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# Degradation of tetracycline antibiotics: Mechanisms and kinetic studies for advanced oxidation/reduction processes

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ABSTRACT

This study involves elucidating the destruction mechanisms of four tetracyclines via reactions with ·OH and solvated electrons ($e^-_{aq}$). The first step is to evaluate the bimolecular rate constants for the reaction of ·OH and $e^-_{aq}$. Transient absorption spectra for the intermediates formed by the reaction of ·OH were also measured over the time period of 1–250 μs to assist in selecting the appropriate wavelength for the absolute bimolecular reaction rate constants. For these four compounds, tetracycline, chlortetracycline, oxytetracycline, and doxycycline, the absolute rate constants with ·OH were $(6.3 \pm 0.1) \times 10^9$, $(5.2 \pm 0.2) \times 10^9$, $(5.6 \pm 0.1) \times 10^9$, and $(7.6 \pm 0.1) \times 10^9$ $M^{-1}$ $s^{-1}$, and for $e^-_{aq}$ were $(2.2 \pm 0.1) \times 10^{10}$, $(1.3 \pm 0.2) \times 10^{10}$, $(2.3 \pm 0.1) \times 10^{10}$, and $(2.5 \pm 0.1) \times 10^{10}$ $M^{-1}$ $s^{-1}$, respectively. The efficiencies for ·OH reaction with the four tetracyclines ranged from 32% to 60%. The efficiencies for $e^-_{aq}$ reaction were 15–29% except for chlortetracycline which was significantly higher (97%) than the other tetracyclines in spite of the similar reaction rate constants for $e^-_{aq}$ in all cases. To evaluate the use of advanced oxidation/reduction processes for the destruction of tetracyclines it is necessary to have reaction rates, reaction efficiencies and destruction mechanisms. This paper is the first step in eventually realizing the formulation of a detailed kinetic destruction model for these four tetracycline antibiotics.



## 1. Introduction

Antibiotic chemicals are one of the largest groups of pharmaceutical compounds used in human and veterinary medicine. Tetracyclines are the second most common antibiotic group, in both production and usage, worldwide (Gu and Karthikeyan, 2005). They are used to treat diseases in humans and are also applied to livestock to prevent disease and promote growth (Halling-Sorensen et al., 1998). Chlortetracycline and oxytetracycline are two of the ten antimicrobials licensed as growth promoters for livestock in the United States (Yang and Calson, 2003).

The annual usage of antibiotics has been estimated to be between 100 000 and 200 000 t globally (Kuemmerer, 2003). Traditionally, these compounds were not regarded as environmental contaminants; however, their occurrence in aquatic systems has become a concern as biological impacts and potential risks to the environment, as well as to human health, have been reported (Hirsch et al., 1999; Boxall et al., 2003; Banik and Hossain, 2006). It has also been shown that residual antibiotics can promote the selection of genetic variants of microorganisms resulting in the occurrence of antibiotic resistant pathogens (Chee-Sanford et al., 2001).

There is increasing evidence that these compounds are ubiquitous in aquatic environments at trace levels, ng $L^{-1}$ to μg $L^{-1}$, and, that the main source is excretion, both in biologically active and inactive forms, as they are not completely metabolized in humans or animals (Halling-Sorensen et al., 1998; Tolls, 2001). In the United States, residues of tetracyclines have been detected at 0.11 μg $L^{-1}$ levels in surface waters while in sewage treatment plants, concentrations of 0.52 μg $L^{-1}$ in influents and 0.17 μg $L^{-1}$ in effluents have been reported (Kolpin et al., 2002; Karthikeyan and Meyer, 2006). In Korea, where most livestock farming relies on tetracycline antibiotics to support intensive breeding operations, a nationwide survey of livestock wastewater treatment plants showed concentrations of chlortetracycline of as high as 2960 and 524 μg $L^{-1}$ in influents and effluents, respectively (NIER, 2007).

Conventional drinking water treatment processes were not designed to remove trace quantities of chemicals such as antibiotics and, given the increasing number of reports of their presence in the environment, it is essential that alternative technologies be developed which effectively degrade or remove these compounds (Stackelberg et al., 2004; Westerhoff et al., 2005; Choi et al., 2008). One such alternative is advanced oxidation/reduction pro-

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cesses (AO/RPs) (Rosenfeldt and Linden, 2004; Song et al., 2008b). AO/RPs are characterized by the production of ·OH as an oxidant and either hydrated electron ($e^-_{aq}$) or hydrogen atom (H·) as reductants. Relatively few reports have appeared relating to the degradation of tetracyclines by AOP oxidation, with the exception of photo-Fenton, or UV/$TiO_2$ processes (Reyes et al., 2006; Bautitz and Nogueira, 2007).

This study reports the destruction mechanisms of four tetracyclines as inferred from the reaction intermediates, from reactions with ·OH and $e^-_{aq}$ in deionized water. To elucidate destruction mechanisms it was necessary to start in pure water solutions before complicating the system with natural organic matter or carbonate/bicarbonate alkalinity, as found in natural waters. This study used liquid chromatography–mass spectroscopy (LC–MS) to propose the destruction mechanism for four tetracyclines.

To provide additional information on their destruction, the absolute bimolecular reaction rate constants for ·OH and $e^-_{aq}$ with four tetracyclines (tetracycline, TC; chlortetracycline, CTC; oxytetracycline, OTC; and, doxycycline, DC) were evaluated. The transient absorption spectra, formed by the reaction of ·OH with the tetracyclines were also measured over the time period of 1–250 μs to assist in choosing the appropriate wavelength for direct kinetic evaluation of the reaction rates. Prior to application of AO/RPs to degradation of tetracylines, another important consideration is the efficiency of reaction for ·OH and $e^-_{aq}$. This efficiency was evaluated for the tetracyclines using steady-state γ-irradiation to simulate both oxidative and reductive processes.

## 2. Experimental methods

### 2.1. Materials

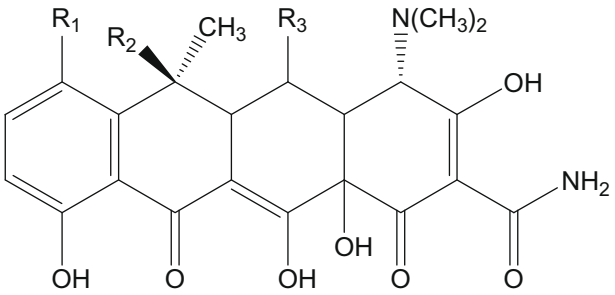
All chemicals were of reagent grade and used without further purification. The tetracycline antibiotics selected for this study were purchased from Sigma–Aldrich (⩾99% purity). The chemical structures of the tetracyclines are shown in Fig. 1. Solutions used in the experiments were prepared with filtered, deionized water from a Millipore Milli-Q system.

### 2.2. Pulse radiolysis and γ-radiolysis

Electron pulse radiolysis experiments were performed at the Radiation Laboratory, University of Notre Dame, with the 8-MeV Titan Beta model TBS-8/16-1S linear accelerator. This irradiation and transient absorption detection system has been described in detail elsewhere (Whitham et al., 1996). Dosimetry for the electron pulse radiolysis was based on the transient absorbance produced in $N_2O$-saturated $1 \times 10^{-2}$ M potassium thiocyanate solutions at $\lambda = 472$ nm, ($G = 5.2 \times 10^{-4}$ m$^2$ J$^{-1}$) with average doses of 3–5 Gy per 2–3 ns pulse (Buxton and Stuart, 1995). All experiments were carried out at pH 7.0 ± 0.1 in the presence of 5 mM phosphate buffer, and at room temperature (22 ± 1 °C). The water system included constant illumination by a Xe arc lamp at 172 nm to keep total organic carbon concentrations below 13 μg L$^{-1}$. The experimental data were determined by averaging 8–12 replicate pulses using a continuous flow method for the solution being studied.

Radiolysis of water (Eq. (1)) generates three highly reactive species (·OH, $e^-_{aq}$, and H·) in addition to the formation of inert or less reactive molecular products ($H_2$, $H_2O_2$, $H^+$). The numbers in parentheses in Eq. (1) are the concentration of each species (*G*-values, μmol J$^{-1}$) (Spinks and Woods, 1964; Buxton et al., 1988).

$$H_2O \rightsquigarrow (0.28)\cdot OH + (0.06)H\cdot + (0.27)e^-_{aq} + (0.05)H_2 + (0.07)H_2O_2 + (0.27)H^+ \quad (1)$$




| Compound \ Functional group | $R_1$ | $R_2$ | $R_3$ |
|---|---|---|---|
| Tetracycline (TC) | H | OH | H |
| Chlortetracycline (CTC) | Cl | OH | H |
| Oxytetracycline (OTC) | H | OH | OH |
| Doxycycline (DC) | H | H | OH |

**Fig. 1.** Chemical structures of the four tetracyclines used in this study.

The reaction of ·OH was studied in nitrous oxide ($N_2O$) saturated aqueous solutions where $e^-_{aq}$ and H· are converted into ·OH (Eqs. (2) and (3)) (Buxton et al., 1988; Song et al., 2008a,b).

$$e^-_{aq} + N_2O + H_2O \rightarrow \cdot OH + OH^- + N_2 \quad k_2 = 9.1 \times 10^9\ M^{-1}\ s^{-1} \quad (2)$$

$$\cdot H + N_2O \rightarrow \cdot OH + N_2 \quad k_3 = 2.1 \times 10^6\ M^{-1}\ s^{-1} \quad (3)$$

The solutions used to study the reactions of $e^-_{aq}$ were pre-saturated with nitrogen in the presence of 0.1 M isopropanol in order to scavenge the ·OH and H·, converting them into relatively inert isopropanol radicals (Buxton et al., 1988; Song et al., 2008a).

Steady state γ-irradiation experiments were performed using a J.L. Shepherd Mark I Model A68 $^{137}$Cs (662 keV γ-radiation) Irradiator. This instrument has a fixed central rod source in a 30 cm diameter, 33 cm high cavity. Samples in glass test tubes were placed reproducibly in a rack at varying distances from the source guide tube to provide dosage rates varying from $4.2 \times 10^3$ to less than $5 \times 10^2$ Gy h$^{-1}$.

### 2.3. HPLC and LC–MS analysis

The degradation of tetracyclines was measured by HPLC using a Phenomenex Gemini C18 column (250 × 4.6 mm, 5 μm). The isocratic mobile phase was 10 mM phosphate buffer solution (pH 3.0):methanol:acetonitrile (80:10:10) at a flow rate of 1 mL min$^{-1}$. UV absorption, at a wavelength of 260 nm, was used for detection.

The LC–MS system used in the study consisted of an Agilent 1100 HPLC Pump and a Waters LCT Classic Mass Spectrometer with electrospray ionization source. A sample volume of 10 μL was applied to a Phenomenex Luna C18 (2) HPLC column (2.0 × 150 mm). The mobile phase was A: 98% $H_2O$ + 2% $CH_3CN$ + 0.2% formic acid and B: $CH_3CN$ + 0.2% formic acid. Gradient elution was 2% of B for 1 min followed by a linear increase to 95% B at 50 min, and then held constant for an additional 7 min. The mass spectral data were obtained in the negative ion mode between *m*/*z* 300 and 1000.

## 3. Results and discussion

### 3.1. Research goal

The ultimate goal of research as described in this paper is to formulate kinetic models which provide a way of utilizing reaction

rate constants and destruction mechanism that are then applied to describing AO/RP treatment alternatives. These data are also necessary, in part, for understanding the photochemically mediate portion of environmental fate in natural waters.

### 3.2. Destruction mechanisms of tetracyclines

The most controlled systems for studying the fundamental radical chemistry of AO/RPs are undoubtedly those employed in radiation chemistry, i.e. pulsed radiolysis and gamma-irradiation. Using accurately calibrated dosimetry it is possible to predict the absolute concentration of the reactive species of interest (Eq. (1)). Thus, in the case of gamma irradiation, the exact dose and therefore the exact concentration of the reactive species can be changed by a combination of distance from the source and the time of irradiation.

The identification of tetracyclines reaction by-products of the γ-irradiation was based on the analysis of the total ion chromatograms and the corresponding mass spectra that were obtained by negative ion electrospray LC–MS. The gamma irradiation experiments were conducted under air-equilibrated 0.5 mM solutions of tetracyclines. The pathways are combined according to the functional group on $R_3$ position of tetracylines Fig. 2 for TC and CTC ($R_3$: –H) and Fig. 3 for OTC and DC ($R_3$: –OH).

Fig. 2 shows the product assignments from γ-irradiation of TC and CTC. The products with MW 460 and 494 were observed for TC and CTC, respectively, which are consistent with the hydroxylation of aromatic ring ('ring 1'). Addition of the electrophilic ·OH to

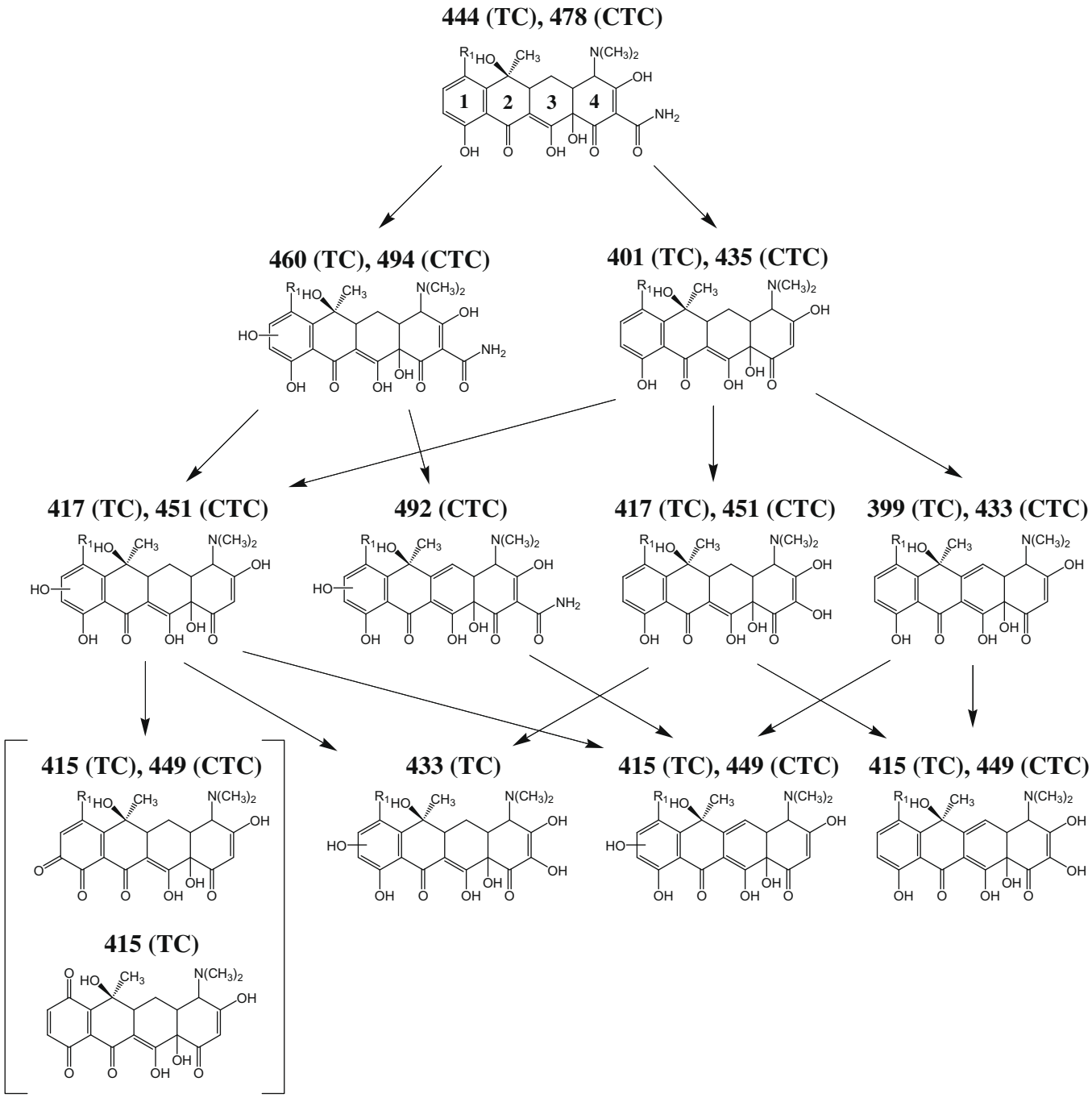



**Fig. 2.** Degradation products and proposed reaction pathways for TC and CTC.

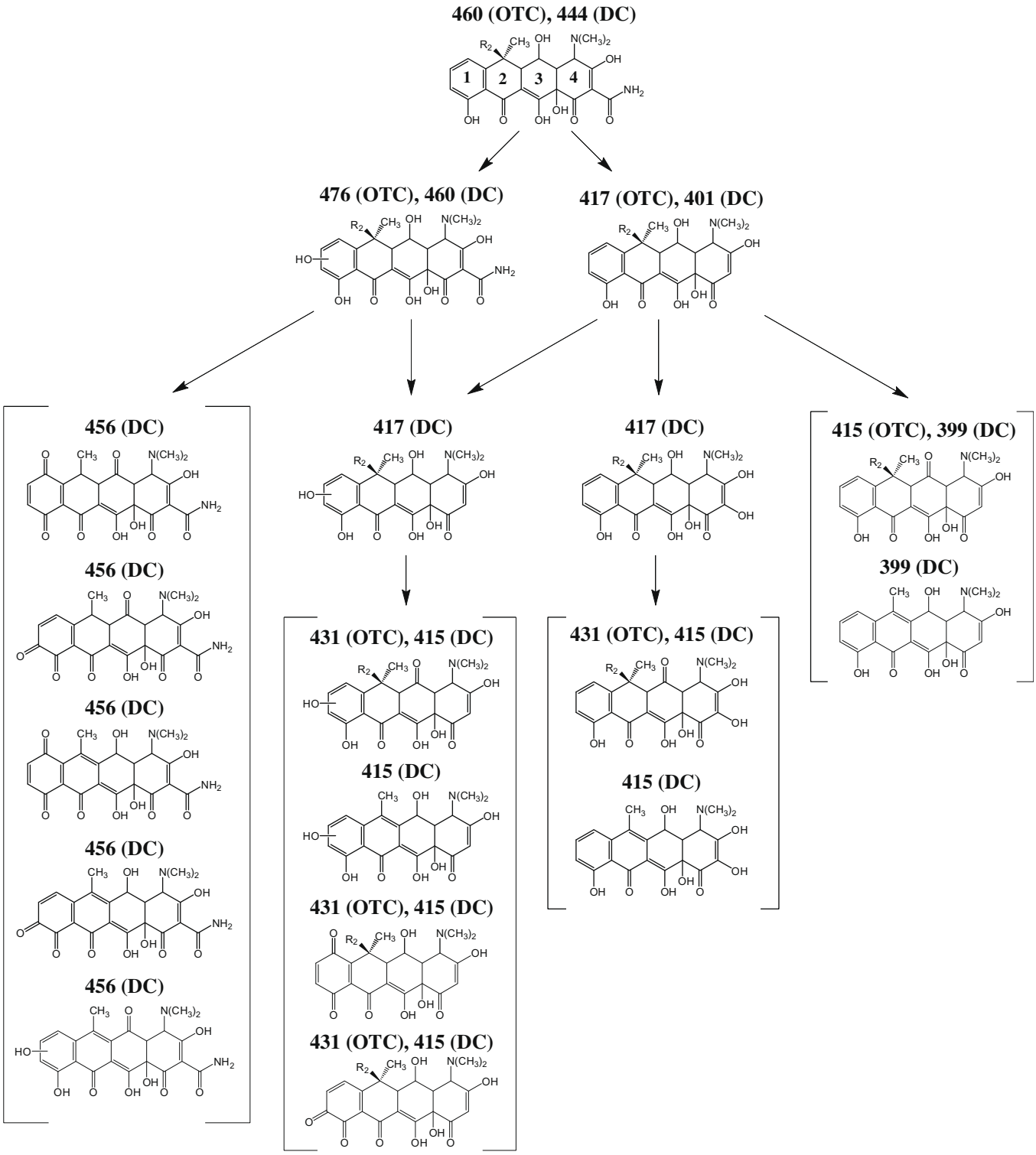
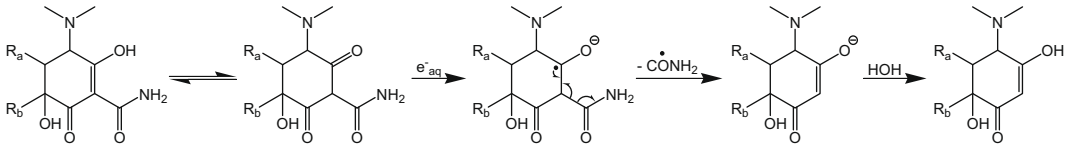



**Fig. 3.** Degradation products and proposed reaction pathways for OTC and DC.

the aromatic ring forms a resonance-stabilized carbon-centered radical with subsequent addition of oxygen, which is followed by the elimination of a hydroperoxyl radical giving a phenolic product (Kurata et al., 1988; Song et al., 2008b). The product with MW 401 (TC) and 435 (CTC) (decrease of 43 atomic mass units from the parent) are attributed to the $e^-_{aq}$ reduction at the ketone position of 'ring 4' with subsequent deamidation as shown in Scheme 1. A keto-enol tautomerization is suggested with the addition of the $e^-_{aq}$ in the keto-tautomer, forming an ion radical with subsequent elimination of formamide radical and enol product (Scheme 1).

Further reaction of these enol products with ·OH results in the formation of products with MW 417 (TC) and 451 (CTC), which are assigned to the products for the hydroxylation of 'ring 1' or 'ring 4'. The products with MW 399 (TC) and 433 (CTC) have two mass units less than the primary products with MW 401 (TC) and 435 (CTC), which suggest these products arise from H-abstrac-



**Scheme 1.**

tion reaction with ·OH. The reaction mechanisms are proposed in the Scheme 2: Hydrogen abstraction atom at 'ring 3' by ·OH resulted in a *tertiary*-carbon centered radical, with subsequently addition of oxygen to produce peroxide radical, followed by the elimination of a hydroperoxyl radical giving alkene products.

Furthermore, the hydroxylation products with MW 417 (TC) and 451 (CTC) could be oxidized to form the quinone products with MW 415 (TC) and 449 (CTC).

The product assignments arising from γ-irradiation of OTC and DC follow the trend of TC and CTC, and are summarized in Fig. 3. The products with MW 476 (OTC) and 460 (DC) and with MW 417 (OTC) and 401 (DC) are assigned to the products for ·OH addition to the 'ring 1' (addition of 16 mass units to the parent molecular weights) and the deamidation products generated by $e^-_{aq}$ reaction with the amide group at the position of 'ring 4' (decrease of 43 mass units to the parent MWs), respectively. The product with MW 456 for DC was attributed to two consecutive oxidations by ·OH reactions (decrease of four mass units), which can be accounted for by any combination of following possible ·OH reactions: (a) the oxidation of –OH group at 'ring 3' to form the ketone product, (b) the formation of quinone at 'ring 1', (c) H-abstraction to form double bond at 'ring 2'. In a similar way, the deamidation products for OTC (MW 417) and DC (MW 401) could also be further oxidized by ·OH, resulting in the formation of products with MW 415 (OTC) and 417, 399 (DC).

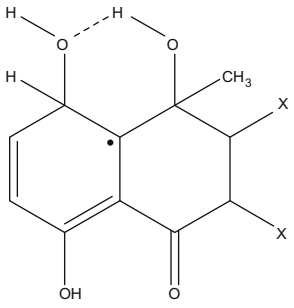
As this was the first study to examine the destruction mechanisms of these compounds in any detail, it was not possible to predict the behavior of the compounds under gamma irradiation conditions. Additional studies will have to be conducted to follow the destruction further; however, this will require synthesis of individual reaction by-products to allow structural elucidation of those further degradation by-products and is beyond the scope of the present study.

### 3.3. ·OH transient spectra

To support the mechanistic studies and obtain absolute bimolecular reaction rate constants of the ·OH with the tetracyclines, transient absorption spectra were obtained in neutral solution (pH 7) in the wavelength range 400–550 nm for up to 250 μs (Fig. 4). Because the four tetracyclines showed a strong self-absorption below 400 nm, transient spectra obtained from electron pulse radiolysis were recorded only at wavelengths higher than 400 nm. As shown in Fig. 4, the spectra of TC, OTC, and DC had very similar shapes with a peak at 430 nm and a shoulder between 450 and 460 nm. The spectra for CTC showed a peak slightly red-shifted ($\lambda_{max}$ = 440 nm), with a shoulder at 470 nm. The red-shift for CTC suggests that the transient intermediate of OH adduct could be stabilized by the electron drawing –Cl substituent at the $R_1$ position (Fig. 1) (Merga et al., 1994).

The addition of ·OH to the electron rich aromatic ring in the tetracyclines was observed in the transient absorption spectra as a red-shifted by 50 nm peak, when compared to the $\lambda_{max}$ of the parent compounds (Sharma et al., 1997). The molar absorptivity ($\varepsilon_{max}$) at the $\lambda_{max}$ for the four tetracyclines was obtained, using a *G*-value of 0.59 μmol $J^{-1}$ for the ·OH (Laverne and Pimblott, 1993), and are listed in Table 1.

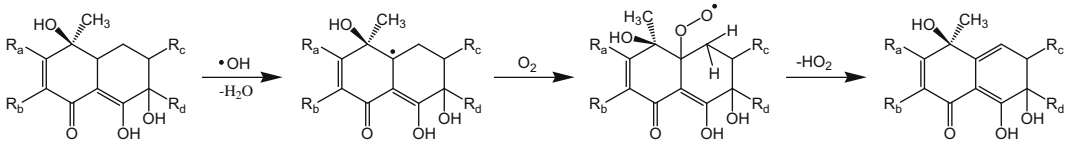
The maximum absorbance of the transient spectra for TC and OTC were observed at 50 and 100 μs, respectively, whereas for CTC and DC they occurred at 5 μs (Fig. 4). The –OH moiety at the $R_2$ position in TC and OTC may have led to the stabilization of a reaction intermediate through hydrogen bonding of the radical as shown below:



Attack of the benzyl moiety at the $R_1$-position would lead to an intermediate that is stabilized by the –OH at $R_2$. However, when this position is chlorinated (CTC) attack is less likely and therefore the radical is not stabilized leading to the observation that the maximum absorption occurs at 5 μs. Similarly for DC the lack of –OH at $R_2$ means that the hydrogen bonded radical shown above cannot be formed. The longer time of maximum absorption for OTC compared with that for TC implies that the –OH moiety at $R_3$ may also have an effect on stabilizing the radical intermediate.

### 3.4. Kinetic measurements

The rate constants for ·OH reaction with the four tetracyclines were determined from the buildup of the maximum absorption of the intermediates at the $\lambda_{max}$. Typical kinetic data for TC, are shown in Fig. Supplementary material (SM)-1. The formation of intermediates exhibited pseudo-first-order kinetics (Fig. SM-1a) and the rate increased linearly with the concentration, 0.1–0.5 mM (Fig. SM-1b). The second-order rate constants of ·OH ($k_{\cdot OH}$) were obtained from the slopes of the linear plots for the pseudo-first-order rate constants ($k_{obs}$) as a function of the concentration of the tetracyclines. The $k_{\cdot OH}$ values obtained for the four tetracyclines in this study are listed in Table 1.



**Scheme 2.**

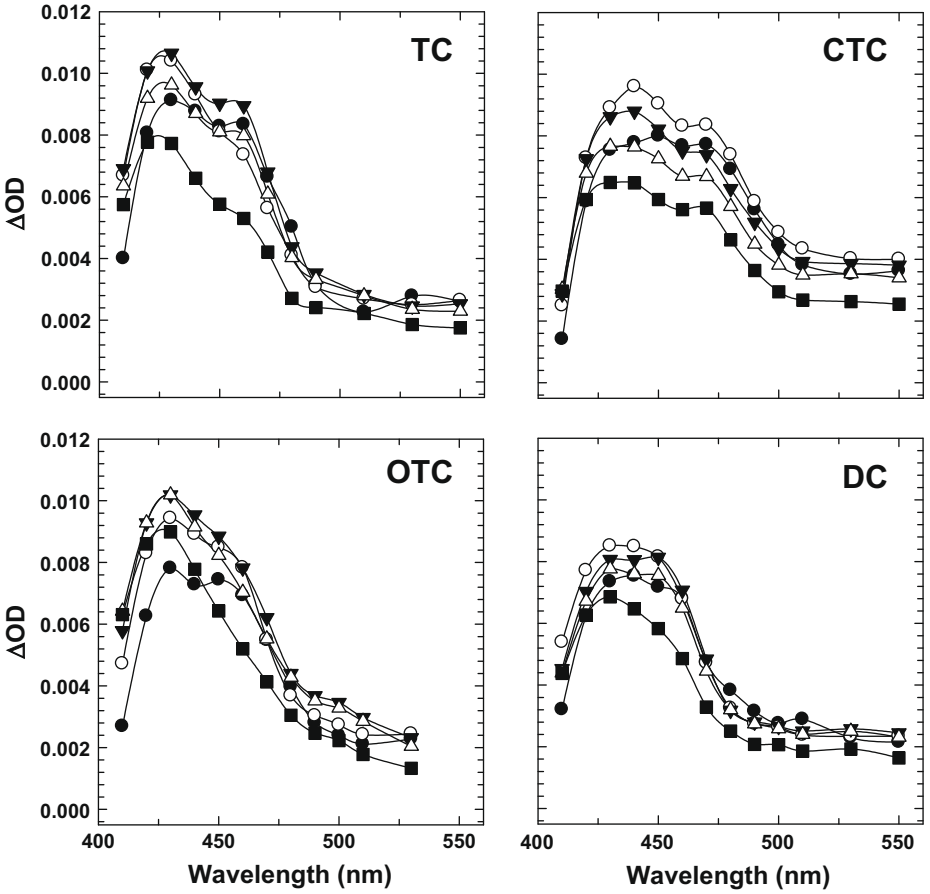



**Fig. 4.** Transient absorption spectra obtained by hydroxyl radical oxidation of TC, CTC, OTC, and DC in $N_2O$-saturated water (pH 7) at room temperature (22 °C) (–●–: 1 μs, –○–: 5 μs, –▼–: 50 μs, –△–: 100 μs, –■–: 250 μs).

**Table 1**
Summary of the transient spectra, second-order rate constants, and degradation efficiencies for the four tetracyclines in this study.

| Parameter/units | Tetracycline | Chlortetracycline | Oxytetracycline | Doxycycline |
|---|---|---|---|---|
| Maximum absorption wavelength (nm) | 430 | 440 | 430 | 430 |
| Molar absorptivity ($\varepsilon_{max}$) of transient species ($M^{-1}$ $cm^{-1}$) | 2814 | 2423 | 2540 | 2282 |
| ·OH rate constant ($10^9$ $M^{-1}$ $s^{-1}$) | 6.3 ± 0.1 | 5.2 ± 0.2 | 5.6 ± 0.1 | 7.6 ± 0.1 |
| $e^-_{aq}$ rate constant ($10^{10}$ $M^{-1}s^{-1}$) | 2.2 ± 0.1 | 1.3 ± 0.2 | 2.3 ± 0.1 | 2.5 ± 0.1 |
| Initial degradation rate (mM $kGy^{-1}$) (air-equilibrated solution) | 0.153 ± 0.008 | 0.254 ± 0.007 | 0.189 ± 0.017 | 0.148 ± 0.005 |
| Initial degradation rate (mM $kGy^{-1}$) ($N_2O$-saturated solution) | 0.212 ± 0.011 | 0.183 ± 0.019 | 0.308 ± 0.028 | 0.178 ± 0.018 |
| Degradation efficiency of ·OH (%) | 40 ± 2 | 32 ± 4 | 57 ± 5 | 32 ± 3 |
| Degradation efficiency of $e^-_{aq}$ (%) | 23 ± 1 | 97 ± 2 | 15 ± 1 | 29 ± 2 |

The rate constant measured for TC $(6.3 \pm 0.1) \times 10^9$ $M^{-1}$ $s^{-1}$, is slightly lower than that reported earlier ($7.7 \times 10^9$ $M^{-1}$ $s^{-1}$), as measured by the competition kinetic method under steady-state γ-radiolysis (Dodd et al., 2006). The values of rate constants for the four tetracyclines are all close, ranging from 5.2 to $7.6 \times 10^9$ $M^{-1}$ $s^{-1}$. Thus, there is no significant effect of the substituents at the $R_1$, $R_2$ and $R_3$ positions on the overall ·OH reaction rate constants. This observation is likely due to the fact that the extended conjugation and multiple unsaturated sites of the tetracyclines lessens the influence of substituent's while providing multiple sites for ·OH attack. The rate constants for tetracyclines are consistent with those reported for the reactions of ·OH with typical aromatic compounds such as benzene ($7.5–7.8 \times 10^9$ $M^{-1}$ $s^{-1}$) and phenol ($6.6 \times 10^9$ $M^{-1}$ $s^{-1}$) but faster than those for trimethylamine ($4.0 \times 10^8$ $M^{-1}$ $s^{-1}$) (Buxton et al., 1988), supporting the fact that the aromatic ring moieties are the main sites of ·OH attack.

In a similar way the rate constants for the reaction of $e^-_{aq}$ with the four tetracyclines were determined by plotting the $k_{obs}$ versus tetracycline concentrations (0.1–0.5 mM). For TC, the plot yielded a straight line with a slope of $k_{e^-_{aq}} = (2.2 \pm 0.1) \times 10^{10}$ $M^{-1}$ $s^{-1}$, which is consistent with that reported previously ($1.9 \times 10^{10}$ $M^{-1}$ $s^{-1}$) (Sabharwal and Kishore, 1994) (Fig. SM-2). The $k_{e^-_{aq}}$ values obtained for the four tetracyclines in this study are given in Table 1.

The rate constants for the reaction of $e^-_{aq}$ with these tetracyclines were similar and ranged from 1.3 to $2.5 \times 10^{10}$ $M^{-1}$ $s^{-1}$. These values are much higher than those for the common functional moieties that compose the tetracyclines including benzene, phenol, amines, and saturated cyclic compounds whose rate constants are in the range of $10^6–10^7$ $M^{-1}$ $s^{-1}$ (Spinks and Woods, 1964; Buxton et al., 1988). Sabharwal and Kishore suggested that the most probable site for the reaction of $e^-_{aq}$ with the TC molecule is at a carbonyl group because the $e^-_{aq}$ is highly reactive toward

molecules containing a carbonyl group adjacent to a double bond, over which the electron can be easily delocalized.

### 3.5. Degradation efficiency

In order to better understand the application of AO/RPs for the degradation of tetracyclines by ·OH and $e^-_{aq}$, estimates of the reaction efficiencies by both species were obtained using steady-state $^{137}$Cs γ-irradiation, as described in Mezyk et al. (2007). The efficiency is defined as follows (Eq. (4)).

$$\text{Reaction efficiency}\,(\%) = 100 \times \frac{(\text{number of solute molecules degraded})}{(\text{number of specific radical reactions})} \quad (4)$$

The concentration of each reactive species produced during γ-irradiation was calculated based on the *G*-values in Eq. (1) and the kinetic rate constants for each reactive species. An example of the data from which degradation efficiencies can be determined is given in Fig. 5. The curved lines show the changes in the concentration of TC, as analyzed by HPLC, resulting from steady-state γ-irradiation under: (a) air-equilibrated and (b) $N_2O$-saturated conditions.

Reaction efficiencies were calculated from the initial slopes (hatched lines in Fig. 5) that were derived from the graphs of the destruction of the tetracyclines by ·OH and $e^-_{aq}$. It was assumed that the ·OH and $e^-_{aq}$ were the only reactive species responsible for degrading the tetracyclines during the γ-irradiation because of the low yields of H·. Since the initial concentrations of tetracyclines (≈0.5 mM) were much higher than those of the reactive species, it was also assumed that at the early stages of the experiments both the ·OH and $e^-_{aq}$ generated reacted only with the parent tetracyclines and did not undergo any secondary reactions with the degradation products. In the $N_2O$-saturated solution, the ·OH should be the sole reactive species responsible for the degradation of tetracyclines, where $e^-_{aq}$ and H· are converted into ·OH (Eqs. (2) and (3)) (Buxton et al., 1988; Song et al., 2008a,b). The initial degradation rate of air-equilibrated could then be derived by a summation of the products of the degradation efficiencies and the yields of the reactants as described by Mezyk et al. (2007):

$$\text{Initial degradation rate}\ (\text{mM kGy}^{-1}) = E_{\cdot OH}Y_{\cdot OH} + E_{e^-_{aq}}Y_{e^-_{aq}} \quad (5)$$

where the $E_{\cdot OH}$ and $E_{e^-_{aq}}$ are the individual degradation efficiencies for the reaction of ·OH and $e^-_{aq}$ with the tetracyclines, and the $Y_{\cdot OH}$ and $Y_{e^-_{aq}}$ are the yields of the reactive species (see Eq. (1)). The details on the calculation of the individual degradation efficiencies for the reaction of ·OH and $e^-_{aq}$ with the tetracyclines are described in the Supplementary material.

Table 1 summarizes the degradation efficiencies of ·OH and $e^-_{aq}$ for the four tetracyclines. The efficiencies for ·OH reaction with the four tetracyclines ranged from 32% to 60%. The efficiencies for $e^-_{aq}$ reaction were 15–29% except for CTC which was significantly higher (97%) than the other tetracyclines in spite of the similar reaction rate constants for $e^-_{aq}$ in all cases. The much higher degradation efficiency for $e^-_{aq}$ reaction with CTC compared to that for the other tetracyclines most probably reflects a difference in mechanism. Presumably $e^-_{aq}$ attachment occurs at the Cl substituent in CTC that is irreversible. This is in contrast to the other tetracyclines where it is likely that radical intermediates are being formed with the $e^-_{aq}$ that are similar to the radical anion observed in the reaction with acetone (Bothe et al., 1977). Such a radical anion can either form a peroxyl radical that can eliminate $\cdot O_2^-$ and collapse back to the starting compound or result in degradation. The fact that this is a reversible reaction that can regenerate the starting material will result in a reduction of the overall degradation efficiency when compared with irreversible $e^-_{aq}$ attachment.

Reaction efficiencies are critical to the formulation of a kinetic model so that forward and backward reactions are considered (Cole et al., 2007). The mechanistic reasoning for less than 100% efficiency is not known and suggests that future studies be conducted. It is possible that ab initio calculations may be the easiest method for developing a better understanding of this concept using transition state theory as a beginning point. The efficiencies that are reported are only for the four parent tetracyclines studied. A complete kinetic model it would require synthesis and evaluation of each major reaction by-products and again is beyond the scope of this study.

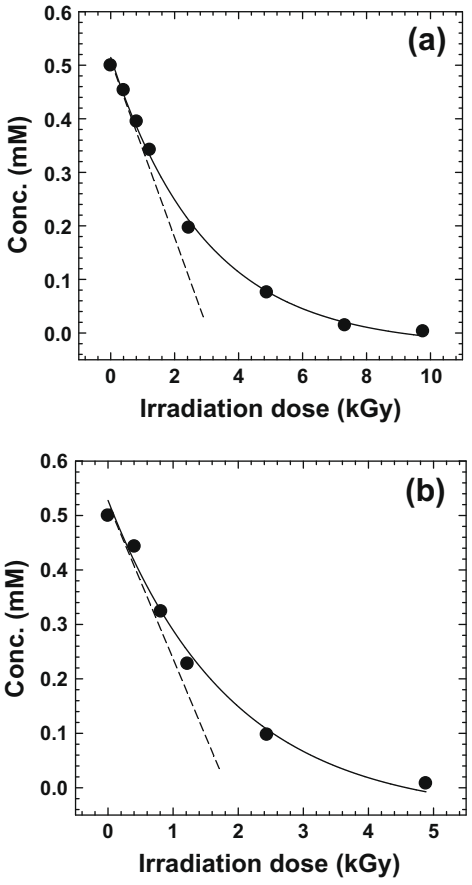



**Fig. 5.** Determination of reaction efficiency of tetracycline using $^{137}$Cs-irradiation (a) in air-equilibrated and (b) in $N_2O$-saturated aqueous solution. Curves are the fitted quadratic function, and the hatched lines correspond to the initial slopes with values of 0.153 ± 0.008 mM kGy$^{-1}$ and 0.212 ± 0.011 mM kGy$^{-1}$, respectively.

## 4. Conclusions

The goal of the degradation of tetracyclines would be to reduce or eliminate their biological effects and to assist in biodegradation of the by-products. It is clear that the destruction mechanisms are complicated; however, it was possible to develop an understanding of the reactions that led to the initial major by-products. The absolute reaction rate constants although very high, have to be taken in the context of the overall reaction efficiencies for the reaction of the ·OH and $e^-_{aq}$ with the parent tetracyclines. The reaction efficiencies were widely different from compound to compound reflecting the location of the attack by the reactive species on the tetracycline studied. Both oxidation and reduction will contribute to degradation; however, the variability in reaction efficiencies suggests that different destruction mechanisms were

operative in these compounds. Therefore, future research will have to be conducted to evaluate the biological activity of these individual compounds or mixtures of reaction by-products from each of these compounds. Another consideration in the application of AO/RPs is to extend these studies to water that would be typically found in water or water reuse treatment and to determine the effect of natural organic matter and the carbonate alkalinity on destruction.

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## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2009.11.024.

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