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# Antibiotic removal from wastewaters: The ozonation of amoxicillin

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**Abstract**

The presence of amoxicillin, a widely used antibiotic, has been documented in Sewage Treatment Plant (STP) effluents. As for other pharmaceuticals, ozonation is proposed as a process for its abatement from these effluents. The results of ozonation experiments on amoxicillin-containing aqueous solutions indicate that ozone attack is mainly directed towards the phenolic ring of the studied molecule leading to the formation of hydroxyderivative intermediates. No direct evidences of attack on sulfur atom with sulfoxide formation are found. A kinetic investigation is carried out allowing the assessment of the kinetics of direct ozone attack and that of OH radicals to amoxicillin.


*Keywords:* Amoxicillin; Sewage Treatment Plant effluents; Ozonation experiments

## 1. Introduction

Recently, the attention of many researchers working in the environmental field was focused on the presence in the environment (and more specifically in waters) of pharmaceuticals as a new class of pollutants [1,2]. Different sources can be indicated to explain the appearance of these xenobiotics in waters and soils [3–5]. It is nowadays completely accepted that the main source is represented by Sewage Treatment Plants (STP) effluents [6,7]. In fact, when drugs are used by humans, after the intake, they are excreted as unmodified molecules or metabolites with urine and faeces. Many pharmaceuticals are only partially removed during biological processes in Sewage Treatment Plants with their consequent release into surface waters [8,9]. Additional inflow into the environment is related with the use of drugs in animal breeding (including aquaculture) and, to a minor extent, with an improper disposal of expired medicines. The concentrations at which these compounds are generally found in waters are quite low and ranges from nanograms to micrograms per liter [10,11]. However, although no direct evidences of any effect on living organisms due to this presence have been so far collected, it is not possible to rule out them a priori. For example in the case of antibiotics, the possibility of inducing resistance in bacterial strains [12–14], which could pass to humans via environmental exposure, is still under debate [15].

In the absence of clear answers about the effects, the precautionary principle would impose to completely prevent pharmaceuticals to enter the environment, a goal hardly achievable from a practical and economical point of view. An acceptable solution could thus be represented by a reduction of the amounts daily discharged into the environment. The adoption of advanced oxidation processes (AOP) in the tertiary treatment section of existing STPs can significantly contribute to this reduction [16–18].

Among the different processes, ozonation can be proposed as a suitable tool for pharmaceutical abatement from STP effluents. Previous investigations by some of the Authors [19,20] and by others [21,22] have already demonstrated that ozone is capable of attacking pharmaceutical species belonging to different therapeutic classes.

In the present work, the attention is focused on amoxicillin, a broad-spectrum antibiotic, widely used in human and veterinary medicine. Its presence in STP effluents has been often documented and recently its concentration up to 120 $ng\,l^{-1}$ has been reported in these effluents during a monitoring campaign in Italy

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**Nomenclature**

| | |
|---|---|
| $f_{AM}$ | UV fraction absorbed by amoxicillin at 254 nm |
| $k_L^o a$ | gas–liquid phase volumetric of mass transfer coefficient without chemical reaction ($0.248\ s^{-1}$) |
| $k_{O_3,\,AM}$ | rate constant of amoxicillin ozonation ($M^{-1}\ s^{-1}$) |
| $k_{O_3,\,PAR}$ | rate constant of paracetamol ozonation ($M^{-1}\ s^{-1}$) |
| $k_{OH,\,AM}$ | rate constant of $HO^\bullet$ attack to amoxicillin ($M^{-1}\ s^{-1}$) |
| $k_{OH,\,BA}$ | rate constant of $HO^\bullet$ attack to benzoic acid ($M^{-1}\ s^{-1}$) |
| $I_o$ | lamp UV-light intensity at 254 nm ($3.03 \times 10^{-6}\ E\ s^{-1}$) |
| $I$ | ionic strength (0.1 M) |
| $l$ | optical pathlength of the reactor (0.201 dm) |
| $V_{sol}$ | volume of the solution for photolytic experiments (0.42 l) |
| $z$ | ratio between the moles of ozone consumed per moles of amoxicillin degradated (dimensionless) |

*Greek symbols*

| | |
|---|---|
| $\varepsilon_{AM}$ | molar extinction coefficient of amoxicillin at 254 nm at pH 5.5 ($1050\ M^{-1}\ cm^{-1}$) |
| $\varepsilon_{BA}$ | molar extinction coefficient of benzoic acid at 254 nm at pH 5.5 ($855\ M^{-1}\ cm^{-1}$) |
| $\varepsilon_{H_2O_2}$ | molar extinction coefficient of hydrogen peroxide at 254 nm ($18.6\ M^{-1}\ cm^{-1}$) |
| $\sigma$ | standard deviation (%) |
| $\phi_{AM}$ | quantum yield of direct photolysis of amoxicillin at 254 nm |

[23]. In previous investigations, some researchers [24] studied the possibility of the abatement of amoxicillin by means of different AOPs but no kinetic constants or oxidation intermediates and products were reported.

The present study aims assessing the reaction kinetics of amoxicillin ozonation and first-stage ozonation intermediates, which could be found in treated waters in the case of an incomplete treatment. This last information is of particular interest when the effluents are submitted to ozonation processes not specifically devoted to the removal of organic pollutants as, for example in water-reuse treatments, the main aim of which is generally to reduce the concentrations of pathogens [25]. With low ozone dosage, pharmaceuticals (and more generally the xenobiotics present) could be converted into compounds even more toxic than the parent species [26].

## 2. Materials and methods

Ozonation runs were performed in a semicontinuous stirred gas–liquid reactor thermostated at 25 °C. An ozonised oxygen stream of 2% by volume, generated by an ozone-generator (Fischer 502) was fed at a flow rate of $36\ l\ h^{-1}$ to the reactor containing the aqueous solution of amoxicillin.

A UV-spectrophotometer equipped with a quartz cell (optical length = $2.0 \times 10^{-2}$ dm) was used to monitor, continuously, the ozone concentration in the outlet gaseous stream ($O_3$ freeboard).

Amoxicillin solutions were buffered, before the ozonation, at desidered pH by adding $H_3PO_4$, $KH_2PO_4$ and $Na_2HPO_4$ salts. The ionic strength was adjusted at a constant value of 0.1 M with addition of NaCl salt. A proper volume (4 ml) of 2-methyl-2-propanol was added to the solution (0.8 l) to prevent any radical mechanism of oxidation. Samples were taken at fixed reaction times and analyzed.

For pH higher than 5.0, the ozonation experiments were carried out in batch mode. Different volumes of a mixture of amoxicillin ($5.0 \times 10^{-4}$ M) and paracetamol ($5.0 \times 10^{-4}$ M) were added to aqueous solutions buffered at desidered pH and containing 2-methyl-2-propanol, previously saturated with ozone ($[O_3]_o = 1.6 \times 10^{-4}$ M). The reaction was quenched by sparging the aqueous solution with nitrogen stream and analyzed by the HPLC.

The substrate and 2-amino-2-(*p*-hydroxyphenyl)acetic acid were analyzed by Hewlett Packard HPLC (HP 1100 L) equipped with a diode array detector and a Phenomenex S5C6 column using a 95:5 buffered aqueous solution:acetonitrile as mobile phase flowing at $1.0\ ml\ min^{-1}$. The buffered aqueous solution was 4 ml $H_3PO_4$ (85% by weight), 50 ml methanol in 1 l HPLC water.

The UV/$H_2O_2$ experiments were carried out in an annular glass reactor equipped with a low-pressure lamp with a monochromatic wavelength emission at 254 nm thermostated at 25 °C. The radiation power (see the legend) was measured by means of $H_2O_2$ actinometric measurements [27]. The total organic carbon was monitored by means TOC analyzer (Shimadzu 5000 A).

The pH of the aqueous solutions was determined using an Orion 960 pH-meter with a glass pH electrode. Amoxicillin and paracetamol were purchased from ICN Biochemicals Inc.

LC–MS analysis of amoxicillin and its degradation products was performed on LCQ-Duo (ThermoFinnigan Inc., USA) equipped with electrospray. MS/MS data were acquired in ESI+ mode (capillary temperature 230 °C; sheath and auxiliary nitrogen gas flows set to 60 and 20; source voltage 4.50 kV, source current 80.00 mA, capillary voltage 29 V) either as direct infusion into the mass spectrometer or recording peaks after the chromatographic separation. The collision energy required to produce the desired quantity of daughter ions was individually optimized. Injection, chromatographic runs and UV spectra recording during chromatographic runs (PDA detector) were performed on Surveyor (ThermoFinnigan Inc., USA). Chromatographic

separation was performed in reverse-phase mode on C18 Hypersil GOLD (5 μm, 150 × 2.2 mm) from Thermo Electron Corporation, USA) using solvents A (water with 0.1% formic acid) and B (methanol). Chromatographic conditions were as follows: isocratic run in 5 min with 95% A and 5%B, then gradient run in 20 min increasing B to 10%.

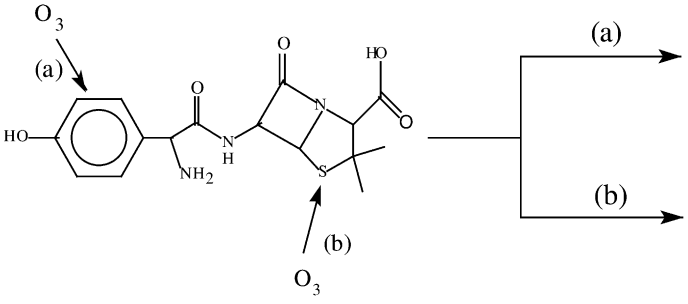
### 2.1. Hydrolytic work-up

An ozonated sample (6 ml), after addition of 4 ml of chloridric acid solution (37% by weight) has been heated by means of an oil bath at 95 °C for 7 h in a cap-screw vial. After cooling, the sample was neutralized with NaOH solution and analyzed by HPLC for determining 2-amino-2-(*p*-hydroxyphenyl)acetic acid.

## 3. Results and discussion

### 3.1. Chemical investigations

A simple analysis of the structure of amoxicillin (AM) indicates that more than one reaction center is present for the ozone electrophilic attack (Fig. 1). In fact, phenolic ring, $-NH_2$ group and sulfur atom may all in principle undergo a reaction with ozone. However, for pH lower than 5.5, one can consider that amine nitrogen is in the protonated form ($pKa_2 = 7.49$), unreactive towards ozone itself. Therefore, it can be expected that the ozone attack, in this pH range, is mainly directed towards the phenolic ring and sulfur atom:



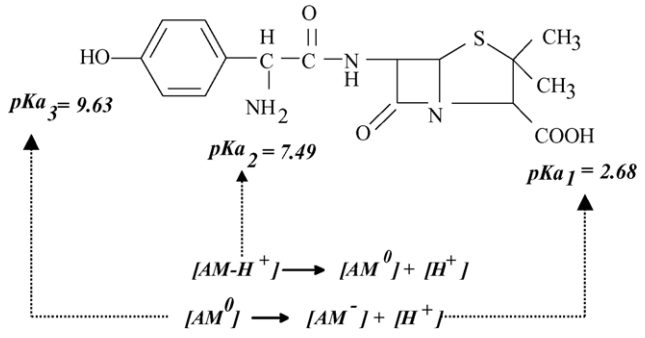




Fig. 1. Amoxicillin (AM) molecular structure and its ionizable groups [43].

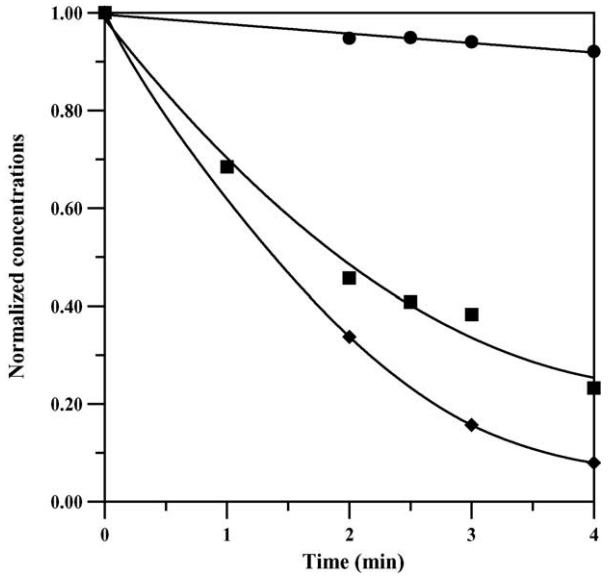
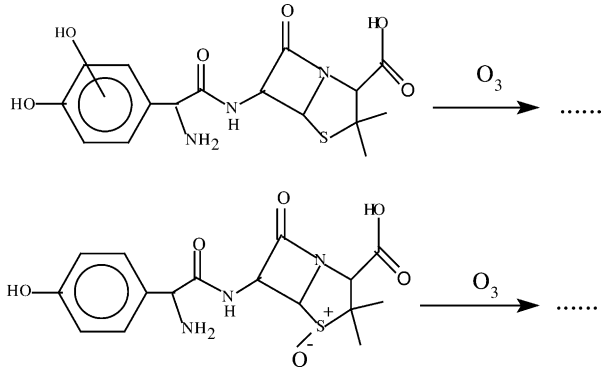



Fig. 2. Ozonation of amoxicillin (♦), TOC measured (●) and 2-amino-2-(*p*-hydroxyphenyl) acetic acid (■) at pH 5.5 without scavenger.

As reported for the penicillin molecules [28], ozone attack on sulfur atom of amoxicillin could result into the formation of two sulfoxide derivatives (*S* and *R* isomers).

Kinetic information available in the literature is dealing only with on the ozone attack to the phenolic rings [29–32] with no indication concerning the sulfur atom. The selectivity for the two pathways (a and b) cannot be thus predicted.




In Fig. 2, the concentration decay of amoxicillin and TOC measurements recorded during an ozonation experiment on an unbuffered solution with an initial pH of 5.5 are reported against the time. For adopted experimental conditions, more than 90% of the substrate is converted after 4 min of ozonation. It is also evident from the diagrams in the figure that only a slight TOC decrease is observed during the first ozonation stages (for longer ozonation times, i.e, 20 min, a TOC removal of 18.2% was recorded).

To throw light on the reaction mechanism, the samples collected during the ozonation experiment have been submitted to a hydrolytic work-up. From the amoxicillin structure, it can

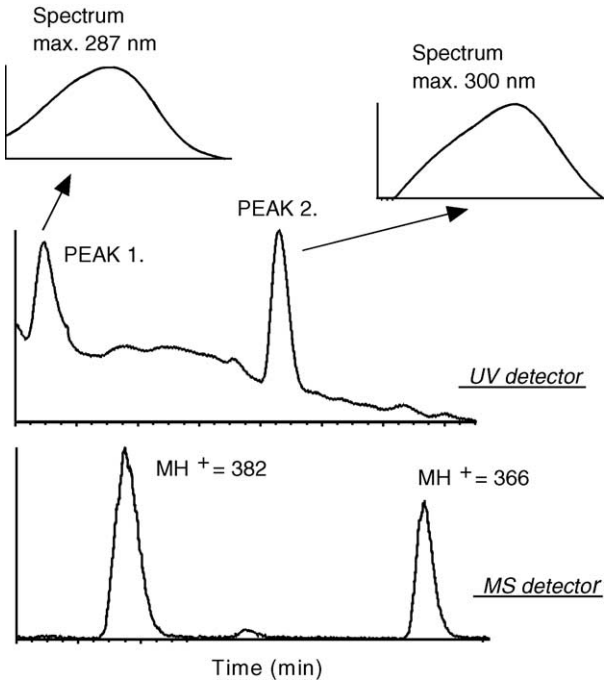
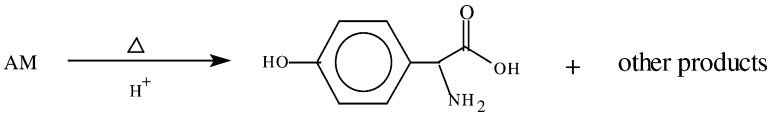



Fig. 3. Chromatogram of the aqueous solution of amoxicillin ozonized for 3 min at pH 5.0. Detection performed by UV and MS, details are given in Section 2.

be expected that heating its aqueous solution in the presence of a mineral acid (HCl) will result in a complete cleavage of the molecule [33].

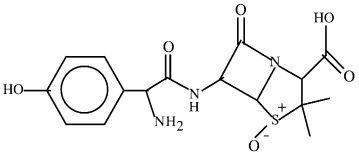
2-Amino-2-(*p*-hydroxyphenyl)acetic acid is one of the products which originate from amoxicillin hydrolysis:



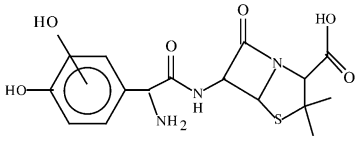

HPLC analysis indicated that for adopted hydrolytic conditions an almost complete conversion of the substrate was achieved on a sample of amoxicillin solution not ozonized (blank), with a yield of 2-amino-2-(*p*-hydroxyphenyl)acetic acid equal to 80.5%. It is evident that during the hydrolytic treatment of an aqueous solution of amoxicillin, previously ozonized, only the molecules which are attacked by ozone in a reaction center different from the phenolic ring give rise to the formation of 2-amino-2-(*p*-hydroxyphenyl)acetic acid.

The results obtained from the hydrolysis of ozonized samples are reported in Fig. 2 (full squares). It is clear from these data that the ozonation of amoxicillin leads in part to the formation of intermediate species, which contain in their structure unmodified phenolic ring. That is, the ozone attack is not completely directed on the phenolic ring but may partially result into the oxidation of sulfur atom.

In an attempt to investigate this possibility, ozonated samples have been analyzed by using a combination of UV and LC/MS/MS techniques. In Fig. 3, the chromatogram obtained on a sample submitted to ozonation for 3 min is shown. In UV (shown detection range 280–320 nm) amoxicillin (due to its relatively low concentration) does not give rise to any distinct peak. Two additional peaks found in UV exhibit different spectra, the first one with $\lambda_{max}$ of 287 nm and the second one with $\lambda_{max}$ of 300 nm. Since no corresponding peaks are observed in MS under the adopted conditions, it means that the compounds are inactive in MS and do not contain free amino group in their structures. This information is not sufficient to identify the compounds responsible for these peaks; however, it is to note that UV absorption with $\lambda_{max}$ of 300 nm is typical of species with nitro or nitroso moiety in their structure. One of the two peaks observed in MS detector belongs to the unreacted protonated amoxicillin (MH+ 366) and the other one (MH+ 382) to its oxidized intermediate. The recorded compound with the molecular weight of 381 u.m.a. is an oxidation intermediate of amoxicillin formed by addition of one oxygen atom. At least three structures deriving from amoxicillin oxidation are consistent with this:




II




IIIa (ortho)

IIIb (meta)

A comparison of the UV spectra (Fig. 4) of the unknown (381 u.m.a.) and amoxicillin indicates that the unknown has UV absorption significantly different from that of amoxicillin with a clear shift to longer wavelengths. This result seems to be consistent with a structure of the unknown compound as III (a or b), since it is well known that the introduction in a phenolic ring of a second hydroxyl group causes a batochromic shift. Additionally, this suggestion is supported by the fragmentation pattern exhibited by its daughter ions (MS/MS). The presence of *m*/*z* 160 produced by the cleavage of the β-lactam (Fig. 5) is typical for protonated amoxicillin [34]. The apperance of several observed ions may be interpreted by the loss of neutral fragments or by the cleavage and loss of aliphatic fragments in side-chains as follows: 365 due to a loss of neutral $NH_3$ typical for penicillins [34]; 350 (loss of neutral $NH_3$ and/or OH– and in addition $–CH_3$); 336 (loss of $H_2O$ and CO); 306 (loss of $H_2O + CO_2 + CH_2–$ or $NH_3 + CO_2 + –CH_3$) and 293 (loss of $CO_2 + NH_3 + 2x\ CH_2–$). Both the results from the UV and

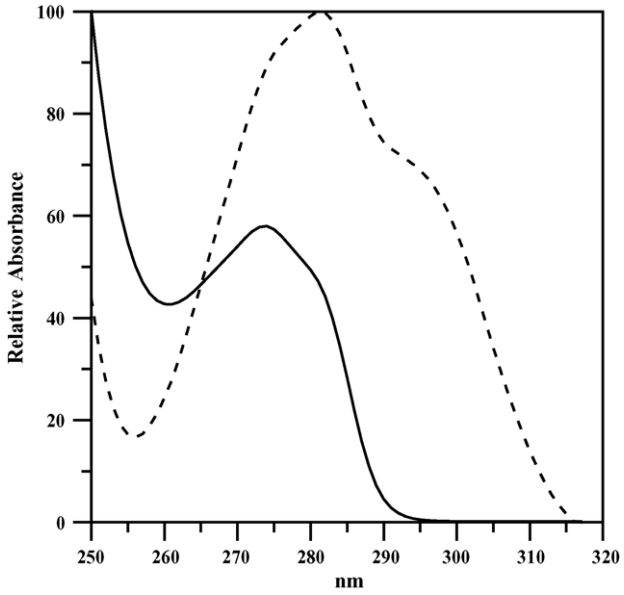



Fig. 4. UV spectrum of amoxicillin (continuous line) and the 381 u.m.a., unknown (dashed line).

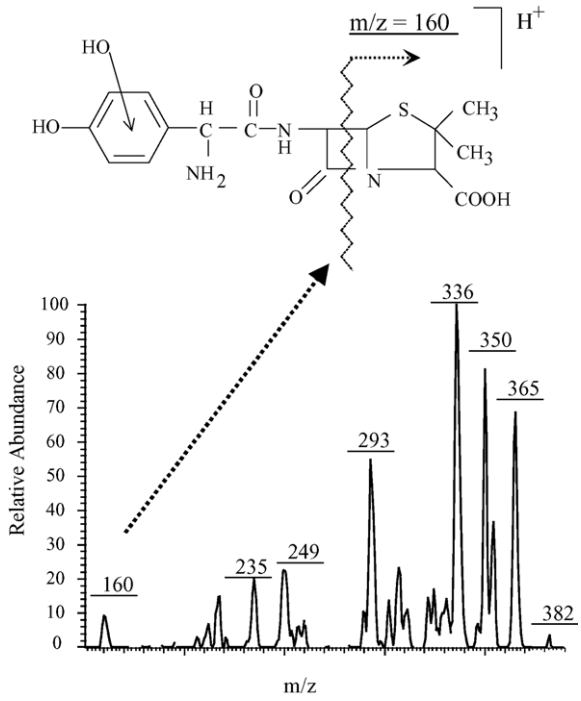



Fig. 5. MS/MS spectrum of the unknown (peak MH+ 382, see Fig. 3). The interpretation of the major daughter ions is given in the text.

especially MS experiments do not support the presence of noticeable quantity of amoxicillin sulfoxide (structure II, above). However, it is not possible to totally rule out its formation in the reacting mixture. However, taking into account other factors, like possible very short lifetime of amoxicillin sulfoxide, its formation in the reacting mixture and the pathway of ozone attacking the sulfur atom cannot be ruled out.

### 3.2. Kinetic investigations

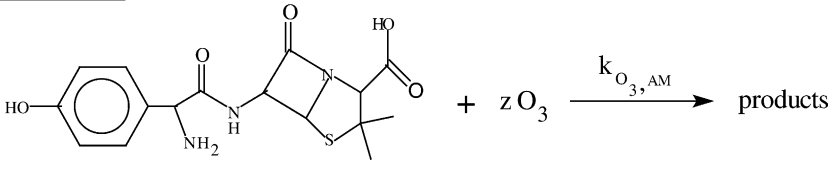
According to accepted mechanisms (direct and radical) for the ozone attack to organic molecules [35,36], the following equation can be written for the rate of oxidation of an organic substrate S [37]:

$$\frac{d[S]}{dt} = -(k_{O_3,S}[O_3][S] + k_{OH,S}[OH][S]) \quad (1)$$

in which $[O_3]$ and [OH] are, respectively, the steady-state OH radical concentration and that of ozone both in the aqueous solution. The use of Eq. (1) to model the ozonation process of a species S requires the adoption of proper kinetic and fluidodynamic models. Some examples of them can be easily found in the literature [38,39]. The use of Eq. (1) requires also the knowledge of the values of kinetic constants, $k_{O_3,S}$ and $k_{OH,S}$, which generally are not known a priori. The remaining part of the present work is dedicated to the evaluation of these constants.

Aiming at simplifying the study, firstly ozonation runs were carried out in the presence of a radical scavenger, *tert*-butyl alcohol (4 ml), to prevent the activation of the radical mechanism of oxidation [29]. Two different experimental approaches were adopted. In fact, in a first attempt to determine the constant, according to a procedure some of the Authors published elsewhere [19,20], the ozonation runs were performed in a semibatch reactor at stirrer speed of 380 rpm and ionic strength equal to 0.1 M by recording the substrate decay and ozone concentration in the freeboard of the reaction against time. Starting concentrations of amoxicillin in the liquid phase and ozone in the gas phase were $1.0 \times 10^{-3}$ and $8.0 \times 10^{-4}$ M, respectively.

The data were analyzed by using the proper fluidodynamic model for the most suitable regime of absorption with reaction [40] and an overall reaction:




With the stoichiometric coefficient $z$ being a constant ($z = a$), or a linear function of the time ($z = a + bt$). The parameters $a$ and $b$ were estimated (Table 1) along with $k_{O_3,AM}$ (Fig. 6, full circles) by means of an optimization procedure [41] aiming at the minimization of the residuals

Table 1
Stoichiometric coefficients at different pH-values

| pH | *a* | *b* |
|---|---|---|
| From semicontinuous runs | | |
| 2.5 | 1.06 ± 0.01 | – |
| 3.0 | 1.22 ± 0.02 | – |
| 3.5 | 1.01 ± 0.05 | 0.65 ± 0.09 |
| 4.0 | 0.91 ± 0.02 | 0.96 ± 0.04 |
| 4.5 | 1.34 ± 0.04 | 0.59 ± 0.05 |
| 5.0 | 1.81 ± 0.05 | 0.43 ± 0.07 |

| pH | *z* |
|---|---|
| From batch runs | |
| 6.0 | 1.89 |
| 7.2 | 2.57 |

between experimental concentrations and those calculated by the model.

As it is expected for molecules, which show an acid–basic character, the reactivity of the system increases at increasing the pH. However, as a result of this behaviour, for pH higher than 5.0 some difficulties were encountered with this procedure being the duration of the runs too short (few experimental concentration points) and the ozone concentration in the freeboard too low.

A different approach was thus chosen by carrying out the runs in batch mode in the presence of a reference species whose kinetics were previously assessed. The reference species used in this work was paracetamol (PAR) [20].

Assuming that the reaction is the first order with respect to both the substrate and ozone, the rate of consumption for amoxicillin and the reference compound can be written as:

$$\frac{\mathrm{d[AM]}}{\mathrm{d}t} = -k_{\mathrm{O_3,\,AM}}[\mathrm{O_3}][\mathrm{AM}] \tag{2}$$

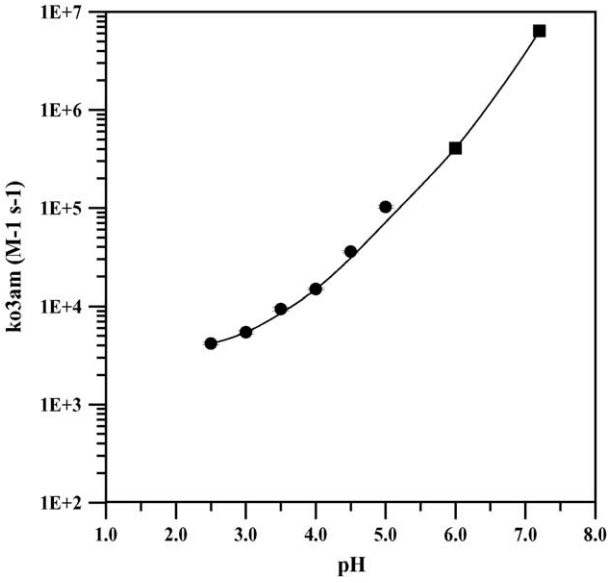



Fig. 6. Kinetic constant values for ozonation of amoxicillin at different pH (●) from the results of semibatch experiments and (■) batch experiments.

$$\frac{\mathrm{d[PAR]}}{\mathrm{d}t} = -k_{\mathrm{O_3,\,PAR}}[\mathrm{O_3}][\mathrm{PAR}] \tag{3}$$

If one divides Eq. (2) by Eq. (3) and integrating between $t = 0$ and $t_R$ the following relationship is obtained:

$$\ln\frac{[\mathrm{AM}]_0}{[\mathrm{AM}]_{t_R}} = \frac{k_{\mathrm{O_3,\,AM}}}{k_{\mathrm{O_3,\,PAR}}}\ln\frac{[\mathrm{PAR}]_0}{[PAR]_{t_R}} \tag{4}$$

A plot of $\ln([\mathrm{AM}]_0/[\mathrm{AM}]_{t_R})$ against $\ln([\mathrm{PAR}]_0/[\mathrm{PAR}]_{t_R})$ yields a straight line whose slope is the ratio of kinetic constants. Since $k_{\mathrm{O_3,\,PAR}}$ can be derived from the literature at pH 6.0 and 7.2 [20], $k_{\mathrm{O_3,\,AM}}$ can be easily calculated (Fig. 6, full squares). In Table 1 are reported the values for the stoichiometric coefficient *z* calculated with this procedure.

The kinetic constant for HO radical attack to amoxicillin (AM) to be used in the Eq. (1) was estimated by means of $H_2O_2$/UV competition experiments, in which the investigated compound was oxidized in the presence of a second chemical species. In the present case benzoic acid (BA) was used as competing species. Preliminary experiments on aqueous solutions containing amoxicillin to which $H_2O_2$ was added did not show any oxidation of the studied substrate.

When an aqueous solution of amoxicillin is irradiated at a wavelength of 254 nm in the presence of both hydrogen peroxide and benzoic acid, the following equation can be used to account for the substrate degradation:

$$\begin{aligned}\frac{\mathrm{d[AM]}}{\mathrm{d}t} = {} & -\phi_{\mathrm{AM}}\frac{I_o}{V_{\mathrm{sol}}}f_{\mathrm{AM}}[1-\exp(-2.3l(\varepsilon_{\mathrm{AM}}[\mathrm{AM}] \\ & +\varepsilon_{\mathrm{BA}}[\mathrm{BA}]+\varepsilon_{\mathrm{H_2O_2}}[\mathrm{H_2O_2}]))] \\ & -k_{\mathrm{OH,\,AM}}[\mathrm{HO}]_{\mathrm{SS}}[\mathrm{AM}]\end{aligned} \tag{5}$$

where $[\mathrm{HO}]_{\mathrm{SS}}$ represents the steady-state HO radical concentration.

The first term in the right hand of the equation (Eq. (5)) accounts for the direct photolytic degradation rate.

The estimation of the quantum yield of the direct photolysis for amoxicillin at 254 nm ($\phi_{\mathrm{AM}}$) was achieved by comparing the concentration data obtained from photolytic runs (pH 5.5) in absence of hydrogen peroxide and those calculated through a numerical integration of Eq. (6):

$$\frac{\mathrm{d[AM]}}{\mathrm{d}t} = -\phi_{\mathrm{AM}}\frac{I_o}{V_{\mathrm{sol}}}[1-\exp(-2.3l\varepsilon_{\mathrm{AM}}[\mathrm{AM}])] \tag{6}$$

A value of $5.71\times10^{-1}$ mol $\mathrm{E}^{-1}$ was obtained for $\phi_{\mathrm{AM}}$. In the presence of hydrogen peroxide and benzoic acid, only a part of the power emitted by the lamp is absorbed by the substrate. Therefore, the term $I_o[1-\exp(\varepsilon_{\mathrm{AM}}[\mathrm{AM}]+\varepsilon_{\mathrm{BA}}[\mathrm{BA}]+\varepsilon_{\mathrm{H_2O_2}}[\mathrm{H_2O_2}])]$, which gives the power absorbed by the solution as a whole, is multiplied by the term:

$$f_{\mathrm{AM}} = \frac{\varepsilon_{\mathrm{AM}}[\mathrm{AM}]}{\varepsilon_{\mathrm{AM}}[\mathrm{AM}]+\varepsilon_{\mathrm{BA}}[\mathrm{BA}]+\varepsilon_{\mathrm{H_2O_2}}[\mathrm{H_2O_2}]} \tag{7}$$

accounting for the fraction of total irradiated power absorbed by the substrate.

The contribution of HO radical attack to the overall degradation can be thus derived from Eq. (8):

$$k_{OH,\,AM}[HO^\bullet]_{SS}[AM] = -\frac{d[AM]}{dt} - \phi_{AM}\frac{I_o}{V_{sol}} f_{AM}[1 - \exp(-2.3l(\varepsilon_{AM}[AM] + \varepsilon_{BA}[BA] + \varepsilon_{H_2O_2}[H_2O_2]))] \quad (8)$$

If the initial rate of degradation of amoxicillin (AM) and benzoic acid (BA) are measured in the competition experiments:

$$k_{OH,\,AM}[HO^\bullet]_{SS}[AM]_0 = -\left(\frac{d[AM]}{dt}\right)_0 - \phi_{AM}\frac{I_o}{V_{sol}} f_{AM}[1 - \exp(-2.3l(\varepsilon_{AM}[AM]_0 + \varepsilon_{BA}[BA]_0 + \varepsilon_{H_2O_2}[H_2O_2]_0))] \quad (9)$$

$$k_{OH,\,BA}[HO^\bullet]_{SS}[BA]_0 = -\left(\frac{d[BA]}{dt}\right)_0 \quad (10)$$

from which:

$$\frac{k_{OH,\,AM}[AM]_0}{k_{OH,\,BA}[BA]_0} = \frac{-(d[AM]/dt)_0 - \phi_{AM}(I_o/V_{sol}) f_{AM}[1 - \exp(-2.3l(\varepsilon_{AM}[AM]_0 + \varepsilon_{BA}[BA]_0 + \varepsilon_{H_2O_2}[H_2O_2]_0))]}{-(d[BA]/dt)_0} \quad (11)$$

By considering that the kinetic constant for HO radical attack to benzoic acid is $4.3 \times 10^9\ M^{-1}\ s^{-1}$ for pH < 3.0 [42] and $5.9 \times 10^9\ M^{-1}\ s^{-1}$ for pH > 6.0 [42], a value of $5.8 \times 10^9\ M^{-1}\ s^{-1}$ for $k_{OH,\,BA}$ was assumed at pH 5.5.

The following values were, respectively, measured for $(d[AM]/dt)_0 = 8.0 \times 10^{-8}\ M\ s^{-1}$ and $(d[BA]/dt)_0 = 1.2 \times 10^{-7}\ M\ s^{-1}$ in a competitive experiment with $[H_2O_2]_0 = 3.0 \times 10^{-2}$ M, $[AM]_0 = 3.0 \times 10^{-5}$ M and $[BA]_0 = 4.0 \times 10^{-5}$ M. From these data, a value of $3.93 \times 10^9\ M^{-1}\ s^{-1}$ for $k_{OH,\,AM}$ is thus calculated.

## 4. Conclusions

The ozonation of amoxicillin has been studied from chemical and kinetic point of view. Chemical investigations showed that the ozonation process is characterized by low degree of mineralisation even for long treatment times. The results indicate that ozone attack is partially directed towards a reaction center different from the phenol ring or the protonated amino group, possibly towards the sulfur atom. The presence of noticeable amounts of stable amoxicillin sulfoxide in the reacting solution was, however, not found. The observed low degree of mineralisation and some indications recorded on the structures of intermediates and products of amoxicillin ozonation strongly suggest the need of further investigations in order to assess their ecotoxicological behaviour. Kinetic investigations allowed to characterize both the direct and radical mechanism of ozone attack to the molecule. It has been found that the kinetic constant for the direct attack is strongly dependent upon the pH of the solutions. Hydrogen peroxide photolysis has been successfully adopted to evaluate the constant for OH radical attack to amoxicillin molecule at pH 5.5 ($k_{OH,\,AM} = 3.93 \times 10^9\ M^{-1}\ s^{-1}$).

It is noteworthy to observe that the value found for $k_{OH,\,AM}$ can be also used for kinetic evaluations in the application of $H_2O_2$/UV system to amoxicillin removal from water.

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