Oxidation kinetics of cyclophosphamide and methotrexate by ozone in drinking water

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1. Introduction

Pharmaceuticals and their degradation by-products are routinely detected in surface waters (Heberer et al., 1997; Calisto and Esteves, 2009; Kummerer, 2009a,b; Mompelat et al., 2009) and even drinking waters (Stackelberg et al., 2004; Jones et al., 2005; Snyder et al., 2007; Benotti et al., 2009). A significant fraction of the pharmaceuticals released into the aquatic environment is attributed to their incomplete removal through conventional wastewater treatment (Halling-Sorensen et al., 1998; Daughton and Ternes, 1999; Joss et al., 2004; Clara et al., 2005; Segura et al., 2007; Carballa et al., 2008; Zhang et al., 2008; Matamoros et al., 2009). Among various classes of pharmaceuticals, cytostatic chemotherapy drugs (also called antineoplastic agents) are of particular environmental concern because they are potentially carcinogenic, mutagenic and genotoxic, even at low concentrations (Zounkova et al., 2007). Cyclophosphamide and methotrexate are widely used at high dose for the chemotherapy of various forms of cancer (bronchial, breast and ovarian cancer, lymphomas, leukemias, etc.) and at low dose for the treatment of autoimmune diseases (e.g., rheumatoid arthritis), and also as immunosuppressants after organ transplantsations (e.g., bone marrow transplantations) (Jolivet et al., 1983; Kuo et al., 2003; Buerge et al., 2006). The excretion of those unchanged parent molecules in patients appears to be around 10–20% (Johnson et al., 2008). According to Buerge et al. (2006), these compounds may reach the aquatic environment via hospital or domestic wastewater collectors on their way to wastewater treatment plants through excretions in the urine and feces of patients under medical treatment.

Concentrations of cyclophosphamide, ifosfamide, 5-fluorouracil, anthracyclines and cancerostatic platinum compounds range from 6 ng L\(^{-1}\) to 145 µg L\(^{-1}\) in hospital wastewater effluents (Kummerer et al., 2000; Lenz et al., 2005; Mahnik et al., 2007). Cyclophosphamide, ifosfamide and methotrexate have been analyzed in untreated and treated municipal wastewater at concentrations in the ng L\(^{-1}\) range (Kummerer et al., 1997; StegerHartmann et al., 1997; Ternes et al., 1998; Buerge et al., 2006; Garcia-Ac et al., 2009).
Antineoplastic agents have also been detected in source waters receiving treated wastewater effluents (≤50 pg L\(^{-1}\)-10 ng L\(^{-1}\)) (Zuccato et al., 2000; Buerg et al., 2006) and occasionally in tap water (≤13 ng L\(^{-1}\)) (Ahern et al., 1990). More recently, a risk assessment study showed that although the risks about the potential concentrations of cyclophosphamide in drinking water may be negligible for healthy adults, more concern may be associated with special subgroup populations, such as pregnant women, their foetuses, and breast-feeding infants, due to their developmental vulnerability (Rowney et al., 2009).

Due to the increase in the demand for chemotherapy drugs in developed countries and their common administration in outpatient treatment departments (Johnson et al., 2008), the concentration of cytostatic agents in municipal wastewaters is expected to increase. Their potential presence in surface waters points to the need to verify the efficacy of drinking water treatment processes to remove such compounds. Among biological, physical and chemical treatments, chemical oxidation using ozone treatment has demonstrated its effectiveness for a wide spectrum of organic micropollutants in both wastewater and drinking water and at various levels of experiments (bench-, pilot- and full-scale) (Ternes et al., 2002; 2003; Huber et al., 2005; Westerhoff et al., 2005; Ikehata et al., 2006; Snyder et al., 2006; Vieno et al., 2007; Gagnon et al., 2008). Ozone reacts with organic contaminants either by the direct reaction of molecular ozone or by the reaction of free radicals (mostly hydroxyl radicals OH\(^*\)) produced by the decomposition of ozone. The rate of OH\(^*\) formation depends on the water matrix, especially the pH, alkalinity and type and content of natural organic matter in drinking water (von Gunten, 2003). Molecular ozone reacts selectively with unsaturated bonds, aromatic systems and amino groups whereas OH radicals react much more indiscriminately (Ljubic and Sabljic, 2002).

To predict the potential removal by ozonation, it is important to determine rate constants of the reaction of micropollutants with ozone and OH radicals. Some rate constants for direct (\(k\(_{01}\)) and indirect (\(k\(_{0m}\)) ozone oxidation have been published and can serve to predict the performance of oxidation (Huber et al., 2003; von Gunten, 2003). Those rate constants can also be estimated by various quantitative models for estimating degradation of chemicals in various environmental compartments (Sabljic and Peijnenburg, 2001). However, few studies have evaluated the efficacy of ozone for the oxidation of cytostatics, which vary in chemical structures and properties that will affect their ability to be oxidized. Pérez-Rey et al. (1999) studied the degradation of 5-fluorouracil, cytarabine, azathioprine and methotrexate with ozone in water using higher concentrations (1.1–2.2 × 10\(^{-3}\) M) than those detected in surface waters. The removal of cyclophosphamide and cisplatin by ozone and advanced oxidation processes (AOP) has been investigated (Venta et al., 2005; Chen et al., 2008; Hernandez et al., 2008) but only the pseudo first-order constants were estimated. Chen et al. (2008) reported the values of the second-order rate constants of irinotecan, tamoxifen and cyclophosphamide in ultrapure water only. A similar study conducted to assess the degradability of 30 pharmaceuticals by AOP showed that O\(_3\) process and O\(_3\)-based/UV-based AOP could efficiently remove a variety of the selected compounds. However, some pharmaceuticals such as cyclophosphamide showed a relatively low degradability (Kim et al., 2008).

The determination of the reaction rate constants in ultrapure and natural water for two commonly used cytostatics, cyclophosphamide and methotrexate, will provide important information for drinking water treatment selection and adjustment. To the best of our knowledge, this is the first paper using very low concentrations of cytostatics representing nicely the environmental concentrations, and presenting the impact of ozone oxidation on cytostatics in a natural water matrix.

2. Objectives of study

In the research project presented hereafter, three objectives were sought:

1. To determine the efficacy of ozone for the degradation of two target cytostatic drugs at bench-scale.
2. To determine the second-order rate constants for the reaction of the selected compounds with molecular ozone and OH radicals in buffered ultrapure water.
3. To assess the impact of water quality on molecular ozone oxidation kinetics using bench-scale assays and natural water matrices representative of conventionally treated surface water.

3. Experimental section

3.1. Chemicals and reagents

Cyclophosphamide and methotrexate (certified purity ≥99%) were purchased from Sigma–Aldrich Canada (Oakville, ON, Canada). Their chemical structures and their main physico-chemical properties are presented in Table 1. The \(pK_a\) value of cyclophosphamide is given as a range because no exact value can be found in the literature (Wang et al., 2009). Methotrexate is a weak organic dicarboxylic acid (Reid et al., 1993). Both substances are hydrophilic (\(log K_{ow} < 2\): low sorption potential). LC–MS grade acetonitrile, water and methanol were obtained from J.T. Baker (Phillipsburg, NJ, USA). Acetic acid (from Fisher-Science, Fair Lawn, NJ, USA) was HPLC-grade reagent and formic acid 98% was purchased from Sigma–Aldrich Canada. L-ascorbic acid (99.0%) and hydrogen peroxide, previously standardized by KMnO\(_4\) titration (Harris, 2003), were purchased from Fisher Scientific (Fair Lawn, NJ). Ultrapure water (18 M\(_2\) cm) was produced with a Millipore™ apparatus. Phosphate buffer and tert-butanol (final concentrations: 0.2 M and 50 mM, respectively) were prepared by dissolution of the commercial compounds in ultrapure water.

3.2. Preparation of standards

Stock solutions (10 mg L\(^{-1}\)) of each cytostatic compound were prepared by weighing and dissolving the corresponding pure powders in ultrapure water. Stock solutions were stored and kept at –15 °C before use in order to prevent microbial degradation. Mixed working solutions containing 50 µg L\(^{-1}\) of the compounds were prepared each time by dilution of the stock solutions in ultrapure water and kept at 4 °C.

3.3. Instrumentation

The on-line SPE procedure for samples and standards to be analyzed are based on the method described by García-Ac et al. (2009). The Environmental Quantification System (EQuan™, Thermo Fisher Scientific, Waltham, MA, USA) was used to carry out the analysis. Also two quaternary LC pumps, an autosampler with a 1 mL-loop (Thermo Fisher Scientific, Waltham, MA, USA) and a TSQ Quantum Mass Spectrometer equipped with an Ion Max API Source (Thermo, Waltham, MA) and an electro-spray ionization (ESI) multimode source using compound-specific fragments. Tube lens and collision energies which are compound-specific appear in Table 2. Method detection limits were 3 and 6 ng L\(^{-1}\) for cyclophosphamide and methotrexate, respectively.
Natural filtered water samples were collected before the ozonation process from a single municipal drinking water treatment plant (DWTP) in the province of Quebec drawing water from the St-Lawrence River. This water was sampled in 10-L polypropylene carboys that had been thoroughly washed and rinsed successively with distilled and ultrapure waters. Samples were filtered through 0.45-μm polyethersulfone membranes and kept at 4°C prior to the ozone experiments. The filtered water had a pH of around 8.04, low turbidity (<0.15 NTU), low natural organic matter (2.55 mg L⁻¹ dissolved organic carbon (DOC)) and moderate alkalinity (80 mg L⁻¹ CaCO₃).

Bench-scale ozonation experiments were performed at 20 ± 1°C. In all experiments, water samples were spiked with target compounds to achieve concentrations of cytostatics between 100 and 200 ng L⁻¹ depending on the compound and the detection limit of the method of determination (LOD) (LOD methotrexate = 6 ng L⁻¹ and LOD cyclophosphamide = 3 ng L⁻¹).

During direct reaction with molecular ozone, ultrapure water was buffered to pH 8.10 with a phosphate buffer to account for the pH of the natural water, while the pH of the natural water was left unbuffered. Tert-butanol was added in buffered ultrapure water and in natural water to quench OH radicals generated by ozone decomposition. This allowed the determination of the second-order rate constants for the reaction of molecular ozone with the target cytostatic drugs (kO₃) in buffered ultrapure and natural water, and assessing the impact of water quality on molecular ozone oxidation kinetics. Stock ozone solutions (50–60 mg L⁻¹ O₃) were prepared by diffusing gaseous ozone, produced with an oxygen-fed generator (Ozone services, BC, CA) through a water-jacketed flask containing ultrapure water chilled at 4°C. The ozonation experiments were initiated by mixing aqueous ozone (6–15 mg L⁻¹) and solutions of cytostatic drugs (100–200 ng L⁻¹).

Aliquots of ozone solutions were injected via a syringe into a continuously stirred 1-L glass reactor containing the water sample and equipped with a floating Teflon lid to prevent degassing. Aliquots of 4 mL were then withdrawn from the test water at regular time intervals for ozone residual analysis. For target compounds analysis, aliquots of 40 mL were withdrawn and transferred into vials containing 400 μL of ascorbic acid (5 g L⁻¹) to quench the residual ozone and prevent microbial degradation (Westerhoff et al., 2005). Target compounds concentrations were analyzed for contact times from 0 to 120 min.

When required, hydrogen peroxide was dosed in buffered ultrapure water at a concentration of 0.25 mg H₂O₂ per mg O₃ prior to the injection of ozone (10 mg L⁻¹) in order to determine the
second-order rate constant for the reaction of cytostatics with OH radicals ($k_{OH}$) and due to the resistance of one of the target compounds to direct ozonation. This concentration was selected as it is in the optimal range to promote AOP (Acero and Von Gunten, 2001). Experiments were also conducted with natural water free from tert-butanol and hydrogen peroxide in order to verify the natural promotion of OH radicals when ozone was applied (10 mg L$^{-1}$). Ozone oxidation reaction was then a combination of direct and indirect reactions with molecular ozone and OH radicals, respectively. In these previous experiments and after ozone injection, samples were collected for ozone residual analysis following the method previously described. Moreover, for pCBA analysis, a sub-sample of 2 mL was collected and analyzed using a HPLC system. Initial ozone consumption was measured as the difference between the ozone dosage and the ozone residual after ~10 s. Initial ozone consumption (mg L$^{-1}$) was then converted in percentage of the ozone dosage for subsequent comparison between ozone experiments.

3.6. Ozone residual and pCBA analysis

At bench-scale, ozone stock solution concentrations and ozone residuals in water were determined according to the standard colorimetric method 4500-O3 using indigo trisulfonate ($\lambda_{600}$ nm = 20 000 M$^{-1}$ cm$^{-1}$). Samples were analyzed at 600 nm with a Varian spectrophotometer (Cary 100, Victoria, Australia) in a 1-cm or 2-cm quartz cell. Para-chlorobenzoic acid (pCBA) was analyzed using a HPLC system (column characteristics: Nucleosil 100-5, RP-C18, 150 mm length, 4.6 μm i.d., from Macherey–Nagel). The detection of pCBA was made using diode array detection at 236 nm (Elite LaChrom, Hitachi). A method detection limit of 0.2 μg L$^{-1}$ was obtained.

3.7. Determination of rate constants for the reaction with molecular ozone

The value of the rate constant with molecular ozone was determined in buffered ultrapure water at pH 8.10 and at ambient pH in natural water in the presence of tert-butanol as hydroxyl radical scavenger. Von Gunten (2003) reported that the kinetics of ozone reactions with organic and inorganic compounds is typically second-order, i.e. first-order both with ozone and the contaminant concentration. The degradation of cytostatics with molecular ozone can be described by:

$$-\frac{d[CD]}{dt} = k_{O3}[O_3][CD]$$

where $CD$ = target cytostatic drug and $k_{O3}$ = reaction rate constant with molecular ozone. The rate constant is obtained from the integration of Eq. (1):

$$\ln \left(\frac{[CD]}{[CD]_0}\right) = -k_{O3} \int_0^t [O_3]dt$$

where $[O_3]dt$ is the time-integrated ozone concentration or ozone exposure CT (oxidant concentration × contact time).

In this study, CT values (mg min L$^{-1}$) were calculated using the integrated CT concept (Barbeau et al., 2005), for which the effective CT at time $t$ (min) is equal to the area under the decay curve at that time. CT values were calculated using the ozone concentration profiles (Eq. (3)) assuming a simple first-order decay:

$$CT_{effective} = \int C(t) \cdot dt = \frac{C_0}{k}[1 - \exp(-k \cdot t)]$$

where $C_0$ = initial ozone residual (mg L$^{-1}$); $C_0$ = initial ozone residual (mg L$^{-1}$) determined from the exponential fit of the relation between the ozone residual and the time (min) and $k$ = ozone first-order decay constant (min$^{-1}$). CT values in mg min L$^{-1}$ were converted in M s for a subsequent comparison of reaction kinetics (M$^{-1}$ s$^{-1}$) with data from the literature.

3.8. Reaction kinetics with OH radicals

A competition kinetics method was used to determine the second-order rate constants for the reaction between the cytostatic drugs and hydroxyl radicals ($k_{OH}$). The rate constants were calculated by following the concentration of an OH$^-$ probe compound, which can indirectly provide the concentration of OH radicals during the ozonation process following the pCBA method (Elovitz and von Gunten, 1999). Final integration of data yields:

$$\ln \left(\frac{[CD]}{[CD]_0}\right) = -\left(\frac{k_{OH}R_{ct} + k_{O3}}{k_{O3}}\right) \int_0^t [O_3]dt$$

where $k_{OH}$ and $k_{O3}$ are the second-order rate constants for the reactions of cytostatic drugs CD with OH$^-$ and O$_3$, respectively; $R_{ct}$ is defined as the ratio between the OH radicals and molecular O$_3$ exposure; ozone exposure (CT value) was calculated as described in Section 3.7.

3.9. Data analysis

The results were analyzed using Statistica software version 7.1 (Statistica 7.0). Unless otherwise mentioned, standard deviations are used to characterize the uncertainty in kinetic constants and statistical significance was set at $p = 0.05$.

4. Results and discussion

4.1. Analytical determination of the chemotherapy drugs

The normalized selected reaction monitoring (SRM) chromatograms for a DWTP natural water sample spiked with methotrexate and cyclophosphamide are illustrated in Fig. 1. Fig. 1a illustrates the good separation of both analytes in an initial solution spiked with 200 ng L$^{-1}$. Since the analysis was conducted using SRM, quantification of each specified ion was not subjected to interferences from the others. After two minutes of reaction with a 10 mg L$^{-1}$ ozone dosage, methotrexate practically disappeared (97% conversion), while for cyclophosphamide a small peak was still observed equivalent to a removal rate of 87% (Fig. 1b). Fig. 1c shows a chromatogram of the same sample taken at 30 min of reaction time. At this time, none of the two compounds were detected taking into account the detection limit of the method of determination of the compounds. From those chromatograms, methotrexate was easier to oxidize than cyclophosphamide. To obtain accurate concentrations of the selected chemotherapy drugs, calibration curves in the range of 50–500 ng L$^{-1}$ were established for both compounds ($R^2 > 0.98$, data not shown).

4.2. Ozone decomposition

Examples of dissolved ozone decay curves in buffered ultrapure water (pH = 8.10) and in DWTP natural water samples are presented in Fig. 2. Assays were performed under the following conditions: (#1) natural water (10 mg L$^{-1}$ O$_3$), (#2) natural water spiked with tert-butanol (10 mg L$^{-1}$ O$_3$), (#3) natural water (10 mg L$^{-1}$ O$_3$) spiked with hydrogen peroxide (2.5 mg L$^{-1}$ H$_2$O$_2$) and (#4) buffered ultrapure water (pH = 8.10) spiked with tert-butanol (10 mg L$^{-1}$ O$_3$). This experimental design enables the calculation of the second-order reaction rates $k_{O3}$ and $k_{OH}$ in buffered ultrapure water and in natural water and the comparison of the efficacy of
conventional ozonation with advanced oxidation in natural water. Ozone dosages typically used in drinking water treatment practice are lower than 1 mg O₃ per mg TOC (Speitl et al., 1993). Due to the high resistance of one of the target compounds to ozonation, high dosages were chosen in order to correctly assess second-order kinetics.

In waters spiked with tert-butanol, ozone was, as expected, more stable (Fig. 2). Ozone stability in the natural water spiked with tert-butanol was slightly higher than the one in the buffered ultrapure water, which could be explained by the moderate alkalinity (80 mg L⁻¹ CaCO₃) of the natural water as an additional presence of carbonate as an OH⁻ scavenger. In natural waters free of tert-butanol, the impact of OH radicals formation on ozone is clearly visible. Finally, the addition of H₂O₂ (0.25 mg H₂O₂ per mg O₃) in natural water significantly accelerated ozone decomposition and no residual ozone was detected beyond 1 min.

Ozone decay was modeled with the first-order kinetic ($R^2 > 0.94$). Ozone decay rates ($k'$) varied over two orders of magnitude (from 0.024 to 5.15 min⁻¹) depending on ozonation conditions. Ozone half-lives were derived from ozone decay rates. They varied from 5.5 min in natural water to 21.1 (±4.2) min and 26.7 (±3.6) min in buffered ultrapure water and in natural water both spiked with tert-butanol, respectively. The addition of hydrogen peroxide (0.25 mg H₂O₂ per mg O₃) lowered ozone half-life to <0.5 min. Overall, initial ozone consumption varied between 33.4% and 54.4% of the ozone dosage.

4.3. Ozone oxidation of target compounds

Methotrexate reacted rapidly with molecular ozone (10 mg L⁻¹ O₃) (data not shown) with removals exceeding 1.4 log. This drug was never detected in any ozonated water samples. Additional
experiments were conducted by spiking 2 μg L⁻¹ of methotrexate and injecting ozone dosages ≤2 mg L⁻¹ O₃. However, removals exceeded 2.0 log at 0.80 mg min L⁻¹ (k_{app} > 3.6 × 10⁷ M⁻¹ s⁻¹, where k_{app} is the apparent rate constant). Methotrexate contains amino groups, an aromatic ring and two N-containing aromatic rings, which are moieties known to quickly react with ozone. Because of the fast oxidation, it was not possible to determine the reaction rate constants with molecular ozone or OH radicals using the same experimental methods applied for cyclophosphamide. Rey et al. (1999) showed that ozonation was effective in removing methotrexate using an initial drug concentration varying between 454 and 1000 mg L⁻¹ (pH 3.0 or 7.0, T = 25 ± 0.1 °C) during semi-batch experiments. According to von Gunten (2003), compounds that react rapidly with molecular ozone at dosages typically used for drinking water treatment have an oxidation kinetics with molecular ozone k_{O₃} > 10⁸ M⁻¹ s⁻¹. A competition kinetics method is under development to confirm these preliminary data and determine the degradation rate using a reference compound. But still, given the fast kinetics, methotrexate represents less environmental and human health issues given its ease of removal.

Removal of cyclophosphamide as a function of ozone exposure (CT) is presented in Fig. 3. In order to estimate the kinetic rate constant k₀, for the reaction between molecular ozone and cyclophosphamide in buffered ultrapure water, tert-butanol was added as a scavenger to exclude the effects of OH radicals formed due to the ozone decomposition. From these experiments, it was observed that the oxidation of cyclophosphamide was limited, therefore indicating a slow reaction between this compound and molecular ozone. The linear relationship (R² = 0.90) yields a calculated rate constant of k_{O₃} = 3.3 ± 0.2 M⁻¹ s⁻¹ (p = 6 × 10⁻⁵) in buffered ultrapure water suggesting that molecular ozone alone would be ineffective in removing this drug unless very high ozone CT values are provided. Under typical water treatment conditions (i.e., pH ~ 6–8), molecular ozone reactions will play a minor role in the degradation mechanism of cyclophosphamide. Molecular ozone reaction is a selective process and cyclophosphamide does not contain unsaturated bonds and aromatic moieties but two electron-withdrawing groups (chloro-substituted double-bond) that lower reactivity of organic molecules toward ozone (von Gunten, 2003). There are no functional groups present in the cyclophosphamide molecule that would be expected to exhibit any more than minimal reactivity toward ozone. Chen et al. (2008) observed similar trends but reported value of the second-order rate constant two orders of magnitude higher (143 M⁻¹ s⁻¹) than the rate constant calculated in this study. The applied ozone concentration was 2 mg L⁻¹ at pH 7.1 with an initial drug concentration between 260 and 1041 μg L⁻¹ while using a 10 mM concentration of tert-butanol. However, the temperature used during the experiments is unknown. The cause for such important difference in second-order rate constants remains unanswered at this time.

In natural water spiked with tert-butanol (R² = 0.90), the kinetic rate constant for the reaction between molecular ozone and the drug cyclophosphamide was 2.9 ± 0.3 M⁻¹ s⁻¹ (p = 8 × 10⁻¹⁰) and was not statistically different than in buffered ultrapure water with tert-butanol (p < 0.05) (Fig. 3). Therefore, the reaction rates determined in ultrapure water can be applied to assess the behaviour of the target compound in natural waters exposed to molecular ozone. This result was previously demonstrated (Huber et al., 2003, 2005) where the authors studied the reaction of selected pharmaceuticals with ozone in natural waters. However, studies with chlorine showed faster cyanoxins degradation rates in natural waters when compared with deionised water (Xagoraraki et al., 2006; Daly et al., 2007), tending to show that the reaction of oxidants and natural organic matter could produce more reactive species than chlorine alone. From the rate constants determined here, it can be predicted that the half-life of cyclophosphamide would have varied from 164 to 190 min with an ozone concentration of about 1 mg L⁻¹ (21 μM).

In natural water (without tert-butanol or hydrogen peroxide), under which condition oxidation reaction is a combination of molecular ozone and OH radicals reactions, the depletion of the drug was higher (Fig. 3). However, a CT value of ~45 mg min L⁻¹ was required to remove 1.4 log (~96%) of cyclophosphamide. This result clearly demonstrated that ozonation only would not be sufficient to completely remove this drug. A CT value of approximately 1.0 mg min L⁻¹ is appropriate for primary disinfection of Giardia (1 log, T ≤ 1 °C) and virus inactivation (~2 log, T ≤ 1 °C) with ozone. Using ozone in buffered solutions and without tert-butanol Venta et al. (2005) showed that only 4% and ~30% of cyclophosphamide was degraded in aqueous solutions after 6 min of reaction time at pH 7 and pH 9, respectively. The greater degradation at pH 9 was explained by the formation of OH radicals by ozone decomposition.

Several studies have previously shown the effectiveness of combining ozone with hydrogen peroxide to enhance the oxidation of trace micropollutants (Ning and Graham, 2008). In our experiments, when OH radicals formation was promoted with H₂O₂ addition, the oxidation of cyclophosphamide was indeed very fast (Fig. 2) in accordance with the results of Venta et al. (2005), who used 6 and 15 min of reaction time with a O₂/H₂O₂ molar concentration ratio of 3:1. However, it was not possible to calculate the second-order rate constant for the reaction of cyclophosphamide with OH radicals in ultrapure water due to the limited number of ozone residual and pCBa data. Therefore, the kinetic rate constant for the reaction between OH radicals and cyclophosphamide (k_{OH}) was assessed using the ozonation experiments performed in natural water (without tert-butanol). The Rₚ obtained, defined as the ratio between the OH radicals and molecular O₃ exposure, was 7.5 × 10⁻⁸ at ~20 °C. The kinetic rate constant for the reaction between OH radicals and cyclophosphamide was therefore calculated as 2.0 × 10⁵ M⁻¹ s⁻¹. Chen et al. (2008) calculated that the second-order rate constant was equal to 2.1 × 10⁸ M⁻¹ s⁻¹. These values fall well within the typical range of reported OH⁻ rate constants, which vary between 10⁷ M⁻¹ s⁻¹ and 10¹⁰ M⁻¹ s⁻¹ (Westerhoff et al., 2005).

Further research is necessary to examine the efficiency of drinking water treatment processes, and especially ozonation, to remove a range of relevant cytostatic drugs as well as their by-products and the toxicity of the ozonated mixture. As an example, an
ozonation by-product of cisplatin was shown to be recalcitrant to reaction with molecular ozone (Hernandez et al., 2008). Moreover, other studies showed the formation of persistent ozonation by-products of two antibiotics (Radjenovic et al., 2009).

5. Conclusion

The oxidation kinetics of cyclophosphamide with molecular ozone and OH radicals were investigated at bench-scale. The second-order rate constant for direct ozone reaction with cyclophosphamide was determined as $3.3 \pm 0.2 \ M^{-1} \cdot s^{-1}$ in ultrapure water buffered to pH 8.10. The impact of the natural water matrix on the efficacy was not statistically significant during direct ozone reaction ($2.9 \pm 0.3 \ M^{-1} \cdot s^{-1}$). Overall process performance was improved for cyclophosphamide in natural water, because of the combination of direct and indirect reactions pathways. The kinetic rate constant for reaction of cyclophosphamide with hydroxyl radicals was estimated at $2.0 \times 10^{9} \ M^{-1} \cdot s^{-1}$. In contrast, methotrexate reacted quickly with molecular ozone ($k_{\text{O}} = 3.6 \times 10^{9} \ M^{-1} \cdot s^{-1}$). This study showed that ozone was very effective to oxidize methotrexate but high CT values would be required to remove cyclophosphamide in natural water matrix. The chemical structure and toxicity of the reaction by-products should be determined.

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