.1°C for 30 d.

Oxytetracycline hydrolysis at different initial concentrations was studied in solutions with pH at 9.06. The initial concentrations of oxytetracycline were 10, 19, 58, 140, and 230 μM and solutions were incubated at 25 ± 0.1°C for 30 days. When investigating the hydrolysis of oxytetracycline at different temperatures, solution pH was 9.06 and the initial concentration of oxytetracycline was 50 μM. The incubation temperatures were 4 ± 0.8 (refrigerator), 15 ± 0.1 (incubator), 25 ± 0.1 (incubator), 35 ± 0.2 (incubator), and 60 ± 1°C (oven).

To investigate the effect of Ca^{2+} on oxytetracycline hydrolysis, oxytetracycline was dissolved in two pH solutions. One was at pH 9.78 adjusted by adding 1 M NaOH and the other was at pH 5.88 without any pH adjustments. Each pH solution was then divided into 3 aliquots and different amounts of $CaCl_2$ were added to achieve $CaCl_2$ concentration at 0, 1, and 10 mM. Solutions were incubated at 25 ± 0.1°C for 26 d.

Photolysis experiments

Oxytetracycline photolysis experiments were conducted in open air under sunlight irradiation on June 28, 2005 in Riverside, CA (34.95°N, 117.40°W). The weather of that day was partially sunny and the temperature during the experiment was 20–27°C. Oxytetracycline solutions at 50 mM were prepared in deionized water or 1 mM $CaCl_2$ solution without any pH adjustments. The pH of both solutions was measured to be 5.85. Oxytetracycline solution without Ca^{2+} addition was transferred into six 125-mL glass serum bottles and bottles were sealed using aluminum caps with Teflon-coating septa. Three of these six bottles were completely covered with 2 layers of aluminum foil, serving as blank controls. Oxytetracycline solution with Ca^{2+} concentration at 1 mM was transferred into other three serum bottles and bottles were then sealed. All nine bottles were then placed under the irradiation of sunlight in an open air. At different irradiation times within 8 h, 1.00 mL sample was taken out from each bottle and delivered into a 2-mL sample vial containing 0.20 mL methanol-85% H_3PO_4 (1:1 in volume) solution. Sample vials were immediately sealed and transferred into a freezer and stored at −21°C until sample analysis.

Oxytetracycline concentration analysis

Samples were analyzed using a Shimadzu LC-2010A high performance liquid chromatograph (HPLC) equipped with an ultraviolet (UV) detector. An Allsphere ODS-2 5μ 250 mm × 4.6 mm column was used for separation and the temperature of column oven was set at 40°C. A gradient mobile phase composed of acetonitrile and water (pH adjusted to 3 using H_3PO_4) was used for elution. Acetonitrile percentage in the mobile phase was 20% from 0 to 4 min, linearly increased to 30% from 4 to 8 min, further increased to 40% from 8 to 9 min, and kept at 40% from 9 to 12 min. From 12 to 13 min, acetonitrile percentage was decreased from 40 to 20% and kept at 20% to 14 min. The flow rate of mobile phase was 1.000 mL min^{-1}. The working wavelength of the detector was set at 360 nm. The sample volume of each injection was 10 μL.

Under this analytical condition, oxytetracycline and three widely reported transformation/degradation products, including 4-epioxytetracycline, α- and β-apo-oxytetracycline, were separated. A typical HPLC spectrum of oxytetracycline standard and these three transformation/degradation products were shown in Figure 1. HPLC spectra of oxytetracycline degradation solution at pH 9.06 without Ca^{2+} after 10 d and at pH 9.78 with 1 mM Ca^{2+} after 10 d were also shown.

Kinetic models

In this study, oxytetracycline degradation kinetics was always fitted using the simple first-order model unless the availability-adjusted first-order model demonstrated a better fitting with a higher regression coefficient. The availability-adjusted first-order model was developed based on the decreasing availability of the target compound with time and has been published in previous studies.[16,20,21] The simple first-order model can be expressed as,

$$\ln C_t = \ln C_0 - kt \quad (1)$$

Where C_0 and C_t are oxytetracycline concentration at time 0 and t day, respectively; k (day^{-1}) is the first-order rate constant. The availability-adjusted first-order model can

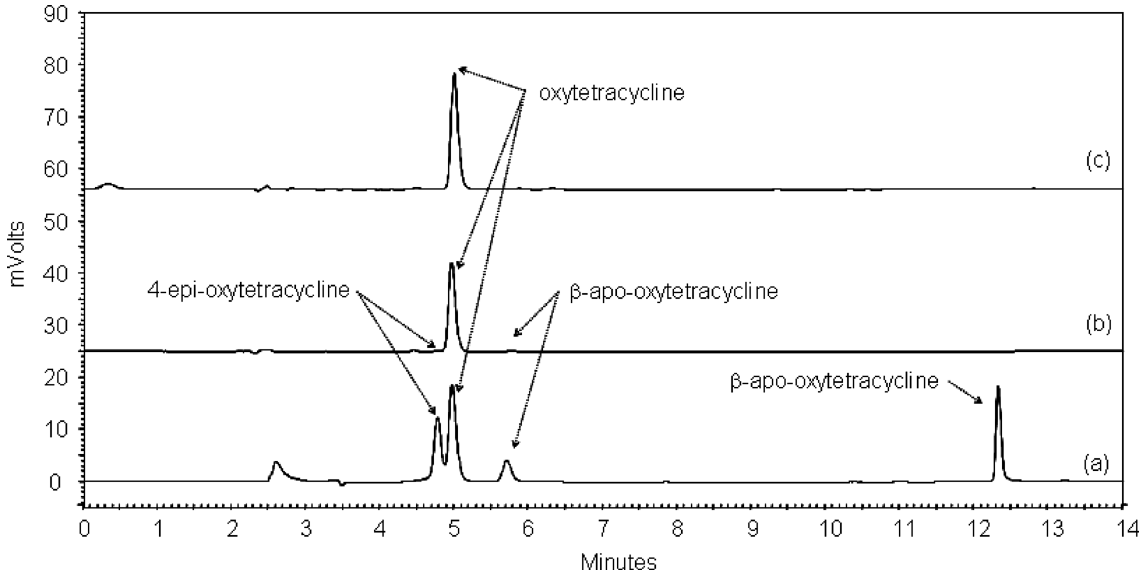

Fig. 1. Typical high performance liquid chromatography (HPLC) spectra of (a) standards, (b) oxytetracycline degradation solution at pH 9.06 without Ca^{2+} after 10 days, and (c) oxytetracycline degradation solution at pH 9.78 with 1 mM Ca^{2+} after 10 days.

be expressed as,

$$\ln C_t = \ln C_0 - \frac{k''}{a}(1 - e^{-at}) \quad (2)$$

where k'' (day^{-1}) is called the availability-adjusted first-order rate constant; and a (day^{-1}) is called availability coefficient. Based on the development of the availability-adjusted first-order model,

$$k'' = k\lambda_0 \quad (3)$$

where λ_0 is the concentration ratio of available oxytetracycline in the total oxytetracycline at 0 day.

Results and discussion

Oxytetracycline hydrolysis with different initial concentrations

For oxytetracycline hydrolysis at pH 9.06 and 25°C with different initial concentrations, a linear correlation was always obtained between ln C_i and t for each investigated initial concentration (Fig. 2). Values of regression coefficients are all greater than 0.99. The linear correlation indicates that oxytetracycline hydrolysis at different initial concentrations follows the simple first-order model. The first-order hydrolysis kinetics of oxytetracycline was also reported by Loftin et al.[19]

Additionally, the values of first-order rate constant, k, obtained for different initial concentrations of oxytetracycline are constant. They are 0.104 ± 0.004, 0.101 ± 0.003, 0.106 ± 0.003, 0.099 ± 0.002, and 0.094 ± 0.001 day^{-1} for the initial concentration of oxytetracycline at 10, 19, 58, 140, and 230 μM, respectively. The constant values of the rate constant at different initial concentrations imply that oxytetracycline hydrolysis at environment relevant concentrations, which are about 10^2 to 10^5 times lower than the experimental concentration in this study,[4,10] may have the similar value of hydrolysis rate constant to those obtained in this study.

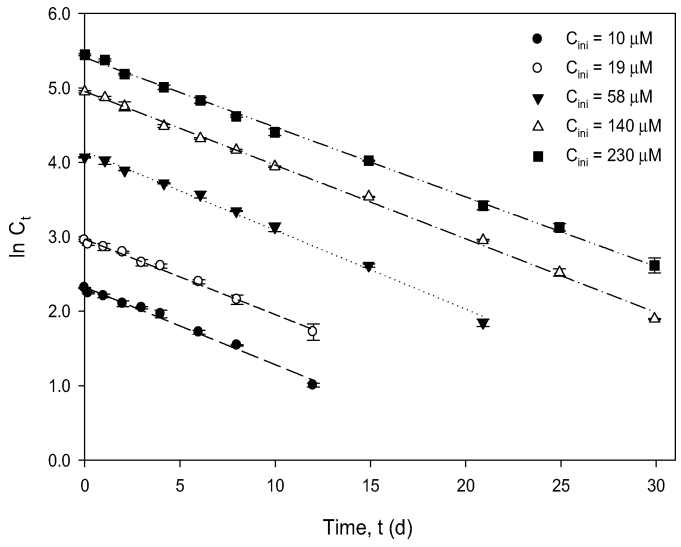

Fig. 2. Linear correlation between ln C_t and t for oxytetracycline hydrolysis at pH 8.96 with different initial concentrations. Points are the means of experiment data and bars are standard deviations. Lines are linear regression results.

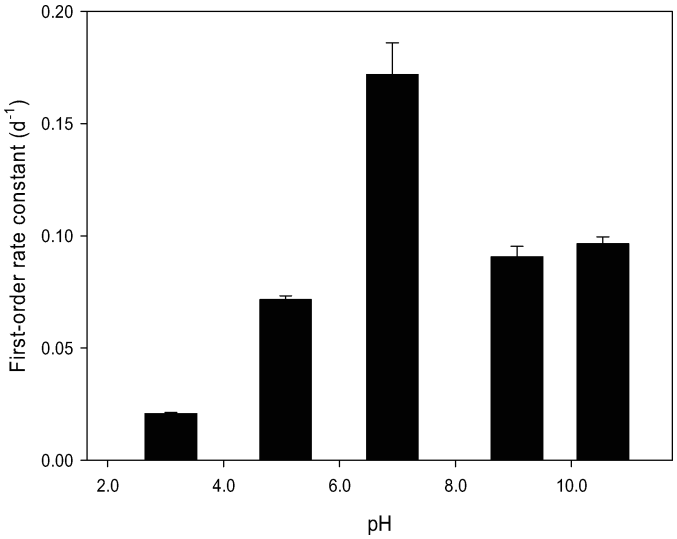

Fig. 3. Hydrolysis rate constant of oxytetracycline at different solution pHs.

Effect of solution pH on oxytetracycline hydrolysis

Notable hydrolysis of oxytetracycline was observed at all investigated pHs. After 6 days of incubation at 25°C, 10.5, 35.5, 72.7, 37.8, and 42.5% of oxytetracycline were hydrolyzed at pH 3.09, 5.07, 6.91, 9.06, and 10.54, respectively. The hydrolytic instability of oxytetracycline at all pHs may be the reason why noticeable decline of oxytetracycline was observed in sterile and acidified manure.[16] However, compared with hydrolysis in aqueous solution, the hydrolysis in manure was much slower because of oxytetracycline adsorption in sterile manure.

At each solution pH, a linear correlation between ln C_t and t was obtained with a regression coefficient greater than 0.99 (data not shown), demonstrating that the first-order kinetics was followed. As illustrated by the changes of rate constant with solution pH (Fig. 3), oxytetracycline hydrolysis appeared greatly depending on solution pH. In addition to the reported observation that acidic conditions favor oxytetracycline stability and alkaline conditions favor oxytetracycline degradation,[19] neutral pH solution was found to be the most favorable to the hydrolytic degradation of oxytetracycline at the pH range of 3.09–10.54. Since the pH of natural water, soil, and fresh animal feces are nearly neutral, the rapid hydrolysis in neutral pH may greatly accelerate the degradation of oxytetracycline in the

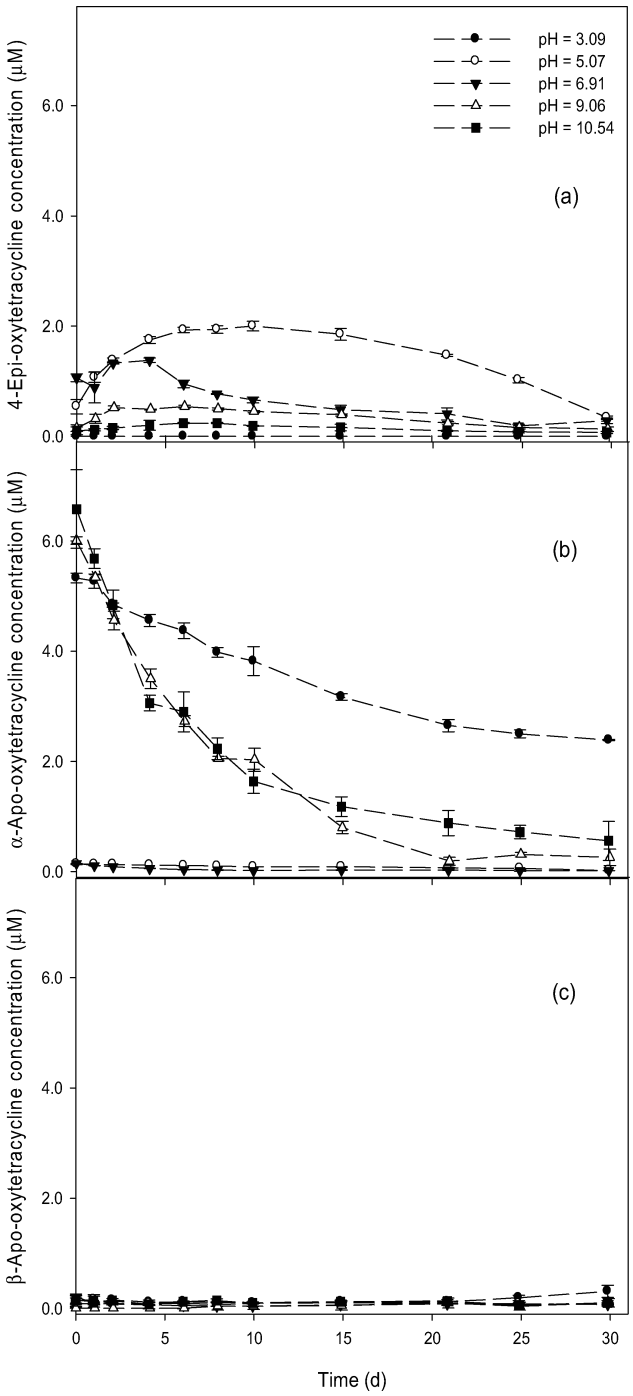

Fig. 4. Concentration changes of (a) 4-epi-, (b) α-apo-, and (c) β-apo-oxytetracycline during oxytetracycline hydrolysis at different pHs. Points are the means of experiment data and bars are standard deviations. Broken lines are links of points.

environment, thus minimizing oxytetracycline contamination. This property of oxytetracycline also implies that pretreatment is needed for the preservation of oxytetracycline in environmental samples. Acidification may significantly slow down oxytetracycline hydrolysis and help to preserve oxytetracycline in samples.

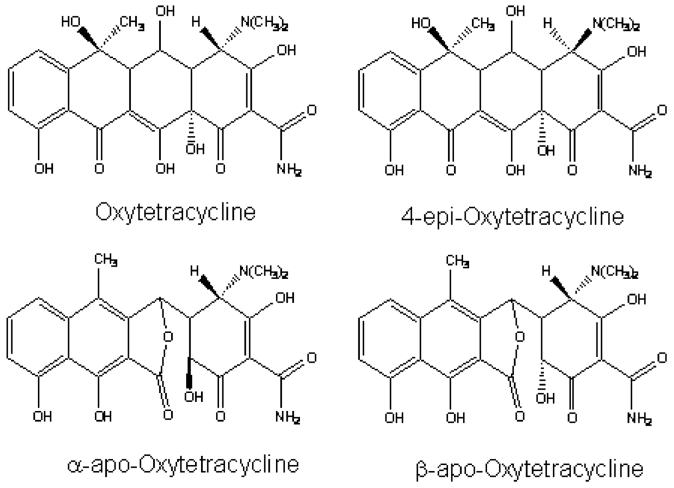

Fig. 5. Chemical structure of oxytetracycline and three degradation/transformation products.

4-Epi-, α-apo-, and β-apo-oxytetracycline are three antimicrobial-active transformation/degradation products of oxytetracycline which have been identified in oxytetracycline degradation process in different media.[22,23] These three products were also identified in oxytetracycline hydrolysis process and their concentration changes are shown in Figure 4. The chemical structures of oxytetracycline and these three products are shown in Figure 5. The existence of acid/base-active phenolic hydroxyl and amine groups in oxytetracycline determines that oxytetracycline may exist in different ionic species at different solution pHs. It is reasonable that certain ionic species are more vulnerable than others and thus are easier to be degraded.

The concentration of 4-epi-oxytetracycline during oxytetracycline hydrolysis was below 2 μM at all investigated pHs. In weak acidic (pH 5.07) and pH neutral (pH 6.91) solutions, 4-epi-oxytetracycline was found to be higher than in other pHs (Fig. 4a). At pH 3.09, no 4-epi-oxytetracycline was detected. This observation illustrates that weak acidic and pH neutral media may particularly favor the formation and/or accumulation of 4-epi-oxytetracycline. It might also be the reason why relatively high concentration of 4-epi-oxytetracycline was detected during the anaerobic digestion of oxytetracycline-contained manure at pH 7.1.[23] The product 4-Epi-oxytetracycline may convert back to oxytetracycline at certain circumstances[24] and it may also be degraded further directly.[25]

The appearance of α-apo-oxytetracycline during oxytetracycline hydrolysis is on the contrary of that of 4-epi-oxytetracycline. Only very low concentrations ($<$0.2 μM) of α-apo-oxytetracycline were observed in weak acidic (pH 5.07) and pH neutral (pH 6.91) solutions during the hydrolysis (Fig. 4b). However, above 5 μM of α-apo-oxytetracycline was detected at concentrations of in the beginning of the hydrolysis in acidic (pH 3.09) and basic (pH 9.06 and 10.54) solutions. With

Table 1. Linear correlation results between natural logarithm value of oxytetracycline concentration (ln C_t) and time (t) during oxytetracycline hydrolysis at different temperatures.

Temperature (°C)	*First-order rate constant,* k *(day*$^{-1}$*)*	*Regression coefficient,* r	*Half-life (day)*	*Arrhenius equation regression results*
4 ± 0.8	0.0059 ± 0.0003	0.98	1.2×10^2	
15 ± 0.1	0.0415 ± 0.0006	0.99	16.7	$\ln k = (33.2 \pm 1.8) - (1.06 \pm 0.06) \times 10^4 \times \frac{1}{T}$
25 ± 0.1	0.106 ± 0.003	0.99	6.5	$r = 0.99$
35 ± 0.2	0.257 ± 0.014	0.99	2.7	Activation energy $E_a = 88.1 \pm 5.0$ kJ/mol
60 ± 1	4.55 ± 0.07	0.99	0.15	

the hydrolysis of oxytetracycline, α-apo-oxytetracycline gradually decreased. This observation indicates that acidic and basic pHs especially favor the formation of α-apo-oxytetracycline.

For all investigated solution pHs, β-apo-oxytetracycline was always detected at very low concentrations ($\leq 0.3\ \mu$M) during the hydrolysis process and no obvious difference in β-apo-oxytetracycline was observed among those tested pHs (Fig. 4c), indicating that solution pH has no noticeable effect on the formation and dissipation of β-apo-oxytetracycline.

Oxytetracycline hydrolysis at different temperatures

The hydrolysis kinetics of oxytetracycline in pH 9.06 solution at different temperatures were found to all obey the simple first-order model. Linear correlation between ln C_t and t was always obtained at each investigated temperature in the range of 4–60°C and correlation results are listed in Table 1. With the increasing temperature, oxytetracycline hydrolysis was dramatically accelerated. The half-life of oxytetracycline at 60 ± 1°C is estimated to be 0.15 d, which is only 0.9% of that at 15 ± 0.1°C. The hastened hydrolysis with increasing temperature greatly supports our previous observation in the degradation of oxytetracycline in animal manure,[16] in which oxytetracycline degradation was also significantly enhanced with incubation temperature and the degradation kinetics was found to deviate from the original kinetics at 15 and 25°C. The temperature effect illustrates that the contribution of hydrolysis to oxytetracycline degradation in moist animal manure may become comparable to that of biodegradation and even dominant with the increasing temperature. Hence, heat treatments may be an effective and low-cost strategy to diminish oxytetracycline contamination in water, animal waste, and food.

Additionally, a linear correlation was also obtained between ln k and $1/T$ based on Arrhenius equation and regression results are shown in Table 1. The activation energy, E_a, for oxytetracycline hydrolysis at pH 9.06 was calculated to be 88.1 ± 5.0 kJ/mol, which is similar to the value obtained at pH 9 by Loftin and coworkers.[19] With an E_a in this magnitude, oxytetracycline hydrolysis would increase by approximately 2 times with every 10°C increase.

Effect of Ca^{2+}

Oxytetracycline can chelate with multivalent cations though its phenolic hydroxyl group and carbonyl groups.[26] The presence of Ca^{2+} (at 1 and 10 mM) was found to play a prominent role in oxytetracycline hydrolysis (Fig. 6). A

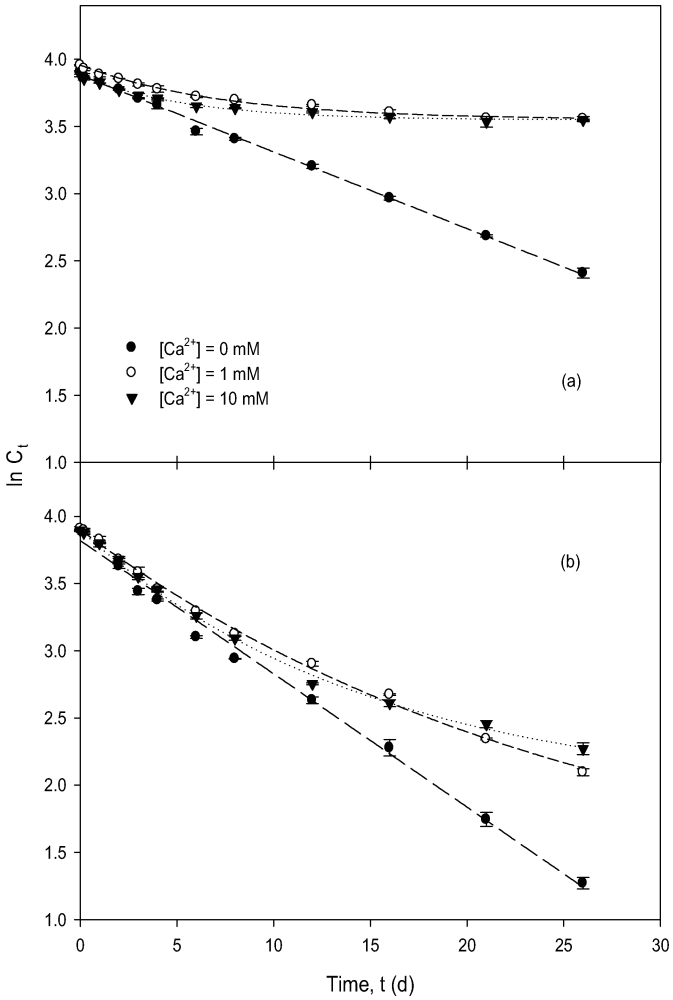

Fig. 6. Correlation between ln C_t and t for oxytetracycline hydrolysis with the presence of different concentrations of Ca^{2+} at (a) initial pH 9.78 (adjusted using NaOH) and (b) initial pH 5.88 (without any pH adjustments). Points are the means of experiment data and bars are standard deviations. Lines are fitting results using Equation 1 or 2.

Table 2. Model fitting results for oxytetracycline hydrolysis with the presence of different concentrations of Ca^{2+} at initial pH 9.78 (adjusted using NaOH) and pH 5.88 (without any pH adjustments).

Initial pH	*Ca^{2+} concentration (mM)*	*Rate constant, k or k″ (day^{-1})*	*Availability coefficient, a (day^{-1})*	*Regression coefficient, r*
9.78	0	$k = 0.0571 \pm 0.0010^a$	0	0.99
	1	$k'' = 0.0551 \pm 0.0030^b$	0.136 ± 0.011	0.99
	10	$k'' = 0.0569 \pm 0.0028^b$	0.175 ± 0.012	0.99
5.88	0	$k = 0.0992 \pm 0.0025^a$	0	0.99
	1	$k'' = 0.107 \pm 0.004^b$	0.0380 ± 0.0042	0.99
	10	$k'' = 0.130 \pm 0.004^b$	0.0660 ± 0.0039	0.99

[a]Fitting using Equation 1. [b]Fitting using Equation 2.

linear correlation was obtained between ln C_t and t for oxytetracycline hydrolysis at pH 9.78 (adjusted using NaOH) and pH 5.88 (without any pH adjustments) both without the presence of Ca^{2+}, indicating that the simple first-order model was followed. However, linear correlation was no longer available when Ca^{2+} was present at 1 or 10 mM. Instead, the availability-adjusted first-order model, i.e. Equation 2, was found to well fit the correlation between ln C_t and t. Model fitting results are listed in Table 2.

The presence of Ca^{2+} not only made oxytetracycline hydrolytic degradation deviate from the simple first-order model, but also markedly slowed down the hydrolysis. The values of the hydrolysis rate constant (k or k'') obtained with different concentrations of Ca^{2+} are quite similar at each pH. However, the value of availability coefficient, a, increases noticeably with the increasing Ca^{2+} concentration, evidencing the decreasing availability of oxytetracycline to hydrolysis with the increasing Ca^{2+}. After 12 days of incubation, 50.6% oxytetracycline was hydrolyzed with no presence of Ca^{2+} at initial pH 9.78. However, only 25.8 and 23.8% was hydrolyzed in the presence of 1 and 10 mM Ca^{2+}, respectively, at the same initial pH. Similar effect of Ca^{2+} was also observed at initial pH 5.88.

Compared with rate constant values at initial pH 9.78 with and without the presence of Ca^{2+}, oxytetracycline hydrolysis rate constant values are much greater at initial pH 5.88. Additionally, the availability coefficient values at pH 5.88 are much smaller than those at pH 9.78. This indicates that the combination of oxytetracycline with Ca^{2+} at initial pH 5.88 is much weaker than that at initial pH 9.78. Hence, Ca^{2+} effect on oxytetracycline hydrolysis greatly depends on solution pH.

Oxytetracycline hydrolysis in deionized water, seawater, and freshwater was investigated by Pouliquen and colleagues.[18] The concentration of Ca^{2+} and Mg^{2+} was 419 and 1386 mg L^{-1} in the seawater, respectively and 17 and 5 mg L^{-1}, in the freshwater, respectively. However, it was concluded in that study that these cations did not play a prominent role in oxytetracycline hydrolysis. The missing of observable effects might be caused by one of the following possible reasons. Firstly, Ca^{2+} and Mg^{2+} in the seawater might have combined with dissolved organic/inorganic matter. Thus readily available concentration of Ca^{2+} and Mg^{2+} was not high enough to affect oxytetracycline hydrolysis. Secondly, the effect of pH was not excluded. The conclusion was drawn based on oxytetracycline hydrolysis in freshwater (pH 7.9) and seawater (pH 8.4). The pH effect might offset the cation effect. Thirdly, the hydrolysis experiments were performed at 8°C. The hydrolysis at this temperature might be not high enough to demonstrate the significant effect of cations.

Photolysis of oxytetracycline

Oxytetracycline has strong absorbance at 200–400 nm,[3] making it susceptible to sunlight irradiation. Rapid photolysis of oxytetracycline was observed under sunlight irradiation (Fig. 7). The degradation with and without the presence of Ca^{2+} was shown to obey the simple first-order model. Obtained values of regression coefficient were all above 0.99 (data not shown). The photolysis rate constant with Ca^{2+} concentration at 0 and 1 mM in 125 mL glass serum bottles was calculated to be 3.61 ± 0.06 and 5.58

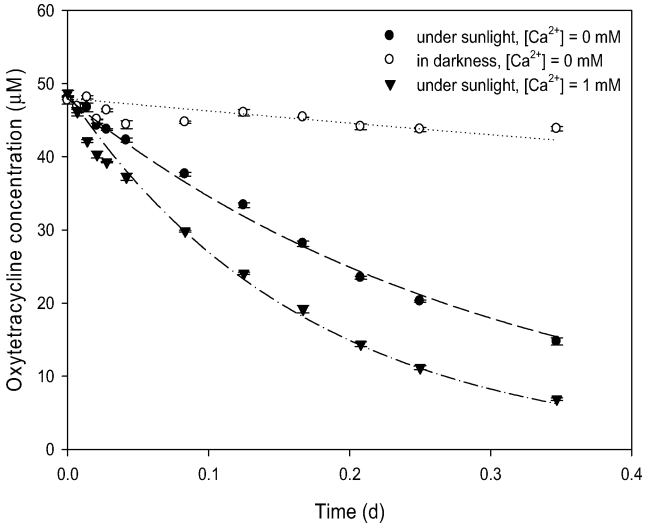

Fig. 7. Photolysis of oxytetracycline with and without Ca^{2+} under irradiation of sunlight (pH 5.85, without any pH adjustments). Points are the means of experiment data and bars are standard deviations. Lines are fitting results using the simple first-order model.

$\pm$ 0.05 day^{-1}, respectively. Correspondingly, the half-life is 0.19 and 0.12 day, respectively. The obtained photolysis rate constant of oxytetracycline is comparable to that of hydrolysis at 60°C. It was noticed that oxytetracycline photolysis was markedly accelerated with the presence of Ca^{2+}. This implies that the chelation of oxytetracycline with Ca^{2+} increases the absorbance of oxytetracycline, making oxytetracycline even more vulnerable to sunlight irradiation. Increase in fluorescence intensity of tetracycline after chelating with metal ions has been reported.[27]

Meanwhile, only slight decrease of oxytetracycline was observed in controls at darkness, indicating that the observed rapid degradation of oxytetracycline under sunlight irradiation was caused mainly by photolysis, not hydrolysis. As listed in Table 2, the hydrolysis rate constant in water without any pH adjustments was measured to be 0.0992 $\pm$ 0.0025 and 0.107 $\pm$ 0.004 day^{-1} with Ca^{2+} at 0 and 1 mM, respectively. Compared with the hydrolytic degradation rate constant, oxytetracycline photolytic degradation at similar conditions (pH, temperature, and Ca^{2+}) is 35.4 and 51.1 times higher, respectively. If quartz or Petridish-like containers were used instead of glass serum bottles, even higher values of photolysis rate constant would have obtained. The overwhelmingly fast degradation under sunlight irradiation demonstrated that photolysis may be a primary degradation pathway for oxytetracycline in shallow transparent water environment with sunlight irradiation.

However, photolysis may lose its dominance in oxytetracycline degradation in turbid water, solid manure, and soil because of low penetration of sunlight in those media.[6] Additionally, photolysis rate may greatly depend on sunlight intensity, which varies with season and latitude. Compared with the photolysis observed in this study, oxytetracycline photolysis in seawater under sunlight irradiation in the study of Lunestad et al.[3] appeared much slower. Oxytetracycline was found to decrease from 50 to 2 mg L^{-1} in seawater after 9 days of exposure in quartz containers at sea level. Based on the obtained first-order rate constant (i.e., 5.58 $\pm$ 0.05 day^{-1}) in this study, a decrease from 50 to 2 mg L^{-1} under conditions of this study needs only 0.58 day. The large difference in photolytic degradation rates between these two studies may result from the following two factors. Firstly, the sunlight intensity under which these two studied were conducted might be quite different. The study of Lunestad et al. was performed in Bergen (60°N), Norway in winter time, while this study was conducted in Riverside (34.95°N), CA, U.S. in summer time. Secondly, the solution media of these two studies were different. Seawater may contain noticeable amount of suspended particles and dissolved organic matters, which may effectively adsorb oxytetracycline and prevent oxytetracycline from photolytic degradation.

Conclusion

Oxytetracycline hydrolysis follows the first-order kinetics at different initial concentrations and occurs in all investigated solution pHs ranging from 3.09 to 10.54. Neutral pH solution was found to favor the hydrolysis the most, followed by alkaline solution. Increasing solution temperature may effectively enhance oxytetracycline hydrolysis, while the presence of Ca^{2+} may greatly slow down the hydrolysis and make the kinetics deviate from the first-order model to the availability-adjusted first-order model.

Oxytetracycline in water under sunlight irradiation degrades very fast with a degradation rate constant comparable to that of hydrolysis at 60°C, indicating that photolysis may be a primary degradation pathway for oxytetracycline in shallow transparent water environment. The presence of Ca^{2+} accelerated oxytetracycline photolysis, implying that oxytetracycline becomes more vulnerable to sunlight irradiation after chelating with Ca^{2+}.

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