Chemosphere 76 (2009) 683–689

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Levofloxacin ozonation in water: Rate determining process parameters and reaction pathway elucidation

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ARTICLE INFO

Article history:
Received 10 February 2009
Received in revised form 23 March 2009
Accepted 24 March 2009
Available online 25 April 2009

Keywords:
Quinolones
Degradation products
Antibacterial activity
Ab initio computations
Fukui functions
Ozonation

ABSTRACT

Ozonation of the quinolone antibiotic levofloxacin was investigated with focus on both the levofloxacin degradation rate and degradation product formation. Degradation was about 2 times faster at pH 10 compared to pH 3 and 7 explained by direct ozonation at the unprotonated N_4', one of the tertiary amines of the piperazinyl substituent. H_2O_2 concentration (2–100 μM) had only limited effect. Liquid chromatography – high resolution mass spectrometry revealed degradation at the piperazinyl substituent and the quinolone moiety, with the relative importance of both pathways being strongly affected by changes in pH. Levofloxacin N-oxide concentrations reached up to 40% of the initial levofloxacin concentration during ozonation at pH 10. Degradation at the quinolone moiety resulted in isatin and anthranilic acid type metabolites, probably formed through reaction with hydroxyl radicals. Ab initio molecular orbital calculations predicted radical attack mainly at C_2 of the quinolone moiety. This is the carbon atom with the largest Fukui function. Reaction with ozone is expected to mainly occur at N_4', characterized by the largest negative charge.

1. Introduction

Fluoroquinolones are synthetic antibiotics inhibiting bacterial DNA synthesis through binding with DNA gyrase and topoisomerase IV enzyme (Hooper, 1999). Nalidixic acid, the first fluoroquinolone, was introduced in 1962. First and second generation quinolones are active against Gram-negative bacteria and atypical pathogens. The latter are pathogens that can cause community-acquired pneumonia (Lee et al., 2002). The activity of third and fourth generation quinolones is extended to Gram-positive and anaerobic bacteria, respectively (Oliphant and Green, 2002). Ciprofloxacin, belonging to the second generation and introduced in 1987, was the mostly prescribed quinolone in Europe in 2003. However, a shift towards levofloxacin and moxifloxacin, introduced in 1996 and 1999, respectively, is noticed (Ferech et al., 2006).

The increased use of quinolones has led to increased bacterial resistance (Jacoby, 2005). This can be partially due to the release of antibiotics into the environment. After administration, quinolones are only partially metabolized and their biotic transformation in the environment is slow (Huang et al., 2001) leading to wastewater treatment plant effluent concentrations up to 5.6 μg L^{-1} for ciprofloxacin (Batt et al., 2006). By consequence, physical-chemical removal technologies, such as advanced oxidation processes (AOPs), are a suitable alternative method for their removal from wastewater. AOPs are characterized by the generation of hydroxyl radicals at ambient conditions. Advanced oxidation of ciprofloxacin has been extensively studied (Dodd et al., 2006; Siminiceanu and Bobu, 2006; Paul et al., 2007; De Witte et al., 2008, 2009). In contrast, literature data on advanced oxidation of more recently introduced quinolones is scarce. In this paper, the ozonation of levofloxacin is discussed for the first time. The effect of process parameters pH and H_2O_2 is tested and degradation products are identified based on UV and high resolution mass spectrometry (HRMS) detection. Reactive sites are predicted based on ab initio molecular orbital calculations. This approach is widely applicable and has proven to be successful for a broad variety of molecules (Geerlings et al., 2003; Hemelsoet et al., 2005; Van Speybroeck et al., 2006).

2. Materials and methods

2.1. Chemicals

Levofloxacin (⩾98%) was delivered by Fluka (Germany). Other chemicals used were of reagents grade and were previously reported (De Witte et al., 2008).

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doi:10.1016/j.chemosphere.2009.03.048

2.2. Experimental setup and analytical procedures

Levofloxacin ozonation was performed in a bubble column containing 1.75 L water buffered with 10.12 mM phosphate buffer (pH 3 and 7) or 2.53 mM borax buffer (pH 10). Initial levofloxacin concentration mounted 45.3 μM (16.4 mg L^{-1}). The experimental setup was identical as recently reported for ciprofloxacin ozonation (De Witte et al., 2008). Based on research on ciprofloxacin ozonation (De Witte et al., 2009), 2–100 μM H_2O_2 was added to the reactor during peroxone experiments. For experiments with radical scavengers, 30.45 mM t-butanol was added.

Ozone in the gas flow was measured by an ozone analyzer (Anseros Ozomat GM) by UV-light absorption at 253.7 nm. For levofloxacin determination, 5 mL liquid samples were taken and analyzed by liquid chromatography (LC)–UV spectroscopy identical to a previously described procedure (De Witte et al., 2009). Quantification of levofloxacin (295 nm) and its degradation products took place at the UV-absorbance maximum ±4.5 nm. For identification of degradation products, 25 mL samples were concentrated by a factor of 125 by solid phase extraction. Compounds were separated by gradient LC and detected by UV and HRMS (De Witte et al., 2008). Comparisons with UV- and MS-spectra of analogous products (De Witte et al., 2008) and the parent compound allowed level 2 or full identification (De Witte et al., in press). Polyethylene glycols (PEG) were used as HRMS internal standard for determination of the accurate mass and chemical formula of the degradation products. An additional energy of 100 V was applied to the electrospray ionization needle (collision induced dissociation, CID) for enhancement of degradation product fragmentation. With CID, PEG ions were not stable as internal standard. They were used as external standard for determination of accurate mass of MS-fragmentation products. If the measured m/z of the protonated compounds deviated less than 5 ppm from the theoretical values in the case of internal standards and less than 15 ppm in the case of external standards, the chemical formula was restrained.

2.3. Ab initio molecular orbital calculations

All ab initio calculations were carried out using the Gaussian 03 software package. Density functional theory (DFT) (Parr and Yang, 1989) was applied due to its excellent cost-to-reliability performance compared to post-Hartree–Fock methods. Geometries were optimized using the B3LYP functional (Lee et al., 1988; Becke, 1993) and 6-31+G(d,p) Pople basis set. Subsequent single-point energy computations were performed using the meta-hybrid BMK functional (Boese and Martin, 2004) in combination with the large 6-311++G(3df,2p) basis set. DFT-based reactivity indicators, and in particular frontier orbital-related properties (i.e., Fukui functions (Fukui, 1973)) were computed at the BMK/B3LYP level of theory. Compared to the frontier orbitals HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) the Fukui functions contain more detailed information, taking also orbital relaxation effects into account. For more explanation on their definition we refer to the handbook of Parr and Yang (1989) or a review of Geerlings et al. (2003). Atomic charges and condensed-to-atoms values of the Fukui function were examined using the Mulliken population scheme (Mulliken, 1955).

3. Results and discussion

3.1. Parameter study

The levofloxacin degradation curve for ozonation at pH 7 is presented as Supplementary material (Fig. S1, $n = 3$) and resulted in a half-life time of 12.8 ± 0.2 min ($n = 3$) and more than 95% and 99% of the initial levofloxacin concentration was removed at 40 and 50 min of ozonation, respectively. The ozone consumption during 60 min of ozonation, calculated from the difference between the inlet and outlet gaseous ozone concentration, mounted 0.61 ± 0.01 mmol compared to 0.44 mmol for the blank experiment without levofloxacin. Half-life time ($t_{1/2}$) as well as levofloxacin degradation rate constants at 10% degradation ($k_{10\%}$) was considered to compare experimental results. For $k_{10\%}$ determination, a quadratic equation was fitted to the levofloxacin data points up to 95% degradation and the first derivative at 10% degradation was calculated.

As can be seen from Table 1, degradation rate constants ($k_{10\%}$) are approximately two times faster at pH 10 compared to pH 7 and 3. Next, levofloxacin degradation is also faster compared to previously reported ciprofloxacin degradation at similar conditions (De Witte et al., 2009). Differences with ciprofloxacin are larger at pH 10 ($t_{1/2}$ = 7.8 versus 13.8 min) compared to pH 7 and 3 ($t_{1/2}$ = 12.8 and 16.0 min versus 15.9 and 17.6 min, respectively). Levofloxacin has a pK_a-value of 6.20 for the carboxylic group, 5.20 for the N'_1-atom and 8.20 for the N'_4-atom of the piperazinyl substituent (Fig. 1) (Lin et al., 2004). Protonated amines are practically unreactive towards ozone whereas the lone electron pair of the unprotonated amine can react fast with ozone, leading to higher degradation rates at higher pH (Muñoz and von Sonntag, 2000). Moreover, the N'_4-atom belongs to a tertiary amine group whereas ciprofloxacin has a secondary amine group at its piperazinyl substituent. Methyl groups are better electron donors than hydrogen

Table 1
Levofloxacin half-life time, degradation rate constants at 10 wt% degradation and ozone consumption during 60 min of ozonation for experiments at 45.3 μM initial levofloxacin concentration and varying pH and H_2O_2 concentration ($O_{3,inlet}$ = 2500 ppm_v = 4.87 mg L^{-1}, T = 27.5 ± 0.1 °C).

pH	H_2O_2 (μM)	$t_{1/2}$ (min)	$k_{10\%}$ [b] (mM min^{-1})	Ozone consumption during 60 min (mmol)
3	–	16.0	1.77 ± 0.05	0.55
7	–	12.8 ± 0.2 [a]	1.96 ± 0.10	0.61 ± 0.01 [a]
10	–	7.8	3.80 ± 0.05	0.65
7	2	10.9	2.62 ± 0.17	0.62
7	10	11.9	2.17 ± 0.04	0.61
7	25	10.6	2.62 ± 0.10	0.62
7	50	11.6	2.31 ± 0.15	0.62
7	100	11.8	2.47 ± 0.12	0.59
3	10	15.6	1.77 ± 0.23	0.55
3	100	16.2	1.81 ± 0.18	0.59
10	10	8.1	3.75 ± 0.40	0.63
10	100	9.9	2.99 ± 0.54	0.65

[a] Standard deviation obtained by three experimental repetitions.
[b] Standard deviation obtained by regression in SPSS 16.

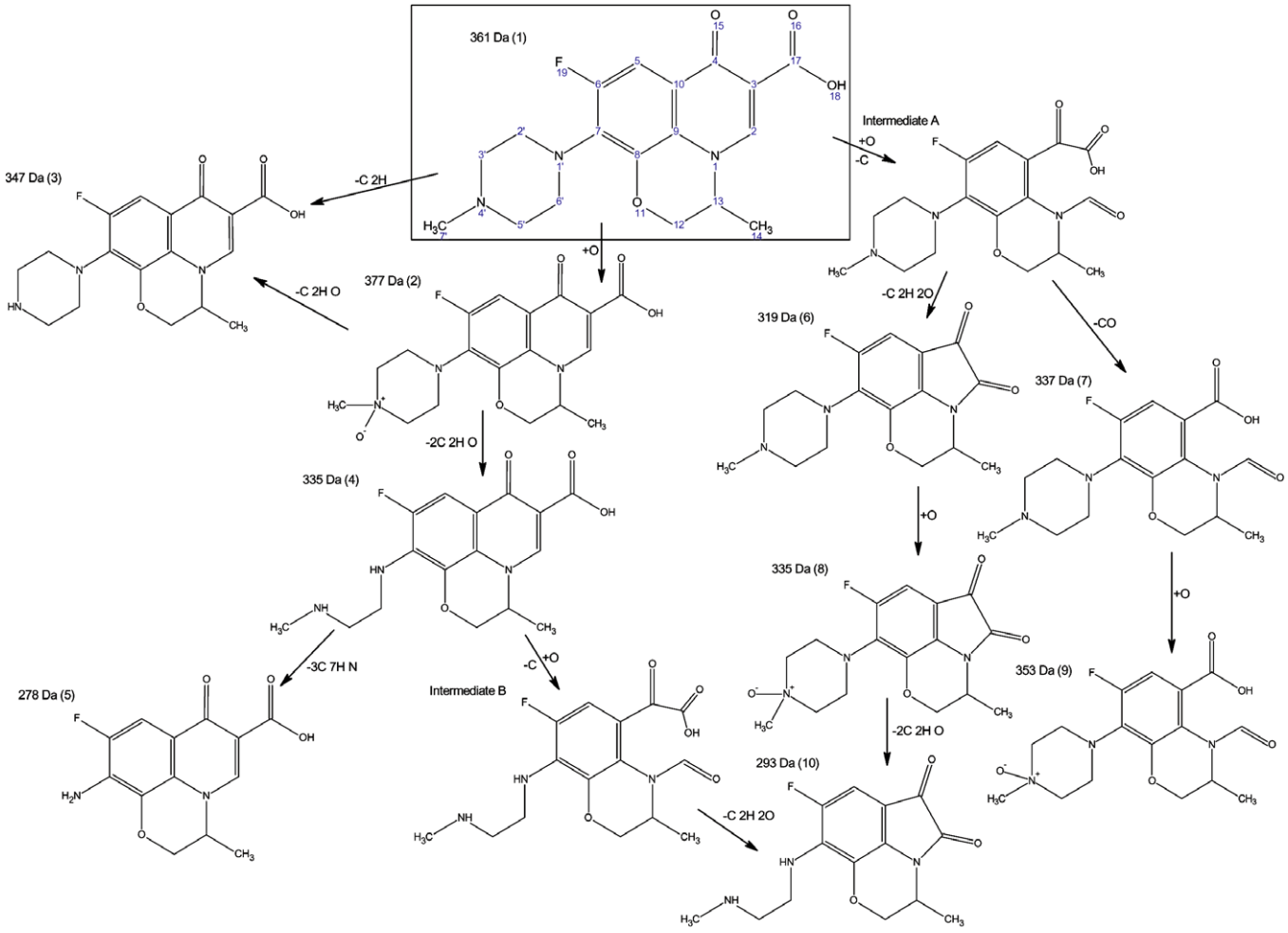

Fig. 1. Possible reaction pathways for levofloxacin ozonation, based on UV and HRMS reaction product analysis. Intermediates A and B were not detected but suggested based on previous work (De Witte et al., 2008).

atoms. This will result in a higher electron density at the N'_4-atom for tertiary amines and, by consequence, faster ozonation rates and larger pH effects for tertiary amines compared to secondary amines. Differences between tertiary and secondary amines are in agreement with Muñoz and von Sonntag (2000), who found rate constants of 4.1×10^6 M^{-1} s^{-1} for triethylamine versus 9.1×10^5 M^{-1} s^{-1} for diethylamine.

At pH 7, addition of 2–100 μM H_2O_2 increased the levofloxacin degradation rate constants ($k_{10\%}$) by 11–34% (Table 1). The effect of H_2O_2 addition on the levofloxacin degradation rate is, however, limited compared to the pH effect. Moreover, no trend in levofloxacin degradation rate as well as ozone consumption is derived as function of the H_2O_2 amount added whereas an optimum concentration could be expected because H_2O_2 can both promote and scavenge hydroxyl radicals (Gogate and Pandit, 2004). The limited effect of H_2O_2 addition may indicate that the radical chain mechanism, described by Staehelin and Hoigné (1985), is of minor importance for levofloxacin degradation compared to direct ozonation because levofloxacin contains several amine groups and an aromatic moiety, both reacting fast with ozone ($10^3 - 10^{11}$ M^{-1} s^{-1}, Muñoz and von Sonntag (2000), Vandersmissen et al. (2008)). A second possibility is that ozonation of amines promotes OH-radical formation (Buffle, 2005), which initiates the radical chain mechanism and reduces the effect of H_2O_2 addition.

At pH 3, addition of 10 and 100 μM H_2O_2 did not affect levofloxacin degradation (Table 1), probably because HO_2^- instead of H_2O_2 plays the major role in OH-radical formation (pK_a of H_2O_2 = 11.8, Beltran (2004)). At pH 10, however, addition of 100 μM H_2O_2 reduced levofloxacin degradation efficiency due to scavenging of hydroxyl radicals at higher HO_2^- concentrations. Buxton et al. (1988) reported reaction constants of 7.5×10^9 and 2.7×10^7 M^{-1} s^{-1} between OH-radicals and HO_2^- and H_2O_2, respectively.

3.2. Reaction pathways

Degradation products are listed in Table 2 and presented in Fig. 1. Compounds 2–5 show a limited change in UV-spectrum compared to the UV-spectrum of levofloxacin (Supplementary material, Fig. S2a). This indicates that the chromophore is unaffected and, by consequence, degradation occurs at the piperazinyl substituent or the oxazinyl group (Fig. 1). Structures can be identified based on both HRMS analysis and MS fragmentation. Compound 2 (377 Da) has a net gain of one oxygen atom compared to levofloxacin. The presence of a MS-fragment m/z 261 ($C_{14}H_{14}N_2O_2F$), similar to levofloxacin MS fragmentation (Supplementary material, Fig. S2b), indicates degradation at the piperazinyl substituent instead of the oxazinyl group. Secondly, MS fragments with loss of OH are typical for N-oxides (Kulanthaivel et al., 2004). Analogously, compound 3 (347 Da) could be identified as a secondary amine due to the net loss of CH_2 and presence of the MS-fragment m/z 261. Compounds 4 and 5 were analogous to previously reported ciprofloxacin degradation products (De Witte et al., 2008).

Table 2
Levofloxacin degradation products[a].

t_R[b] (min)	$[M+H]^+$ measured	Nominal mass (Da)	Molecular formula	Error[c] (ppm)	Difference with levofloxacin		Degradation site moiety[d]	No.
3.82	365.159	364	$C_{14}H_{23}O_7N_3F$	−1.21	$-C_4$	$+H_3O_3$		
9.45	**338.152**	**337**	**$C_{16}H_{20}O_4N_3F$**	**1.83**	**$-C_2$**		**Qui**	**7**
11.49	312.135	311	$C_{14}H_{18}O_4N_3F$	−1.06	$-C_4H_2$			
12.05	338.152	337	$C_{16}H_{20}O_4N_3F$	2.63	$-C_2$			
15.23	**320.141**	**319**	**$C_{16}H_{18}O_3N_3F$**	**0.04**	**$-C_2H_2O$**		**Qui**	**6**
15.88	**294.126**	**293**	**$C_{14}H_{16}O_3N_3F$**	**2.70**	**$-C_4H_4O$**		**Pip, Qui**	**10**
17.69	**354.145**	**353**	**$C_{16}H_{20}O_5N_3F$**	**−3.77**	**$-C_2$**	**+O**	**Pip, Qui**	**9**
18.59	376.129	375	$C_{18}H_{18}O_5N_3F$	0.18	$-H_2$	+O		
20.34	**336.135**	**335**	**$C_{16}H_{18}O_4N_3F$**	**0.32**	**$-C_2H_2$**		**Pip**	**4**
20.68	354.145	353	$C_{16}H_{20}O_5N_3F$	−3.40	$-C_2$	+O		11
20.88	*362.151*	*361*	*$C_{18}H_{20}O_4N_3F$*	*−1.44*	*Levofloxacin*			
21.68	314.068	313	$C_{13}H_{12}O_7NF$	0.74	$-C_5H_8N_2$	$+O_3$		
21.77	**348.137**	**347**	**$C_{17}H_{18}O_4N_3F$**	**3.67**	**$-CH_2$**		**Pip**	**3**
22.99	**336.136**	**335**	**$C_{16}H_{18}O_4N_3F$**	**0.44**	**$-C_2H_2$**		**Pip, Qui**	**8**
27.73	**378.146**	**377**	**$C_{18}H_{20}O_5N_3F$**	**0.25**		**+O**	**Pip**	**2**
29.63	326.151	325	$C_{15}H_{20}O_4N_3F$	−0.31	$-C_3$			
40.48	298.073	297	$C_{13}H_{12}O_6N_1F$	0.93	$-C_5H_8N_2$	$+O_2$		
41.47	**279.078**	**278**	**$C_{13}H_{11}O_4N_2F$**	**0.21**	**$-C_5H_9N$**		**Pip**	**5**
42.32	364.130	363	$C_{17}H_{18}O_5N_3F$	−0.10	$-CH_2$	+O		

[a] The numbering follows the reaction pathway (Fig. 1), except for compound 11 for which no structure was identified.
[b] HPLC retention time based on MS detection.
[c] Difference between measured and theoretical mass.
[d] pip = Piperazinyl and qui = quinolonic moiety.

N-oxide formation was not detected during ciprofloxacin oxidation (De Witte et al., 2008). Probably, the electron donating capacity of a methyl group compared to a hydrogen atom substituted at N_4' of the piperazinyl affects not only reaction rate but also degradation product formation. According to Muñoz and von Sonntag (2000), ozonation of tertiary amines leads to ozonide ammonium zwitterions mainly followed by loss of dioxygen resulting in compound 2. The secondary amine (compound 3) can be formed together with formaldehyde through dissociation of the ozonide ammonium zwitterion into the ozonide radical ion and the amine radical cation or through reaction of a tertiary amine with hydroxyl radicals (Muñoz and von Sonntag, 2000). Compounds 4 and 5 are formed after multiple reactions, leading to enhanced degradation at the piperazinyl substituent.

Compounds 6–10 have UV spectra different from levofloxacin which indicates degradation at the chromophore, i.e. the quinolone moiety. Based on UV-spectrum, molecular formula and MS fragmentation, these degradation products were found to be isatin (compounds 6, 8 and 10) or anthranilic acid analogues (compounds 7 and 9). Similar degradation products were identified during ciprofloxacin ozonation (De Witte et al., 2008). Formation of isatin and anthranilic acid analogues by means of hydroxyl radicals is possible through formation of intermediates A and B (Fig. 1) whereas direct ozonation can also lead to anthranilic acid analogues (Karl et al., 2006; De Witte et al., 2008).

In conclusion, nine levofloxacin ozonation products were identified indicating degradation at the piperazinyl substituent and the quinolone moiety. No degradation products were found corresponding to degradation at the oxazinyl group (Fig. 1). However, the structure could not be identified for every degradation product, detected by HPLC-MS (Table 2).

3.3. Effect of pH, H_2O_2 and t-butanol on degradation product formation

Without sample concentration, LC–UV peak areas could be determined for seven degradation products at all investigated process conditions and different ozonation times. The chromatographic separation for samples at 20 min ozonation at pH 7 is given in Supplementary material, Fig. S3.

For 2 out of the 7 compounds, no molecular formula could be obtained with HRMS using the threshold error value of 5 ppm. The maximum concentration for the two unidentified compounds were observed at pH 3 and 7 (data not shown). For the other compounds, maximum peak areas are plotted in Fig. 2. Addition of 10 μM H_2O_2 at pH 3, 7 and 10 does not affect degradation product concentrations (Fig. 2). pH, on contrary, is an important parameter with respect to degradation product formation. Compounds 8 and 11 are mainly formed at pH 7 while addition of the radical scavenger t-butanol in excess at pH 7 excludes their formation. Compound 8 shows degradation at the quinolone moiety. Compound 11 was not identified but reveals a strong change in UV-spectrum compared to levofloxacin, also suggesting degradation at the quinolone moiety. The results suggest that degradation at the quinolone moiety is mediated by OH-radicals in agreement with previously reported ciprofloxacin degradation (De Witte et al., 2008). At pH 3, no OH-radicals are expected (Rivas et al., 2005). At pH 10, the N_4'-atom of the piperazinyl is unprotonated and becomes an important reactive centre for ozone probably hampering high concentrations of compounds 8 and 11 at this pH.

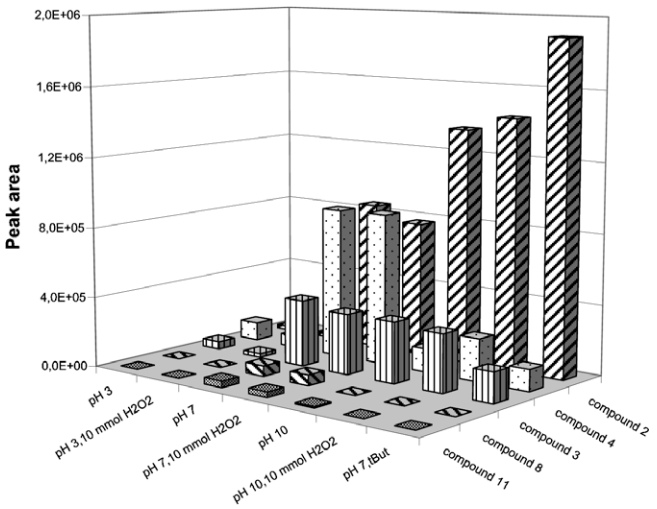

Fig. 2. Maximum peak area of compounds 2, 3, 4, 8 and 11 during levofloxacin ozonation as a function of pH, H_2O_2 and t-butanol concentration. The peak area of compound 2 (N-oxide) was divided with a factor 5 for reasons of visibility.

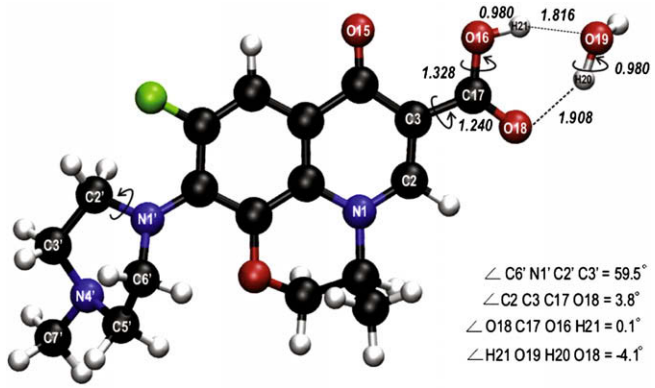

Fig. 3. Optimized geometrical structure of the levofloxacin molecule. Important bond lengths (in Å) and dihedrals (in degrees) are indicated.

Table 3
Mulliken atomic charges and condensed-to-atoms Fukui functions f^- and f^0, corresponding with an electrophilic and radical attack, respectively.

	Charges	f^-	f^0
N1	−0.843	0.051	0.102
C2	0.155	−0.027	**0.128**
O15	−0.759	0.057	0.082
O16	−0.791	0.034	0.054
C17	0.698	0.007	−0.028
O18	−0.638	0.007	0.017
N'_1	−0.802	**0.175**	0.102
N'_4	**−0.941**	0.048	0.031
C'_7	−0.407	−0.004	−0.004

Compounds 2 and 3 contain the same chromophore as levofloxacin while the UV-spectrum of compound 4 is only slightly shifted (data not shown). Therefore, concentration estimations can be based on the levofloxacin UV response factor. Concentrations of the N-oxide compound 2 reach up to 0.3, 11.4 and 18.1 μM at pH 3, 7 and 10, respectively. Addition of t-butanol at pH 7 increased concentrations up to 25.4 μM. These results indicate that compound 2 formation is favored by direct ozonation at the unprotonated tertiary amine while its degradation is partially affected by the presence of OH-radicals.

Compounds 3 and 4 reveal equal or lower concentrations at pH 10 (up to 0.9 and 0.4 μM, respectively) compared to pH 7 (up to 1.0 and 2.3 μM, respectively) while t-butanol addition at pH 7 reduces their concentration to 0.5 and 0.3 μM (Fig. 2). This indicates that direct ozonation at the partially unprotonated N'_4-atom can not be the main reaction pathway at pH 7: hydroxyl radicals are suggested to be more important for formation of these products at this pH.

High concentration of the N-oxide compound 2 proves that direct ozonation is important for levofloxacin ozonation. Moreover, the levofloxacin degradation rate as a function of pH can be linked to N-oxide formation. However, the influence of t-butanol addition on the formation of compounds 3, 4, 8 and 11 reveals that the radical chain mechanism also contributes to levofloxacin degradation, affecting degradation at the quinolone moiety. Since the carbonyl and carboxyl at the quinolone moiety are essential for binding at the DNA gyrase or topoisomerase IV target (Chu and Fernandes, 1989), the radical chain mechanism is likely to inactivate the drug instantly.

3.4. Rationalization of reactivity by ab initio molecular orbital calculations

It was investigated whether the experimentally observed oxidation products can be further rationalized in terms of ab initio computations. A conformational analysis was performed, rotating the carboxyl group on one hand and varying the planarity of the piperazinyl substituent on the other. The latter leads to a twist boat configuration for the ring substituent (dihedral angle of 59.5°, Fig. 3), whereas the quinolone moiety and carboxyl functional group accords with an almost planar substructure (maximal dihedral angle of 3.8°, Fig. 3). The most stable conformer was ultimately obtained taking into account an explicit hydrogen molecule, forming an external hydrogen bridge with the hydrogen-atom of the carboxyl group. The optimized geometry of the gas-phase levofloxacin molecule with an explicit water solvent molecule is depicted in Fig. 3.

The reactivity and site selectivity of the optimized geometry was examined using DFT-based reactivity indicators. According

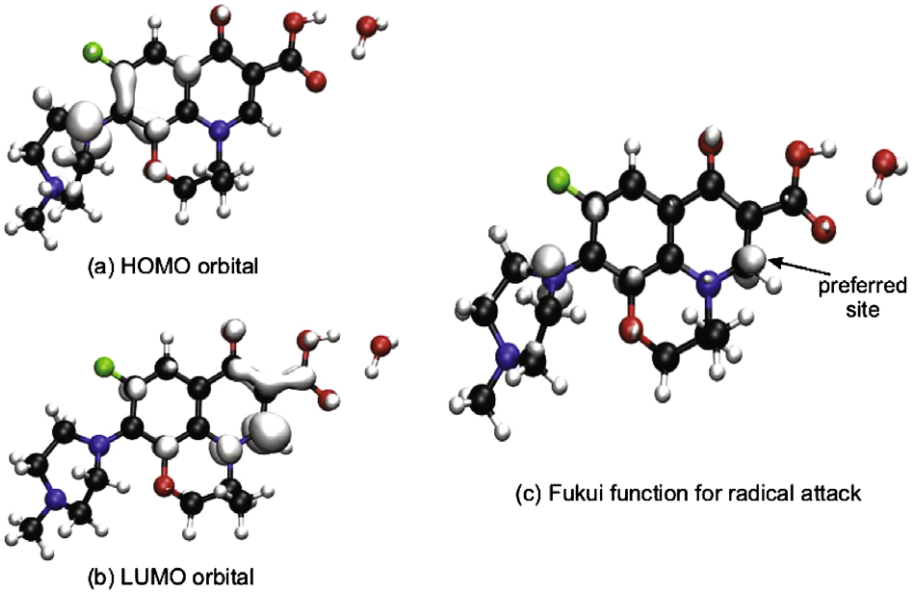

Fig. 4. Three-dimensional iso-surfaces of the frontier orbitals (a, b) and the Fukui function for radical attack (c) for the levofloxacin molecule.

to the early work of Klopman (1968), reactions can be classified as predominantly frontier-orbital- or charge-controlled.

The radical Fukui function f^0 is used to examine the interaction with hydroxyl radicals. The preferred site is identified by the maximal value of the Fukui function. The condensed-to-atoms values (Table 3) indicate enhanced reactivity at the levofloxacin C_2 and (to a lesser extent) the N_1 and N_1'-atom. Reactions occurring at the nitrogen atoms can, however, be affected by steric hindrance effects which are not encapsulated in the definition of the reactivity indicators. The three-dimensional iso-surfaces of the HOMO and LUMO frontier orbitals and the radical Fukui function are shown in Fig. 4. It can be seen that the combination of the HOMO and LUMO (taking the mean average) can be regarded as an initial approximation of the Fukui function. Compared to the condensed-to-atoms results, the f^0 iso-surface has the advantage to disregard the effect of the population analysis scheme. The C_2-atom is clearly found to be a suitable site for radical attack. This is in agreement with the formation of isatin and anthranilic acid analogues through degradation at the quinolone moiety. These products were excluded when the radical scavenger t-butanol was added. Moreover, Karl et al. (2006) proved that degradation of the quinolone enrofloxacin by hydroxyl radicals with formation of isatin and anthranilic acid analogues proceeds through breaking of the C_2–C_3 bond. By consequence, radical interactions of levofloxacin with hydroxyl radicals can be modeled as frontier-orbital controlled.

Interaction with ozone corresponds to an electrophilic attack. The electrophilic Fukui function f^- does not support the experimental observations as the f^- values indicate N_1' to be the most reactive centre (Table 3). However, due to the less favorable overlap between the frontier orbitals of levofloxacin and ozone, the interaction will be dominated by charge/charge effects and is thus expected to occur at the sites with the highest electron density. The Mulliken scheme shows the largest negative charge at the N_4'-atom of the piperazinyl susbstituent, which is indeed the most reactive centre towards ozone. Ozone nor OH-radicals reactions are predicted at the oxazinyl group, confirming the lack of products with degradation at this part of the molecule.

4. Conclusions

Levofloxacin ozonation at different pH and different H_2O_2 amounts revealed strong influence of pH on levofloxacin degradation rate as well as reaction pathways whereas H_2O_2 addition had only limited effect. At pH 10, the tertiary amine at the piperazinyl substituent is unprotonated leading to fast ozonation at this site of the molecule and high concentrations of the N-oxide degradation product. At pH 7, degradation at the quinolone moiety is also observed, probably mediated by reaction with hydroxyl radicals. This was confirmed by ab initio molecular orbital calculations which predicted the carbon atom of the quinolone moiety with the largest value of the Fukui function as the most reactive centre for radical attack.

Acknowledgements

We acknowledge financial support from the Flemish Government for the MAT 95XP-Trap in the framework of the Flemish investment support for heavy research equipment. This work is supported by the Fund for Scientific Research-Flanders (FWO) and the Research Council of Ghent University.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2009.03.048.

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