Journal of Environmental Monitoring

Cite this: *J. Environ. Monit.*, 2012, **14**, 1853

www.rsc.org/jem

PAPER

Effects of adsorption onto silica sand particles on the hydrolysis of tetracycline antibiotics†‡

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Received 30th November 2011, Accepted 22nd March 2012
DOI: 10.1039/c2em10961a

Due to high usage of tetracycline antibiotics, concerns have been raised about their environmental fate. In this study, potential changes in the pseudo-first-order hydrolysis rate constants for three tetracyclines, tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC), were evaluated by measuring the rate constants in batch and column leaching experiments. The first-order hydrolysis rate constants were measured at pH 5, 7, and 9 using batch tests. The values were highest at pH 7 for all three tetracyclines (0.0030 ± 0.0004, 0.0042 ± 0.0001, and 0.0323 ± 0.0003 h^{-1} for TC, OTC, and CTC, respectively), indicating relatively short environmental half-lives of tetracyclines. Interestingly, it was found that the rates of degradation of the parent tetracyclines were much faster when silica sand was present in a batch solution or when the solution was passed through a silica column. For example, the ratios of the first-order degradation rate constants obtained in the column experiments to those in batch experiments were 13.2, 2.1, and 2.0 for TC, OTC, and CTC at a volumetric flow rate of 0.08 mL h^{-1}, with an observed tendency for this ratio to increase with an increased flow rate. This indicates that the silica surface may serve as a catalyst for hydrolysis and that the actual environmental half-lives of tetracycline antibiotics could be shorter than those estimated from laboratory hydrolysis rate constants using the standard batch protocol. Furthermore, the toxicity of the column effluent containing hydrolysis metabolites was assessed using bioluminescence inhibition in *Vibrio fischeri*. It was estimated that the toxicity of the metabolites of CTC was lower than that of their parent compound, whereas the toxicity of metabolites of TC and OTC was as high as or higher than that of their parent compounds.

Introduction

Tetracyclines are some of the most widely used veterinary antibiotics. For example, their estimated annual sales in South Korea alone amount to over 4000 tons.[1] Because the tetracycline antibiotics that are used will end up in the environment and residual tetracyclines may disturb bacterial communities,[2,3] many studies have been conducted to evaluate their environmental fate in soil and water environments.[4–12]

Researchers have revealed that tetracyclines predominantly adsorb onto mineral surfaces or onto soil organic matter *via* cation exchange or ion bridging.[4,7,12] Values of the distribution

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† Published as part of a special issue dedicated to Emerging Investigators.

‡ Electronic supplementary information (ESI) available: Changes in aqueous concentration of tetracyclines for the determination of homogeneous hydrolysis rate constants (Fig. S1), determination of dispersivity of the column using chloride anion (Fig. S2), all breakthrough curves of tetracyclines at various flow rates (Fig. S3), and bioluminescence inhibition curves using *Vibrio fischeri* (Fig. S4). See DOI: 10.1039/c2em10961a

Environmental impact

In risk assessment of a chemical, parameters determining its environmental fate are usually obtained from laboratory experiments. For example, the hydrolysis rate constant for a hydrolysable chemical is measured following a standard batch protocol. Because many chemicals may be present in the environment adsorbed to soil surfaces, the environmental half-lives estimated from batch experiments might not reflect real environmental conditions. In this article, we demonstrate that the hydrolysis rate constants for three commonly used tetracycline antibiotics can be enhanced when aqueous solutions containing the tetracyclines flow through a sand column. This indicates that the mineral surfaces may play a role of a catalyst and half-lives of tetracyclines in the soil environment would be shorter than those estimated from laboratory batch experiments.

coefficient between soil and water (K_d) have been reported to be of the order of 10^2–10^4 L kg^{-1} for various field soils and sediments,[6,8] clay minerals,[10] and biosolids.[13] Because of their high K_d values, it is thought that the majority of the tetracyclines released into the soil environment are likely to associate with soil surfaces and/or organic matter with limited mobility in the soil environment. It has also been reported that they are transformed into epimers, iso-tetracyclines or dehydrated products *via* hydrolysis pathways under environmentally relevant conditions[14–18] and that they undergo photo-degradation[11,14,15] and biotransformation.[19] Aqueous half-lives of parent tetracyclines have been reported between 20 h and 50 days around pH 7.[14–17]

Both adsorption and hydrolysis studies are generally conducted in a laboratory to obtain the parameters that determine the environmental fate, including the distribution coefficient between soil and water (K_d) or adsorption isotherms and pseudo-first-order rate constants at a given pH. Although individually obtained values of K_d and transformation rate constants can be used for the prediction of the environmental fate and for assessing the mobility of tetracyclines in soil environments, it is not clear that these two processes occur independently in the environment. Experimental determination of K_d may be affected by potential hydrolysis in the solution and the sorption may change the rate of hydrolytic transformation. Previous studies on the fate of hydrolysable organic chemicals have shown that the adsorption of organic contaminants onto mineral surfaces may accelerate or decelerate their transformation rate.[20–23] For example, the rate constants of endosulfan hydrolysis were higher in the presence of suspended solids such as α-FeOOH, TiO_2 and SiO_2.[21] Similar observations were also reported for the hydrolysis of other organic esters.[23] On the other hand, sorptive preservation has been observed for 2,2-dichloropropane when it sorbed in the micropores of hydrophilic solids.[20] Therefore, the hydrolysis half-lives of tetracyclines in the soil environment may not be the same as those obtained from laboratory tests in which chemicals undergo homogeneous hydrolysis in a batch reactor. To the best of our knowledge, potential catalytic effects of mineral surfaces on the transformation of tetracyclines have not been reported, despite the possibility of this process affecting the overall fate of tetracyclines in soil environments.

Consequently, the changes in hydrolytic transformation of tetracycline antibiotics were evaluated upon their adsorption onto soil surfaces. For this purpose, we selected the three most widely used tetracyclines, namely tetracycline (TC), oxytetracycline (OTC) and chlortetracycline (CTC), as model compounds and used silica sand as a model soil. The hydrolysis rate constants and adsorption coefficients onto silica surfaces were then measured. Pseudo-first-order rate constants of hydrolysis were obtained in a batch system at pH 5, 7, and 9. For the determination of K_d, column leaching experiments were conducted. A one-dimensional transport model through a porous medium with first-order decay and retardation was used for the derivation of the retardation factor and the degradation rate constant. The rate constants obtained using the column leaching experiment were compared to those obtained *via* batch hydrolysis tests to evaluate the potential catalytic effects of silica surfaces on the overall transformation rates. An acute *Vibrio fischeri* bioluminescence inhibition test was also conducted to monitor the changes in the acute toxicity of tetracyclines and their transformation products that formed in the silica column.

Materials and methods

Chemicals and materials

Analytical grade hydrochloride salts of three tetracycline antibiotics – tetracycline (98%), oxytetracycline (97%), and chlortetracycline (93%) – were purchased from Sigma-Aldrich (St Louis, MO, USA). All salts used in the preparation of buffer solutions were of high purity and were purchased from Sigma-Aldrich or Dae-Jung (Siheung, Republic of Korea). Silica sand (40–150 mesh) was purchased from Sigma-Aldrich and used without any further treatment.

Batch hydrolysis

100 mL aqueous solutions each containing a tetracycline were freshly prepared by dissolving the tetracycline in buffer solutions maintained at pH 5 (10 mM citrate), pH 7 (10 mM phosphate), and pH 9 (10 mM tetraborate). The initial concentration was approximately 100 μM. The triplicate solutions were placed in the dark at 25 °C. After pre-determined time intervals, a 500 μL solution was taken and transferred into a vial containing 500 μL of methanol with 1% (v/v) hydrochloric acid in order to inhibit hydrolysis by lowering the solution pH. Samples fixed with the acid were stored at −18 °C until they were quantified using HPLC. The pseudo-first-order hydrolysis rate constants for the three tetracyclines were obtained using a linear regression between the logarithmic concentration and time.

Batch adsorption tests

Batch adsorption tests were conducted to evaluate the adsorption capacity of silica sand for the three tetracyclines. Solutions of each tetracycline (*ca.* 200 μM) were prepared by dissolving them in 10 mM ammonium acetate buffer at pH 7. Immediately following the preparation of the aqueous solution, a 1 mL aliquot was transferred into a 2 mL Eppendorf tube containing 500 mg of silica sand that was agitated at 150 rpm and 25 °C in the dark using a shaking incubator. Each tube was taken after the pre-determined time interval. After allowing the sand particles to settle, the supernatant was filtered through a 0.45 μm hydrophilic PTFE filter (Advantec, Tokyo, Japan). The filtrate was then immediately analysed by HPLC.

Column leaching experiments

A series of column leaching experiments was conducted using the experimental device shown in Fig. 1. A glass chromatography column (100 mm × 6.6 mm i.d.) was filled with 3.8 g of silica sand. The resulting porosity of the medium was measured as 0.39. The exterior of the column was wrapped with aluminium foil to minimize potential photolysis. The sand column was wetted by allowing the phosphate buffer solution (10 mM, pH 7) to flow through it. Solution A containing approximately 200 μM tetracycline antibiotics was freshly prepared and 0.5% concentrated HCl was added to the buffer solution in order to minimize the potential degradation of tetracyclines during storage. Solution B

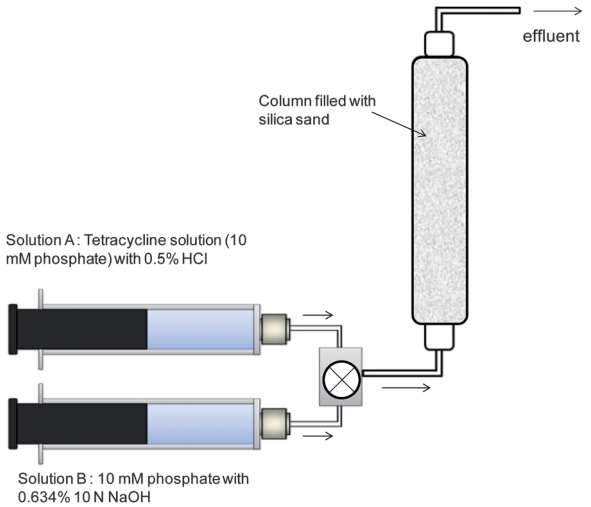

Fig. 1 A schematic diagram of the experimental device used for the column leaching experiment.

was prepared by adding a corresponding amount of strong base (0.634%, 10 N NaOH). The pH of the solution, after mixing solution A and B at the same flow rate (1 : 1), reverted to the original pH of the phosphate buffer solution (pH 7). Both solutions A and B were pumped at the same rate to produce overall volumetric flow rates of 0.08, 0.2, 0.6, and 3.0 mL h^{-1} using a KDS-100 syringe pump (KD Scientific, Holliston, MA, USA).

The mixed solution was collected to determine the influent concentration of tetracyclines before entering the column and the column effluent was collected after designated time intervals. Both the influent and effluent solutions were fixed with the same volume of methanol containing 1% (v/v) HCl and stored at −18 °C until HPLC analysis.

The dispersivity (α_x, m) of the sand medium was measured using a 50 mg L^{-1} NaCl solution. The salt solution was passed through the column at 1 mL min^{-1} using a Waters 626 HPLC pump. The change in the electrical conductivity with respect to time was monitored using a Waters 431 conductivity detector (Waters, Milford, MA, USA). The longitudinal dispersion coefficient (D_x, m^2 h^{-1}) and α_x were calculated using:[24]

$$D_x = \frac{u_x L}{8}\left[J(0.84) - J(0.16)\right]^2 \quad (1)$$

$$\alpha_x = \frac{D_x}{u_x} \quad (2)$$

where u_x is the longitudinal seepage velocity of water (m h^{-1}), L is the length of the column (m), and $J(0.16)$ and $J(0.84)$ represent the values of the pore volume function $((u_x t/L - 1)/(u_x t/L)^{0.5})$ when the ratio of the effluent concentration to influent concentration (C/C_0) equals 0.16 and 0.84, respectively.

The analytical solution for the concentration of tetracyclines at the exit of the column considering adsorption and first-order transformation is given by[25]

$$C = \frac{C_0}{2}\exp\left[\frac{(u_x - v)L}{2D_x}\right]\mathrm{erfc}\left[\frac{RL - vt}{2\sqrt{D_x R t}}\right] + \frac{C_0}{2}\exp\left[\frac{(u_x + v)L}{2D_x}\right]\mathrm{erfc}\left[\frac{RL + vt}{2\sqrt{D_x R t}}\right] \quad (3)$$

and

$$v = u_x\sqrt{1 + \frac{4kD_x}{u_x^2}} \quad (4)$$

where t is the time (h), C_0 is the concentration of tetracyclines entering the column (mg L^{-1}), k is the first-order degradation constant (h^{-1}), and R is the dimensionless retardation factor that is dependent on the linear adsorption coefficient (K_d, L kg^{-1}):

$$R = 1 + \frac{K_d \rho}{n} \quad (5)$$

where ρ and n are the bulk density (kg L^{-1}) and porosity of the medium, respectively.

After obtaining time-course changes in the effluent concentration, values of C/C_0 were plotted against the bed volume and best-fit values of k and R were calculated using a least-squares analysis.

Instrumental analyses

All three tetracyclines were quantified using an HPLC system equipped with a Waters 515 pump (Milford, MA, USA), an autosampler (Waters 717+), and a photodiode array detector (Waters 996). Samples were separated on a SunFire™ C8 column (2.1 × 150 mm, 3.5 μm particle size; Waters) at ambient temperature and the concentration of the three tetracyclines was quantified at 355 nm. The mobile phase was 85% 30 mM oxalic acid (A) and 15% acetonitrile (B) with a flow rate of 0.25 mL min^{-1}. The chromatographic retention times were 5.4, 4.4, and 13.7 min for TC, OTC, and CTC, respectively.

Acute *Vibrio fischeri* toxicity

Changes in the acute toxicity of the influent and the effluent in the column experiments were also assessed by the luminescence inhibition by *Vibrio fischeri* (strain NRRL B-11177) using a Microtox® Model 500 (Strategic Diagnostics Inc., Newark, DE, USA). The bacterium was purchased in freeze-dried form from Strategic Diagnostics Inc. The influent and the effluent taken at the outlet of the column were subjected directly to the bioassay. The concentrations of TC and OTC in the influent were 100 μM and that of CTC was 200 μM. The flow rate was 0.08 mL h^{-1}. The luminescence inhibition was recorded after 5 and 15 min of exposure following the manufacturer's experimental protocol. The effective dilution of 50% inhibition (ED_{50}) was obtained after processing the data using Microtox Omni software (Strategic Diagnostic Inc.), according to the test protocol provided by the manufacturer.

Results and discussion

Hydrolysis of tetracycline antibiotics

Table 1 shows the pseudo-first-order hydrolysis rate constants obtained in the batch experiments with the corresponding aqueous half-lives of the three tetracyclines at pH 5, 7, and 9. Changes in the aqueous concentration with time are also shown in Fig. S1, ESI‡. The rate constants for OTC and CTC were greatest at pH 7, followed by values at pH 9 and pH 5. However, the hydrolysis rate constants for TC were similar at pH 5 and pH

Table 1 Pseudo-first-order rate constants (k_h) and half-lives ($t_{1/2}$) for tetracycline (TC), oxytetracycline (OTC), and chlortetracycline (CTC) as a function of pH at 25 °C and reported values in the literature

	This study			Literature		
	pH 5	pH 7	pH 9	pH 5	pH 7	pH 9
TC						
k_h (h^{-1})	0.0030 (±0.0001)	0.0030 (±0.0004)	0.0018 (±0.0003)			
$t_{1/2}$ (h)	231.7 (±8.9)	235.7 (±29.4)	398.9 (±49.5)	354[a]	116[a]	231[a]
OTC						
k_h (h^{-1})	0.0012 (±0.0002)	0.0042 (±0.0001)	0.0026 (±0.0001)			
$t_{1/2}$ (h)	611.2 (±97.7)	165.1 (±2.3)	271.2 (±12.3)	171[a]	41[a], 156[b], 337[c]	155[a]
CTC						
k_h (h^{-1})	0.0037 (±0.0002)	0.0323 (±0.0003)	0.0237 (±0.0005)			
$t_{1/2}$ (h)	189.9 (±8.9)	21.5 (±0.2)	29.3 (±0.6)	293[a]	16[a]	<6[a]

[a] Values obtained at 22 °C (ref. 16). [b] From ref. 15. [c] From ref. 14.

7. The hydrolysis half-life at pH 7 was shortest for CTC (<1 d). However, for TC and OTC, half-lives were approximately 7–10 days at pH 7. The range of hydrolysis half-lives agreed well with those reported previously in the literature,[14–17] though the literature values varied by one order of magnitude for OTC. The effects of pH on the hydrolysis of TC also agreed well with the generally reported trend.[16] The hydrolysis half-lives of the three tetracyclines indicate that risk assessment should include both the parents and their transformation products for such species.

Batch adsorption

Fig. 2 shows changes in the concentration of (a) TC, (b) OTC, and (c) CTC with respect to incubation time when 500 mg of silica sand was added to a 1 mL aqueous solution. Dashed lines represent the expected decrease in the aqueous concentration of the tetracyclines according to the first-order degradation with the rate constant previously obtained. If the adsorption occurs instantaneously or at least much faster than hydrolysis, the curves should show an initial fast decrease in the concentration of aqueous solution due to adsorption, followed by a gradual decrease. However, the observed decrease in the aqueous concentration fitted well to an exponential decay as observed in the batch hydrolysis experiment, thereby indicating that the transformation of tetracyclines may be accelerated in the presence of silica surfaces. Because of the continuous decrease in the aqueous concentrations, we were unable to reliably quantify the distribution coefficients between silica sand and water in the batch experiments, but they were quantified using column leaching experiments.

Column leaching experiments

A representative breakthrough curve of chloride anion at the volumetric flow rate of 1 mL min^{-1} is shown in Fig. S2, ESI‡. The dispersivity of the medium under our experimental conditions was estimated to be 0.416 cm using eqn (1) and (2).

An exemplary breakthrough curve for CTC at the flow rate of 0.08 mL h^{-1} is shown in Fig. 3. The long-dashed line indicates the expected breakthrough curve for a compound that is non-reactive and does not adsorb onto silica surfaces, such as chloride anion used in the present study. The short-dashed and solid lines represent the best-fit curve of the concentration in the effluent to the incoming concentration (C/C_0) with respect to the number of bed volumes using eqn (3), after fitting for only the retardation factor (R), and for both R and the hydrolysis rate constant (k_h'), respectively. All other breakthrough curves for CTC at different volumetric flow rates and for TC and OTC are shown in Fig. S3, ESI‡. As shown in Fig. 3 and S3‡, measured concentrations of all three tetracyclines at apparent steady state were much lower than those expected if one assumes that the k_h values are the same as those obtained in the batch hydrolysis experiments using a homogeneous aqueous solution. For example, the hydrolysis rate constant for CTC at the flow rate of 0.080 mL h^{-1} was estimated to be 0.0636 h^{-1}, which is much larger than that obtained in the hydrolysis experiment at pH 7 (by a factor of 1.97, see Table 2), indicating an accelerated hydrolysis of CTC in the silica sand column.

Values of retardation factors (R) and first-order hydrolysis rate constants (k_h') obtained from the column leaching experiments at four different flow rates are listed in Table 2. The retardation factor ranged from 1.45 to 1.76 for TC, from 1.53 to 2.11 for OTC, and from 1.47 to 2.23 for CTC. The corresponding values of K_d were 0.11–0.19, 0.13–0.27, and 0.12–0.30 L kg^{-1} for TC, OTC, and CTC, respectively. The obtained values of K_d ranged within a factor of two. These values of K_d are lower by some orders of magnitude than those obtained using field soils, probably as a result of high sorptive capacity of clay minerals and organic matter.[6,8,12,13]

The ratio (r) of the hydrolysis rate constant (k_h') obtained under the flowing conditions to that obtained in static water was much higher than unity at all flow rates, thus showing the catalytic effects of silica sand on hydrolysis (Table 2). The values of k_h' increased by a factor of 2–48, though C/C_0 values at steady state did not significantly differ from unity at the slowest flow rate (3 mL h^{-1}, see Fig. S3‡). In addition, this ratio increased with increasing flow rate, implying that the catalytic effects of the silica surfaces may be affected by shear stress near the surfaces. Increased shear stress is likely to enhance mass transfer of the tetracyclines between the solution and surfaces by lowering the thickness of the aqueous boundary layer.[26] Thus, these observations can be explained by assuming that the heterogeneous hydrolysis rate is much faster than the homogeneous rate. They also imply that the half-lives of tetracyclines in soil environments not only depend on the heterogeneous rate constants but also on the rate of exchange between the dissolved and adsorbed species under the specific

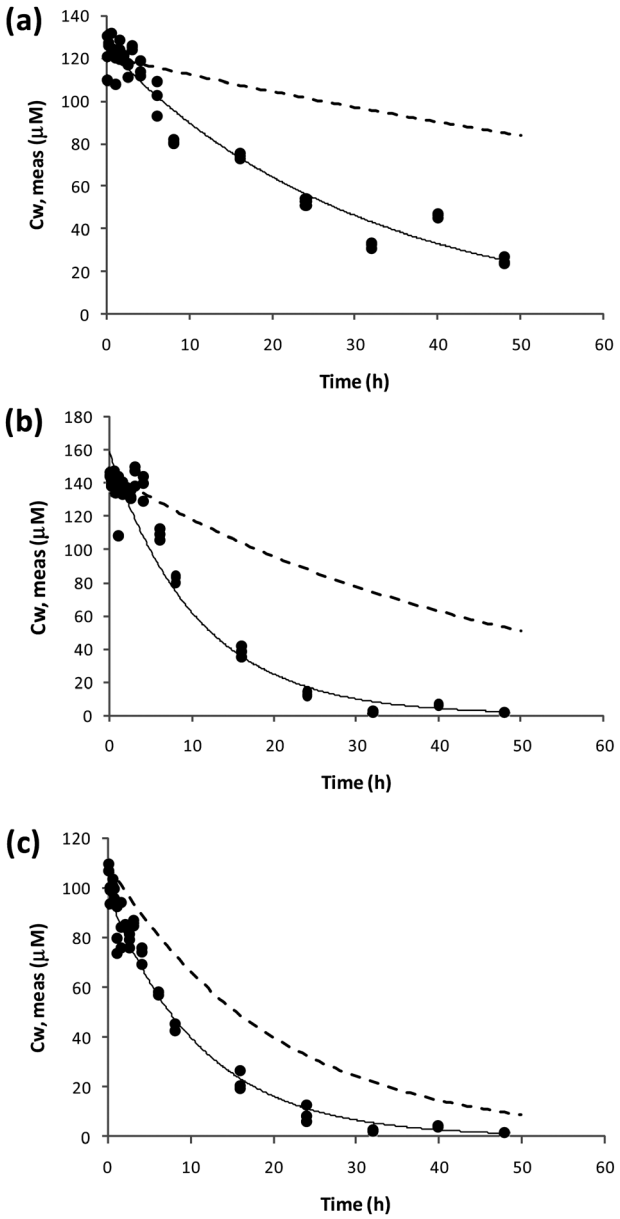

Fig. 2 Changes in the aqueous concentration of (a) tetracycline, (b) oxytetracycline, and (c) chlortetracycline in a test vessel containing 500 mg of silica sand per mL of the solution. Dashed lines indicate predicted changes in aqueous concentration with the measured pseudo-first-order hydrolysis rate constants without silica sand. Solid lines indicate best-fit using an exponential decay curve.

flowing condition. Typical hydraulic conductivity of coarse sand aquifer is known: 0.003–20 m h^{-1}.[27] Thus, the seepage velocity used in this study, 0.006 m h^{-1} at the slowest flow rate, would be considerably higher than the actual flow velocity of groundwater in a sand aquifer. However, potential increase in hydrolysis may be expected because a significant increase in k_h was observed at the slowest flow rate especially for tetracycline. Another environmental process affected by the heterogeneous hydrolysis reaction would be the water infiltration through a sandy layer. Because the infiltration rate could be comparable to those tested in the present study, it is highly likely that the apparent hydrolysis rate is significantly enhanced during this event.

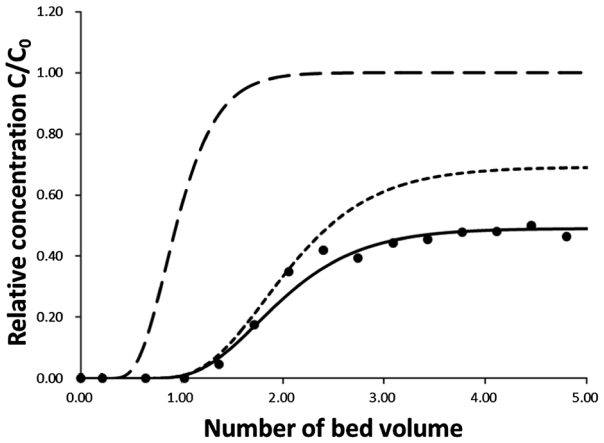

Fig. 3 Changes in the relative concentration (C/C_0) with respect to the number of bed volumes passing through the sand column for chlortetracycline with a flow rate of 0.08 mL h^{-1} as an example. Dotted line indicates the expected breakthrough curve for a tracer (Cl^-). Short-dashed line and solid line represent the modelled breakthrough curve by fitting only the retardation factor and both the retardation factor and the degradation rate constant in eqn (3). Long-dashed line indicates the theoretical breakthrough curve for a tracer.

Table 2 Retardation factors (R), first-order hydrolysis rate constants (k_h') and the ratio (r) of the hydrolysis rate constants (k_h')

	R	K_d (L kg^{-1})	k_h' (h^{-1})	r (k_h'/k_h)
TC				
3 mL h^{-1}	1.45	0.11	0.1322	44.06
0.6 mL h^{-1}	1.76	0.19	0.0916	
0.2 mL h^{-1}	1.57	0.14	0.0742	24.75
0.08 mL h^{-1}	1.74	0.18	0.0397	13.25
OTC				
3 mL h^{-1}	1.53	0.13		
0.6 mL h^{-1}	2.11	0.27	0.1283	30.56
0.2 mL h^{-1}	1.69	0.17	0.0192	4.56
0.08 mL h^{-1}	1.74	0.18	0.0086	2.05
CTC				
3 mL h^{-1}	1.47	0.12	0.1846	5.71
0.6 mL h^{-1}	1.60	0.15	0.0948	2.94
0.2 mL h^{-1}	1.94	0.23	0.0747	2.31
0.08 mL h^{-1}	2.23	0.30	0.0636	1.97

Hydrolysis of organic chemicals occurs *via* many different reaction mechanisms. The importance of acid- and base-catalyzed pathways is well known and interfacial mechanisms could also be important.[28] Complicated physicochemical interactions may change the reactivity of the reaction sites and transformation pathways by altering the activation energy. Three major transformation pathways of tetracyclines are reversible epimerization at the C-4 position,[29] dehydration at the C-5a hydrogen and C-6 hydroxy group in tetracyclines and their epimers,[30] and the cleavage of C-6 hydroxy group to form isotetracyclines[31] (Fig. S4‡). Because those reactions are catalysed by H^+ or OH^-, local accumulation of ions near the silica surface may affect the overall transformation rates. In addition, the reaction pathways depend on types of tetracycline as well as environmental conditions such as pH and the presence of metal cations.[14–18,32] Because the primary focus of this study was to quantitatively evaluate the changes of apparent hydrolysis rate

constants in the presence of silica sand particles due to the change in flow rate, the reaction pathways and mechanisms of surface-catalyzed hydrolysis were not investigated.

As shown in Table 2, the catalysis effects of the silica surface may significantly vary even for structurally similar tetracyclines, indicating that the ratio of the homogeneous to heterogeneous hydrolysis rate constants may vary significantly. Although the enhanced hydrolysis rate of tetracyclines was observed in the presence of silica sand, one should be careful when extrapolating these laboratory results for the estimation of half-lives of tetracyclines in soil environments. Researchers reported many important aspects that may influence their fate in soil environments including oxidative degradation with naturally occurring manganese oxides[33,34] and changes in hydrolysis rate constants in the presence of dissolved organic matter[32] or cations.[34] Further research is needed to provide mechanistic understanding of surface-catalyzed hydrolysis and on the importance of the contribution of other surfaces such as clays and organic matter to hydrolysis rate constants.

Change in acute toxicity to *Vibrio fischeri*

Table 3 summarizes the bioluminescence inhibition of the influent and column effluent at 0.08 mL h^{-1} for the three tetracyclines with values of 50% effective dilution (ED_{50}). Because the concentration of each tetracycline in the influent was known, the corresponding EC_{50} values for 15 min experiments were 54, 87, and 25 μM for TC, OTC, and CTC, respectively. They agree well with literature values obtained under similar experimental conditions. For example, Kim *et al.*[35] reported EC_{50} values of TC to be 81 μM for a 15 min experiment, and Park and Choi[36] reported 189 and 27 μM for OTC and CTC, respectively, under the same experimental conditions.

For TC and OTC, the ED_{50} values in the effluent were larger than those in the influent for both the 5 and 15 min experiments (Table 3 and Fig. S5‡), indicating that the hydrolysis metabolites formed in the sand column were likely to be more toxic to *Vibrio fischeri*. In contrast, the ED_{50} values for CTC effluent were lower than those of the influent with the level close to that found for the remaining parent compound. Although the formed metabolites were not identified and the contribution of each metabolite to the acute toxicity requires further evaluation, it is shown that the toxicity of hydrolysis metabolites may or may not be higher than that of their parent compounds (for the three structurally similar tetracyclines herein). Halling-Sørensen *et al.*[2] showed that various transformation products of tetracyclines have antibacterial potency as high as their parent compounds. For example, the EC_{50} of 5a,6-anhydrotetracycline for the growth inhibition of sludge bacteria was lower than that of TC. The lower ED_{50} of the column effluent may be explained by the presence of toxic metabolites such as anhydrotetracyclines, although further investigations are needed. Consideration of the toxicity of tetracycline metabolites is needed in order to evaluate the ecotoxicological effects of released tetracyclines on non-target organisms in the environment.

Table 3 Acute toxicity of the column influent and effluent at 0.08 mL h^{-1} using the luminescence inhibition by *Vibrio fischeri*[a]

	5 min	15 min
TC		
Influent	0.7782 (0.3010, 2.012)	0.5416 (0.0901, 3.253)
	EC_{50} 77.82 μM	EC_{50} 54.16 μM
Effluent	0.5239 (0.3176, 0.8641)	0.3064 (0.0908, 1.033)
OTC		
Influent	0.8831	0.8659
	EC_{50} 88.31 μM	EC_{50} 86.59 μM
Effluent	0.7652	0.7926
CTC		
Influent	0.2335 (0.1869, 0.2919)	0.1245 (0.0900, 0.1722)
	EC_{50} 46.71 μM	EC_{50} 24.91 μM
Effluent	1.237	0.7645

[a] Values in parentheses indicate the lower and the upper 95% confidence intervals. Confidence intervals were not obtained for those samples with limited number of data points used for fitting dose–response curves.

Conclusions

Although the independently obtained hydrolysis rate constants and adsorption coefficients for adsorption onto soil surfaces are used for evaluating the fate of chemicals in soil environments, this study showed that the hydrolysis rate constants of tetracycline antibiotics may change when adsorption onto silica surfaces occurs simultaneously. Although the mechanism governing the changes in hydrolysis rate constants needs to be revealed, potential catalytic effects emanating from the surfaces may have importance for assessing the environmental fate of hydrolysable chemicals. This study provides an example that a simple linear combination of environmental processes may not reflect the actual environmental fate of chemicals.

Acknowledgements

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean Government (MEST) (no. 2008-0062072).

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