Ozonation and advanced oxidation by the peroxone process of ciprofloxacin in water

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ABSTRACT

A bubble reactor was used for ozonation of the antibiotic ciprofloxacin. Effects of process parameters ozone inlet concentration, ciprofloxacin concentration, temperature, pH and H_2O_2 concentration were tested. Desethylene ciprofloxacin was identified, based on HPLC–MS analysis, as one of the degradation products. Formation of desethylene ciprofloxacin was highly dependent on pH, with the highest concentration measured at pH 10. Radical scavengers *t*-butanol and *para*chlorobenzoic acid were added in order to gain mechanistic understanding. Radical species other than hydroxyl radicals were suggested to occur at acidic pH which can explain fast ciprofloxacin ozonation at pH 3.

1. Introduction

The antibiotic ciprofloxacin is the most widely prescribed quinolone in Europe [1]. It is a second generation quinolone. They are designed to expand the activity against gram-negative bacteria but have only limited gram-positive activity.

Because pharmaceuticals are made to interfere with biological systems, even low concentrations can be harmful after prolonged exposure. Pharmaceuticals can exhibit toxic effects on cells, organs, organisms and populations. Specifically for antibiotics, the increased use and exposure during the last decades can increase bacterial resistance against them, favored by low concentrations [2]. Moreover, exposure to one compound can lead to resistance against a whole class of antibiotics.

Ciprofloxacin was found in wastewater treatment plant (WWTP) influents typically at concentrations of 313–568 ng l^{-1} [3,4] and up to 124.5 µg l^{-1} [5] in raw sewage hospital water. Because of its high wastewater–sludge partition coefficient (log $k_d \sim 4$), 79–92% of the initial concentration is removed in a WWTP. However, Kümmerer et al. [6] found that ciprofloxacin was not biodegradable in closed bottle tests. Because of the low biodegradability, ciprofloxacin could still be found in WWTP effluents by Golet et al. [3,4] at concentrations of 61–106 ng l^{-1}. Concentrations up to 514 ng l^{-1} and 5.6 µg l^{-1} were detected by other authors [7,8]. Advanced oxidation processes (AOPs), which generate hydroxyl radicals, are a promising tool for

removal of persistent pharmaceutical compounds. Ozonation can also be seen as an AOP because ozone decomposes into hydroxyl radicals at higher pH.

Until now, only few authors described degradation of quinolones by AOPs while none of them identified degradation products. Balcioğlu and Ötker [9] described ozonation of enrofloxacin mixed with 10% organic and inorganic additives, next to ozonation of ceftriaxone sodium and penicillin VK. The authors studied the influence of pH, initial COD, H_2O_2 and biodegradability although they did not measure enrofloxacin itself but BOD, COD, TOC and UV$_{254}$ values next to O_3 and H_2O_2 concentrations. In a second article, the authors also investigated the influence of ozonation on enrofloxacin adsorption on zeolite [10].

Andreozzi et al. [11] investigated the toxicity of a mixture of pharmaceuticals containing the quinolone ofloxacin on algae and invertebrates. Recently, Dodd et al. [12] determined the second order rate constants of 14 pharmaceuticals, including ciprofloxacin, with ozone and hydroxyl radicals. Other radical species were not taken into account.

In this research, ozonation of ciprofloxacin is investigated. By our knowledge, no parameter study has been carried out so far for this compound. The effect of ciprofloxacin concentration, pH and H_2O_2 concentration is studied. Focus lays on ozone consumption and on the compound degradation itself, in contrast with the enrofloxacin parameter study of Balcioğlu and Ötker [9]. Next, influence of temperature and ozone concentration is tested as well. In order to gain more insight in the degradation mechanism, hydroxyl radical scavengers *t*-butanol and *para*chlorobenzoic acid (*p*CBA) were added. Finally, a first degradation product was identified.

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2. Materials and methods

2.1. Chemicals and stock solutions

Ciprofloxacin HCl was purchased from MP biomedicals Inc. Desethylene ciprofloxacin was kindly provided by Bayer. t-Butanol (≥99%, GC) and pCBA (99%) came from Fluka and Acros Organics, respectively. All stock and buffer solutions were prepared with deionized water. All chemicals used for solutions were of reagents grade and were used without further purification.

2.2. Experimental setup

Ozonation of ciprofloxacin was done in a temperature controlled (6.0–62 °C) bubble reactor with a height of 56.5 cm and an inner and outer diameter of 10.4 and 13.2 cm, respectively. Ozone was generated in dry air by a LAB2B ozone generator (Ozonia) and after flow adjustment dosed through a sintered glass plate at the bottom of the reactor. The reactor contained 1.75 l of deionized water spiked with 7.5–45 mg/l of ciprofloxacin (22.64–135.81 μM). 5 ml liquid samples were taken by means of a liquid syringe with a 30 cm long needle through a septum on top of the reactor. Immediately after sampling, the samples were flushed with nitrogen for 3 min at 15 ml min^{-1} in order to remove residual ozone.

At standard conditions, the ozone inlet concentration was 2500 ppm$_v$ with a gas flow rate of 120 ml min^{-1}, the initial ciprofloxacin concentration was 45.27 μM and the reactor was at 27.5 °C. The water was buffered by a 10.12 mM phosphate buffer (pH 3 and 7) or a 2.5 mM borax buffer (pH 10). For the peroxone experiments, H_2O_2 was added in concentrations of 2–990 μmol l^{-1}. Experiments with H_2O_2 but without ozonation revealed that ciprofloxacin did not react with H_2O_2. If radical scavengers were applied, t-butanol and pCBA concentrations were 30.45 and 0.0033 mM, respectively.

2.3. Analytical procedures

Ozone in the gas flow was measured by an ozone analyser (Anseros Ozomat GM) by UV-light absorption at 253.7 nm. Ciprofloxacin and desethylene ciprofloxacin were measured by liquid chromatography with a photodiode array detector (Surveyor, Thermo Finnigan). A Luna C18(2) column (150 mm × 3.0 mm, 3 μm, Phenomenex) was used as stationary phase with a mobile phase containing 87.5% water (0.1% formic acid) and 12.5% acetonitrile. Quantification took place at 278 ± 4.5 nm (ciprofloxacin) and 276 ± 4.5 nm (desethylene ciprofloxacin). Analysis of pCBA was done on the same column with a 75% water (0.1% phosphoric acid)–25% acetonitrile mobile phase with quantification at 234 ± 4.5 nm. Aqueous hydrogen peroxide concentrations were determined by a spectrophotometric method based on 2,9-dimethyl-1,10-phenanthroline (DMP) and Cu(II) [13]. pH measurements were done by a Jenway 3310 electrode.

LC–MS analyses were performed on a Surveyor HPLC system (Thermo Finnigan) coupled to a MAT 95XP-Trap (Thermo Finnigan) equipped with a TSQ/SSQ 7000 atmospheric pressure ionisation source. Analyses were done in positive ionisation mode by electrospray ionisation (ESI). Polyethylene glycol (PEG) was used as reference for high resolution MS. An additional energy of 100 V was applied to the ESI-needle to identify MS fragmentation products by collision induced dissociation (CID). Ciprofloxacin and desethylene ciprofloxacin stock solutions for direct inlet MS analysis were prepared in methanol (0.5% formic acid) and also analysed by low and high resolution MS as well as by CID-MS.
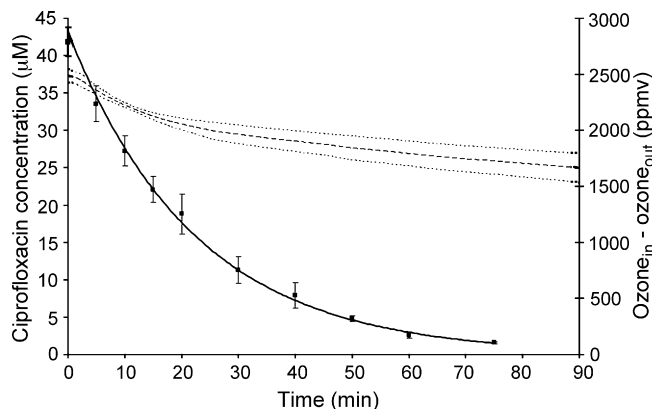
Fig. 1. Ozonation of ciprofloxacin at standard conditions: (1) ciprofloxacin concentration versus time (■) with standard deviation, (2) fitting of first order degradation kinetics to ciprofloxacin data points (full line) and (3) ozone consumption profile versus time with 68.2% confidence intervals (dotted lines).

2.4. Statistical procedures

To check if the pseudo-first order reaction constants $k_{1,cipro}$ for each experiment are equal, the natural logarithm of ciprofloxacin concentration was plot against time and the equality of the slopes was tested by means of a linear regression (SPSS15.0, $\alpha = 0.05$).

3. Results and discussion

3.1. Influence of process parameters

3.1.1. Experiments at standard conditions

The ciprofloxacin degradation and ozone consumption profiles, which represent the ozone inlet minus the ozone outlet concentration, are shown in Fig. 1 for standard conditions ($n = 3$). A ciprofloxacin half life time of 15.90 min was found with 95% degradation reached between 60 and 75 min, indicating ciprofloxacin degradability by ozonation at pH 7. After 90 min of ozonation, 0.841 ± 0.036 mmol of ozone was consumed compared to 0.517 mmol for the blank experiment without ciprofloxacin.

If all data points up to 95% ciprofloxacin degradation are considered, pseudo-first order reactions kinetics are observed (Eq. (1)):

$$\ln\frac{[A]_\tau}{[A]_0} = -k_{1,cipro}\int_0^\tau dt = -k_{1,cipro}\tau \tag{1}$$

in which $[A]_0$ stands for the ciprofloxacin concentration at time 0 and $[A]_\tau$ for the ciprofloxacin concentration at reaction time τ. A pseudo-first order reaction constant $k_{1,cipro}$ of 0.0453 ± 0.0030 min^{-1} was found for the experiments at standard conditions.

The pseudo-first order constants observed for each experiment are function of the concentration of ozone, hydroxyl radicals and other oxidative species, e.g. $O_2^{\bullet-}$, $O_3^{\bullet-}$, $HO_2^\bullet$ and $HO_4^\bullet$. By this, the pseudo-first order reaction constants given cannot be seen as fundamental reaction kinetics. However, they allow reliable comparisons between the different experiments.

3.1.2. Influence of ozone inlet concentration

The ozone inlet concentration was varied from 660 to 3680 ppm$_v$. The $k_{1,cipro}$ values varied from 0.0081 ± 0.0004 min^{-1} (660 ppm$_v$) to 0.0660 ± 0.0029 min^{-1} (3680 ppm$_v$) while the ozone consumption during 90 min of ozonation increased from 0.254 (660 ppm$_v$) to 1.253 mmol O_3 (3680 ppm$_v$) (Table 1). This faster

Table 1
Pseudo-first order reaction constants and ozone consumption during 90 min of ozonation for experiments at 45.27 µM initial ciprofloxacin concentration

Inlet ozone concentration (ppm$_v$)	Temperature (°C)	pH	H$_2$O$_2$ (µmol l^{-1})	$k_{1,cipro}$ (min^{-1})	Ozone consumption during 90 min (mmol)
660	27.5	7	–	0.0081 ± 0.0004	0.254
2010	27.5	7	–	0.0343 ± 0.0012	0.778
2500	27.5	7	–	0.0453 ± 0.0030	0.841 ± 0.036
3260	27.5	7	–	0.0613 ± 0.0022	1.102
3680	27.5	7	–	0.0660 ± 0.0029	1.253
2500	6.0	7	–	0.0549 ± 0.0027	0.884
2500	13.4	7	–	0.0413 ± 0.0037	0.877
2500	21.0	7	–	0.0436 ± 0.0030	0.877
2500	27.5	7	–	0.0453 ± 0.0030	0.841 ± 0.036
2500	35.4	7	–	0.0561 ± 0.0044	0.897
2500	62.0	7	–	0.0382 ± 0.0022	0.927
2500	27.5	3	–	0.0567 ± 0.0032	0.770
2500	27.5	7	–	0.0453 ± 0.0030	0.841 ± 0.036
2500	27.5	10	–	0.0515 ± 0.0018	0.882
2500	27.5	7	2	0.0496 ± 0.0011	0.841
2500	27.5	7	10	0.0505 ± 0.0014	0.885 ± 0.015
2500	27.5	7	50	0.0514 ± 0.0009	0.832
2500	27.5	7	100	0.0422 ± 0.0013	0.826
2500	27.5	7	360	0.0450 ± 0.0011	0.882
2500	27.5	7	990	0.0362 ± 0.0011	0.850
2500	27.5	3	10	0.0518 ± 0.0021	0.761
2500	27.5	7	10	0.0505 ± 0.0014	0.885 ± 0.015
2500	27.5	10	10	0.0462 ± 0.0010	0.953

ciprofloxacin degradation as well as higher ozone consumption at higher ozone inlet concentrations can be explained by the higher amount of reactive species expected.

Secondly, when the pseudo-first order constants $k_{1,cipro}$ are plotted against the gaseous ozone inlet concentrations (Fig. 2), a linear relationship was found ($y = (1.81 \pm 0.04)10^{-5}x$, $R^2 = 0.997$), which goes through the origin (SPPS15.0, regression, $\alpha > 0.05$). Because ciprofloxacin is non-volatile (Henry coefficient = $5.09 \times 10^{-19}(25\,°C)$, [14]), reactions in the gas phase are negligible. By consequence, the ozone concentration in the gas phase is linear to the amount of reactive species reacting with ciprofloxacin in the liquid phase.

A plot of dosed versus consumed ozone during 90 min also showed a linear relation with intercept equal to zero ($y = (0.787 \pm 0.019)x$, $R^2 = 0.996$) which means that the ozone elimination rate (ER) is first order in ozone inlet concentration (ER = $k[O_3]_{gas}$). This indicates that in this system, ciprofloxacin degradation is limited by ozone supply rather than reaction kinetics.
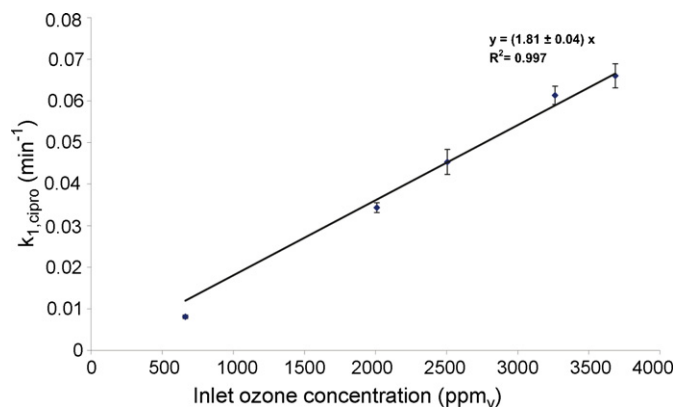
Fig. 2. Ciprofloxacin degradation reaction constants versus ozone inlet concentration (45.27 µM ciprofloxacin concentration, pH 7, $T = 27.5\,°C$).

3.1.3. Influence of initial ciprofloxacin concentration

The initial ciprofloxacin concentration was varied from 22.64 to 135.81 µM. The fastest ciprofloxacin degradation ($k_{1,cipro} = 0.0940 \pm 0.0075$ min^{-1}) can be found at the lowest initial ciprofloxacin concentration (22.64 µM), as can be seen from Table 2. This is in agreement with the results of Balcioğlu and Ötker [9] in their study on enrofloxacin ozonation. Moreover, if only the experiments at 22.64–90.54 µM initial ciprofloxacin concentration are taken into account, a straight line can be plotted between the ciprofloxacin degradation half life time ($t_{1/2}$) and ciprofloxacin concentration ($y = 0.3615x$, $R^2 = 0.998$) which goes through the origin (SPSS15.0, regression, $\alpha > 0.05$).

From the standard experiments, it was concluded that ciprofloxacin degradation versus time is first order in ciprofloxacin concentration (pseudo-first order kinetics) which would result in constant half life times when changing ciprofloxacin concentration ($t_{1/2} = \ln 2/k_{1,cipro}$). In previous paragraph, however, a linear relationship between ciprofloxacin concentration and half life time was proven, suggesting zero order kinetics ($t_{1/2} = A_0/2k_{0,cipro}$ with A_0, the initial ciprofloxacin concentration and $k_{0,cipro}$ the zero order constant). The way of ozone supply can explain this duality between first and zero order kinetics. Initially, there is no ozone in the reactor and the ciprofloxacin degradation is only dependent on the ozone supply, leading to zero order kinetics in ciprofloxacin concentration ($dA/dt = k_{0,cipro}$). As the experiment goes on, more ozone has entered the bubble reactor and degradation products are formed, leading to overall pseudo-first order kinetics in ciprofloxacin concentration ($dA/dt = k_{1,cipro}A$). The zero order kinetic constant $k_{0,cipro}$ is only valid in the initial phase.

In Table 2, $k_{0,cipro}$ values, measured by calculating the slope of the ciprofloxacin degradation curves over the first 10 min, equal $1.62 \pm 0.06\,\mu M\,min^{-1}$. If $k_{0,cipro}$ values are calculated from $t_{1/2}$, there is 10–15% deviation compared to the $k_{0,cipro}$ values because at the half life time, the reactions are not zero order any longer.

3.1.4. Influence of temperature

The effect of temperature (6.0–62.0 °C) on the $k_{1,cipro}$ values and ozone consumption data is shown in Table 1. Significant

Table 2
Reaction constants, half life times and ozone consumption during 90 min of ozonation for ozonation experiments with different initial ciprofloxacin concentration at 2500 ppm$_v$ ozone inlet concentration and $T = 27.5\,^{\circ}$C at pH 7

Ciprofloxacin concentration (μM)	$k_{1,cipro}$ (min^{-1})	$t_{1/2}$ (min)	$k_{0,cipro}$ (μM min^{-1})	Ozone consumption during 90 min (mmol)
22.64	0.0940 ± 0.0075	8.59	1.695	1.018
45.27	0.0453 ± 0.0030	15.90	1.536	0.841
67.91	0.0364 ± 0.0011	24.08	1.616	0.851
90.54	0.0333 ± 0.0023	33.21	1.624	0.905
135.81	0.0219 ± 0.0005	37.09	1.635	0.974

faster ciprofloxacin degradation could be noticed at 6.0 and 35.4 $^{\circ}$C compared to the other experiments. However, no clear trend in ciprofloxacin degradation as well as ozone consumption could be found.

Elevating the temperature can exert effects on Henry coefficients, reaction kinetics and diffusion kinetics, making it difficult to distinguish clear trends. The Henry coefficient of ozone is higher at higher temperature (2.23 at 6 $^{\circ}$C to 28.18 at 62 $^{\circ}$C, [15]) leading to less ozone in the liquid phase. However, because of a higher diffusion speed of ozone in the liquid phase and faster reaction kinetics, ozone mass transfer and ciprofloxacin degradation can also get faster at higher temperature.

3.1.5. Effect of pH on ozonation experiments

Experiments were performed at different pH (3, 7 and 10). As a first observation, it can be seen in Table 1 that the ozone consumption is higher at higher pH. This can be explained by the higher rate of ozone decomposition at higher pH, for instance by reaction of ozone with hydroxyl anions which can lead to highly reactive hydroxyl radicals [16].

Secondly, the fastest ciprofloxacin degradation was observed at pH 3 ($k_1 = 0.0567 \pm 0.0032$ min^{-1}). This is in contrast with Balcıoğlu and Ötker [9], who observed the fastest enrofloxacin degradation at pH 7, which is, according to the authors, mediated by the higher hydroxyl radical concentration. At pH 3, however, the least ozone is consumed and no hydroxyl radical formation is expected, according to the ozone decomposition model presented by Staehelin, Hoigné and Bühler (SHB-model, [17]). Next to the concentration of ozone and radical species, degradation is also influenced by the reactivity of the substrate, e.g. its degree of protonation. Direct ozonation occurs fast at uncharged N-atoms. Dodd et al. [12] concluded that direct ciprofloxacin ozonation preferentially takes place at the N$_4$-atom of the piperazinyl substituent. At pH 3, however, this N-atom is protonated (p$K_a \sim 8.24$–8.95) as well as the N$_1$-atom of the piperazinyl substituent (p$K_a \sim 5.05$). The N-atom of the quinolone moiety (p$K_a \sim 3.64$) is partially protonated [18]. Direct ozonation can also take place at the quinolone moiety. However, according to Dodd et al. [12], these reaction constants are several orders of magnitude lower than at the N$_4$-atom of the piperazinyl substituent. The fast ciprofloxacin degradation at pH 3 compared to the experiments at pH 7 and 10, in which the degree of protonation is less, can only be explained if direct ozonation at the quinolone moiety is as fast as the ozonation rate of deprotonated N-atoms or if other reactive species enhance degradation at low pH.

Third, ciprofloxacin degradation was significantly slower at pH 7 ($k_{1,cipro} = 0.0453 \pm 0.0030$ min^{-1}) than at pH 3 and 10. During the first 10 min of ciprofloxacin degradation, however, no difference in degradation speed was noticed. Possibly, ozonation at pH 7 leads to other ratios between the ciprofloxacin degradation products that compete with ciprofloxacin for oxidative species after prolonged ozonation.

3.2. H$_2$O$_2$ addition

3.2.1. Effect of H$_2$O$_2$ concentration

For peroxone experiments, H$_2$O$_2$ concentrations of 2–990 μmol l^{-1} were added to the standard ozonation experiments at pH 7. Addition of 2, 10 and 50 μmol l^{-1} H$_2$O$_2$ led to 9.5–13.5% increase in ciprofloxacin degradation rate compared to the experiment at standard conditions without H$_2$O$_2$ (Table 1). However, adding more H$_2$O$_2$ did not increase the degradation rate (100 and 360 μmol l^{-1} H$_2$O$_2$), it even lowered degradation (990 μmol l^{-1} H$_2$O$_2$) by 20%. Addition of H$_2$O$_2$ promotes OH-radical formation [19], which can explain the increased ciprofloxacin degradation when 2–50 μmol l^{-1} H$_2$O$_2$ is initially added. On the contrary, H$_2$O$_2$ can also inhibit oxidation between organic compounds and hydroxyl radicals by scavenging the hydroxyl radicals. This becomes important at higher H$_2$O$_2$ concentrations.

No trend could be found in the ozone consumption profiles for the experiments at different H$_2$O$_2$ concentration, while it is expected to have higher ozone consumption when H$_2$O$_2$ is added. This is in contrast with the blank experiments without ciprofloxacin, were addition of 10 and 100 μmol l^{-1} H$_2$O$_2$ resulted in 0.567 and 0.653 mmol of ozone consumption, respectively. For the blank experiment without H$_2$O$_2$, 0.517 mmol of ozone was consumed during 90 min of ozonation.

Next, no H$_2$O$_2$ consumption was measured in the experiments with 50, 100 and 360 μmol l^{-1} H$_2$O$_2$ addition (SPSS15.0, regression, $\alpha > 0.05$). This indicates that H$_2$O$_2$ degradation rate and H$_2$O$_2$ formation rate are equal. Hydroxyl radicals can react fast with each other with formation of H$_2$O$_2$ ($k = 5 \times 10^9$ M^{-1} s^{-1}). Moreover, ozonation of organic compounds can also lead to H$_2$O$_2$ formation [20].

As it was concluded in previous paragraph, addition of small H$_2$O$_2$ amounts (2–50 μmol l^{-1}) before starting ozonation increased ciprofloxacin degradation at pH 7. Because initially there is no ozone in the reactor, the H$_2$O$_2$/O$_3$ ratio is infinite and gets lower the more ozone is dosed. The optimal H$_2$O$_2$/O$_3$ ratio is around 0.33 [19]. In a rough attempt to keep the H$_2$O$_2$/O$_3$ ratio more constant, the initial H$_2$O$_2$ concentration of 2 μmol l^{-1} H$_2$O$_2$ was increased by 2 μmol l^{-1} H$_2$O$_2$ every 10 min during 90 min. This led to a significant faster ciprofloxacin degradation ($k_1 = 0.0536 \pm 0.0014$ min^{-1}) compared to the experiments with 2 ($k_1 = 0.0496 \pm 0.0011$ min^{-1}) and 10 μmol l^{-1} ($k_1 = 0.0505 \pm 0.0014$ min^{-1}) initial H$_2$O$_2$ concentration.

3.2.2. Effect of pH for peroxone experiments

Analogous to the ozone experiments, the pH was changed for the peroxone experiments (10 μmol l^{-1} H$_2$O$_2$ initially added). The results are presented in Table 1. As with the ozone experiments, there is a higher ozone consumption at higher pH. Next to $^-$OH, HO$_2^-$ (p$K_a = 11.8$) also reacts fast with ozone ($k = 2.8 \times 10^6$ M^{-1} s^{-1}, [21]).

When comparing the peroxone and ozone experiments, the ozone consumption at pH 7 and 10 is higher when H$_2$O$_2$ is added.

This can also be explained by the fast reaction between HO_2^- and ozone. At pH 3, no increase in ozone consumption could be seen when H_2O_2 was added, probably because of the negligible amount of HO_2^- present.

Considering the ciprofloxacin degradation, no significant difference between the pseudo-first order reaction constants was observed for the peroxone experiments at pH 3 and 7. At pH 10, when the amount of HO_2^- is higher, the $k_{1,cipro}$ value is significantly lower, probably because HO_2^- and ^-OH compete with ciprofloxacin for reaction with hydroxyl radicals.

By comparing the peroxone experiments with the ozone experiments, significant faster ciprofloxacin degradation can be seen at pH 7 when adding H_2O_2. This is expected because of the enhanced generation of reactive hydroxyl radicals. At pH 3 and 10, on the contrary, there was a significant slower degradation. Possibly, H_2O_2 or HO_2^- competes with ciprofloxacin for ozone or scavenges radical species.

3.3. Mechanistic study

3.3.1. Effect of t-butanol addition

The fastest ciprofloxacin degradation was found at pH 3. Although no hydroxyl radicals are expected at this pH according to the SHB-model [17], *t*-butanol was added in excess (30.45 mM) in order to completely rule out the effect of hydroxyl radicals. *t*-Butanol has reaction constants with hydroxyl radicals of $6 \times 10^8\,M^{-1}\,s^{-1}$ and with ozone $<0.003\,M^{-1}\,s^{-1}$ [22]. Surprisingly, *t*-butanol addition led to 16% faster ciprofloxacin degradation ($k_1 = 0.0656 \pm 0.0032\,min^{-1}$ vs. $0.0567 \pm 0.0032\,min^{-1}$). This can be explained by changes in overall transfer coefficient (k_La). Lopez-Lopez et al. [23] found that *t*-butanol addition to ozonation processes leads to smaller gas bubbles and increases in interfacial area, resulting in increased k_La. Moreover, changes in bubble pattern could be seen visually in the experiments with *t*-butanol. These results show that *t*-butanol is not the ideal scavenger to exclude presence of hydroxyl radicals at pH 3, although the increase in ciprofloxacin degradation after *t*-butanol addition again suggests the importance of direct ozonation or radical species, other than hydroxyl radicals, at low pH.

3.3.2. Effect of pCBA addition

*p*CBA, which has a pK_a value of 3.98 [24], is known as a probe for hydroxyl radicals and therefore typically applied at low concentrations. It was added at a concentration of 0.0033 mM to have better understanding of hydroxyl radical formation. It has a slow reaction speed with ozone ($k_{O_3} < 0.15\,M^{-1}s^{-1}$, [25]; $k_{O_3} = 4.5\,M^{-1}s^{-1}$, [26]) while the *p*-chlorobenzoate anion is reported to have fast reaction speed with hydroxyl radicals ($k_{OH^\bullet} = 5.2 \times 10^9\,M^{-1}\,s^{-1}$, [24]). At pH 3, i.e. when *p*CBA can be found in its protonated form, no hydroxyl radicals are expected [24]. Ozonation experiments with *p*CBA were performed at pH 3, 7 and 10 with and without ciprofloxacin. In Fig. 3, *p*CBA degradation versus time is presented for each experiment.

First, it can be seen that *p*CBA degrades faster at each pH when ciprofloxacin is absent, indicating competition between *p*CBA and ciprofloxacin for reactive species.

In absence of ciprofloxacin, *p*CBA degradation is faster at pH 7 than at pH 10, probably because at pH 10 more hydroxyl radicals are scavenged by radical scavengers. At pH 3, i.e. when no hydroxyl radicals are expected, *p*CBA degrades the slowest. After 5 min of ozonation, 54.2% of *p*CBA was degraded at pH 3 compared to 86.0 and 71.3% at pH 7 and 10, respectively. Considering the high reaction rate constant between *p*CBA and hydroxyl radicals and the higher amount of hydroxyl radicals at pH 7 and 10, the slow reaction constant of *p*CBA with ozone cannot explain the small difference
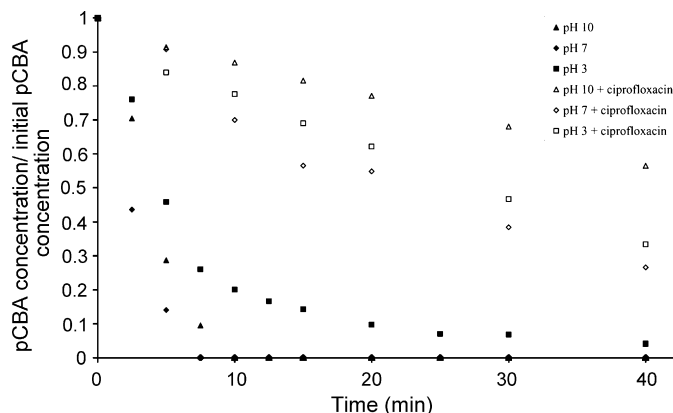

Fig. 3. Relative *p*CBA concentration (initial concentration 0.0033 mM) versus time during ozonation processes at pH 3, 7 and 10 with and without 45.27 μM ciprofloxacin (2500 ppm$_v$ ozone, $T = 27.5\,°C$).

between the experiment at pH 3 and those at pH 7 and 10. This strengthens the suggestion that radical species are present during ozonation at low pH, first reported by Rivas et al. [26] in their study on *p*CBA ozonation. Pi et al. [25] proved the formation of H_2O_2 from *p*CBA ozonation at pH 7 and 8. Although no prove can be given, radical species may possibly be created at pH 3 from ozonation of *p*CBA itself.

In absence of ciprofloxacin, a slower *p*CBA degradation was found at pH 3 compared to pH 10 while the inverse can be seen when ciprofloxacin is present (Fig. 3). This indicates a higher concentration of radical species at pH 3. In previous paragraphs, it was already suggested that radical species are present at pH 3. Possibly, the presence of ciprofloxacin and its degradation products enhances the concentration of radical species. Buffle [27] already proved that ozone can decompose rapidly into radical species during the initial phase, i.e. during the first 20 s of ozonation. This initial phase is controlled by direct reaction of ozone with organic molecules rather than by the autocatalytic chain reaction which is predominant in the second phase.

3.3.3. Desethylene ciprofloxacin identification

During chromatographic analysis of ozonation experiments, two clear peaks next to some minor peaks could be identified: a first peak eluting at retention time (R_t) = 8.24 min, corresponding with ciprofloxacin, and a second peak at R_t = 6.09 min which had a molecular weight (MW) +1 of 306.

Analysis of ciprofloxacin (MW + 1 = $C_{17}H_{19}O_3N_3F$) stock solutions gave several fragmentation products for which an MS fragmentation pattern could be proposed (Fig. 4a), analogous to Volmer et al. [28]. The fragmentation product with MW +1 = 263 was not reported by Volmer et al. [28].

Using high resolution mass spectrometry, the molecular formula for the compound with MW +1 = 306 was identified as $C_{15}H_{17}O_3N_3F$, which indicates a loss of C_2H_2 compared to ciprofloxacin. Based on the fragmentation products, desethylene ciprofloxacin was suggested to be this degradation compound of ciprofloxacin. As can be seen from the proposed fragmentation pattern (Fig. 4b), fragmentation products with MW +1 = 288, 263, 245, 217 and 203 can also be found on the ciprofloxacin fragmentation pattern while the products with MW +1 = 289 and 235 have similar functionalities as the ciprofloxacin fragmentation products. Finally, the identity of desethylene ciprofloxacin was undoubtedly proven based on retention time and by MS analysis of desethylene ciprofloxacin standard solutions.
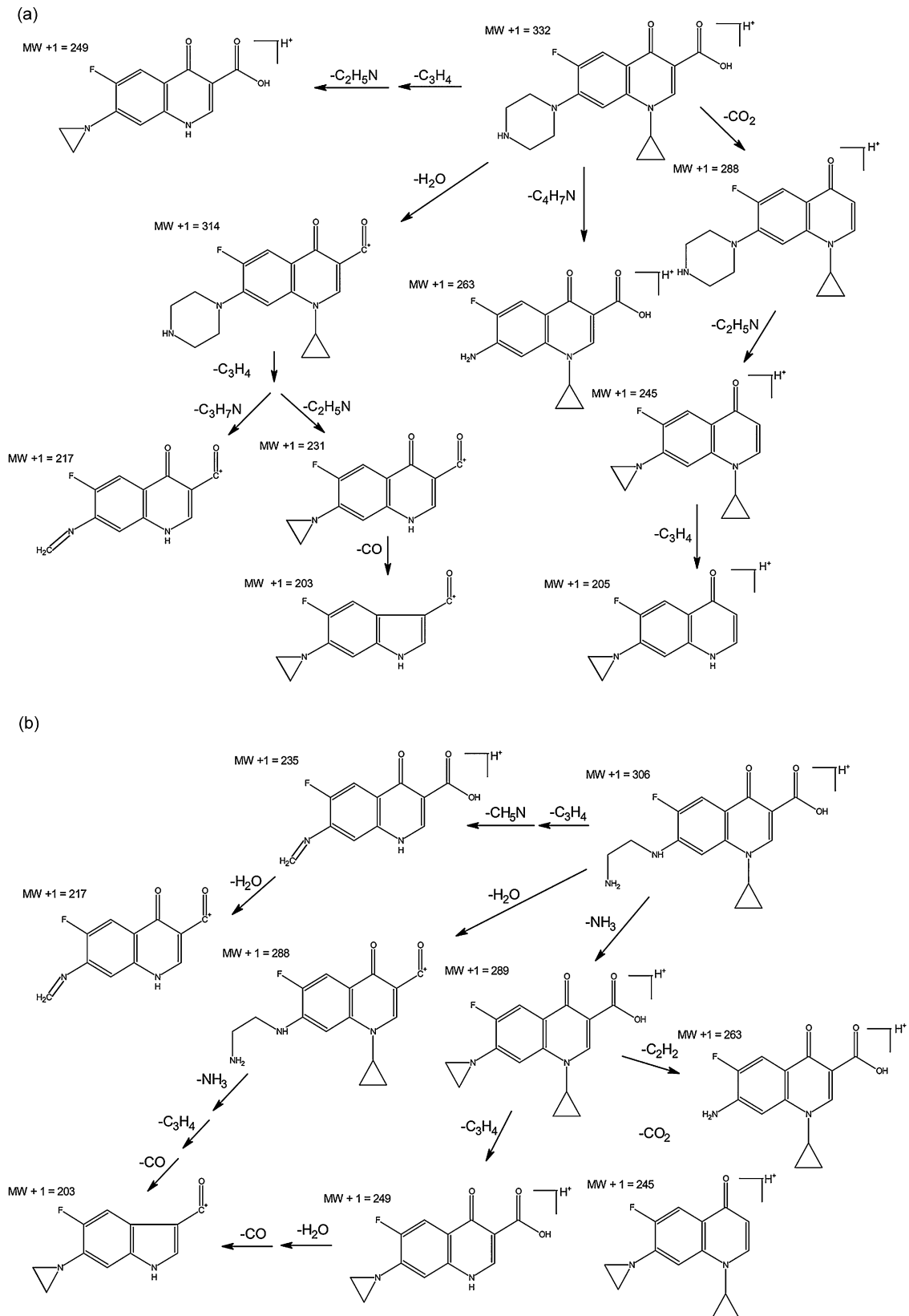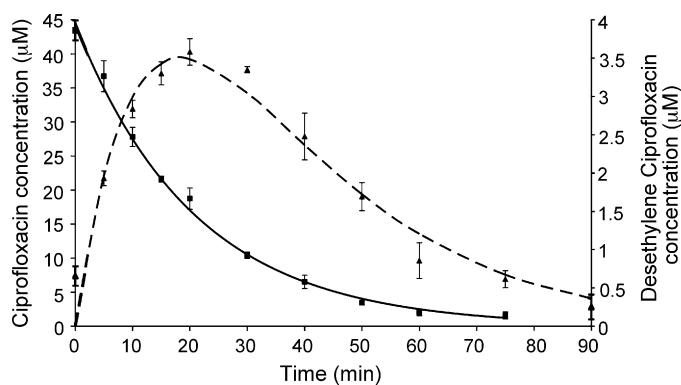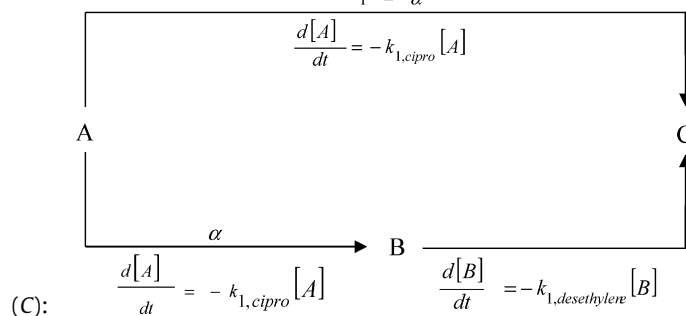
Fig. 4. MS fragmentation pattern of (a) ciprofloxacin and (b) desethylene ciprofloxacin.

Fig. 5. Peroxone oxidation of ciprofloxacin: evolution of ciprofloxacin (■, left Y-axis) and desethylene ciprofloxacin (▲, right Y-axis) concentration and modelling of first order kinetics to ciprofloxacin (full line) and desethylene ciprofloxacin (dotted line) data points during ozonation at 2500 ppm$_v$ ozone inlet concentration, 45.27 μM ciprofloxacin concentration and $T = 27.5\,^\circ$C at pH 7 (10 μmol l^{-1} H$_2$O$_2$).

3.3.4. Effect of process parameters on desethylene ciprofloxacin formation

Fig. 5 shows the change of desethylene ciprofloxacin concentration versus time for the standard peroxone experiment at pH 7 (10 μmol l^{-1} H$_2$O$_2$). As can be seen, a maximum concentration of 3.58 μM is reached at 20 min. In order to make an estimation on the percentage of desethylene ciprofloxacin formed, desethylene ciprofloxacin formation was modelled considering first order reaction kinetics for desethylene ciprofloxacin degradation, described by a pseudo-first order constant $k_{1,\text{desethylene}}$. A fraction (α) of ciprofloxacin (A) was considered to degrade to desethylene ciprofloxacin through intermediate products. A fraction ($1 - \alpha$) reacts to other products (C) while desethylene ciprofloxacin (B) itself also degrades to several oxidation products

$$\frac{d[A]}{dt} = -k_{1,cipro}[A]$$

$$\text{A} \qquad\qquad\qquad\qquad \text{C}$$

$$\frac{d[A]}{dt} = -k_{1,cipro}[A] \xrightarrow{\alpha} \text{B} \qquad \frac{d[B]}{dt} = -k_{1,desethylene}[B]$$

(C):

Values for $k_{1,\text{desethylene}}$ and α were modelled by fitting formula (4) to the data points:

$$[B] = \frac{k_{1,\text{cipro}}\alpha[A]_0}{k_{1,\text{desethylene}} - k_{1,\text{cipro}}}(e^{(-k_{1,\text{cipro}}t)} - e^{(-k_{1,\text{desethylene}}t)}) \qquad (4)$$

Table 3 summarizes the $k_{1,\text{cipro}}$, $k_{1,\text{desethylene}}$ and α values for several ozonation experiments. For the standard ozonation experiment at pH 7 and the standard peroxone experiment at pH 7 (10 μmol l^{-1} H$_2$O$_2$), $k_{1,\text{desethylene}}$ values are found to be higher than $k_{1,\text{cipro}}$ values, indicating that desethylene ciprofloxacin degrades faster than ciprofloxacin during ozonation.

When the ozone inlet concentration is increased from 660 to 3260 ppm$_v$, $k_{1,\text{desethylene}}$ values increase, similar to the ciprofloxacin degradation constants $k_{1,\text{cipro}}$. Experiments at different ciprofloxacin concentration and temperature also revealed similar trends between $k_{1,\text{cipro}}$ and $k_{1,\text{desethylene}}$ values. Increasing ciprofloxacin concentration led to lower $k_{1,\text{desethylene}}$ values while no clear trend could be found in function of temperature. At higher ciprofloxacin inlet concentrations, higher maximum

desethylene ciprofloxacin concentrations were obtained at longer ozonation times: a maximum desethylene ciprofloxacin concentration of 1.50 μM was obtained at 10 min of ozonation when the ciprofloxacin concentration was 22.64 μM, while 135.81 μM ciprofloxacin led to 8.93 μM desethylene ciprofloxacin at 50 min ozonation. A plot of maximum desethylene ciprofloxacin concentration versus ciprofloxacin inlet concentration showed a linear relationship ($y = 0.0648 \pm 0.0008x$, $R^2 = 0.998$) which goes through the origin (SPSS15.0, regression, $\alpha > 0.05$).

Remarkably, the cumulative desethylene production from ciprofloxacin mounted 14–25% for all experiments at pH 7. This means that 75–86% of ciprofloxacin degrades to other degradation products (C).

H$_2$O$_2$ addition (10 μmol l^{-1}) only proves to have a small effect on the α value at pH 10, with an increase from 52 to 64%. The most important parameter, however, is the pH. At pH 3 and 7, only 21–25% of ciprofloxacin is degraded to desethylene ciprofloxacin compared to 52–64% at pH 10. This could be expected because at pH 10, both N-atoms of the piperazinyl substituent are deprotonated, which can lead to direct ozonation at N-atoms [12]. Secondly, the enhanced hydroxyl radical concentration (SHB-model; [17]) can also lead to enhanced desethylene ciprofloxacin formation.

At pH 7, the N$_4$-atom of the piperazinyl substituent is protonated while the N$_1$-atom is not. Surprisingly, no enhanced α value was found compared to pH 3, where both N-atoms of the piperazinyl substituent are protonated. Dodd et al. [12] mentioned that the N$_4$-atom is the most reactive centre of ciprofloxacin for ozonation, which seems to be confirmed by these findings. The N$_1$-atom of the piperazinyl substituent belongs to the aromatic moiety of ciprofloxacin. This seems to stabilize the free electrons of the deprotonated N-atom which makes the N$_1$-atom of the piperazinyl

Table 3
Ciprofloxacin and desethylene ciprofloxacin reaction constants and α values for experiments at 2500 ppm$_v$ ozone inlet concentration, 45.27 μM ciprofloxacin concentration and $T = 27.5\,^\circ$C at pH 7, unless specified otherwise

Inlet ozone concentration (ppm$_v$)	$k_{1,\text{cipro}}$ (min^{-1})	$k_{1,\text{desethylene}}$ (min^{-1})	α
660	0.0081 ± 0.0004	0.0081	0.20
2010	0.0343 ± 0.0012	0.0438	0.24
2500	0.0453 ± 0.0030	0.0609 ± 0.0010	0.21 ± 0.04
3260	0.0613 ± 0.0022	0.0706	0.17
3680	0.0660 ± 0.0029	0.0580	0.14
Ciprofloxacin concentration (μM)	$k_{1,\text{cipro}}$ (min^{-1})	$k_{1,\text{desethylene}}$ (min^{-1})	α
22.64	0.0940 ± 0.0075	0.0999	0.19
45.27	0.0453 ± 0.0030	0.0609 ± 0.0010	0.21 ± 0.04
67.91	0.0364 ± 0.0011	0.0518	0.19
90.54	0.0333 ± 0.0023	0.0254	0.16
135.81	0.0219 ± 0.0005	0.0215	0.17
Temperature ($^\circ$C)	$k_{1,\text{cipro}}$ (min^{-1})	$k_{1,\text{desethylene}}$ (min^{-1})	α
6.0	0.0549 ± 0.0027	0.0523	0.16
13.4	0.0413 ± 0.0037	0.0667	0.15
21	0.0436 ± 0.0030	0.0467	0.16
27.5	0.0453 ± 0.0030	0.0609 ± 0.0010	0.21 ± 0.04
35.4	0.0561 ± 0.0044	0.0717	0.23
62.0	0.0382 ± 0.0022	0.0489	0.25
pH	$k_{1,\text{cipro}}$ (min^{-1})	$k_{1,\text{desethylene}}$ (min^{-1})	α
3	0.0567 ± 0.0032	0.0523	0.25
3, peroxon	0.0492 ± 0.0023	0.0497	0.25
7	0.0443 ± 0.0018	0.0609 ± 0.0010	0.21 ± 0.04
7, peroxon	0.0505 ± 0.0014	0.0616 ± 0.0088	0.24 ± 0.01
10	0.0515 ± 0.0016	0.0562	0.52
10, peroxon	0.0462 ± 0.0010	0.0797	0.64
3, t-butanol	0.0656 ± 0.0068	0.0479	0.09

substituent less susceptible for an electrophilic attack by ozone molecules.

Because no hydroxyl radicals are expected at pH 3 according to the SHB-model [17] and because direct ozonation at protonated N-atoms can be neglected [12], it is remarkable that still 25% of ciprofloxacin degrades to desethylene ciprofloxacin at this pH. Moreover, when t-butanol is added as radical scavenger, the α value is still 9%. Again, this suggests that radical species, other than hydroxyl radicals, are partially responsible for ciprofloxacin degradation.

The highest ciprofloxacin reaction constant was found at pH 3 although less desethylene ciprofloxacin (25%) is formed at this pH. The high degradation rate has to be explained by other reactions pathways. For a comprehensive view on ciprofloxacin degradation, further research is needed to clarify different degradation pathways and their relative importance in function of pH.

4. Conclusions

- Ciprofloxacin ozonation in a bubble reactor where ozone supply rather than reaction kinetics is rate limiting could be described by first order kinetics.
- The highest degradation rate was obtained at the highest ozone concentration (660–3680 ppm$_v$) and lowest ciprofloxacin concentration (22.64–135.81 µM) tested. No effect of reactor temperature was found (6.0–62.0 °C).
- Addition of small amounts of H_2O_2 (2–50 µmol l^{-1}) increased ciprofloxacin degradation while high amounts (990 µmol l^{-1}) decreased degradation rates at pH 7 because of the competitive effect of promoting hydroxyl radical formation versus scavenging hydroxyl radicals by HO_2^-.
- Addition of t-butanol as radical scavenger at pH 3 increased ciprofloxacin degradation. Probably, changes in bubble pattern lead to an enhanced mass transfer coefficient.
- Desethylene ciprofloxacin could be identified as the major ciprofloxacin degradation product: at pH 10, when N-atoms are deprotonated and hydroxyl radicals are expected, 52–64% of ciprofloxacin concentration degrades to desethylene ciprofloxacin.
- At pH 3, when N-atoms are protonated and no hydroxyl radicals are expected, the fastest ciprofloxacin degradation could be found next to 25% formation of desethylene ciprofloxacin. Considering relatively fast degradation of pCBA at pH 3 compared to pH 7 and 10, the presence of reactive species other than hydroxyl radicals and ozone, is thought to be partially responsible for ciprofloxacin degradation at low pH.

Acknowledgement

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