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Tetracycline degradation by ozonation in the aqueous phase: Proposed degradation intermediates and pathway

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ABSTRACT

During the ozonation of tetracycline (TC) in aqueous media at pHs 2.2 and 7.0, the effects of pH variations, protonation and dissociation of functional groups and variation in free radical exposure were investigated to elucidate the transformation pathway. Liquid chromatography–triple quadrupole mass spectrometry detected around 15 ozonation products, and uncovered their production and subsequent degradation patterns. During ozonation at pH 2.2, the TC degradation pathway was proposed on the basis of the structure, ozonation chemistry and mass spectrometry data of TC. Ozonation of TC at the C11a–C12 and C2–C3 double bonds, aromatic ring and amino group generated products of *m*/*z* 461, 477, 509 and 416, respectively. Further ozonation at the above mentioned sites gave products of *m*/*z* 432, 480, 448, 525 and 496. The removal of TOC reached a maximum of ≈40% after 2 h of ozonation, while TC was completely removed within 4–6 min at both pHs. The low TOC removal efficiency might be due to the generation of recalcitrant products and the low ozone supply for high TC concentration. Ozonation decreased the acute toxicity of TC faster at pH 7.0 than pH 2.2, but the maximum decrease was only about 40% at both pHs after 2 h of ozonation. In this study, attempts were made to understand the correlation between the transformation products, pathway, acute toxicity and quantity of residual organics in solution. Overall, ozonation was found to be a promising process for removing TC and the products initially generated.

1. Introduction

Antibiotics, comprising a significant amount of pharmaceutical compounds, are used as human and veterinary treatments and as growth promoters in livestock-farming [1]. In Europe during 1999, approximately 8500 and 4700 metric tons of antibiotics were used as human and veterinary medicines, respectively, with the global production and use of tetracycline (TC) ranking second [2]. The environmental concentrations of these antibiotics are very low, but sustained (≈ng L^{-1} or ≈μg L^{-1}). Recent studies investigating the environmental presence and management of antibiotics have indicated considerable adverse effects on humans and ecological systems; therefore, the investigation of new resistant bacterial strains is of utmost importance [3]. In a US study, the concentrations of TC residues found in the influent and effluent of wastewater treatment plants ranged between 1.1–0.32 and 0.075–0.41 μg L^{-1}, respectively [4]. Low concentrations of TC can be reduced by 60–90% using conventional activated sludge treatment, typically operating with solid-retention times of several days, which results in prolonged exposure of wastewater-borne bacteria to antibiotics. This condition may favor the evolution of low-level antibacterial resistance in affected bacterial communities [5]. The removal of TC from real wastewater using UV treatment was not as effective as previously thought [4]; whereas, ozonation is effective in the removal of antibiotics and recalcitrant pollutants from wastewater, even for dissolved organic carbon levels of 23 mg C/L [5–9]. Therefore, ozonation for the removal of TC from water, the generation of transformation products and the oxidation pathway, which are affected by variations in the pH, were investigated.

Triple quadrupole mass spectrometry was employed to identify and quantify the transformation products generated during ozonation. Triple quadrupole mass spectrometry works using three sets of parallel rods, i.e., quadrupole, hexapole and quadrupole. The first quadrupole separates ions into precursor ions that are fragmented into product ions in the hexapole, which are separated by the second quadrupole. In this process, a precursor ion is selected from the first quadrupole, and its fragments scanned by the second quadrupole, resulting in the mass spectrum of that precursor ion. This is referred as the 'product ion (PI)' mode. This mass spectrum can be used to identify a compound's fingerprint. The second option in triple quadrupole mass spectrometry involves the selection of a precursor ion from the first quadrupole, with its product ion from the second quadrupole selected and quantified. This is called 'mul-

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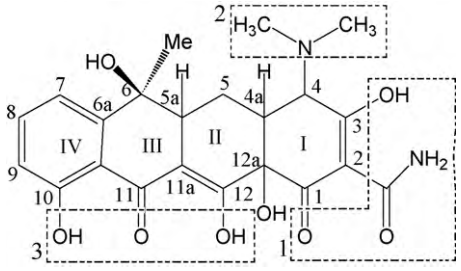


Fig. 1. Chemical structure and pKa of tetracycline due to combinations of different groups. '1' = 3.3, '2' = 7.7, '3' = 9.7.

tiple reactions monitoring (MRM)' and is the most sensitive mode used in quantitative analysis [10].

Ozone reacts with electron rich functional groups of target molecules: double bond, amino group and aromatic ring, etc. The reaction of ozone with olefins at double bond sites has been well established, and is reported to be independent of certain variables [5]. The reaction of ozone with TC at the double bond site and the two products formed during this reaction have been reported in the literature [11]; these are discussed in Section 3.1.3. The reaction of ozone with amines has also been suggested in the literature, with a complex reaction pathway depending on the pH. In this study, the diverse products of TC ozonation were further identified than those in previous research.

TC has pKa values of 3.3 (combination '1'), 7.7 (combination '2') and 9.7 (combination '3') (Fig. 1) [5,12]. Under acidic conditions, the hydroxyl group at ring 'I' would be dominantly non-dissociated and the amide group being in the protonated form. This non-dissociated, protonated form would decrease the electron densities on the C2–C3 double bond, and the amide and ketone groups at C1 via conjugation. In turn, the decreased electron densities would lower the probability of ozone attack at these sites. At a pH above the pKa of combination '1', the dissociation of protons from the functional groups would affect the rate and pathway of the reaction. In this study, pHs 2.2 and 7.0 were selected as they are above and below the pKa of the combination '1' of groups at TC, respectively. The variations in toxicity, UV_{254} and total organic carbon (TOC) were monitored to understand their correlation with the transformation products, the pathway of their formation and the quantity of residual organics in solution.

2. Experimental

2.1. Chemical and stock solutions

All chemicals in this study (dichloromethane and sodium nitrite) were of analytical grade and used without further purification. TC, with purity more than 98.0%, was from Sigma–Aldrich Fluka. The acetonitrile and water used as the mobile phase in HPLC were from J.T. Baker, "Baker Analyzed® HPLC Solvent." Water was used within 2 weeks of opening the bottle.

2.2. Experimental system

Ozonation of aqueous solutions of TC was performed in a column-type reactor, with dimensions of 37 cm × 7.5 cm and a capacity ≈1.5 L (Fig. S1 given in Supporting Information). An ozone generator (Ozonia, LAB2B) produced 10 mg min^{-1} ozone when fed with pure oxygen at a flow rate of 2 L min^{-1}, which was subsequently bubbled through water using a glass tube with a sintered end. The TC solution (0.5 mM) was prepared in de-ionized water. The pH was adjusted using either phosphate buffer (0.008 M) or phosphoric acid (2.5 ml conc. phosphoric acid/L). Samples were collected in test tubes containing $NaNO_2$ solution to quench the oxidation reaction. To estimate the removal of TC due to physical phenomenon during ozonation, TC solution at pH 7.0 was sparged with oxygen, with samples taken to monitor the variation in the TC concentration.

2.3. Analytical procedures

The ozone content in the solution was estimated using the Indigo method [13]. TC solution samples were taken during ozonation and placed directly into a test tube containing $NaNO_2$ solution (0.002 M). These samples were acidified with phosphoric acid and extracted by solid phase extraction (SPE) using an Oasis HLB cartridge (Waters) and subsequently eluted with methanol, which gave TC recovery above 97% [14]. These concentrated samples were analyzed by HPLC (Agilent 1200) equipped with an Agilent SB-C18 column (50 mm length, 4.6 mm internal diameter and 1.8 μm particle size maintained at 20 °C) and triple quadrupole mass spectrometer (Agilent 6410). The following isocratic HPLC operating conditions were employed: Eluent A, water (20 μM ammonium formate in 0.3% formic acid); Eluent B, acetonitrile (pure), with 40% B, at a flow rate of 0.4 ml min^{-1}, with the analysis stopped after 4 min. Gradient separations of the same samples were performed by HPLC to facilitate the detection of new signals. The gradient solvent was as follows: B = 20% (0 min), 20% (1 min), 80% (3 min), 80% (4 min), 20% (4.1 min) and 20% (7 min). The HPLC UV detector was set at 280, 360 and 485 nm for the appropriate detection of TC and its oxidation products. TC was estimated via triple quadrupole mass spectrometry via the multiple reactions monitoring (MRM) mode, under the following conditions: ion mode = +ve, precursor ion = 445.1, product ion = 410.0, fragmentor = 100 V, collision energy = 20 V, gas = N_2, gas temperature = 350 °C, and gas flow rate 8 L min^{-1}. For the detection of TC ozonation products the same samples were used in the detection and quantification steps. New signals in the MS2 scan mode were detected and their m/z values obtained. The fragmentation pattern of each new signal was obtained by analysis of the samples in the MS product ion (PI) mode. The fragmentor and collision energies were varied to optimize the sensitivity, and the product ion with the highest sensitivity selected. Each sample was analyzed in the MS MRM mode for every new signal and quantified by integrating the signal area. As pure standards could not be prepared, only peak area integration was used to assess the production and decomposition of the oxidation products (Specific MRM conditions optimized for each signal are given in Supporting Information).

UV spectra, UV_{254} (Thermo Electron Corporation, Genesys6 UV spectrometer) and TOC (analytik Jena, multiN/C 3000) were measured and recorded using samples diluted three times with distilled water. The toxicities of the samples before and during ozonation were assessed using a bioluminescent assay with marine photobacteria, *Vibrio fischeri*, according to ISO 11348 (1994) [15,16]. The Microtox® Acute Toxicity bioassay system (Azur Environmental) was used to assess the toxicities of the samples, with the method described by the manufacturer followed throughout. The sample was applied in its original state without dilution. The bioluminescence was measured just before sample application and after a 30 min incubation time.

The toxicity was calculated as follows:

$$\text{Relative toxicity} = \left(\frac{\text{control luminescence} - \text{sample luminescence}}{\text{control luminescence}}\right)$$

All sample tests and control experiments were performed in triplicate, with the averages and standard deviations reported in this study.

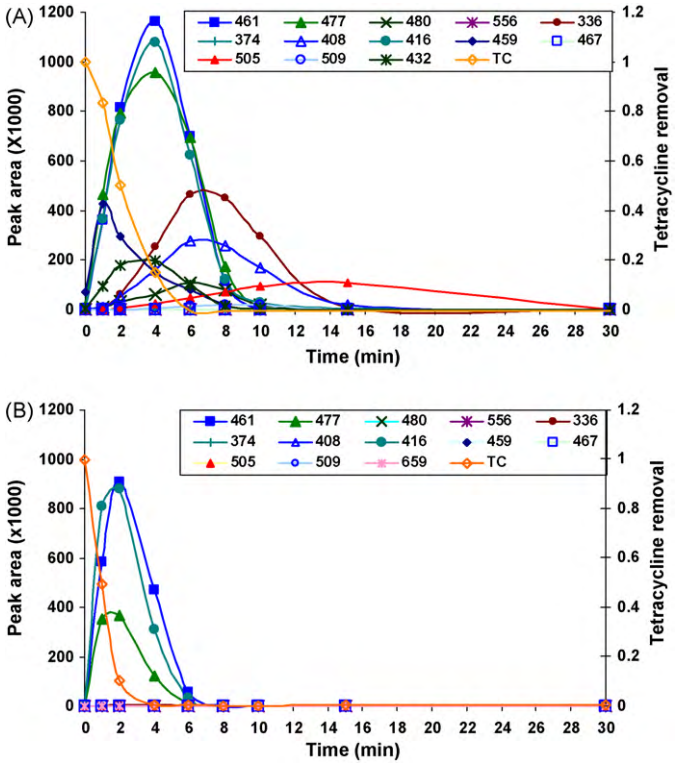


Fig. 2. Tetracycline removal with ozonation and pattern of products generation and degradation indicated by *m*/*z*. Conditions; TC = 0.5 mM, temperature = 20 °C, O_3 supply = 10 mg min^{-1}, (A) pH 2.2 ± 0.1, (B) pH 7.0 ± 0.1.

3. Results and discussion

3.1. Tetracycline ozonation

3.1.1. TC removal at different pHs

At pH 2.2, TC was completely removed after 6 min of ozonation (Fig. 2), but after 4 min at pH 7.0. Bubbling of only oxygen was unable to remove TC, excluding any physical process as being responsible for the removal of TC during the ozonation process. The increase in reaction rate at higher pH may have been due to the deprotonation and dissociation of TC, but can also be affected by the enhanced generation of free radicals during ozonation. During ozonation at pH 2.2, the reaction was dominantly with TC, which has a hydroxyl group on the ring 'I' in the non-dissociated from. Above pH 3.3, the dissociation of the hydroxyl group on the ring 'I' of the TC structure (Fig. 1) increases the reaction rate [5]. In addition, the tertiary amine group on ring 'I' of the TC structure becomes deprotonated at higher pH, which also causes an increase in rate of TC decomposition. At higher pH, hydroxyl radicals are generated from the decomposition of ozone, which is catalyzed by the hydroxyl ions in the aqueous media and the functional groups on the TC molecule. These hydroxyl radicals are much stronger oxidant than ozone itself [17] and can increase the rate of reaction, though the faster rate of the reaction of ozone with TC (10^5–10^7 M^{-1} s^{-1}) minimizes the effect of free radicals during the removal of TC [5]. The reactions of the TC ozonation products will be discussed in later sections.

3.1.2. New compounds generated during ozonation

At pH 2.2, total ion chromatograms (TIC) of the samples collected at reaction times of 0 and 4 min were compared and the TC ozonation products were identified (TIC's for these samples at different retention times are given in Supporting Information). More than 15 major and minor compounds with unique *m*/*z* ratios and MS fragmentation patterns were detected. The products at pH 2.2 were detected with *m*/*z* values of 416, 461, 477, 336, 408, 374, 432, 459, 467, 480, 483, 505, 509, 525, 527 and 556. When ozonation was performed at pH 7.0, only three compounds were detected, with *m*/*z* values of 416, 461 and 477. The other compounds generated were at too lower concentrations to be quantified.

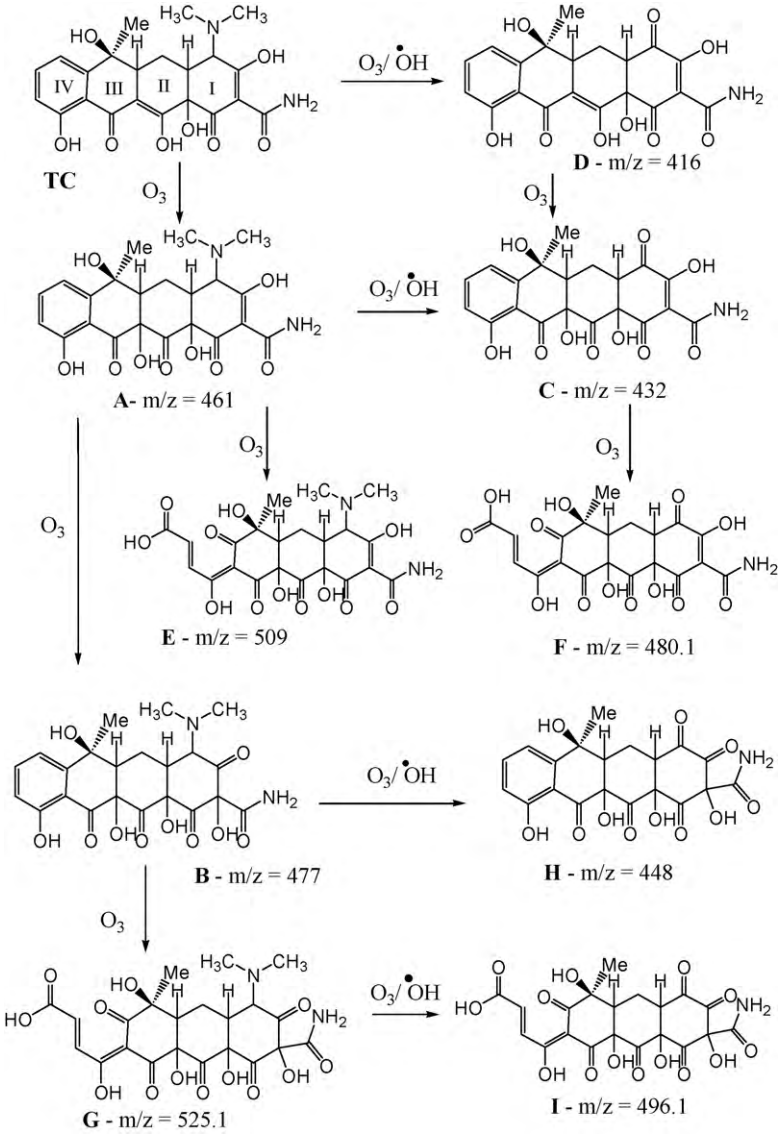
At pH 2.2, the decreased electron densities on the C2–C3 double bond, amide group and ketone group at C1, due to the non-dissociated and protonated form of TC, would lower the probability of ozone attack at these sites. Therefore, the TC degradation rate during ozonation at lower pH would decrease. At pH 7.0, the dissociation of protons from the functional groups would alter the rate of reaction, especially in the case of the amino group. Under acidic conditions, the dominant pathway of TC degradation and its ozonation products would be direct ozonation. These site-specific reactions of ozone with TC and its products would result in specific final products at sufficient concentrations to be detected and quantified.

Conversely, at higher pH, such as pH 7.0, the products of ozonation would be at low concentrations because the TC and its degradation products would be degraded more rapidly by direct ozonation [5]. Moreover, the free radicals will non-specifically react with the ozonation products of TC and are able to attack any site of the structure, producing a number of final low molecular weight products. The analytical system used in this study was unable to extract these products from the sample, which made them difficult to identify and quantify. Based on the integrated peak areas of the new compounds from all samples, a definite pattern of their generation and subsequent decomposition was able to be established (Fig. 2).

3.1.3. Tetracycline ozonation pathway

The new signals detected by HPLC/quadrupole MS during ozonation at pH 2.2 indicated that ozonation was taking place through a specific mechanism. One such mechanism (Scheme 1) could be proposed on the basis of the chemistry of TC, and the ozonation mechanisms and the MS spectra of TC products (MS spectra and fragmentation patterns of individual products are given in Supporting Information). During ozonation, two compounds were detected, with *m*/*z* values of 461 and 477. These compounds have been reported in a previous study [11]. For convenience, in this study, these compounds were designated as 'A' and 'B'. During the initial period of TC ozonation, three kinds functional groups will compete for ozone; double bond, amine group, and phenolic groups. 2-(3-Methylbutyryl)-5,5-dimethyl-1,3-cyclohexandione (MBDCH); a surrogate for the C11a–C12 double bond on TC, reacts with ozone, with a rate constant of 10^6 M^{-1} s^{-1} [5]. The direct ozonation of non-protonated amino groups takes place with a rate constant of 10^3–10^6 M^{-1} s^{-1}. The protonated amino groups react with ozone at a rate constant of approximately <0.1 M^{-1} s^{-1} [18]; although both pHs during the ozonation reactions were below the pKa of the amine group on TC. Ozonation of phenol is a pH dependent reaction. At pH 3, the rate constant of the ozone phenol reaction has been reported to be 1.3×10^3 M^{-1} s^{-1} [19], which lead to the conclusion that the C11a–C12 double bond on TC molecule was the most reactive site for direct ozonation at pH 2.2 as a result of the neighboring electron donating functional groups.

During classical ozonolysis, double bonds cleave completely to give carboxyl groups. When olefins are sterically hindered, which prevents the easy 1,3-dipolar cycloaddition of ozone, the partial cleavage of the C–C double bond leads to the formation of epoxide and singlet oxygen and yields the corresponding carbonyl compound [20,21]. When ozone reacts with the C11a–C12 double bond of TC, the epoxide groups which open to leave behind a hydroxyl and a ketone group at positions C11a and

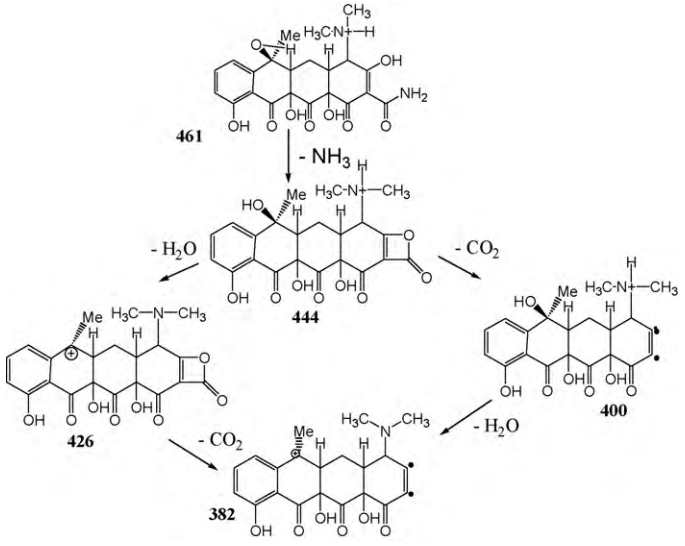


Scheme 1. Pathway of tetracycline degradation during ozonation: TC = 0.5 mM, temperature = 20 °C, pH 2.2 ± 0.1, O_3 supply = 10 mg min^{-1}.

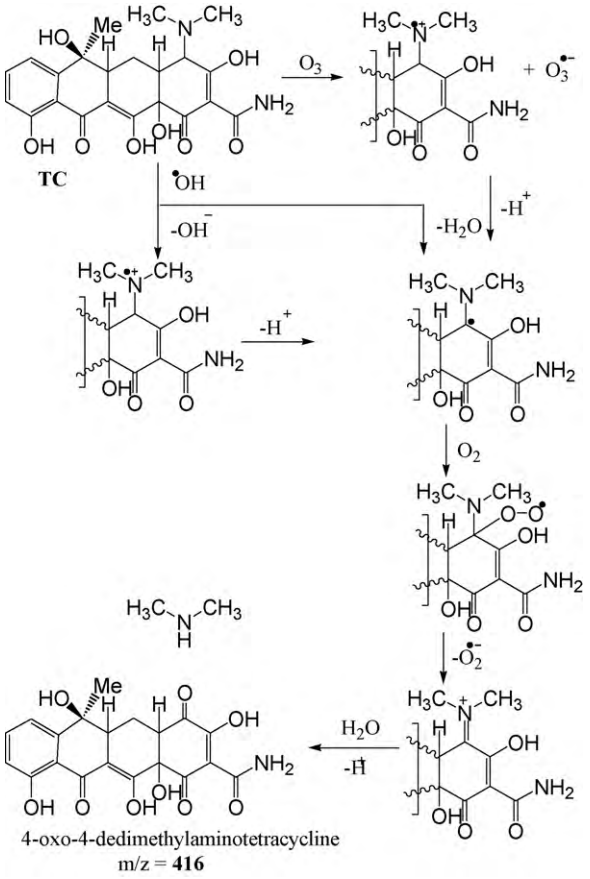
C12, respectively, result. Therefore, for the *m*/*z* value of 461, 11a-hydroxo-12-oxotetracycline was proposed as the compound. Scheme 2 (MS Spectra in Supporting information) indicated that fragmentation of 'A' yielded the ion with an *m*/*z* value of 444 on the loss of NH_3, which further fragmented to the ion with an *m*/*z* value of 400 on the loss of CO_2 and to the ion with an *m*/*z* value of 426 on the loss of H_2O. The two ions with *m*/*z* values of 400 and 426 yielded the ion with an *m*/*z* value of 382 on the losses of H_2O and CO_2, respectively. This fragmentation pattern supported that 'A' was the structure of the ion with an *m*/*z* value of 461.

The next vulnerable group for the attack of ozone was the double bond in the C2–C3 position. This bond has a lower electron density than the previous double bond. In the same way ozone could have attacked this bond to leave behind an epoxide, which opened to give a hydroxyl group and a ketone group at the C2 and C3 positions, respectively. For this signal, with an *m*/*z* value of 477, 2,11a-dihydroxo-3,12-dioxotetracycline was proposed as the compound.

The next group on TC attacked by ozone would be the dimethyl amino group at the C4 position. Ozone reacts with only free amino groups, whose concentration will depend on the reaction pH when the reaction is below the pKa of the amino group [18]. Gener-



Scheme 2. Proposed fragmentation pattern of compound 'A' with *m*/*z* 461.



Scheme 3. Pathway of tetracycline oxidation by free radicals to 4-oxo-4-dedimethlyaminotetracycline (m/z = 416) by different mechanisms.

ally, during the ozonation of tertiary amines in aqueous media, two primary products result, aminoxide (90%) with singlet oxygen, and N-centered amine radicals (10%) with the ozonide radical (Scheme 3) [22]. The latter is more important when considering free radical pathways. Ozonide decomposes to give a hydroxyl radical when protonated at low pH (e.g., by water) [23], and the N-centered amine radical deprotonates at the carbon to yield a carbon-centered radical. This conversion depends on the neighboring groups and the stereochemistry of the deprotonated carbon, because the tendency of a C-centered radical to have a planar conformation can exert high strain, which inhibits deprotonation [23]. For TC, no such strain exists on the C4 for the formation of a planar conformation. This was proven by finding that the atoms bonded at the C4 position make all three bond angles 120°, as calculated using 'CS Chem 3D pro'. In addition, a TC molecule has three conjugated double bonds (one olefin and two oxo groups on ring 'I'). This conjugation will extend to the C-centered free radical group, which may stabilize and enhance its formation. The reaction of a t-amine with the hydroxyl radical might also directly generate C-centered free radicals, similar to the aforementioned mechanism. These C-centered free radicals react with di-oxygen at a diffusion-controlled rate ($k \approx 2 \times 10^9$ dm^3 mol^{-1} s^{-1}), resulting in the formation of a peroxyl radical. This radical is very short lived and yields both a Schiff-base and superoxide. Superoxide radicals react with and decompose ozone to hydroxyl radicals. These mechanisms indicate that t-amine and phenolic groups increase hydroxyl radical exposure ($\int HO^{\bullet} dt$) until they are completely consumed [24]. The Schiff-base then decomposes to give an aldehyde or ketone and a secondary amine. While the addition of a *tert*-butyl alcohol reduces the yield of the free amine and; where its measurement makes sense, also that of the corresponding aldehydes [20]. The above discussion indicates that; even at low pH, some amines can react during ozonation via a free radical pathway. Some compounds containing amino groups, like EDTA, iminodiacetic acid and clarithromycin, react even in low pH environments [23,25,26].

Davies et al. reported that the dimethyl amino group on TC was oxidized to a ketone group on exposure of an aqueous solution of TC to UV light. On further dehydration, a compound was produced, termed the 'red compound' [27]. The dimethyl amino group at the C4 position of TC can react during ozonation via the mechanisms mentioned in the previous paragraph (Scheme 3) [23]. These mechanisms are different from the previously reported photochemical oxidation [27]. Upon oxidation of the amino group on TC to a keto group, a molecule with an m/z value of 416 should be produced. In our study, the 'red compound' was not detected. The reaction rate of tertiary amino groups during direct ozonation should be very slow [26]. For TC ozonation, stabilization of free radicals on TC molecule due to conjugation extending to the double bond (C2–C3 position), amide group (C2) and keto group (C1) (Scheme 3), suggested that oxidation of the dimethyl amino group on TC was more feasible through the C-centered radical generated by the reaction of TC with free radicals.

In Fig. 2a and b, the maximum concentration achieved for each compound depended on both the production and decomposition rates. Therefore, at pH 2.2, the maximum concentration for the signal with an m/z value of 416 occurred after 4 min of ozonation due to the slower subsequent decomposition. At pH 7.0, a smaller fraction of TC was protonated and; in addition, hydroxyl radicals were at a higher concentration causing faster production and subsequent decomposition. The rate of production of the product with an m/z value of 416 during the first minute of ozonation increased 2-fold on increasing the pH from 2.2 to 7.0 (data not shown here). This would be due to deprotonation of the amino group and increased free radical attack. Based on above facts, for this m/z value of 416, 4-oxo-4-dedimethylaminotetracycline was suggested as the compound, and referred to as 'D' in this work. The supporting data of the MS fragmentation pattern is given in Supporting Information.

The double bond at the C11a–C12 position of 'D' might be attacked by ozone via mechanisms similar to that involved in the production of 'A'. This could generate a compound with an m/z value of 432, which was detected in this work. The same compound could also be generated if 'A' was attacked by ozone or free radicals at the dimethyl amine groups in the C4 position. However, as shown in Fig. 2a, the quantity of 'B' was higher than that of 'C', indicating that 'A' decomposed to 'B' via a faster reaction. Therefore, it could be concluded that most of 'A' decomposed by direct ozonation to 'B', and that the generation of 'C' was predominantly through 'D'. The reaction of 'D' with ozone was more feasible than the reaction of 'A' with free radicals due to presence of the three sites that dominantly react with ozone. The MS spectrum was supported by the expected fragmentation pattern of this proposed molecule. Therefore, 4,12-dioxo-11a-hydroxo-4-dedimethylaminotetracycline was suggested as the compound for the m/z value of 432; referred to as 'C' in this study (MS data given in Supporting Information).

The next group that might be attacked by ozone was the aromatic ring of TC. If this ring was attacked at the C6a–C7 bond, the benzene ring of TC could open up; thus, resulting in a ketone group at the C6a position and a carboxylic group at the C7 position. This should give a signal at an m/z value of 525, which was found in this study. An MS fragmentation pattern for the MS spectrum of the signal observed in this work has been proposed in Supporting Information. The signal at an m/z value of 46 in the MS spectrum of this compound provided evidence of the presence of a carboxylic group. Therefore, 2,11a-dihydroxo-3,6a,12-trioxotetracycline-8-carboxylic acid was proposed as the compound for the m/z value of 525; referred to as 'G' in this study.

The compounds proposed above could further decompose during ozonation, with similar mechanisms to those discussed above. The aromatic ring of Compound 'C' might be attacked by ozone at the C6a–C7 bond, which would result in a ketone group and a carboxylic group at the C6a and C7 positions, respectively, as proposed in the generation of 'G', and should have an *m*/*z* value of 480, which was also found in this study. This compound showed a signal at an *m*/*z* value of 46 in the MS spectrum, indicating the presence of a carboxylic group. Therefore, 4,6a,12-trioxo-11a-hydroxo-4-dedimethylaminotetracycline-8-carboxylic acid was proposed as the compound for this signal; referred to as 'F' in this study. 'F' could only be detected at pH 2.2, because direct ozonation might be involved in the conversion of 'D' to 'C' and then to 'F'. At pH 7.0, ozonation via free radicals would be a dominant reaction; therefore, 'F' could not be detected at this pH. 'A' could be attacked by ozone at the aromatic ring, with a reaction similar to that of 'C', resulting in a ketone group and a carboxylic group at the C6a and C7 positions, respectively. This would yield a signal with an *m*/*z* value of 509, which was detected in this study. The MS spectrum detected the signal with an *m*/*z* value of 46, indicating fragmentation of the carboxylic groups from the compound. Based on the above facts, 11a-hydroxo-12,6a-dioxotetracycline-8-carboxylic acid was proposed as the compound responsible for this signal; referred to as 'E' in this study. Compound 'B' could be attacked by ozone at position C4, generating a ketone group, resulting in a signal at an *m*/*z* value of 448, which was found in this study. The MS spectrum and fragmentation patterns supported this proposed structure and; therefore, 2,11a-dihydroxo-3,4,12-trioxo-4-dedimethylaminotetracycline was proposed as the compound for the signal with an *m*/*z* value of 448; referred to as 'H' in this study. By the same mechanism, the attack of ozone on 'G' would give an *m*/*z* value of 496, which was detected in our study. The MS spectrum of this compound showed a signal at an *m*/*z* value of 46, which provided evidence of the formation of carboxylic groups. Therefore, 2,11a-dihydroxo-3,4,6a,12-tetraoxo-4-dedimethylaminotracycline-8-carboxylic acid was proposed as the compound for signal with an *m*/*z* value of 496; referred to as 'I' in this study.

During the ozonation of TC at pH 2.2, compounds with *m*/*z* values of 336 and 408 were detected, but these were not detected during ozonation at pH 7.0. This indicated that these compounds were formed as a result of the ozonation of TC or from products of the oxidation of TC. The life-time of the products was very short due to the reaction with free radicals at higher pH; these two compounds could not be detected at pH 7.0. The structures for these compounds could not be identified in this study. Further work will be required for a more detailed investigation of these structures.

3.2. Removal of total organic carbon

At pH 2.2, ozonation removed 15% of the TOC after 30 min, while only 39% was removed in the same reaction time at pH 7.0 (Fig. 3). The reason for this increased TOC removal was the increased generation of hydroxyl radicals at the higher pH. The first product of the ozonation of TC was degraded further by free radicals at pH 7.0. In the first 15 min of the process, the TOC removal rate was found to be very high, but dropped with increasing ozonation time, indicating an increase in recalcitrant pollutants on ozonation. Further study will be required employing higher ozone and lower TC concentrations.

3.3. Changes in relative toxicity

Variation in the relative acute toxicity during the ozonation of a tetracycline solution was monitored at pH 2.2. The toxicity

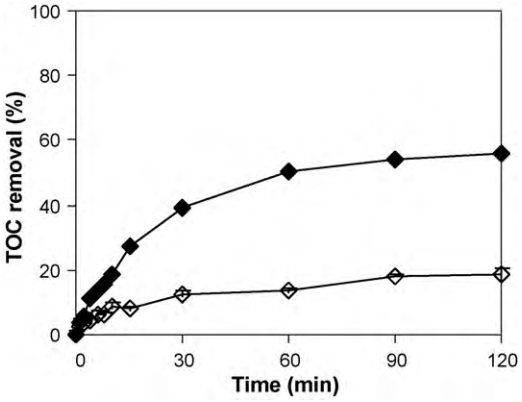


Fig. 3. Total organic carbon removal during ozonation of tetracycline at different conditions: TC = 0.5 mM, temperature = 20 °C, O_3 supply = 10 mg min^{-1}, ozonation at pH 2.2 ± 0.1 (◊), ozonation at pH 7.0 ± 0.1 (♦).

was found to be little affected during the first 15 min of ozonation (Fig. 4), but further ozonation decreased the relative acute toxicity from 1.0 to 0.68. At pH 7.0, the relative toxicity began to decrease from the first minute of ozonation. In both cases, the maximum removal of the relative toxicity was about 40% after 2 h of ozonation. At pH 2.2, compounds 'A', 'B', 'D', 'E' and 'G' took around 15 min to decompose, but the toxicity remained at around 1.0 during that time. The removal trends of TC, and the product generation, subsequent degradation and proposed structures of some of these products were compared to gain a better understanding of the mechanisms. The products retaining the three-ring structure of TC were found to have similar toxicities to TC. The ozonation products of TC have been reported to cause stoichiometric elimination of antibacterial activity (i.e., loss of 1 mole equiv. of potency per mole of parent compound consumed) [28]. This comparison showed that TC products exhibited high acute toxicity at high concentrations, but when in low concentrations showed no delayed toxicity. In this study, the variation in UV_{254} was examined during ozonation. The absorbance at 254 nm decreased sharply during the first 15 min of ozonation, which was almost independent of pH because the oxidation of the aromatic ring was predominantly via direct ozonation. The toxicity was found to not really be related to UV_{254} because the aromatic ring was not the sole toxic functional group in TC, with other combinations of functional groups proving even more toxic.

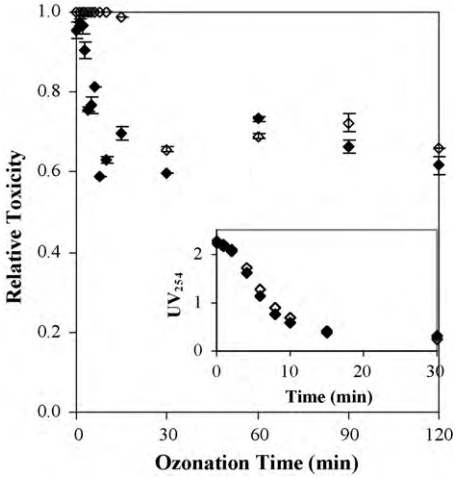


Fig. 4. Relative toxicity and UV_{254} variations during ozonation of tetracycline at different conditions. In-set graph shows the variation of UV_{254} during ozonation: TC = 0.5 mM, temperature = 20 °C, O_3 supply = 10 mg min^{-1}, ozonation at pH 2.2 ± 0.1 (◊), ozonation at pH 7.0 ± 0.1 (♦).

From this study, it was concluded that the degradation products of direct ozonation were more toxic than those generated via the free radical pathway.

4. Conclusion

Ozonation was found to be a very effective process for the degradation of tetracycline, with its complete removal within 4–6 min of ozonation. Liquid chromatography–triple quadrupole mass spectrometry was used to identify the products of tetracycline ozonation. At pH 2.2, around 15 products were detected, including two that have already been identified in the literature. At pH 7.0, only 3 products were detected. The reason for this was that at pH 7.0 the free radicals would non-selectively react with the ozonation products of tetracycline, resulting in many low molecular weight final products at very low concentrations, which could not be extracted and detected by the analytical procedures used in this study.

A pathway of tetracycline degradation based on its structure, the ozonation chemistry and mass spectrometry data has been proposed in this study. It was found that ozone attacked the double bonds, aromatic ring and amino group to give product with m/z values of 461, 477, 509 and 416, respectively. Further selective ozonation of these products under acidic conditions took place, resulting products with m/z values of 432, 480, 448, 525 and 496. A kinetic pattern for the degradation of tetracycline, the generation of products and their subsequent degradation against ozonation time was established. The removal of total organic carbon increased with increasing pH value. The relative acute toxicity decreased faster at pH 7.0 than at pH 2.2, but the maximum toxicity removals at both pHs were similar. Further study will be required with a higher supply of ozone and a lower concentration of tetracycline.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2010.05.063.

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