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

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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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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.