OXIDATION KINETICS OF ETHINYLESTRADIOL BY OZONE

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Abstract: The study of ozonation was carried out initially on concentrated solutions of ethinylestradiol (EE2 $\approx$ 30μmol.L^{-1} or 10 mg.L^{-1}) to evaluate the effectiveness of this treatment in terms of conversion rate of EE2 and its mineralization. Moreover, this research studied the reactivity of ozone at low ethinylestradiol concentrations close to those found in wastewater (some ng.L^{-1}). Different parameters were studied mainly the influence of pH, the ozone dose, and the initial concentration of EE2 on the ozonation degradation mechanism and on mineralization. The degradation and mineralization of the molecule are strongly pH dependent. Ozone degrades mainly ethinylestradiol through molecular mechanism against the mineralization of the molecule and its byproducts through a radical mechanism. Mineralization of ethinylestradiol is divided into two phases. In the first phase, TOC disappears rapidly due to the oxidation of phenol and ethinyl functions of the molecule. In the second phase, disappearance of TOC becomes less rapid. It reaches 84% after 3 hours of photodegradation suggesting the opening of different carbocycles. The dose of ozone necessary to degrade the phenol ring of ethinylestradiol, which is responsible for the estrogenic activity of the molecule, corresponds to 1200 µmol.L^{-1} for 37 µmol.L^{-1} of ethinylestradiol, or a weight proportion of 5 g ozone per gram of EE2 at neutral pH. The initial concentration of EE2 has a role on the stoichiometry of the reaction between ozone and ethinylestradiol: when the concentration of EE2 decreases, the overall consumption of ozone per mole of EE2 increases. This is due to the self-decomposition reaction of ozone which becomes competitive.
Keywords: Ozone, Endocrine Disruptors, Pesticides Removal.

1.   Introduction

Recent studies indicate the presence of very low level (in the order of ng.L^{-1} - µg.L^{-1}) of

pharmaceuticals, hormones, and other organic compounds as well as their metabolites in the

aquatic environment [1-2-3-4-5]. These chemicals include a wide range of molecules such as

pesticides, phthalates, alkylphenols, antibiotics, estrogens, and natural hormones. Some of

these compounds have been widely used in household, pharmaceuticals, and industrial

processes (i.e. textile, paper, metal working fluids, detergents, and polymeric material production). The effect of these micropollutants on the environment is extremely negative on fauna and flora [6]. Moreover, some of these substances are biotransformable in the environment, leading to metabolites or by-products that are more dangerous than the original compounds. Nowadays, many governmental and non-governmental organizations are considering this problem and setting up directives and legal frameworks to protect and improve the quality of fresh water resources. Among these compounds, the endocrine disrupting chemicals (EDCs) are considered as emerging environmental contaminants, that, in very small concentrations, may cause disruption of endocrine systems and affect the hormonal control of development in aquatic organisms in wildlife [7-8]. Steroid hormones are compounds that contribute the most to the estrogenic activity of aquatic systems. As shown in **Figure 1,** steroids have different structures: oestradiol (A), oestrone (B), oestriol (C), and 17 α-Ethinylestradiol (D). All steroids are naturally excreted and join the natural environment via sewage treatment plants.
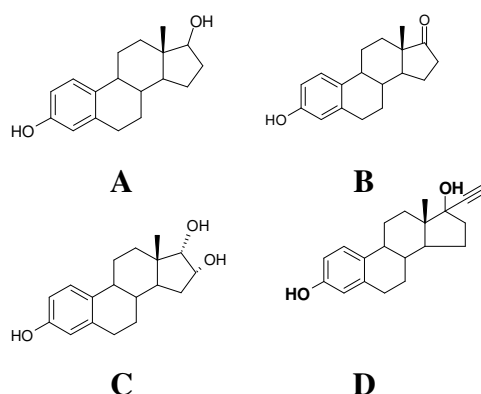

A   **B**

C   **D**

Figure 1: Major endocrine disruptors' structures.

There is a relevant number of studies on the influence of anthropogenic compounds on the environment especially these steroid hormones since they have estrogenic effects at concentrations of the order of ng.L^{-1}[9-10]. In fact, steroid hormones have estrogenic effects several thousand times higher than the synthetic chemicals as 4-nonylphenols and phthalates. The synthetic female hormone 17 α-ethinylestradiol, EE2, is found in the contraceptive pill [11]. EE2 is excreted naturally and it joins the natural environment. The classical treatment of water does not remove this molecule. This leads to contamination of the aquatic environment and therefore a modification of the estrogenic activity of fish. In this study EE2 is selected because it is a prominent endocrine disrupting pharmaceutical and because of its low

observed effect concentration (LOEC) of 0.1 ng.L^{-1}. In addition, this compound is one of the least polar estrogen (log K_{ow} : 4.2), which makes it one of the most easily bioaccumulative molecule by biological organisms. EE2 might be the most important endocrine disruptor in water treatment plant effluents together with the octyl and nonylphenols [12]. The main objective of the current paper is to develop an effective oxidation process using ozone for a total elimination of endocrine disruptors. This study contains ozonation of EE2, mechanism of ozonation, mineralization of EE2 and its metabolites, and develops a method for quantifying the concentration of O$_3$ in surface and treated waters.

2   Experimental Section

2.1   Materials and reagents

17 α-ethinylestradiol (EE2) is purchased from Sigma Aldrich. Water used in the experiments is treated by Milli-Q water purification system. Acetonitrile is of HPLC grade. The solid phase extraction (SPE) cartridges OASIS with 200 mg LiChrolut-EN adsorber are purchased from Merck (Darmstadt, Germany).

2.2   Ozonation procedure

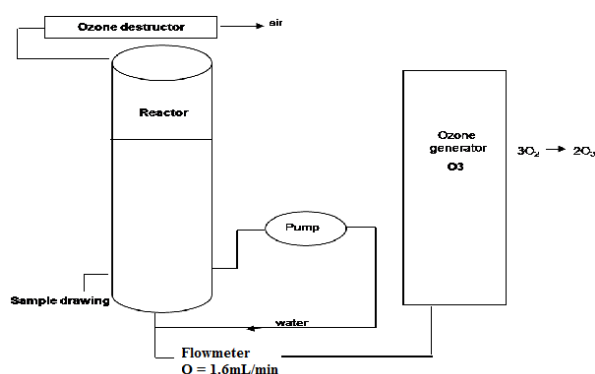Experimental set up for ozonation is shown in **Figure 2**.

Figure 2: Schematic of the ozonation pilot plant.

The pilot plant consists of a cylindrical glass reactor (450 mm high water column, 5000 mL maximum capacity), an ozone generator 5LO Trailigaz model, the flow of liquid is made with a magnetic drive pump, a valve adjustment to adjust the speed from 0 to 60 L.h^{-1} and an ozone destructor. Ozone is produced from pure oxygen and fed into the reactor through a porous glass diffuser located at the bottom of the reactor to produce bubbles. Production of ozone is controlled by changing the power input of ozone generator. The gas flow rate is adjustable by a valve. In all ozonation experiments, the power of the ozone generator is set at 50W with gas flow rate of 1.6 L.h^{-1}.

2.3   Sample preparation

2.3.1   For the high concentration of EE2

An ozonated water solution is prepared by bubbling ozonized air in a phosphate buffer solution (ionic strength $I = 10^{-2}$ mol/L, pH = 2 to 12). This solution was used to adjust a variety of vials containing a constant volume of ethinylestradiol solution with different concentrations. For an initial concentration of EE2 of 20 µmol/L, the concentration of ozone varies from 0 to 30 µmol/L. The remainder of ethinylestradiol was measured by HPLC after 2 hours of reaction and the concentration of ozone added was controlled by colorimetry using indigo carmine.

2.3.2   For the low concentration of EE2

The principle of preparation is similar as above. After 2 hours of oxidation, samples are extracted using solid phase extraction (SPE) cartridges in order to monitor low concentrations of EE2 between 4 ng.L^{-1} and 4 µg.L^{-1}. The aqueous sample (200 mL) from the oxidation experiments passes through the preconditioned SPE cartridge. Conditioning is performed with 10 mL acetonitrile and 10 mL distilled water successively. The analytes are eluted with 5 ml acetonitrile. The solvent is evaporated by a gentle stream of nitrogen and the residue dissolves immediately in 200 µl acetonitrile. Thus the analytes are concentrated to a factor of 1000. The analysis is done by HPLC / fluorescence (λ exc. = 210 nm, λ issue. = 309 nm).

2.3.3   Sample preparation of the TOC

The sample is prepared by spiking EE2 in distilled water in the reactor. The initial concentration of EE2 is about 24 µmol.L^{-1}. Ozone gas contacted the EE2 solution. Samples are withdrawn at different intervals for 2 hours to determine the total organic carbon.

2.4   Analytical methods

Residual EE2 is determined using high-performance liquid chromatography (HPLC, Hewlett-Packard, 1050 series) with a UV detector set at 220 nm for the high concentrations of EE2, and a fluorescence detector (HP 1064A) (λ exc = 210 nm, λ issue = 309 nm) for the low concentrations of EE2. The eluent is acetonitrile: water (70:30, v/v). Concentration of dissolved ozone in water is measured by the indigo method [13]. The absorptivity of the different samples at 600 nm is determined using a SAFAS spectrophotometer UV-visible. pH is measured with a Tacussel LPH330T pH-meter equipped with a Radiometer Analytical combined electrode and previously calibrated with standard buffers (pH 4 and 7). The total organic carbon (TOC) is measured by a direct injection of the filtered samples into a

Shimadzu-5050A TOC analyzer provided with a NDIR detector and calibrated with standard solution of hydrogen potassium phthalate.

3   Results and discussions

The study of ozonation is conducted with a high concentration of ethinylestradiol of 24 $\mu mol.L^{-1}$ prepared in ultra-pure water in order to estimate the efficiency of this treatment in terms of conversion rates and mineralization of the substrate.

3.1 Ozonation and mineralization of 17α-ethinylestradiol in column

Knowing the removal efficiency of the EE2 molecule by ozonation is important but not sufficient to assess the quality of the water. It is important to monitor not only the disappearance of the original molecule but also the impact of generated by-products to ensure there are no negative effects. In this context and in order to determine whether estradiol is completely oxidized, total organic carbon (TOC) and the concentration of EE2 are monitored for different ozonation time. The study of the ozonation is carried out by continuous injection of a flow of ozone gas at a rate of 1 $mmol.min^{-1}$ to a 5 liter concentrated solution of ethinylestradiol ([EE2] = 33 $\mu mol.L^{-1}$) prepared in ultrapure water buffered to pH 6.7. The evolution of the concentration of EE2 and TOC is expressed in terms of C_t/C_0 where C_t is the concentration of EE2 at time t and C_0 is the initial concentration of EE2.
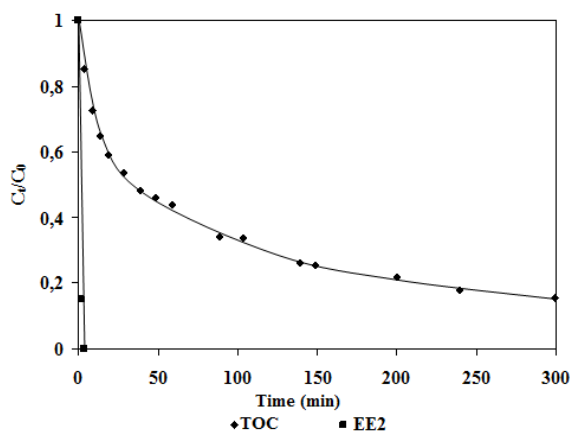

Figure 3: Degradation of TOC and EE2 in function of the ozonation time**.**

As shown in **Figure 3**, the kinetics of disappearance of ethinylestradiol is extremely fast since it is completely degraded after two minutes of ozonation. This rapid phase is due to the high concentration of ozone in the gas stream at the inlet of the column since the ratio is: 1 mmol ozone to 100 µmol EE2. Eventhough ozonator was adjusted to its lowest capacity, it was not possible to follow the kinetics of degradation of EE2 in the ozonation column. On the other hand, TOC degradation results, reported in **Figure 3,** show that after 20 minutes, 40% of the initial organic carbon is converted to CO_2 and it takes more than 3 hours to reach a

mineralization superior to 80%. The difference between the TOC and the EE2 concentrations indicates a generation of intermediate molecules that are not detected by HPLC. These intermediate molecules can be polar such as carboxylic acids whose retention time on a reverse phase is very short. They are non-aromatic molecules and can not be detected by the diode array detector. Referring to **Figure 3**, the mineralization of ethinylestradiol can be divided into two phases:

- The first phase between 0 and 20 minutes when the disappearance of the TOC is rapid and almost linear. During this time of ozonation, ethinylestradiol loses 40% of TOC which is equivalent to 8 carbon atoms. The analysis of the degradation mechanism of this estrogen has not been studied. However, in bibliographical studies, it is likely that the first phase of ozonation is the oxidation of the ethinyl and phenol molecule leading to fragments similar to those revealed in **Figure 4** by Metcalfe *et al.* **[14]**
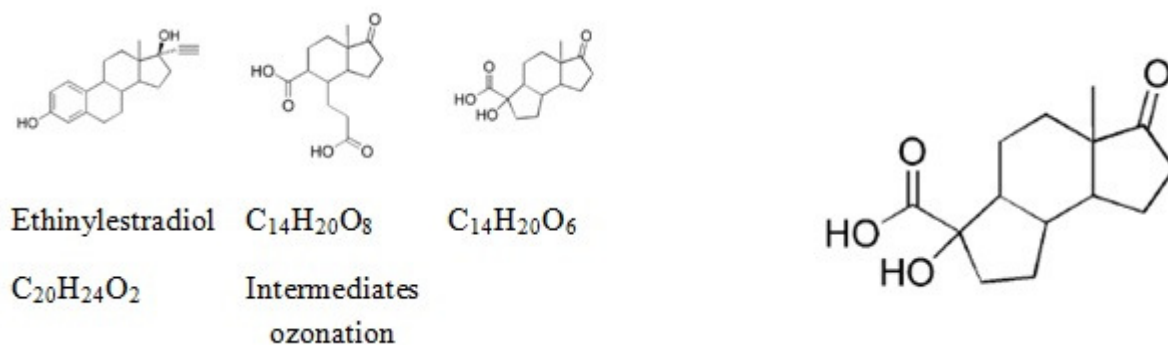
Ethinylestradiol $C_{14}H_{20}O_8$ $C_{14}H_{20}O_6$

$C_{20}H_{24}O_2$ Intermediates
                     ozonation

Figure 4: Intermediate molecules during EE2 ozonation.

-The second phase begins at *t* superior to 20 minutes. The organic carbon continues its decrease. After 3 hours, the molecule loses the equivalent of 16 carbon atoms. At this stage ethinylestradiol has undergone many transformations. These transformations will open up different carbon rings of the molecule with the possible formation of aliphatic carboxylic acids which require larger oxidation time. The expression of the variation of the TOC based on the ozone dose transferred is used to determine the effective amount of ozone needed in the reaction. Thus, **Figure 5** shows that at pH 6.7 it takes about 2000 μmol.L^{-1} ozone to mineralize 50% of ethinylestradiol at 33 μmol.L^{-1}, approximately 100 mg ozone to 3 mg of ethinylestradiol. This result is comparable to that obtained by Canton *et al.* **[15]** during mineralization of 100 mg.L^{-1} of phenol by ozone at pH 8. These authors showed in their experimental conditions, that it took 7200 mg.L^{-1} to mineralize 50% of phenol solution, an amount about four times greater than the amount found in this experiment. These results indicate that the amount of ozone required to mineralize completely organic compound is

very important. However, the goal of ozonation treatment is not in the case of ethinylestradiol to achieve complete mineralization of the molecule but rather to reduce its estrogenic activity. After our literature review, the phenol or aromatic ring is probably the site responsible for the estrogenic activity of this molecule. The dose of ozone to be introduced is 1200 µmol/L (58 mg.L^{-1}) for 11 mg.L^{-1} of ethinylestradiol (5 g of ozone per gram of EE2 at neutral pH).
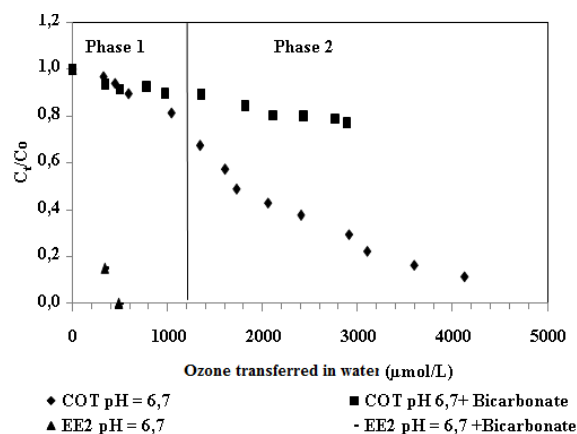

Figure 5: EE2 and TOC degradation versus concentration of ozone transferred in water in the presence and in the absence of bicarbonate (pH = 6.7, [EE2]$_0$ = 33 µmol/L).

To determine the mechanism of ozonation for the removal of ethinylestradiol and TOC, the inhibitory effect of bicarbonate ions was studied using an EE2 solution of 33 µmol.L^{-1} containing 10^{-2} mol.L^{-1} radical scavengers. The results reported in **Figure 5** show that:

- For EE2, degradation is not affected by the presence of radical scavengers. This result reveals that ozone reacts directly on EE2 and probably on the unsaturated molecules functions (the phenolic group or ethinyl radical) to produce oxidized compounds (EE2ox). The introduction of bicarbonate ions in the medium does not affect this reaction either:

$$EE2 + O_3 \rightarrow EE2_{ox}$$
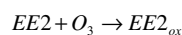
- For the TOC, a sharp slowdown of the mineralization of the molecule is observed when bicarbonate ions are present in the solution. For 3000 µmol.L^{-1} ozone transferred, only 20% of the total carbon of the molecule are converted to CO_2. This result clearly indicates that the mineralization of EE2 occurs through a radical mechanism. The introduction of bicarbonate ions in the medium inhibits the oxidation reaction of the intermediate products (IP) produced by oxidation of EE2 by competing reactions:

$$HCO_3^- + {}^\circ OH \rightarrow HCO_3^\circ + OH^-$$

$$PI + {}^\circ OH \rightarrow PI^{\circ +} + HO^-$$
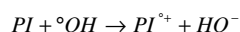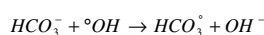
This radical mechanism can explain the good performance of ozonation on the EE2 molecule that has many functions that are not reactive with the molecule of ozone. Ozone has an oxidizer effect in eliminating the initial EE2 molecule rapidly, nevertheless total mineralization requires a very large dose of ozone. Since the first reaction steps are fast, it is possible that the reaction between EE2 and O_3 forms 6 to 7 major intermediates.

3.2 Ozonation of 17α-ethinylestradiol in vial

Ozonation of ethinylestradiol follows a reaction kinetics of the second order with a partial order of 1 with respect to the concentration of EE2 and compared to the ozone concentration Deborde *et al.* [16]

$$v = -\frac{d[EE2]}{dt} = k_{EE2/O_3}[EE2][O_3]$$

With: $k_{EE2/O_3} = 1.83 \times 10^5$ L.mol^{-1}.s^{-1} at neutral pH and 3.6×10^9 L.mol^{-1}.s^{-1} at basic pH.

These kinetic constants lead to very high initial concentrations of EE2 and ozone equimolar 30 µmol/L. Given the extremely important reactivity of ozone with ethinylestradiol (half-reaction time of 0.18 s at neutral pH), it was not possible to follow the kinetics of ozonation reaction of this molecule. Increased doses of ozone are added to ethinylestradiol solution and analyses of the residual organic carbon were made.

3.3 Study of ozonation of ethinylestradiol

The study of the ozonation of ethinylestradiol is performed in a series of 5 to 6 vials containing a constant volume of a buffered solution of EE2. Volumes of flasks were adjusted from a solution of ozone (initial concentration of O_3 varies from 0 to 30 µmol.L^{-1}). After adding ozone, the vials are shaken vigorously and stored in the dark for 120 minutes before analyzing the residual concentration of EE2. **Figure 6** shows the results obtained during ozonation of EE2 solution at 22 µmol.L^{-1} and at pH = 3.
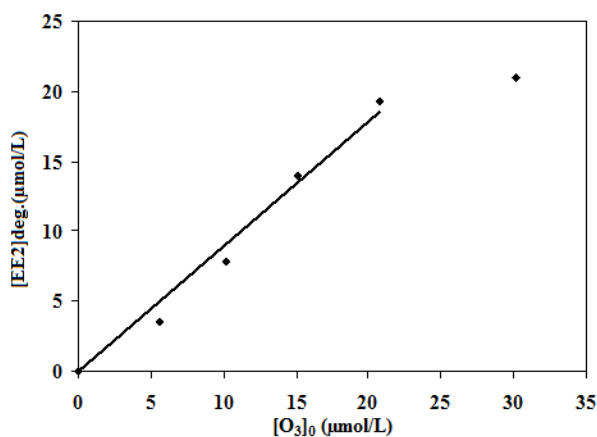

Figure 6: Influence of ozone dose on the degradation of EE2.

In this figure, two phases can be distinguished:

- The first phase characterizes the initial concentrations of ozone located between 2 and 20 μmol.L^{-1}. In these mixing conditions, the concentration of EE2 is in excess relative to the concentration of ozone which is observed after a sufficiently long reaction time. In this phase the concentration of EE2 degraded varies linearly with the initial concentration of ozone introduced. The slope of the line determines to the stoichiometry of the overall reaction of ozonation. For these operating conditions, the observed stoichiometry is close to 1 (value found to be 1.13) indicating that each one mole of ozone reacts with one mole of EE2.

- The second phase dictates the initial concentrations of ozone greater than 20 μmol.L^{-1}. Under these conditions, ethinylestradiol is degraded, ozone is in excess and the curve tends towards an asymptote corresponding to the initial concentration of EE2. This phase is complicated to understand.

Therefore, only the first phase is studied. The evolution of the global consumption of ozone is monitored by varying operating conditions. Generally, O_3 consumption is calculated until a decomposition of 80% of EE2. Beyond that value, there is no more linearity and intermediate products generated compete with EE2 molecule. The influence of pH and the presence of radical scavengers on the overall consumption of ozone during the ozonation reaction of ethinylestradiol is studied.

3.3.1 Ozonation mechanism

Different doses of ozone were added to four sets of 25 μmol/L EE2 with pH ranging from 3 to 11.6. The results reported in **Figure 7** show that for all pH values of the solution, the amount of degraded EE2 increases linearly with the concentration of O_3 introduced. From the slope of the lines, the overall stoichiometry of the reaction is determined and expressed in terms of the ratio of mole of ozone consumed per mole of EE2 degraded. The results shown in **Figure 8** reveal that at acidic pH, the stoichiometry of the reaction tends to 1, meaning that 1 mole of ozone reacts with one mole of EE2. On the other hand, at neutral and basic pH, reaction stoichiometry increases toward 1.8 moles of ozone per mole of EE2 degraded. These results are comparable to those published by Huber *et al.* **[17]** who obtained a stoichiometry of 1.8 moles of ozone per mole of EE2 during ozonation of a solution of 10 μmol/L of EE2 at pH = 8. This stoichiometry greater than 1 can be explained:

- Either by a reaction between O_3 and the intermediate products (I.P.) generated from the oxidation of EE2. These I.P. are extremely reactive with molecular ozone at basic pH:

$$EE2 + O_3 \rightarrow PI$$

$$PI + O_3 \rightarrow PI_{ox}^{\circ}$$
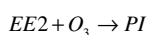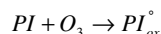
- or a reaction between O_3 and the hydroxide ions to decompose into radicals [18]. The stoichiometry is of 0.66 mol hydroxyl radicals per 1 mol of decomposed ozone which will lead to excessive consumption of ozone resulting in the elimination of the original molecule of ethinylestradiol through a radical mechanism.
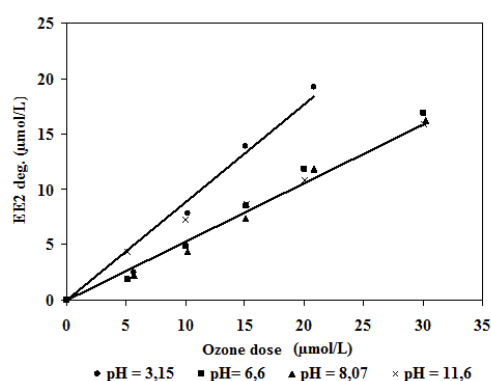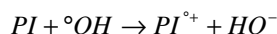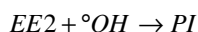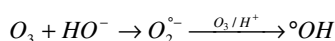
$$EE2 + O_3 \rightarrow PI$$

$$O_3 + HO^- \rightarrow O_2^{\circ -} \xrightarrow{O_3 / H^+} {}^{\circ}OH$$

$$EE2 + {}^{\circ}OH \rightarrow PI$$

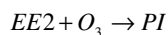$$PI + {}^{\circ}OH \rightarrow PI^{\circ +} + HO^-$$

Figure 7: Influence of pH on the degradation of EE2 in ozone dose function.
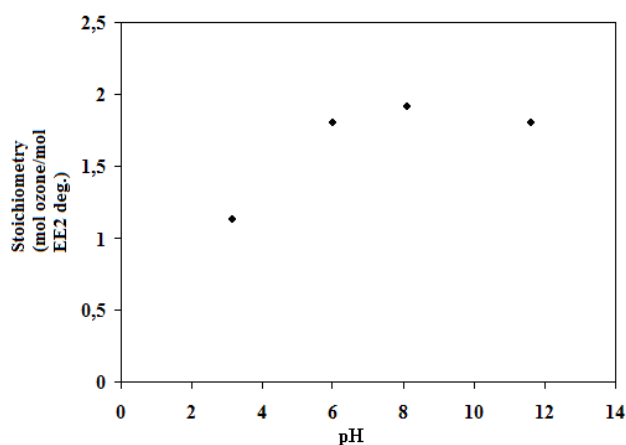

Figure 8: Effect of pH on the stoichiometry of the EE2 ozonation.

To verify the role of pH in the reaction mechanism, we have studied the evolution of the stoichiometry of the reaction in the presence of radical scavengers. For these tests, tert-butanol (BuOH) was chosen as an inhibitor of ozone radical reaction. The tests were carried out by preparing a mixture containing 24 µmol/L EE2 and varying the concentrations of ozone from 0 to 30 µmol.L^{-1}, fixing tert-butanol concentration to 10^{-2} mol.L^{-1}. **Figure 9** shows that, at all pH, the stoichiometry of the reaction between ozone and EE2 is lower when

tert-butanol is present in the medium. It was revealed that at acidic pH, there is little inhibition with an overall stoichiometry close to 1. Thus, for these processing conditions, ozone reacts almost exclusively with ethinylestradiol molecule by molecular pathway according to the reaction:

$$EE2 + O_3 \rightarrow PI$$
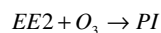
For pH close to neutrality and in a basic medium, inhibition of the reaction, due to tert-butanol, is important but not complete. For example at pH = 11, we observed a stoichiometry of 1.4 mol of ozone per mole of EE2 in the presence of tert-butanol, while it was 1.8 without radical scavengers. This stoichiometry change ensures that the consumption of ozone at basic pH is due to a radical decomposition of ozone. The presence of radical scavengers stabilizes ozone in water by limiting the propagation reactions. Ozone becomes available to react with the molecule of EE2:
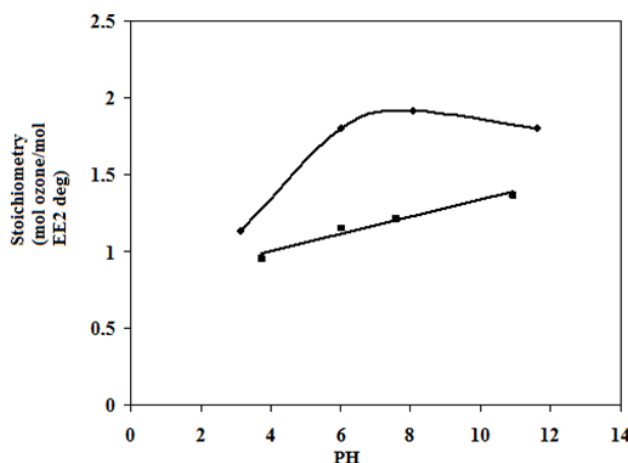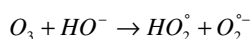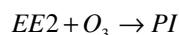
$$EE2 + O_3 \rightarrow PI$$

$$O_3 + HO^- \rightarrow HO_2^{\circ} + O_2^{\circ -}$$

Figure 9: Influence of radical scavengers presence (▪) and absence (●) on the stoichiometry of the EE2 ozonation at different pH.

3.4 Influence of the concentration of EE2 on the reaction stoichiometry

In all treatment processes, the effect of pollutant concentration is of major importance. Ethinylestradiol is a pollutant detected at very low concentrations in wastewater (in the order of ng.L^{-1}) [19]. It is important to study the reaction of ozonation at concentrations similar to those encountered in these effluents. Nonetheless, the majority of studies published in the literature [20] describes ozonation of EE2 at a concentration range between 0.1 and 10 µmol/L (30 - 3000 µg.L^{-1}) which cannot be compared to concentrations found in the environment. To better understand the influence of pollutant concentration on the ozonation

reaction, a series of EE2 solutions (initial concentrations between 1 and 16,500 $nmol.L^{-1}$) was ozonated at pH = 7.5 ± 0.2. An example of the degradation of EE2 to 13 nmol/L is shown in **Figure 10.**
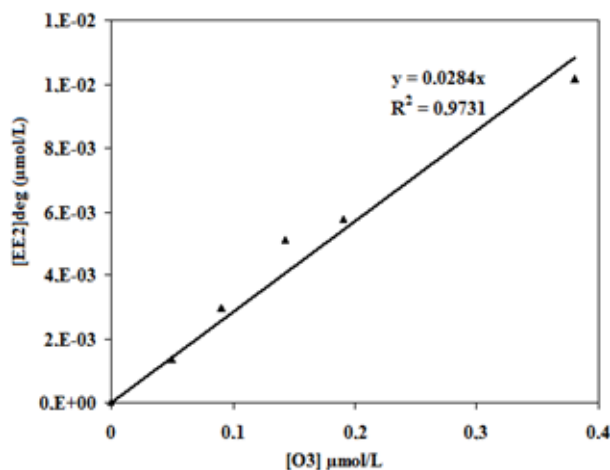

Figure 10: Ozonization of a solution of EE2 to 13 nmol/L (pH = 7.6).

For the studied concentration range, the overall reaction stoichiometries expressed in moles of ozone consumed per mole of degraded EE2 are reported in **Table 1**.

Table 1: Evolution of the overall reaction stoichiometry depending on the initial concentration of EE2 at neutral pH.

$[EE2]_0$ nmol/L	Reaction stoichiometry (Number of moles of ozone consumed per mole of degraded EE2)
16,500	1.72
980	5.34
74	13.3
60	17
29	21.3
13	38.4
1.34	125

These results show that the overall consumption of ozone per mole of degraded EE2 increases exponentially when the concentration of EE2 in the medium decreases **Figure 11**. At neutral pH and in the absence of scavenging radicals, there is a change in the

stoichiometry from 1.8 mol of ozone consumed per mole of degraded EE2 (with initial EE2 concentration of 16 µmol.L^{-1}) to 125 moles of ozone consumed per mole of degraded EE2 (with initial EE2 concentration of 1 nmol.L^{-1}).
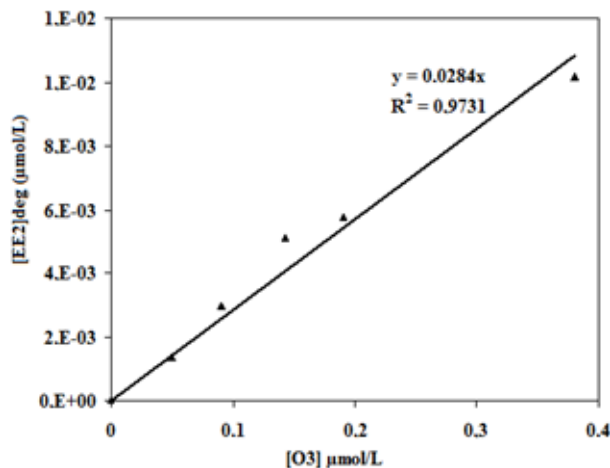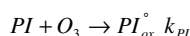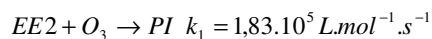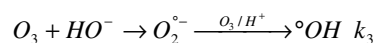

Figure 11: Ozonation stoichiometry versus the EE2 initial concentration.

The overall rate of decomposition of ozone is the result of:

- The rate of the reaction between ozone and ethinylestradiol and its reaction products, at neutral pH:

$$EE2 + O_3 \rightarrow PI \quad k_1 = 1{,}83.10^5 \, L.mol^{-1}.s^{-1}$$

$$PI + O_3 \rightarrow PI_{ox}^{\circ} \quad k_{PI}$$

- And the autodecomposition of ozone in water:

$$O_3 + HO^- \rightarrow O_2^{\circ -} \xrightarrow{\ O_3/H^+\ } {}^{\circ}OH \quad k_3$$

The overall rate of decomposition of ozone in water can be written as:

$$v = -\frac{d[O_3]}{dt} = k_1[O_3][EE_2] + \sum k_{PI}[O_3] + [PI] + k_3[O_3]$$

For EE2 concentrations of order of micromoles per liter, the reaction between O_3 and EE2 is favored because its kinetics is very fast. As a result, a stoichiometry close to 1 is observed. On the other hand, for lower concentrations of EE2, the reaction rate decreases between EE2 and O_3 and the reactions involved in the autodecomposition of ozone become competitive. To obtain an EE2 degradation rate equivalent to that in higher concentrations, a greater amount of ozone is needed. The representation of stoichiometry of the overall reaction versus the initial concentration of EE2, on a logarithmic scale, dictates the existence of a linear

relationship (insert in **Figure 11)**. This linearity verified for 4 log EE2 concentration (10 nmol.L^{-1} – 10,000 nmol.L^{-1}) allows to extrapolate this relationship to EE2 concentrations close to those found in environmental aqueous matrices. Thus, for ultrapure water buffered to pH 7 and contaminated by 1 ng.L^{-1} (3.10^{-12} mol.L^{-1}) of EE2, an ozone dose of 2 µg.L^{-1} could be added to decontaminate it. This very low dose confirms that ozone is an oxidant suitable for the elimination of this type of pollutant in water.

4. Conclusions and discussions

-The kinetics of degradation of the molecule ethinylestradiol EE2 by ozone is extremely fast ($t_{1/2} < 2$minutes).

-Ozone is an unstable reactant.

-Mineralization of ethinylestradiol is divided into two phases: a first phase where the TOC disappearance is fast indicating that the first step corresponds to the oxidation of phenol and ethinyl functions of the molecule. A second phase where the disappearance of TOC is less rapid, reaching 84% after 3 hours, suggesting the opening of different carbon rings of the molecule and requiring a longer oxidation time for their elimination.

- Degradation and mineralization of the molecule are highly dependent on pH. The study of the effect of pH and the presence of radical scavengers leads to conclude that the ozone degrades ethinylestradiol mainly by molecular mechanism, whereas mineralization of the molecule and its byproducts follows a radical mechanism.

- The ozone dose required to degrade the phenol ring of the ethinylestradiol, responsible for its estrogenic activity, has been determined via mineralization studies. This O_3 dose corresponds to 1200 µmol/L for 37 µmol/L of ethinylestradiol (5 g of ozone per gram of EE2 at neutral pH).

- The initial concentration of EE2 plays a major role in the stoichiometry of the reaction between ozone and ethinylestradiol: when the concentration of EE2 decreases, the overall consumption of ozone per mole of EE2 increases. This is due to the autodecomposition of ozone.

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