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Elimination of Pharmaceuticals during Oxidative Treatment of Drinking Water and Wastewater: Application of Ozone and Chlorine Dioxide

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Summary

In recent years, various studies have reported the occurrence of a large number of pharmaceuticals in surface water, but also in groundwater. Surface water and groundwater are widely used as water resources for drinking water. Therefore, the widespread occurrence of pharmaceuticals may have a negative impact on the purity of drinking water. In Europe, a typical treatment train for surface water comprises several different treatment steps including oxidative treatment with chlorine, ozone (O_3), or chlorine dioxide (ClO_2). In contrast to surface water, groundwater is often subjected to a single treatment step that may consist of treatment with chlorine, ClO_2, or ultraviolet radiation.

The goal of the present thesis was to assess the potential of O_3 and ClO_2 for the oxidation of pharmaceuticals and hormones during water treatment. For this purpose, second-order rate constants for the reaction of selected environmentally relevant pharmaceuticals with O_3 (k_{O3}) and ClO_2 (k_{ClO2}) were determined in bench-scale experiments using pure water. At pH 7, apparent k_{O3} and k_{ClO2} for the antiphlogistic diclofenac, the estrogen 17α-ethinylestradiol (EE2), and the sulfonamide antibiotic sulfamethoxazole were >5000 M^{-1} s^{-1} (half-lives <10 s for an oxidant concentration of 1 mg L^{-1}), indicating that these compounds are efficiently transformed during treatment with either O_3 or ClO_2. The macrolide antibiotic roxithromycin and the antiepileptic carbamezpine were very reactive toward O_3 (k_{O3} > 5000 M^{-1} s^{-1}), but were much less or not reactive toward ClO_2. None of the remaining compounds bezafibrate, ibuprofen, diazepam and iopromide showed an appreciable reactivity toward ClO_2, and only the first two pharmaceuticals exhibited a significant reactivity toward O_3.

Hydroxyl radicals ($^{\bullet}OH$), formed by O_3 decay, can also contribute to the oxidation of pharmaceuticals during ozonation. Second-order rate constants for the reaction of the selected pharmaceuticals with $^{\bullet}OH$ (k_{OH}) ranged from 3.3 to

9.8×10^9 M^{-1} s^{-1}. Due to the relatively high $^{\bullet}OH$ rate constants, O_3 refractive pharmaceuticals will also be oxidized to a significant extent during ozonation. It was shown that a faster oxidation of such compounds can be achieved by applying advanced oxidation processes (AOPs) like the O_3/H_2O_2 process.

Experiments performed using natural waters demonstrated that k_{O3}, k_{OH}, and k_{ClO2}, which were determined in pure aqueous solution, could be applied to predict the behavior of pharmaceuticals spiked to natural waters. For ClO_2, this could also be shown for concentrations in the ng/L-range.

Oxidative treatment results in the transformation of pharmaceuticals, but does not lead to full mineralization. Taking EE2 as an example, it was tested whether its oxidation products formed during ozonation still exhibit the pharmacological effect (estrogenic activity) of the parent compound. For this purpose, a recombinant yeast estrogen screen was used. The results showed that the estrogenic activity of EE2-containing solutions is reduced by a factor of 200 to 500 by ozonation. These findings demonstrate that the modifications in the structure of EE2 caused by oxidation were significant enough to substantially reduce the activity of the oxidation products. The identification of oxidation products of EE2 with the help of LC-MS/MS and model compounds proved that ozonation destroys the phenolic moiety of EE2, which is essential for the binding of EE2 to the estrogen receptor.

To reduce the release of pharmaceuticals and hormones in the aquatic environment, ozonation could be applied to wastewater effluents. Pilot-scale experiments performed in a municipal wastewater treatment plant demonstrated that relatively low O_3 doses ($\geq$2 mg L^{-1}) are required to oxidize fast-reacting pharmaceuticals such as macrolide and sulfonamide antibiotics, diclofenac, naproxen, 17α-ethinylestradiol and natural estrogens. It could also be shown

that suspended solids have only a minor effect on the oxidation of the investigated compounds.

In light of the high reactivity of many pharmaceuticals to O_3 and $^{\bullet}OH$ and the successful application of ozonation in pilot-scale, it can be concluded that ozonation is a promising tool for the control of pharmaceuticals in water treatment. In contrast, ClO_2 is only effective in removing specific, however important, classes of compounds like macrolide and sulfonamide antibiotics and estrogens.

Zusammenfassung

In den letzten Jahren haben diverse Studien gezeigt, dass Spurenkonzentrationen zahlreicher Arzneimittel in Oberflächenwässern und teilweise auch im Grundwasser nachweisbar sind. Oberflächenwasser und Grundwasser sind die wichtigsten Ressourcen für die Trinkwassergewinnung. Das weit verbreitete Auftreten von Arzneimittelrückständen stellt deshalb eine Gefahr für die Reinheit des Trinkwassers dar. In Europa wird Oberflächenwasser normalerweise mehrstufig aufbereitet, wobei typischerweise entweder Chlor, Ozon (O_3) oder Chlordioxid (ClO_2) als Oxidations- und Desinfektionsmittel eingesetzt werden. Im Gegensatz dazu wird Grundwasser häufig nur einer Desinfektion mit Chlor, ClO_2 oder Ultraviolett-Bestrahlung unterzogen.

Das Ziel der vorliegenden Doktorarbeit war es, das Potential von O_3 und ClO_2 für die Oxidation von Arzneimitteln während der Wasseraufbereitung abzuschätzen. Zu diesem Zweck wurden in Laborexperimenten mit Reinstwasser Geschwindigkeitskonstanten 2. Ordnung für die Reaktion von O_3 (k_{O3}) und ClO_2 (k_{ClO2}) mit ausgewählten umweltrelevanten Arzneimitteln bestimmt. Für das Schmerzmittel Diclofenac, das Östrogen 17α-Ethinylestradiol (EE2) und das Sulfonamidantibiotikum Sulfamethoxazol waren die scheinbaren k_{O3} und $k_{ClO2} > 5000\ M^{-1}\ s^{-1}$ bei pH 7 (dies entspricht Halbwertszeiten <10 s für eine Oxidationsmittelkonzentration von 1 mg L^{-1}). Dieser Wert zeigt, dass die genannten Substanzen sowohl bei der Anwendung von O_3 als auch ClO_2 effizient oxidiert werden. Das Makrolidantibiotikum Roxithromycin und das Antiepileptikum Carbamazepin reagierten sehr schnell mit O_3 ($k_{O3} > 5000\ M^{-1}\ s^{-1}$) aber nur relativ langsam respektive gar nicht mit ClO_2. Von den übrigen Verbindungen Bezafibrat, Ibuprofen, Diazepam und Iopromid reagierte keine

messbar mit ClO_2 und nur die ersten zwei zeigten eine signifikante Reaktivität gegenüber O_3.

Hydroxylradikale ($^{\bullet}OH$), die als Folge des Ozonzerfalls entstehen, können während der Ozonung ebenfalls zur Oxidation von Mikroverunreinigungen beitragen. Die Bestimmung der Geschwindigkeitskonstanten 2. Ordnung für die Reaktion ausgewählter Arzneimittel mit $^{\bullet}OH$ (k_{OH}) ergab Werte zwischen 3.3 und 9.8×10^9 M^{-1} s^{-1}. Aufgrund dieser relativ hohen Geschwindigkeitskonstanten können während der Ozonung auch Arzneimittel, die nicht direkt mit O_3 reagieren, zu einem guten Teil oxidiert werden. Es wurde auch gezeigt, dass durch die Anwendung von weitergehenden Oxidationsprozessen (AOPs, z.B. O_3/Wasserstoffperoxid) die Oxidation solcher Substanzen beschleunigt werden kann.

Experimente in natürlichem Wasser habe gezeigt, dass $k_{O3,}$ k_{OH} und k_{ClO2}, welche in reinen Lösungen bestimmt wurden, angewendet werden können, um die Oxidation von Arzneimitteln in natürlichem Wasser vorherzusagen. Zusätzlich konnte für ClO_2 gezeigt werden, dass dies auch für realistische Konzentrationen im ng/L-Bereich gilt.

Oxidative Aufbereitung führt zu einer Transformation von Arzneimitteln aber nicht zur vollständigen Mineralisation. Am Beispiel von EE2 wurde getestet, in welchem Ausmass die pharmakologische (östrogene) Aktivität der Ausgangsverbindung von den während der Ozonung gebildeten Oxidationsprodukten behalten wird. Zu diesem Zweck wurde eine Hefe-Östrogen-Test (YES) verwendet. Die Resultate zeigten, dass die östrogene Aktivität von EE2-Lösungen durch die Behandlung mit O_3 um einen Faktor 200 bis 500 vermindert wurde. Diese Ergebnisse demonstrieren, dass die durch die Oxidation verursachten Veränderungen in der Struktur von EE2 bedeutend genug sind, um die Aktivität der Substanz beträchtlich zu vermindern. Die

Identifikation von Oxidationsprodukten mittels LC-MS/MS und Modellverbindungen bewies, dass die Ozonung zur Zerstörung der Phenolgruppe von EE2 führt, welche sehr wichtig für das Binden von EE2 an den Östrogenrezeptor ist.

Mit der Anwendung von O_3 in der Abwasserreinigung sollte es möglich sein, den Eintrag von Arzneimitteln und Hormonen in die aquatische Umwelt zu vermindern. Pilotexperimente, die in einer kommunalen Abwasserreinigungs-anlage durchgeführt wurden, bestätigten, dass relativ kleine O_3 Dosen ($\geq$2 mg L^{-1}) ausreichen, um die schnell reagierenden Arzneimittel zu über 90% zu oxidieren. Zusätzlich zeigten die Experimente, dass suspendierte Stoffe keinen grossen Effekt auf die Oxidation dieser Stoffe haben.

In Anbetracht der hohen Reaktivität vieler Arzneimittel gegenüber O_3 und $^{\bullet}OH$ sowie der erfolgreichen Anwendung von O_3 im Pilotmassstab, kann die Schlussfolgerung gezogen werden, dass die Ozonung sehr geeignet ist für die Verminderung der Arzneimittelkonzentration in der Trinkwasseraufbereitung oder Abwasserreinigung. Im Gegensatz dazu ist ClO_2 nur effektiv für die Oxidation spezifischer, jedoch besonders wichtiger Arzneimittelklassen, wie der Makrolid- und Sulfonamidantibiotika sowie der Östrogene.

1

General Introduction

1.1 Pharmaceuticals in the Aquatic Environment

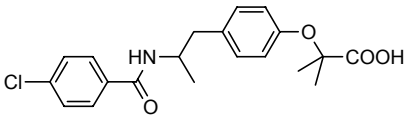
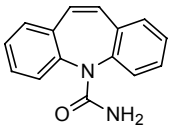
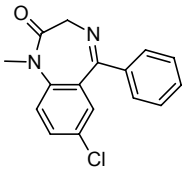
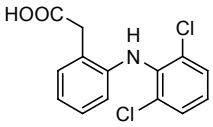
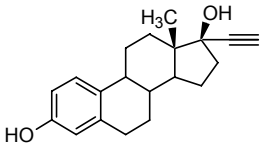
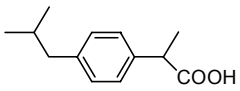
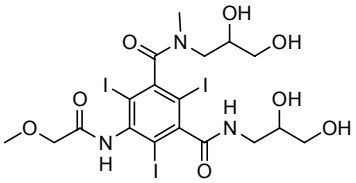
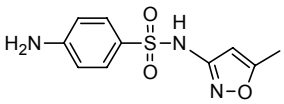
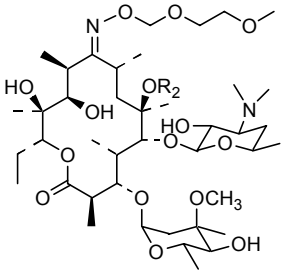
Various studies have recently shown that a large number of pharmaceuticals are ubiquitously present in surface waters that are influenced by wastewater effluents (*1-3*). Human or veterinary use is the major source for these pharmaceuticals. Table 1-1 lists the annual consumption of selected human pharmaceuticals for Switzerland and Germany. Table 1-2 reports the use and chemical structure of these compounds, which were investigated in the present thesis.

Table 1-1. Annual Consumption of Selected Pharmaceuticals in Switzerland and Germany[a]

Compound	**Switzerland (2000)**		**Germany (2001)**	
	7.3 Mio inhabitants		82.4 Mio inhabitants	
	kg/year	mg/(p × y)	kg/year	mg/(p × y)
bezafibrate	1574	216	26000	315
carbamazepine	4065	558	78000	946
diazepam	40	5	440	5
diclofenac	3887	530	49000	594
17α-ethinylestradiol	4	0.5	11	0.1
ibuprofen	15714	2160	128000	1553
iopromide	11000	1509	130000	1577
sulfamethoxazole	2572	353	47000	570
roxithromycin	149	20	6200	75

[a] source: ref (*4*)

Table 1-2. Selected Pharmaceuticals

Compound (use)	pK_a / log K_{ow} [a]	Structure
bezafibrate (lipide regulator)	pK_a: 3.6 log K_{ow}: 4.25	
carbamazepine (antiepileptic/analgesic)	pK_a: - log K_{ow}: 2.45	
diazepam (tranquilizer)	pK_a: - log K_{ow}: 2.82	
diclofenac (antiphlogistic)	pK_a: 4.2 log K_{ow}: 4.6	
17α-ethinylestradiol (oral contraceptive)	pK_a: 10.4 log K_{ow}: 3.9	
ibuprofen (antiphlogistic)	pK_a: 4.9 log K_{ow}: 3.5	
iopromide (X-ray contrast media)	pK_a: - log K_{ow}: -2.33	
sulfamethoxazole (sulfonamide antibiotic)	pK_a: 5.7 log K_{ow}: 0.48-0.89	
roxithromycin (macrolide antibiotic)	pK_a: 8.8 log K_{ow}: 2.75	

[a] source: ref (*5*)

Ingested pharmaceuticals are often excreted unchanged by the organism or in the form of easily cleavable conjugates. In the case of human use, these compounds are transported through the sewer system to the wastewater treatment plants (WWTPs). Conjugates can be cleaved and reform the parent compound by this way. Consequently, a substantial share of the consumed pharmaceuticals reaches WWTPs.

Table 1-3. Median (Maximum) Concentrations of Selected Pharmaceuticals in the Influent and Effluent of German WWTPs, and in Rivers[a]

Compound	Influent [ng L^{-1}]	Effluent [ng L^{-1}]	River [ng L^{-1}]
bezafibrate	4900 (7500)	2200 (4600)	350 (3100)
carbamazepine	2200 (3000)	2100 (6300)	250 (1100)
diazepam	< LOQ	< LOQ (40)	n.d.
diclofenac	3500 (28000)	810 (2100)	150 (1200)
17α-ethinylestradiol	3.4[b]	1 (4)[c]	< LOQ
ibuprofen	5000 (14000)	370 (3400)	70 (530)
iopromide	13000 (22000)	750 (11000)	100 (910)
sulfamethoxazole	1370 (1700)	400 (2000)	30 (480)
roxithromycin	830 (1000)	100 (1000)	< LOQ (560)

n.d. not detectable (< detection limit)

[a] source: ref (*5*)

[b] average concentration of 2 Swiss and 1 German WWTP (*6*)

[c] source: ref (*7*)

Table 1-3 gives an overview of the concentrations of selected pharmaceuticals detected in the influents and effluents of WWTPs in Germany. The fact that also wastewater effluents contain pharmaceuticals demonstrates that conventional wastewater treatment does not lead to a complete elimination of all these compounds. In a conventional activated sludge treatment, micropollutants can

be removed through biological degradation, sorption onto sludge or stripping into the air during aeration processes. For pharmaceuticals, the latter process does usually not lead to a significant removal because the relatively polar pharmaceuticals generally exhibit a very low volatility (*5*). Similarly, sorption onto sludge results for most pharmaceuticals only in a minor removal, because the sorption coefficients (K_d) of these compounds are typically relatively low (K_d < 500 L kg^{-1} (*5*)). In contrast, a significant elimination through biodegradation could be observed for many of the investigated pharmaceuticals for sludge ages >4 d (*8,9*). Generally, the degradation increased with increasing sludge age. However, even at higher sludge ages most of the compounds were not completely degraded and some pharmaceuticals (e.g., carbamazepine) were not degraded at all.

The concentration of pharmaceuticals in surface water is typically significantly lower than in effluents (see Table 1-3). The fact that pharmaceutical concentrations in streams and rivers are positively correlated with the fraction of the discharge that consists of treated wastewater (*1*) demonstrates that dilution is an important factor governing surface water concentrations. Because many pharmaceuticals are degraded to some extent in WWTPs, it can be assumed that biodegradation takes also place in the aquatic environment. Consequently, the observed pharmaceutical concentration in surface waters can be described as steady-state concentration, which is a function of the continuous input of pharmaceuticals, dilution and biodegradation.

The occurrence of pharmaceuticals in groundwater has also been reported (*10,11*). Due to dilution and degradation during bank filtration or soil passage, fewer compounds and lower concentrations of pharmaceuticals are found in groundwater than in surface water (*12*). First of all, groundwater influenced by contaminated surface water or groundwater recharged with treated wastewater is

affected. A further reason for the presence of pharmaceuticals in groundwater could also be the land application of sludge or manure contaminated with human and veterinary pharmaceuticals, respectively.

1.2 Drinking-Water Treatment

Surface water and groundwater are the principle water resources used for drinking water production. Therefore, the occurrence of pharmaceuticals in these resources could have a negative impact on the purity of drinking water. Whether the presence of pharmaceuticals in drinking water at levels found in surface water (< 1 μg/L) can produce adverse health effects is unclear up to date. However, based on precautionary principles, the concentration of these compounds in drinking water should be as low as possible to minimize the risk of unpredictable long-term effects.

Pharmaceutical concentrations in finished drinking water will depend on raw water quality and the applied treatment processes. The primary objectives of surface water treatment are:

- removal of particles
- elimination of undesired dissolved organic compounds (e.g., micropollutants and compounds causing color, taste, or odor problems)
- disinfection

Usually a combination of different treatment processes has to be applied to reach the different objectives. Table 1-4 shows, which processes are commonly applied to achieve the respective objectives.

Typically, a treatment train for surface water starts with a particle removal process followed by an oxidation and primary disinfection process. The next treatment step can consist of activated carbon filtration, either operated as a

biofilter or in an adsorptive mode. Especially after ozonation, a biological filtration is required. As a last step chlorine, chlorine dioxide, or chloramine are dosed to the water as final disinfectants.

TABLE 1-4. Treatment Objectives of Surface-Water Treatment and Commonly Applied Processes

Treatment objectives	Removal of suspended solids	Elimination of dissolved organic compounds	Disinfection
Targets	inorganic and organic particles, microorganisms	color, taste and odor compounds micropollutants DOC AOC and DBPs (formed during oxidative treatment)	viruses, bacteria, protozoa
Processes	coagulation / sedimentation flocculation/ filtration microfiltration ultrafiltration	oxidation with chlorine, ozone, or chlorine dioxide adsorption by activated carbon (GAC, PAC) biological filtration (activated carbon, slow sand filtration)	treatment with chlorine, ozone, or chlorine dioxide ultraviolet radiation ultrafiltration

abbreviations: AOC, assimilable organic matter; DBPs, disinfection by-products; GAC, granular activated carbon; PAC, powdered activated carbon

In recent years, the application of membranes in drinking water treatment has steadily increased. Ultrafiltration (UF) can be used as an additional barrier for microorganisms or to replace filtration as well as oxidative disinfection steps. In conjunction with powdered activated carbon (PAC, Cristal®-Process), UF can also be applied to remove specific micropollutants. The application of nanofiltration and reverse osmosis membranes is usually restricted to specific problems.

In contrast to surface water, groundwater requires less treatment, because it generally exhibits a lower turbidity and a better hygienic quality. Therefore, treatment is often limited to a single step that usually consists of disinfection by chlorine, chlorine dioxide or ultraviolet radiation (*13,14*). In Switzerland, it is also common practice to distribute groundwater without any treatment (*15*). Bank filtrate, a groundwater in close contact with surface water, may require more extensive treatment than higher quality groundwater.

A major objective of the European Union project POSEIDON was to assess the conventional and advanced treatment processes mentioned above with respect to their potential for removing pharmaceuticals. The main focus of the project was on processes already applied to remove dissolved organic compounds like oxidation processes (ozone, chlorine dioxide) and adsorption to activated carbon (GAC filtration, UF/PAC). Among the oxidation processes, chlorination was not studied because this process was already investigated by other research groups. Biological filtration was not investigated either, except for bank filtration. Table 1-5 lists the processes investigated in the POSEIDON project. Within the framework of this study, the present thesis focused on oxidative treatment with ozone, advanced oxidation processes (AOPs) and chlorine dioxide. In addition to the application of these processes for drinking water treatment, the use of ozonation for wastewater treatment was investigated as well.

1.3 Ozonation

1.3.1 Drinking Water

The application of ozone for the treatment of surface water is widespread in Europe, whereas in the USA the number of plants is still quite low despite the recent growth in ozone use. Table 6-1 gives an overview of the use of ozone in

Europe and North America. The most important reasons for the use of ozone are disinfection and/or oxidation. With respect to oxidation, treatment objectives encompass removal of iron and manganese as well as taste and odor control, decolorization, and oxidation of micropollutants (*16*). These objectives can often be achieved more effectively with ozone than with other oxidants like chlorine or chlorine dioxide. Furthermore, ozonation usually produces much less halogenated disinfection by-products than chlorination (*17*). The major drawback of ozonation is the formation of bromate as a consequence of high bromide concentrations (>50 μg L^{-1}) in the raw water (*18*). Bromate is a potential human carcinogen (*19*). Typical ozone dosages used for conventional drinking water production range from 1 to 3 mg L^{-1} for good quality raw waters (*16*).

TABLE 1-5. Drinking-Water Treatment Processes Investigated in the POSEIDON Project

Investigated Processes	bench-scale	pilot-scale	full-scale
bank filtration			X
coagulation/filtration	X	X	X
ozonation	X	X	X
advanced oxidation processes (AOPs)	X	X	
GAC filtration	X		X
ultrafiltration/PAC		X	
nanofiltration		X	
treatment with chlorine dioxide	X		

TABLE 1-6. Estimated Number of Drinking-Water Plants Using Ozone in Europe and North America for 1997

Country	Number of Plants	Reference
France	700	(*20*)
Germany	400	(*21*)
USA	200	(*22*)
Switzerland	100	(*20*)
Canada	70	(*23*)
United Kingdom	50	(*24*)
BENELUX	20	(*25*)

1.3.2 Municipal Wastewater

Only a limited number of WWTPs in the world uses ozonation for treatment. Most of these plants are located in the Japan, Korea, the USA and Germany (*26*). Ozone doses applied in wastewater treatment typically range from 2-15 mg L^{-1}. The primary objective of wastewater ozonation is disinfection. In various states of the USA, WWTPs are required to disinfect the treated wastewater before releasing it to receiving waters (*27*). In Europe, wastewater discharged into bathing waters has occasionally to be disinfected to meet the guideline values of the EU bathing water quality directive (*28*). Disinfection is usually also mandatory, if wastewater is used for agriculture or other reuse purposes. However, disinfection is normally performed applying chlorination, treatment with chlorine dioxide or ultraviolet radiation rather than ozonation. With the growing importance of water reuse, ozonation might become a more interesting option because ozone is not only a disinfectant but also a powerful oxidant, which can oxidize various classes of micropollutants. Furthermore, in the context of water reuse, it may also be important to avoid the formation of

halogenated disinfection by-products, which are formed to a significantly larger extent during chlorination.

1.3.3 Ozone Chemistry

Properties. The structure of ozone has been described as a resonance hybrid of the four canonical forms depicted in Figure 1-1 (*29*). Table 1-7 reports some important properties of ozone. Comparing its Henry constant with the respective constant of oxygen (720 atm M^{-1} at 20 °C (*30*)) shows that the water solubility of ozone is much higher than that of oxygen. This is important for treatment because ozone is produced in the gas phase and has to be transferred to the aqueous phase. Ozone absorbs UV light over a relatively broad range from 200-300 nm with a maximum absorption at 258 nm. Therefore, ozone in the gas phase or in pure aqueous phase can be easily measured spectrophotometrically. However, some uncertainty is associated with the decadic molar extinction coefficient ε(258 nm). The commonly applied values range from 2900 M^{-1} cm^{-1} (*31*) to 3300 M^{-1} cm^{-1} (*32*). In the present study a value of ε(258 nm) = 3000 M^{-1} cm^{-1} was used.

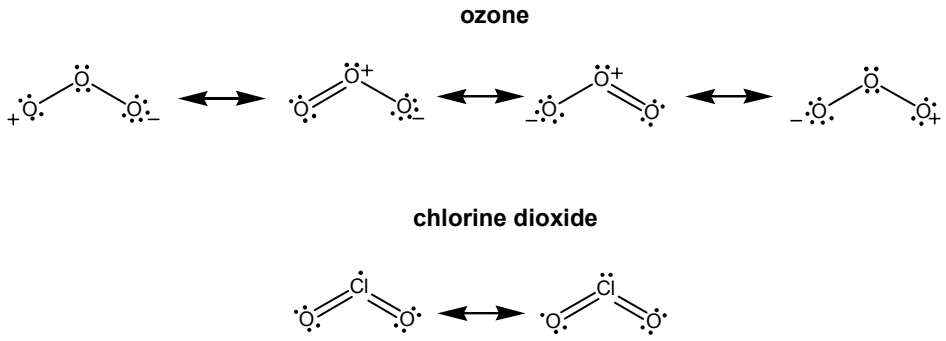


FIGURE 1-1. Chemical structures of ozone and chlorine dioxide.

Ozone has a relatively high two electron reduction potential. However, the significance of this value for the comparison with other water relevant oxidants (ClO_2, HOCl, $^{\bullet}OH$) is relatively limited because the reaction mechanisms vary

strongly among the different oxidants. Furthermore, the kinetics of electron transfer reactions is usually governed by the one electron reduction potentials (*33*).

TABLE 1-7. Selected Properties and Safety Data of Ozone and Chlorine Dioxide

Properties	**Ozone (O_3)**	**Chlorine Dioxide (ClO_2)**
molecular weight	48.0 g mol^{-1}	67.45 g mol^{-1}
melting point	- 193 °C [a]	- 59 °C [b]
boiling point	- 112 °C [a]	11 °C [b]
Henry constant (20 °C)	100 atm M^{-1} [c]	~ 0.8 atm M^{-1} [b]
diffusion coefficient	1.7×10^{-9} m^2 s^{-1} [d]	1.7×10^{-9} m^2 s^{-1} [b]
UV absorption	ε(258nm) = 3000 $M^{-1}cm^{-1}$	ε(359nm) = 1200 $M^{-1}cm^{-1}$ [e]
instant odor threshold	40 μg m^{-3} [c]	≥ 275 μg m^{-3} [g]
permissible exposure limit (averaged over 8 h workshift)	0.1 ppm (200 μg m^{-3}) [f]	0.1 ppm (275 μg m^{-3}) [f]
Swiss air pollution standard	120 μg m^{-3}	-
redoxpotential in aqueous solution	E_0^H = 2.07 V [c]	E_0^H = 1.15 V [f]
	$(O_3)_{gas} + 2H^+ + 2\ e^- = (O_2)_{gas} + H_2O$	$(ClO_2)_{gas} + e^- = ClO_2^-$
explosion hazard	> 12 – 17% (v/v) in oxygen [g]	> 300 g m^{-3} in air (= 8 g/L in H_2O at 20 °C) [b]

sources: [a] (*40*), [b] (*41*), [c] (*16*), [d] (*42*), [e] (*43*), [f] (*44*), [g] (*45*)

Ozone is a highly toxic gas. Exposure to ozone can irritate eyes, nose, and throat. Breathing ozone can irritate the lungs and cause coughing and/or shortness of breath (*34*). At higher exposures, it can cause headaches, upset stomach, vomiting, and pulmonary edema. Chronic exposure to ozone may lead to lung damage. The permissible exposure limit over a 8 h work shift is 0.1 ppm. The characteristic odor of ozone can be smelled below this concentration.

However, the ability to smell ozone may decrease after adaptation. The Swiss air pollution standard is 120 μg m^{-3}. This value should not be exceeded for more than 1 h per year. For gaseous ozone-oxygen mixtures, lower explosion limits ranging from 12-17% (v/v) are reported. This implies that ozone cannot be compacted and stored. Surprisingly, the explosive properties of ozone do in practice not affect the production of ozone-oxygen mixtures up to an ozone content of 25%.

Kinetics. Ozone reacts with a large number of inorganic and organic compounds (*16,35-39*). The fact that rate constants for the reaction with ozone range over several orders of magnitude demonstrates that ozone is a very selective oxidant. With respect to inorganic compounds, the reaction of ozone with iodide, sulfide, and sulfite approaches nearly diffusion controlled rates. The protonated species of the latter two compounds are also reactive toward ozone, but the rate constants are several orders of magnitude lower. Nitrite, cyanide, deprotonated hydrogen peroxide, and bromide are further inorganic nucleophiles that are reactive toward ozone. Furthermore, ozone oxidizes Mn(II) and Fe(II). With respect to organic compounds, ozone is particularly reactive toward phenols, amines, compounds exhibiting C=C double bonds, and activated aromatic compounds (e.g., polyaromatic compounds and benzene rings substituted with an alkoxy group or several aliphatic moieties).

With many inorganic compounds ozone reacts by an apparent oxygen transfer mechanism. Reactions with organic compounds usually proceed through ozone addition followed by fast rearrangement, which can result in the release of oxygen. Ozone reacts rarely by electron transfer reactions. Exceptions are the reactions of ozone with amines and phenols (*46,47*). Also, the reaction of ozone with chlorite seems to proceed by a one electron transfer mechanism, as indicated by the formation of chlorine dioxide (*39*).

Product Formation. Oxidation products formed during ozonation of organic compounds in aqueous solutions are relatively well known for the reaction of ozone with double bonds and phenols. For most other functional groups relatively little information is available. Reaction of ozone with double bonds proceeds through the well-established Crigee mechanism, which results in a cleavage of the double bond under the formation of ketones, aldehydes and/or carboxylic acids (*29,48*). Ozonation of phenols results first in the formation of benzoquinones, hydroquinones and muconic acid derivatives (*47*). Further oxidation leads to cleavage of the cyclic products and finally yields various acids and aldehydes (e.g., formic acid, glyoxylic acid, glyoxal) (*49*). The reaction of ozone with tertiary amines seems to yield aminoxides (probably only a transient product) or secondary amines and the corresponding aldehydes (*46*).

Ozone Decay and Hydroxyl Radicals. The stability of ozone in aqueous solution strongly depends on water quality parameters, mainly DOC, alkalinity, pH, and temperature. Various reactions of ozone with the water matrix (e.g., with hydroxide ions) lead to the formation of hydrogen peroxide. Deprotonated hydrogen peroxide reacts quickly with ozone, producing superoxide. The subsequent quick reaction of superoxide with ozone triggers a reaction sequence that ultimately yields hydroxyl radicals. Hydroxyl radicals react extremely fast with different components of the water matrix. As a result, also organic peroxyl radicals are formed that decay by releasing superoxide, which reacts with ozone to hydroxyl radicals. This radical-type chain reaction cycle is described in detail in ref (*16*).

The hydroxyl radical is a powerful but non-selective oxidant. It reacts at nearly diffusion controlled rates with various inorganic and organic components of the water matrix (*50*). Therefore, hydroxyl radicals can also contribute to the oxidation of micropollutants. However, their efficiency is most often limited by the scavenging effect of the water matrix. In ozone-based advanced oxidation

processes (AOPs), the formation of hydroxyl radicals during an ozonation process is accelerated by increasing the pH of the water, by dosing hydrogen peroxide, or by the application of UV radiation. This can ensure a faster oxidation of compounds that do not exhibit an appreciable reactivity with ozone directly. However, it has to be emphasized that the application of AOPs does not increase the overall oxidation capacity of an ozonation process (*51*). The use of an AOP only allows one to exploit the full oxidation capacity of a system within shorter time frames than in conventional ozonation processes.

A comprehensive overview of products formed by hydroxyl radical reactions in aqueous solutions is given by von Sonntag and Schuchmann (*52*). Hydroxyl radicals react with saturated organic compounds principally by H-abstraction. In the presence of oxygen, this reaction leads to the formation of peroxyl radicals, which decay either through release of superoxide or bimolecular termination. In both cases, the main products are ketones, aldehydes, and alcohols. With unsaturated compounds the reaction can proceed through H-abstraction or hydroxyl addition. In case of benzene, hydroxyl addition can lead to ring cleavage or the formation of phenol.

If results from lab-scale studies on ozonation should be applied to water treatment under realistic conditions, it is indispensable to distinguish between oxidation by ozone and by hydroxyl radicals. In lab-scale experiments, ozone reactions can be distinguished from hydroxyl radical reactions using a scavenger compound, which quenches hydroxyl radicals without promoting the chain reaction described above. Examples for scavengers are *tert*-butyl alcohol, acetone, or the inorganic ions bicarbonate and carbonate (*53*).

1.4 Treatment with Chlorine Dioxide

In Europe, chlorine dioxide is widely used for the disinfection of relatively high quality water, such as groundwater or treated surface water. Dosing of chlorine dioxide to the finished water will protect the drinking-water distribution system from microbiological recontamination and fouling. In this case, ClO_2 residuals are often kept < 0.05 – 0.1 mg L^{-1} (*54*). In the USA, ClO_2 is rather used for the preoxidation of surface waters. Common ClO_2 dosages range from 1 to 1.4 mg L^{-1} (*54,55*). Furthermore, ClO_2 is used for the disinfection of wastewater.

Compared to disinfection with chlorine, chlorine dioxide has several advantages: it is more effective than chlorine for the inactivation of protozoa, its biocidal properties are not influenced by pH, and it does not react with ammonia. Furthermore, chlorinated and brominated disinfection by-products are not formed under proper generation condition (*56*). Compared to ozone, chlorine dioxide is more stable in water and provides residuals, which are required for the protection of distribution systems. However, ozone is more effective for the inactivation of microorganisms. Used for preoxidation, chlorine dioxide can control taste and odor and oxidize iron and manganese. However, the application of ClO_2 is limited by the formation of chlorite, which is considered a blood poison. The USEPA standard of 1 mg L^{-1} for chlorite limits chlorine dioxide dosages to approximately 1.4 mg L^{-1}. In Switzerland, chlorine dioxide can only be used for preoxidation in combination with other oxidants (e.g., chlorine) due to a more stringent chlorite standard of 0.2 mg L^{-1}.

1.4.1 Chlorine Dioxide Chemistry

Chlorine dioxide is a stable free radical (for structure see Figure 1-1). Selected properties of chlorine dioxide are listed in Table 1-7. Its water solubility is considerably higher than that of ozone. Therefore, highly concentrated solutions of chlorine dioxide could be produced. However,

chlorine dioxide is explosive in the gas phase at concentrations >300 g m^{-3}. Such concentrations build up in the headspace of aqueous solutions with chlorine dioxide concentrations >8 g/L at 20 °C. In the gas phase or in pure aqueous solution, chlorine dioxide can be determined spectrophotometrically due to its UV absorbance with a maximum at 359 nm.

Chlorine dioxide is a highly toxic gas. The permissible exposure limit for a 8-hour workshift is 0.1 ppm. Exposure can irritate the eyes, nose and throat. Breathing chlorine dioxide can irritate the lungs causing coughing and/or shortness of breath. Higher exposure can cause pulmonary edema (*34*).

Chlorine dioxide reacts fast with the nucleophilic inorganic compounds nitrite, iodide, sulfide, sulfite and hydrogen peroxide anion, but also with Fe(II) and Mn(II) (*43*). Because chlorine dioxide does not oxidize chloride and bromide, halogenated disinfection by-products typically formed in the presence of hypochlorous or hypobromous acids are not produced during treatment with chlorine dioxide. Additionally, chlorine dioxide is also unreactive toward hypochlorous acid so that treatment with a mixture of chlorine dioxide and chlorine is a viable process for disinfection and oxidation. With respect to organic compounds, chlorine dioxide exhibits a high reactivity toward phenoxide ions, neutral tertiary amines and certain polyaromatic compounds (*43,57*). Figure 1-2 compares the rate constants for the reaction of ozone and chlorine dioxide with four organic chemicals representative for the respective compound classes.

Most of the inorganic and organic reactions of chlorine dioxide result in its reduction to chlorite through one-electron transfer. The relatively few studies that have been performed on organic oxidation products formed during treatment with chlorine dioxide have been summarized by Rav-Acha (*58*). Phenolic compounds are normally oxidized to benzoquinones, which are

sometimes reduced to hydroquinones after treatment. To what extent and by which mechanisms chlorinated benzoquinones are formed is unclear. *p*-Benzoquinones are also a major oxidation products of the reaction of chlorine dioxide with aniline and its derivatives (*59*). Treatment of tertiary amines with chlorine dioxide usually leads to a dealkylation. The resulting products are a secondary amine and the respective aldehyde (*60*).

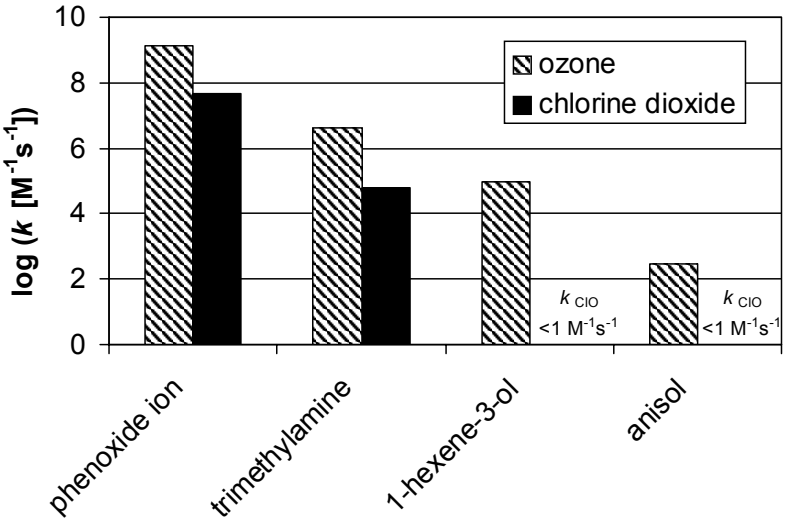


Figure 1-2. Comparison of rate constants for the reaction of ozone and chlorine dioxide with four chemicals representative for phenoxide ions, tertiary amines, compounds exhibiting C=C double bonds, and alkoxy benzenes. For trimethylamine, the absolute rate constant for the neutral species is given.

1.5 Kinetic Concepts

Major parts of the present thesis deal with oxidation kinetics. First, this section provides a short introduction to the kinetic concept on which this thesis is based, second, it presents the basic principles of the methods used for the determination of kinetic constants, and third, it explains the prediction of the oxidation of pharmaceuticals during water-treatment processes.

The oxidation of pharmaceuticals (P) during treatment with an oxidant (Ox) can be described by the following reaction:

$$P + \eta\, Ox \xrightarrow{k_{Ox}} products \quad (1)$$

where k_{Ox} is the second-order rate constant and η is the stoichiometric factor that determines the moles of Ox consumed per mole of converted P. If the reaction rate is first order with respect to the oxidant and target compound concentrations, the loss of P and Ox per time is given by the following rate laws:

$$-\frac{d[P]}{dt} = k_{Ox} \cdot [P] \cdot [Ox] \quad (2)$$

$$-\frac{1}{\eta}\frac{d[Ox]}{dt} = k_{Ox} \cdot [P] \cdot [Ox] \quad (3)$$

If P is an acid that reacts according to

$$PH \leftrightarrows P^- + H^+ \quad (4)$$

eq 2 can be modified to include the specific rate constants for the reaction of the oxidant with the acid ($k_{Ox,PH}$) and the respective anion (k_{Ox,P^-}) of the target compound P:

$$-\frac{d[P]_{tot}}{dt} = (\alpha \cdot k_{Ox,PH} + (1-\alpha) k_{Ox,P^-}) \cdot [P]_{tot} \cdot [Ox] \quad (5)$$

The degree of dissociation (α) can be calculated with the help of the dissociation constant (K) and the pH:

$$\alpha = \frac{1}{1 + \frac{K}{[H^+]}} \quad (6)$$

Equations for basic compounds can be developed accordingly.

For pH-dependent reactions, the overall reactivity of a compound at a certain pH can be expressed by the apparent second-order rate constant (k_{app}) that is defined as:

$$k_{app} = \alpha \cdot k_{Ox,PH} + (1-\alpha) \cdot k_{Ox,P^-} = \frac{1}{1+\frac{10^{-pK}}{10^{-pH}}} \cdot k_{Ox,PH} + (1-\frac{1}{1+\frac{10^{-pK}}{10^{-pH}}}) \cdot k_{Ox,P^-} \qquad (7)$$

To determine $k_{Ox,P-}$ from measured values of k_{app}, k_{app} is plotted versus α. The slope of the resulting straight line yields $k_{Ox,P-}$ (for an example see Figure 1-3 (a)). To predict the pH dependency of a reaction over a larger pH range, k_{app} can be calculated based on $k_{Ox,PH}$ and $k_{Ox,P-}$. To illustrate this pH dependency, the logarithm of k_{app} can be plotted versus pH (for an example see Figure 1-3 (b)).

1.5.1 Determination of Rate Constants under Pseudo-first-order Conditions

For the determination of accurate and precise second-order rate constants, pseudo-first- order conditions with either P or Ox in excess are most appropriate because they allow a direct measurement of the rate constants. For $[Ox]_0 >> [P]_0$ it follows that $[Ox]_t \approx \text{const} \approx [Ox]_0$. Using this simplification, the solution of eq 2 yields

$$\ln\left(\frac{[P]}{[P]_0}\right) = -k_{obs} \cdot t \quad \text{with} \quad k_{obs} = k_{Ox} \cdot [Ox]_0 \qquad (8)$$

where k_{obs} is the pseudo-first-order rate constant. Applying above specified conditions, the decrease of P as a function of time can be determined experimentally. To evaluate the results, the left side of eq 8 has to be plotted versus t. The slope of the resulting straight line represents k_{obs}, which can be converted to a second-order rate constant by dividing k_{obs} by $[Ox]_0$. Figure 1-4 depicts a logarithmic first-order plot for the reaction of ozone with bezafibrate. Experiments can also be conducted with $[P]_0 >> [Ox]_0$ by monitoring the decay of Ox. However, in this case $k_{obs} = \eta\ k_{Ox}\ [P]_0$. Therefore, the stoichiometric coefficient has to be known to determine the intrinsic second-order rate constant. With the presented method, second-order rate constants up to 1000-10000 $M^{-1}\ s^{-1}$

can be measured using batch experiments. For acidic or basic compounds the determined rate constants can often be extrapolated, if the pK_a of the target compound and the pH dependency of the reaction rate is known (see Figure 1-3).

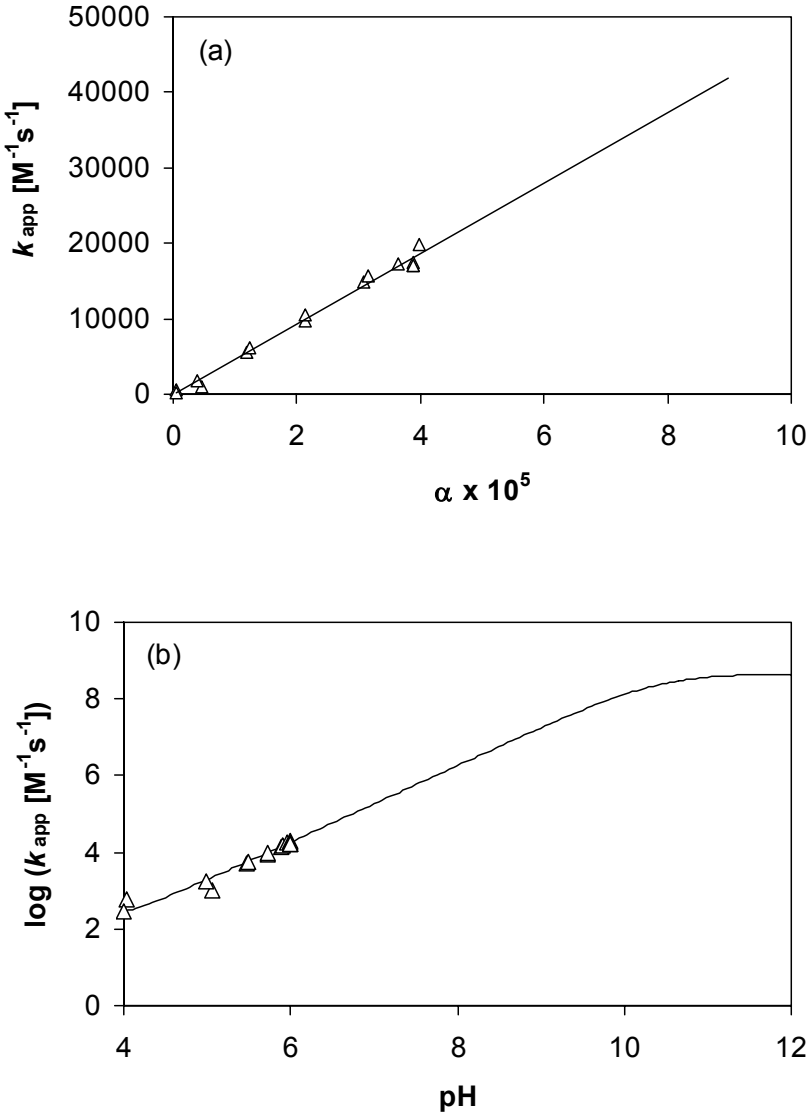


Figure 1-3. The pH dependency of the apparent second-order rate constant (k_{app}) for the reaction of EE2 with chlorine dioxide. (a) k_{app} plotted versus the degree of dissociation (α) of EE2. b) Log k_{app} plotted versus pH. The triangles represent measured data, the line in a) was obtained by linear regression, and the line in (b) represents the prediction of k_{app} based on a pK_a of 10.4, the fitted $k_{Ox,P-}$, and an estimated rate constant of $k_{Ox,PH}$ ≈ 100 $M^{-1}s^{-1}$ for neutral EE2.

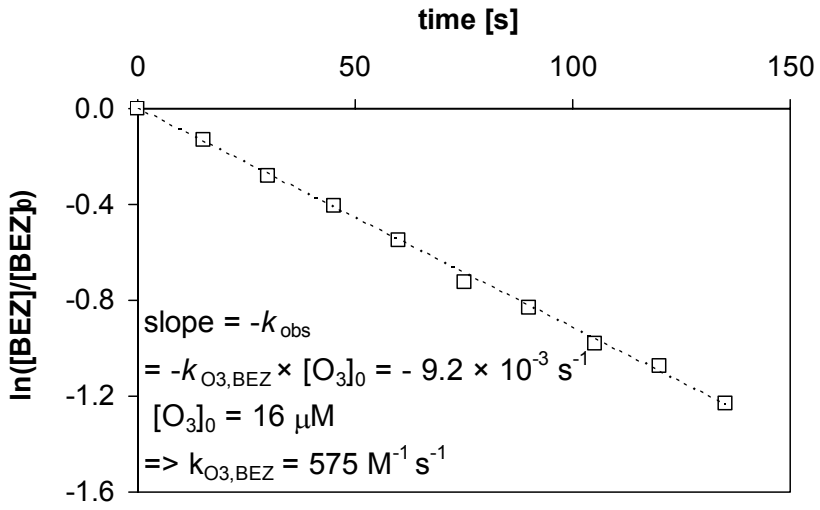


Figure 1-4. Logarithmic first-order decrease of bezafibrate (BEZ) at $[O_3]_0$ = 16 μM.

1.5.2 Competition Kinetics: Method 1

For the determination of rate constants > 1000-10000 M^{-1} s^{-1} more sophisticated apparatus (e.g., stopped-flow or quench-flow systems) have to be used. However, the application of such methods is only relatively easy as long as the oxidant, the target compound or an oxidation product can be monitored spectrophotometrically. Due to spectral interferences of these compounds, especially in case of aromatic compounds, this is often not possible. A relatively simple alternative to such systems is competition kinetics. If neither compound is in excess, the solution of eq 2 can be written as:

$$\frac{1}{k_{Ox}} \ln\left(\frac{[P]}{[P]_0}\right) = -\int [Ox]dt \qquad (9)$$

where $\int [Ox]dt$ is the oxidant exposure (concentration integrated over time). If besides P and Ox a reference compound R is present in a batch experiment, R will experience the same oxidant exposure as P. Therefore, we can write:

$$\frac{1}{k_{Ox}}\ln\left(\frac{[P]}{[P]_0}\right)=\frac{1}{k_{Ox,R}}\ln\left(\frac{[R]}{[R]_0}\right) \quad \longrightarrow \quad \ln\left(\frac{[P]}{[P]_0}\right)=\frac{k_{Ox}}{k_{Ox,R}}\ln\left(\frac{[R]}{[R]_0}\right) \qquad (10)$$

where $k_{Ox,R}$ is the second-order rate constant for the reaction of R with the oxidant. To obtain varying oxidant exposures and, consequently, varying extents of parent compound oxidation, the oxidant dose or the reaction time can be varied. For a series of such experiments, a plot of $\ln\left(\frac{[P]}{[P]_0}\right)$ versus $\ln\left(\frac{[R]}{[R]_0}\right)$ yields a straight line with the slope $\frac{k_{Ox}}{k_{Ox,R}}$. If $k_{Ox,R}$ is known, k_{Ox} can be easily calculated. Eq 10 was successfully applied to determine hydroxyl radical and ozone rate constants. Figure 1-5 and 1-6 illustrate the evalution of rate constants according to this competition kinetic method for ozone and hydroxyl radical reactions, respectively.

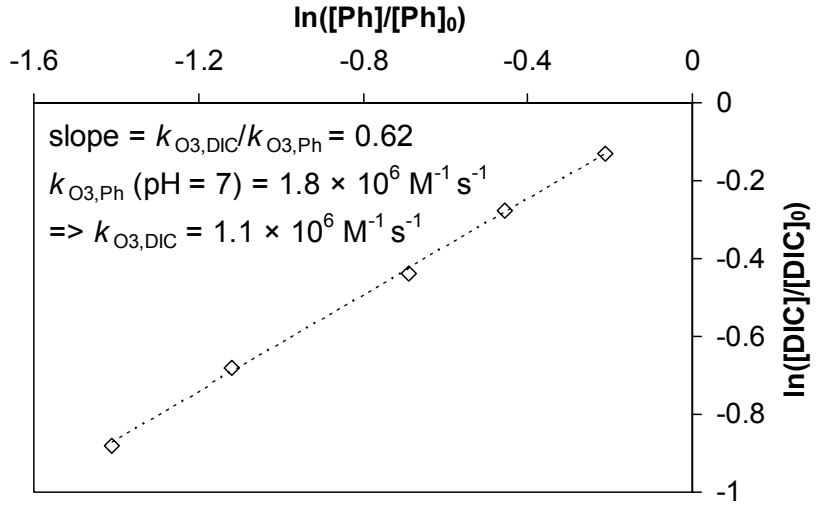


Figure 1-5 Evaluation of the second-order rate constant for the reaction of diclofenac (DIC) with ozone at pH 7. Phenol (Ph) was used as reference compound.

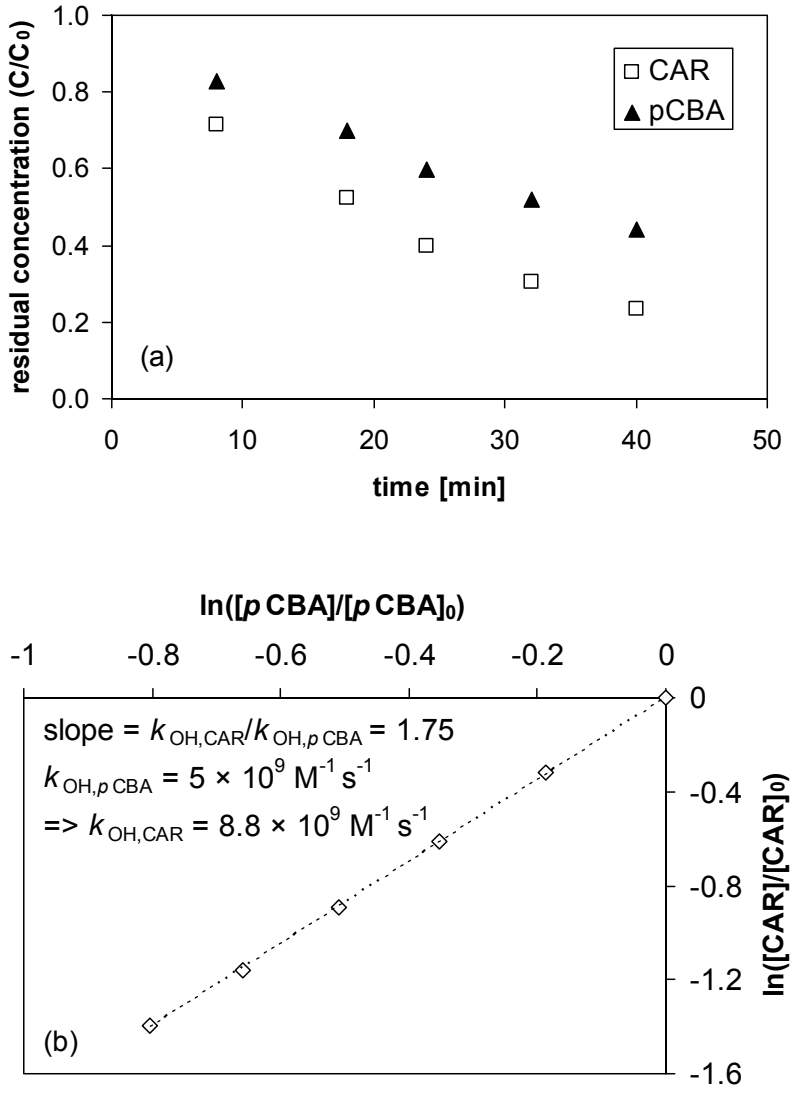


FIGURE 1-6 Evaluation of the second-order rate constant for the reaction of carbamezepine (CAR) with hydroxyl radicals. *para*-Chlorobenzoic acid (*p*CBA) was used as reference compound. (a) Decrease of CAR and *p*CBA as a function of time. (b) Double logarithmic plot of the residual concentrations of CAR versus *p*CBA.

1.5.3 Competition Kinetics: Method 2

If the investigated pharmaceutical compound or the reference compound cannot be easily determined, another approach developed by Muñoz and von

Sonntag (*61*) can be used under the condition that either P or R produces a stable oxidation product that can be measured. In the following, we assume that R produces a measurable oxidation product (OR). For a given initial ozone dose under the condition $[Ox]_0 << [R]_0$ and $[P]_0$, the ratio of the OR yields of a batch system with R in the presence of P ($[OR]_{R+P}$) and in its absence ($[OR]_R$) will be

$$\frac{[OR]_{R+P}}{[OR]_R} = \frac{\eta_R \cdot k_{Ox,R} \cdot [R]}{\eta_R \cdot k_{Ox,R}[R] + \eta_P \cdot k_{Ox}[P]} \tag{11}$$

where η_R and η_P are the stoichiometric coefficients for the reaction of the oxidant with R and P, respectively. Rearranging eq 11 yields

$$\frac{[OR]_R}{[OR]_{P+R}} = \frac{\eta_R \cdot k_{Ox,R} \cdot [R] + \eta_P \cdot k_{Ox} \cdot [P]}{\eta_R \cdot k_{Ox,R} \cdot [R]} = 1 + \frac{\eta_P \cdot k_{Ox} \cdot [P]}{\eta_R \cdot k_{Ox,R} \cdot [R]} \tag{12}$$

For the determination of a rate constant, experiments with varying [P]/[R] ratios and at least one experiment without P have to be conducted. Plotting the left side of eq 12 versus [P]/[R] ratio will yield a straight line with the slope $\frac{\eta_P \cdot k_{Ox}}{\eta_R \cdot k_{Ox,R}}$ from which k_{Ox} can be calculated. In Figure 1-7, the application of this method is illustrated taking the determination of the ozone rate constant of carbamazepine as an example.

1.5.4 Predicting the Extent of Oxidation for Micropollutants

The solution of eq 2 can be used to predict the oxidation of pharmaceuticals in the presence of a single oxidant:

$$\frac{[P]}{[P]_0} = e^{-k_{Ox} \cdot \int [Ox] dt} \tag{13}$$

The precondition for such predictions is that k_{Ox} and the oxidant exposure are known. For oxidants like ozone and chlorine dioxide, the exposure can be calculated by measuring the time-dependent oxidant concentration in the investigated water and integrating the concentrations over time. In the case of

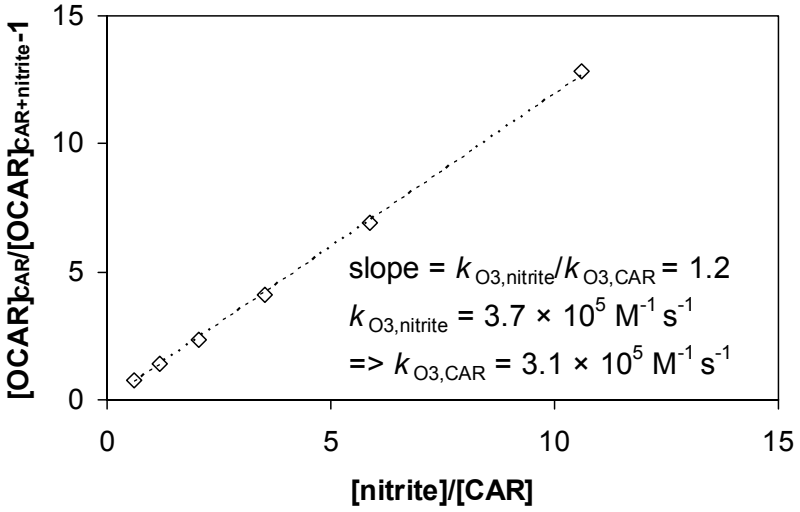


FIGURE 1-7. Evalution of the second-order rate constant for the reaction of ozone with carbamazepine (CAR). Nitrite was used as reference compounds. Instead of measuring nitrate, the oxidation product of nitrite, the oxidation product of carbamazepine (OCAR) was determined due to a simpler analysis method. Therefore, carbamezepine represents R and nitrite P in eq 12. Stoichiometric coefficients = 1 for both compounds.

hydroxyl radicals ($^{\bullet}$OH), concentrations are so low that they cannot be measured directly. However, it is possible to determine hydroxyl radical exposures with the help of a probe compound (R) for which the rate constant with $^{\bullet}$OH (k_{OH}) is known (*62*). The calculation of the exposure is performed using a rearranged form of eq 13.

$$\int [^{\bullet}OH]dt = -\frac{1}{k_{OH}} \ln\left(\frac{[R]}{[R]_0}\right) \quad (14)$$

In ozonation processes, at least two oxidants, namely ozone and hydroxyl radicals are present at the same time. In this case, predictions of the extent of oxidation of P can be made using the following equation:

$$\ln\left(\frac{[P]}{[P]_0}\right) = -k_{O3} \cdot \int [O_3]dt - k_{OH} \cdot \int [^{\bullet}OH]dt \quad (15)$$

Elovitz and von Gunten (*39,62*) found out that the ratio of the exposures is usually constant for most of the duration of an ozonation process:

$$\frac{\int [^{\bullet}OH]dt}{\int [O_3]dt} = \frac{[^{\bullet}OH]}{[O_3]} = R_c \qquad (16)$$

Combining eq 15 and eq 16 yields:

$$\ln\left(\frac{[P]}{[P]_0}\right) = -(k_{O3} + k_{OH} \cdot R_C) \cdot \int [O_3]dt \qquad (17)$$

Under the condition that R_c is constant, it is possible to calculate the contribution of hydroxyl radicals to the overall oxidation of a pharmaceuticals compound by the following equation:

$$f(^{\bullet}OH) = \frac{k_{OH} \cdot R_C}{k_{O3} + k_{OH} \cdot R_C} \qquad (18)$$

Estimating the fraction of a pollutant oxidized by hydroxyl radicals can be important to assess the product distribution of an ozonation process.

1.6 Objectives

The major objective of the present thesis was to assess oxidative treatment with ozone and chlorine dioxide with respect to its potential for the elimination of pharmaceuticals present in drinking water resources or in wastewater. The specific objectives were:

- to determine rate constants for the reaction of ozone, hydroxyl radicals and chlorine dioxide with the selected pharmaceuticals.
- to test whether the rate constants determined in pure aqueous solutions can be applied to predict the oxidation of pharmaceuticals in natural waters.

- to identify oxidation products of a relevant compound during ozonation.
- to test these oxidation products for remaining pharmacological effects.
- to conduct pilot-experiments in wastewater to investigate the behavior of pharmaceuticals under realistic treatment conditions and to identify the crucial parameters governing the efficiency of the oxidation process.

1.7 Outline

The results are presented in the following way (in brackets the corresponding publication):

Chapter 2 reports the second-order rate constants determined for the reaction of ozone and hydroxyl radicals with the selected pharmaceuticals. Further, experiments are presented that compare the predicted and measured oxidation of selected pharmaceuticals in natural waters. (Huber, M. M.; Canonica, S.; Park, G-Y.; von Gunten, U.: Oxidation of Pharmaceuticals during Ozonation and Advanced Oxidation Processes, *Environ. Sci. Technol.* **2003**, 37, 1016-1024.)

In Chapter 3, determined and estimated second-order rate constants for the reaction of chlorine dioxide with various pharmaceuticals are presented. The determined rate constants were verified under realistic treatment conditions by simulating preoxidation of lake water or disinfection of groundwater. Furthermore, a comparison of apparent rate constants for the reaction of ozone, chlorine dioxide and chlorine with selected pharmaceuticals is shown. (Huber, M. M.; Korhonen, S.; Ternes, T. A.; von Gunten, U.: Oxidation of Pharmaceuticals during Water Treatment with Chlorine Dioxide, *Water Res.* **2004**, submitted.)

Chapter 4 illustrates the effect of ozonation on pharmacological effects of pharmaceuticals. 17α-Ethinylestradiol was selected for this purpose because of its high biological activity and its ecotoxicological relevance. It is also shown, which oxidation products are formed during ozonation of this synthetic hormone. (Huber, M. M.; Ternes, T. A.; von Gunten, U.: Removal of Estrogenic Activity and Formation of Oxidation Products during Ozonation of 17α-Ethinylestradiol, *Environ. Sci. Technol.* **2003**, 38, 5177-5186.)

Chapter 5 presents the pilot-scale application of wastewater ozonation. This chapter focuses on the effect of ozone dosage and suspended solids on the oxidation process and the predictability of pharmaceutical oxidation in wastewater. (Huber, M. M.; Göbel, A.; Joss, A.; et al.: Oxidation of Pharmaceuticals during Ozonation of Municipal Wastewater Effluents: A Pilot Study, *Environ. Sci. Technol.* **2004**, submitted.)

In Chapter 6, the results of the present thesis are summarized and put into relation with the results for other treatment processes that were investigated in the POSEIDON project.

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2

Oxidation of Pharmaceuticals during Ozonation and Advanced Oxidation Processes

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Abstract

This study investigates the oxidation of pharmaceuticals during conventional ozonation and advanced oxidation processes (AOPs) applied in drinking water treatment. In a first step, second-order rate constants for the reactions of selected pharmaceuticals with ozone (k_{O3}) and hydroxyl radicals (k_{OH}) were determined in bench-scale experiments (in brackets apparent k_{O3} at pH = 7 and T = 20 °C): bezafibrate (590 ± 50 M^{-1} s^{-1}), carbamazepine (~3 × 10^5 M^{-1} s^{-1}), diazepam (0.75 ± 0.15 M^{-1} s^{-1}), diclofenac (~1 × 10^6 M^{-1} s^{-1}), 17α-ethinylestradiol (~3 × 10^6 M^{-1} s^{-1}), ibuprofen (9.6 ± 1 M^{-1} s^{-1}), iopromide (<0.8 M^{-1} s^{-1}), sulfamethoxazole (~2.5 × 10^6 M^{-1} s^{-1}), and roxithromycin (~7 × 10^4 M^{-1} s^{-1}). For five of the pharmaceuticals the apparent k_{O3} at pH 7 was >5 × 10^4 M^{-1} s^{-1}, indicating that these compounds are completely transformed during ozonation processes. Values for k_{OH} ranged from 3.3 to 9.8 × 10^9 M^{-1} s^{-1}. Compared to other important micropollutants such as MTBE and atrazine, the selected pharmaceuticals reacted about two to three times faster with hydroxyl radicals. In the second part of the study, oxidation kinetics of the selected pharmaceuticals was investigated in ozonation experiments performed in different natural waters. It could be shown that the second-order rate constants determined in pure aqueous solution could be applied to predict the behavior of pharmaceuticals dissolved in natural waters. Overall it can be concluded that ozonation and AOPs are promising processes for an efficient removal of pharmaceuticals in drinking waters.

2.1 Introduction

In recent years, there has been growing concern about the occurrence of pharmaceuticals in the aquatic environment. Comprehensive review articles on the environmental relevance of pharmaceuticals have recently been published by Halling-Sørensen et al. (*1*), and Daughton and Ternes (*2*). Moreover, several studies have reported the occurrence of a great variety of pharmaceuticals in surface waters (*3-5*).

Surface water is widely used as water resource for drinking water. Therefore, the widespread occurrence of pharmaceuticals in surface waters may pose a problem to water utilities. Only a few pharmaceuticals have been detected in drinking waters so far (*6,7*). Concentrations were typically in the lower nanogram/L-range. Up to now, there has been no proof that very low concentrations of pharmaceuticals have any adverse health effects. Nevertheless, based on precautionary principles, drinking water should be free from these compounds to minimize the risk of unpredictable long-term effects. Hence, it is important to assess water treatment processes with regard to their potential for removing pharmaceuticals. Only limited information is available concerning this question. In a recent study, the removal of some selected pharmaceuticals during drinking water treatment was investigated in lab, pilot and full-scale experiments (*8*). It was demonstrated that among different treatment steps only ozonation and filtration with granular activated carbon were effective in removing pharmaceuticals. The potential of ozonation and advanced oxidation processes (AOPs) for removing pharmaceuticals was confirmed in another study (*9*). However, both studies were performed using natural water samples, yielding case-specific information on the removal efficiency. To assess the removal efficiency of ozonation and AOPs in different natural waters, it is indispensable to determine the rate constants for the reaction of pharmaceuticals

with the oxidants, i.e., ozone (O_3) and hydroxyl radicals ($^{\bullet}OH$). In addition, information about oxidant concentrations is required.

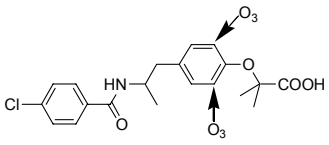
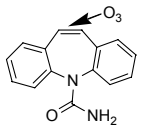
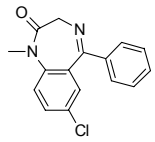
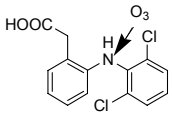
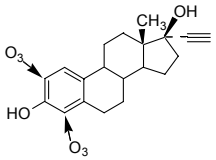
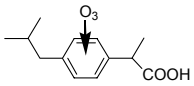
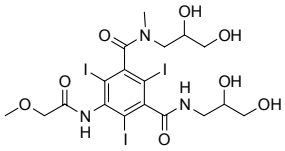
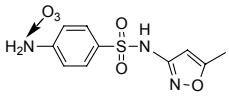
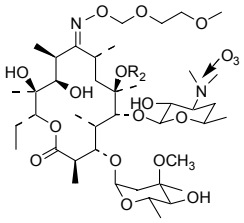
The aim of the research presented here was to assess the potential of ozonation and AOPs for the oxidation of pharmaceuticals. A list of nine pharmaceuticals was selected based on consumption and environmental relevance (see Table 2-1). A major task of this study was to establish a database with second-order rate constants for the reactions of the selected pharmaceuticals with O_3 and $^{\bullet}OH$. The determination of the rate constants was carried out in bench-scale systems in pure aqueous solution. To predict the oxidation of micropollutants during ozonation processes, Elovitz and von Gunten (*10*) developed the R_{ct}-concept, which allows the prediction of the time-dependent transformation of a compound based on rate constants and oxidant behavior. The R_{ct}-concept was successfully applied to predict the oxidation of atrazine (*11*) and MTBE (*12*) and the formation of their oxidation products during ozonation and AOPs. In the present study, this concept was applied to the selected pharmaceuticals in order to show the effect of the water matrix on the oxidation efficiency. To apply the R_{ct}-concept, experiments were performed with surface and ground waters under realistic treatment conditions.

2.2 Materials and Methods

2.2.1 Standards and Reagents

Bezafibrate, carbamazepine, diclofenac, ibuprofen, sulfamethoxazole and roxithromycin were obtained from Sigma-Aldrich with a purity higher than 99% (roxithromycin > 91%). Iopromide and 17α-ethinylestradiol were provided by Schering/Berlin, Germany. Diazepam was offered by Roche AG/Basel, Switzerland. Stock solutions of these pharmaceuticals were prepared with Milli-Q water (Millipore). All chemicals used for solutions (buffer, eluents, etc.) were

TABLE 2-1. Selected Pharmaceuticals (arrows show the sites of the molecules where ozone attack can be expected)

Compound	Bezafibrate	Carbamazepine	Diazepam
Use	lipid regulator	antiepileptic/analgesic[a]	tranquilizer
Structure			
Compound	Diclofenac	17α-Ethinylestradiol	Ibuprofen
Use	antiphlogistic	ovulation inhibitor	antiphlogistic
Structure			
Compound	Iopromide	Sulfamethoxazole	Roxithromycin
Use	contrast medium	antibiotic	antibiotic
Structure			

[a] Used in the treatment of nerve pain

reagent grade and were used without further purification. Ozone (O_3) stock solutions (~1 mM) were produced by sparging O_3-containing oxygen through Milli-Q water that was cooled in an ice bath (*13*). For some experiments, O_3 stock solutions were prepared without cooling to obtain less concentrated solutions.

TABLE 2-2. Water Quality Parameters

	pH	DOC ($mg\ L^{-1}$)	alkalinity ($mM\ HCO_3^-$)
lake water, Zurich (LZ water)	7.9	1.2	2.6
bank filtrate River Seine, Paris (RS water)	7.8	1.3	4.1
well water, Porrentruy (WP water)	7.2	0.8	5.8
lake water, Finland (LF water)	7.5	3.7	0.7

2.2.2 Natural Water Systems

To simulate real treatment conditions, experiments were performed using four natural waters that differed in dissolved organic carbon content (DOC) and alkalinity. They covered the range of typical raw waters used for drinking water production in Europe (for water quality parameters, see Table 2-2): (i) raw water from Lake Zurich, Switzerland collected from a depth of 30 m was obtained from a drinking water plant in Zurich (LZ water); (ii) bank filtrate from River Seine was received from a drinking water plant in Paris, France (RS water); (iii) filtered well water was provided by a drinking water plant in Porrentruy, Switzerland (WP water); (iv) flocculated, sandfiltered water from a lake in Finland was received from Tampere University of Technology, Finland (LF water). All waters were filtered (0.45 μm cellulose nitrate) upon arrival and stored at 4 °C until use.

2.2.3 Analytical Methods

All pharmaceuticals and *para*-chlorobenzoic acid (*p*CBA) used as reference compound for the determination of $^{\bullet}OH$ rate constants and R_c-values were determined by high-performance liquid chromatography (HPLC, Hewlett-Packard, 1050 series) equipped with a variable wavelength detector. Eluents

consisted of 10 mM phosphoric acid and methanol or acetonitrile. Depending on compounds and experiments, isocratic or gradient elutions were used with varying eluent ratios (column: Nucleosil 100, 5 μm C18, Machery-Nagel). The sample volumes injected varied from 25 to 250 μL depending on concentrations analyzed. Quantification limits of about 0.05-0.1 μM (10-40 μg L^{-1}) were achieved. The 95% confidence intervals for a single measurement were typically ± 3-10% and the recoveries in natural waters ranged from 95% to 100%. Dissolved O_3 was determined with the indigo method (*13*) or spectrophotometrically by measuring the absorbance at 258 nm (ε = 3000 M^{-1} cm^{-1}).

2.2.4 Determination of Rate Constants for the Reaction of Pharmaceuticals with Ozone

All experiments were performed in Milli-Q water using *tert*-butyl alcohol (10-50 mM) as $^{\bullet}OH$ scavenger. The solutions were adjusted to the desired pH with phosphate buffer (5 or 50 mM). If not stated otherwise, experiments were carried out at 20 °C. The second-order rate constants were determined under conditions where either O_3 or the target compound was in excess.

Ozone in Excess. Experiments with *bezafibrate, diazepam and ibuprofen* were conducted with O_3 in excess at pH 6. As a reaction vessel, 500 ml glass bottles with a dispenser system mounted onto the screwtop were used (*14*). For *bezafibrate*, the kinetic runs were started by adding 10 mL of the O_3 stock solution to the solution containing *bezafibrate* (1 μM), yielding a final O_3 concentration of 20 μM (1mg L^{-1}). After 20 s, the first sample (5 mL) was withdrawn with a dispenser system. Subsequently, sampling was performed in 15 s time intervals. The O_3 residual was quenched immediately by adding 0.1 mL of a fresh sodium sulfite solution (24 mM). After 2.5 min the last sample was directly transferred into a UV-cell without adding sulfite and O_3 was

determined spectrophotometrically. Preliminary experiments performed under the same conditions showed that O_3 decrease is < 5% during the sampling period of 2.5 min. Therefore, it was assumed that the O_3 concentration remained constant during the experiments. The samples were immediately analyzed by HPLC. To determine the activation energy for the reaction of O_3 with bezafibrate, the same experiments were carried out at 5, 10, and 15 °C.

For *diazepam* and *ibuprofen*, the procedure for bezafibrate had to be adapted to higher O_3 concentrations (0.2 and 0.1 mM, respectively) and longer sample intervals (10 and 5 min, respectively) making it necessary to measure the O_3 decay. In this case, the data were evaluated by plotting pharmaceutical concentrations versus O_3 exposure, i.e., O_3 concentration integrated over time. The slope of the resulting straight line represented the rate constant.

Pharmaceuticals in Excess. Second-order rate constants for *iopromide* and *roxithromycin* as well as the *activation energy for ibuprofen* were determined under conditions where the pharmaceutical compounds were in excess. In this case, the O_3 decrease was monitored instead of the disappearance of the target compound. Due to the absorption of *iopromide* and *ibuprofen* in the range of 258 nm, O_3 could not be quantified by measuring the direct UV absorption at 258 nm. Therefore, the indigo method was applied for these experiments (*15*). To determine the *activation energy of ibuprofen*, experiments were carried out at 5-25 °C. Since *roxithromycin* exhibits no UV absorption at 258 nm, the O_3 concentration could be determined by measuring its UV absorption. Kinetic runs were performed in spectrophometric cells of 35 mL volume with Suprasil® quartz window and 10 cm optical pathlength. The cell was mounted in a temperature-stabilized metal block inside the spectrophotometer (Kontron Instruments, Uvikon 940). The O_3 decay was monitored directly in the spectrophotometer with a sampling interval of 1.2 s. Due to the pH-dependent rate constant, pH values ranging from 4.5 to 3.3 were selected. The resulting O_3

half-lives ranged from 0.2 to 5 min. The experimental setup did not allow the measurement of shorter half-lives, which would occur at higher pH values

Competition Kinetics. The methods described above are limited to rate constants that are lower than about 1000 M^{-1} s^{-1}. Only pH-dependent rate constants can be extrapolated to higher values. In this study, competition kinetics was used to determine high rate constants. For *17α-ethinylestradiol*, phenol ($k_{O3,phenolate}$ = 1.4 × 10^9 M^{-1} s^{-1}, pK_a = 9.9 (*16*)) was selected as a reference compound because a similar reaction mechanism and a similar rate constant were expected. The experiments were carried out in 25 mL serum vials at pH 6 with solutions containing equal concentrations of *17α-ethinylestradiol* and reference compound (4 μM). Different understoichiometric concentration levels of O_3 ranging from 1.5 to 7.5 μM were added with a glass syringe to a series of serum vials. During O_3 injection the solutions were vigorously stirred. Remaining concentrations of target and reference compound in the serum vials were then analyzed by HPLC. The data were evaluated based on eq 1, where k_{O3}(R) and k_{O3}(M) are the rate constants for the reference (R) and target compound (M), respectively. The different O_3 doses are represented by n.

$$\ln\left(\frac{[\mathrm{M(n)}]}{[\mathrm{M(0)}]}\right) = \ln\left(\frac{[\mathrm{R(n)}]}{[\mathrm{R(0)}]}\right)\frac{k_{\mathrm{O3}}(\mathrm{M})}{k_{\mathrm{O3}}(\mathrm{R})} \qquad (1)$$

The apparent rate constant k_{O3}(M) could be determined from a plot of ln([M(n)]/[M(0)] versus ln([R(n)]/[R(0)] with k_{O3}(M)/k_{O3}(R) as the slope of the straight line. To confirm the results, the same experiments were also performed with the pairs *17α-ethinylestradiol*/4-chlorophenol and phenol/4-chlorophenol. The second-order rate constant for the dissociated 17α-ethinylestradiol was calculated from the measured apparent rate constant based on the assumption that the reactivity of the neutral 17α-ethinylestradiol can be neglected at pH 6.

A very similar experimental setup was used to determine the rate constants for *diclofenac* and *sulfamethoxazole*. Phenol was chosen as the reference compound. In contrast to the method mentioned above, the ratio of the target compound to the reference compound was varied from 1:3 to 3:1 and experiments were carried out at pH 7. Preliminary experiments showed that at this pH the apparent rate constants of phenol was similar to the rate constants of the target compound. Phenol reacts about 10 times faster at pH 7 than at pH 6. To check whether competition kinetics can be applied under these conditions, additional experiments were conducted at pH 6.7 for *diclofenac* and pH 7.3 for *sulfamethoxazole*. At pH 6.7 phenol reacts slightly more slowly with O_3 than *diclofenac,* whereas at pH 7 phenol reacts slightly faster. Correspondingly, the reactivity of phenol at pH 7.3 is higher than the one of sulfamthoxazole and lower at pH 7. The variation of pH resulted in only small changes for the calculated rate constants showing that the results are consistent. Competition kinetics between *diclofenac* and *sulfamethoxazole* directly could not be performed due to secondary reactions between a product of *sulfamethoxazole* and *diclofenac*.

A method developed by Muñoz and von Sonntag (*17*) was adapted to determine the rate constant of *carbamezepine*. A constant O_3 dose (10 μM) was added to solutions containing 80 μM carbamazepine and varying concentrations of the reference compound nitrite (3.7×10^5 M^{-1} s^{-1} (*18*)) or buten-3-ol (7.9×10^4 M^{-1} s^{-1} (*19*)), respectively. The product formed through the reaction of O_3 with *carbamazepine* was monitored (the structure of this product is not known, its UV spectra is similar to that of carbamazepine). The rate constant was then derived by comparing product formation in the presence and in the absence of the reference compound. The experiments were carried out at pH 7 in 25 mL serum vials. Ozone was added with a glass syringe while the reaction solution was vigorously stirred.

2.2.5 Determination of Rate Constants for the Reaction of Pharmaceuticals with Hydroxyl Radicals

Competition kinetics was used to determine the second-order rate constants for the reaction with hydroxyl radicals ($^{\bullet}OH$). The reference compound was *p*CBA exhibiting a rate constant of $k_{OH} = 5\times10^{9}$ M^{-1} s^{-1} (*20*). All experiments were carried out with Milli-Q water at 25 °C and the pH was kept constant at 7 using 5 mM phosphate buffer. For most experiments, $^{\bullet}OH$ was generated by photolysis of H_2O_2 at 313 nm. These experiments were performed in quartz tubes using a merry-go-round photoreactor (DEMA model 125, Hans Mangels GmbH, Bornheim-Roisdorf, Germany) equipped with a medium-pressure mercury lamp (Hanau model TQ718) driven at a power of 500 W. A UVW-55 glass band-pass filter (supplied by DEMA) was used to eliminate radiation of wavelengths shorter than 308 nm in order to minimize direct photolysis of the pharmaceuticals. Further details about the irradiation equipment are given elsewhere (*21,22*). For compounds undergoing direct photolysis, $^{\bullet}OH$ was generated with γ-radiolysis. The experiments were performed in a ^{60}Co γ-radiation source with lead shielding (type GAMMACELL, Atomic Energy of Canada; dose rate for water = 2.1 $kGyh^{-1}$ ± 20% in the center of source with lead shielding). Solutions were saturated with a 4:1 mixture of N_2O and O_2 (*23*). N_2O is necessary to convert solvated electrons into $^{\bullet}OH$. The rate of $^{\bullet}OH$ formation was about 0.1 μM s^{-1}. Initial pharmaceutical and reference compound concentrations were 1 μM in UV/H_2O_2 experiments and 10 or 50 μM in γ-radiolysis experiments. In both experiments, samples were repeatedly irradiated for constant time intervals. Between irradiation periods, samples were withdrawn for HPLC analysis. The data were evaluated based on eq 2, where k_{OH}(R) and k_{OH}(M) are the rate constants for the reference (R) and target compound (M), respectively. The irradiation time is represented by t.

$$\ln\left(\frac{[M(t)]}{[M(0)]}\right) = \ln\left(\frac{[R(t)]}{[R(0)]}\right)\frac{k_{OH}(M)}{k_{OH}(R)} \quad (2)$$

For iopromide, $^{\bullet}OH$ was also generated by the O_3/H_2O_2 system. Instead of repeated sampling, different O_3 doses were added to solutions with varying ratios of iopromide and *p*CBA (from 1:3 to 3:1).

2.2.6 Ozonation of Natural Waters

Two different experimental setups were used to investigate the oxidation of pharmaceuticals during the ozonation of natural waters. Pharmaceuticals with rate constants $k_{O3} > 10^3$ M^{-1} s^{-1} were investigated with the setup for fast-reacting compounds, pharmaceuticals with rate constants <100 M^{-1} s^{-1} with the setup for slow-reacting compounds. Bezafibrate was tested with both setups.

Fast reacting compounds. RS or LF water (Table 2-1) was buffered to pH 8 by adding 10 mM borate buffer and adjusting the pH with HCl. The water was then spiked with one pharmaceutical compound (0.5 µM) and transferred into five 25 mL serum vials. In a next step, O_3 doses of 0.1, 0.2, 0.5, 1, and 2 mg L^{-1} were injected into the vials while the water was vigorously stirred for a few seconds. As soon as O_3 was completely consumed (up to 10 h for RS water), residual pharmaceutical concentrations were measured with HPLC. All experiments were performed at 10 °C. To determine bromate formation under the applied conditions, one experiment was carried out without adding a pharmaceutical compound. Instead, 50 µg L^{-1} bromide was added to LF water to achieve a bromide level of 60 µg L^{-1}. RS water already contained about 60 µg L^{-1} bromide and no spiking was necessary. Bromate was determined according to a method developed by Salhi and von Gunten (*24*).

Slow reacting compounds. Natural water samples were buffered to pH 8 by adding 10 mM borate buffer and adjusting the pH with HCl. The water was then spiked with one pharmaceutical compound (0.5 µM) and the probe compound

*p*CBA (0.25 μM), which does not react with O_3 directly. The spiked concentrations were low enough to avoid significant changes in O_3 half-lives or $^{\bullet}OH$ scavenging-capacities of the investigated waters. The experiments were carried out at 10 °C in order to mimic realistic treatment conditions. Amber bottles (500 mL) equipped with a dispenser system (*14*) served as reaction vessels. The experiments were started by adding 2 mg L^{-1} O_3 and, subsequently, samples were withdrawn in regular time intervals. The reaction was stopped with indigo for residual O_3 measurements. For pharmaceutical and *p*CBA analysis, O_3 was quenched with sodium sulfite. For analysis methods with gradient elution, samples had to be adjusted to acidic pH by adding HCl before analysis.

2.3 Results and Discussion

2.3.1 Rate Constants for the Reaction of Selected Pharmaceuticals with Ozone

Second-order rate constants for the reaction of pharmaceuticals with ozone (O_3) have been determined to assess the oxidation of these compounds during conventional ozonation or ozone-based AOPs. Table 2-3 and Figure 2-1 summarize the results. Measurements were performed at least three times and the errors given are 95% confidence intervals. For competition kinetics, errors are larger and more difficult to estimate, partly due to the errors induced by the use of reference compounds. We expect rate constants measured with this method to vary up to a factor of two. Methods monitoring the O_3 decay (indigo, UV) yield a rate constant which differs from the second-order rate constant by the stoichiometric factor η, which defines the number of O_3 molecules consumed per molecule of target compound under the experimental conditions.

According to (*15*) values for η range from 1 to 2.5. Deviations from η = 1 can be caused by fast side reactions of O_3 with products of the primary reactions.

TABLE 2-3. Second-order Rate Constants for the Reaction of Ozone with the Investigated Pharmaceuticals

Compound	pK_a	Method[a]	k_{O3} (T = 20 °C)[b] (M^{-1} s^{-1})	Reactive Species
Bezafibrate	3.6	HPLC	590 ± 50	dissociated
Carbamazepine	-	CK	~3×10^5	neutral
Diazepam	-	HPLC	0.75 ± 0.15	neutral
Diclofenac	4.2	CK	~1×10^6	dissociated
17α-Ethinylestradiol	10.4	CK	~7×10^9	dissociated
Ibuprofen	4.9	HPLC/indigo	9.6 ± 1	dissociated
Iopromide	-	indigo	<0.8	neutral
Sulfamethoxazole	5.7	CK	~2.5×10^6	dissociated
Roxithromycin	8.8	UV	$(4.5 \pm 0.5) \times 10^6$	neutral

[a] HPLC: decrease of target compound
indigo: measurement of O_3 decrease with indigo method
UV: direct measurement of O_3 at 258 nm
CK: competition kinetics

[b] Rate constants for the most reactive species given in the last column

Figure 2-1 shows half-lives ($[O_3]$ = 1 mg L^{-1}) and apparent second-order rate constants as a function of pH. Ozone rate constants typically depend on speciation. Generally, deprotonated species react faster with the electrophilic O_3, because they are stronger nucleophiles (*16*). The rate constants given in Table 2-3 refer to the most reactive species, which are listed in the last column of the table. For most pharmaceuticals, the reactive species correspond to the predominant species in the pH range from 5 to 10. As a consequence, their

apparent rate constants are stable in this pH range. However, rate constants for 17α-ethinylestradiol and roxithromycin depend strongly on pH because their pK_a values are higher than 8 and the deprotonated phenolic group of 17α-ethinylestradiol and the non-protonated amine of roxithromycin react many orders of magnitude faster than their protonated forms.

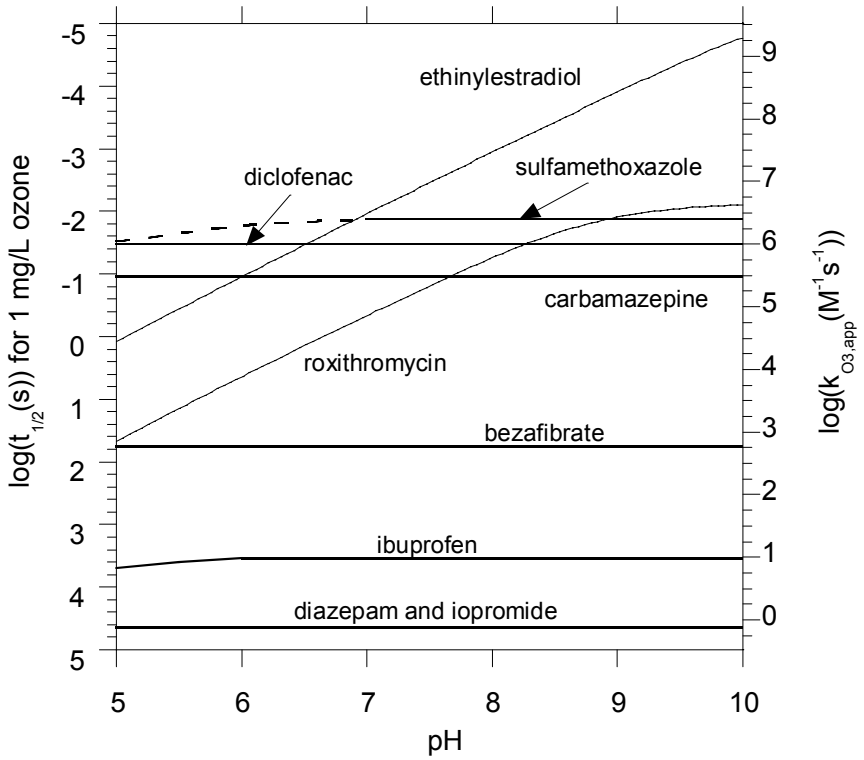


FIGURE 2-1. Half-lives and apparent second-order rate constants for the reactions of the investigated pharmaceuticals with O_3 as a function of pH at 20 °C. The half-lives are calculated for an O_3 concentration of 1 mg L^{-1} (20 μM) neglecting reactions with $^{\bullet}OH$.

Oxidation reactions with O_3 are highly selective reactions. As a result, rate constants range over about 10 orders of magnitude (*25,26*). Among the pharmaceuticals investigated, the dissociated form of 17α-ethinylestradiol

exhibited the highest rate constant reacting at nearly diffusion-controlled rate. Experiments with 17β-estradiol instead of 17α-ethinylestradiol yielded the same rate constant (data not shown) and a similar product, demonstrating that the reaction takes place at the phenolic group and not at the ethinyl group. This implied that the rate constant for 17α-ethinylestradiol is pH dependent. Knowing the pK_a (10.4), the rate constant for the dissociated form could be extrapolated. This rate constant is in accordance with rate constants for other phenolic compounds.

For diclofenac and sulfamethoxazole the rate constants are 1×10^6 and 2.5×10^6 M^{-1} s^{-1}, respectively. These compounds react more slowly than dissociated 17α-ethinylestradiol, however, apparent rate constants at pH 7 are in the same order of magnitude. The main reaction sites are the aromatic amino groups (see Table 2-1). Since protonated amino groups do not react with O_3 (*16*), the reactivity of amines is strongly related to their pK_a values. Compared to other amines diclofenac and sulfamethoxazole react particularly fast because the pK_a values of the amino groups are < 3. This means that at pH > 5, the non-protonated amine (reactive species) is the predominant species. By contrast, the amine group in roxithromycin has a pK_a of 8.8 and at pH < 8 the non-reactive protonated amine is predominant, leading to a diminished reactivity. The rate constant for sulfamethoxazole might be weakly pH dependent at pH < 7 due to the protonation of the dissociated sulfonamide group (pK_a = 5.7). The high reactivity of carbamezepine can be assigned to the reaction of O_3 at the double bond which connects the two phenyl moieties (see Table 2-1). The rate constant is slightly higher than for other olefins, but in very good agreement with the rate constants for styrene (3×10^5 M^{-1} s^{-1} (*15*)).

For water treatment conditions (pH 7-8, [O_3] = 1 mg L^{-1}) half-lives for 17α-ethinylestradiol, carbamazepine, roxithromycin, diclofenac and

sulfamethoxazole are <0.5 s. This indicates clearly that, for these pharmaceuticals, the parent compound is completely transformed during ozonation processes and ozone-based AOPs. The remaining pharmaceuticals have considerably lower rate constants. The intermediate reactivity of bezafibrate is caused by the R-oxy substituent (-O-C$(CH_3)_2$COOH) on one of the aromatic rings. R-oxy substituents have a similar activating effect on aromatic rings as hydroxy substituents (*15*). However, R-oxy substituents cannot be deprotonated and consequently the overall rate constant at pH > 4 is much lower. Nevertheless, this compound will also be largely transformed by most ozonation processes. Ibuprofen reacts with a rate constant of 9.6 M^{-1} s^{-1}. The low rate constants can be explained by the absence of reactive groups and an aromatic ring that is only slightly activated, similar to toluene (*15*). Due to the low rate constant, direct reactions with O_3 will play a minor role during ozonation processes and the oxidation of this compound will mainly be caused by $^{\bullet}OH$ originating from O_3 decay. The reaction of O_3 with iopromide is very slow which only allows an estimate of an upper limit. Iopromide exhibits three nitrogen atoms as amides. In contrast to amines, amides have a very low reactivity to O_3. The rate constant for the reaction of diazepam with O_3 is also very low. Therefore, during ozonation processes direct O_3 reactions are in both cases less important than oxidation by $^{\bullet}OH$.

For two pharmaceuticals with intermediate to low O_3 rate constants, activation energies were determined to calculate their rate constants at temperatures below 20 °C. Ibuprofen exhibited an activation energy of 57 ± 8 kJ mol^{-1} and bezafibrate one of 39 ± 6 kJ mol^{-1}. Typically, activation energies for reactions with O_3 range from 35 to 50 kJ mol^{-1} (*15*).

TABLE 2-4. Examples of Pharmaceuticals, Personal Care Products and Endocrine Disrupters for which High Ozone Rate Constants are Expected

Compound/Class	Use/Origin	Reactive Group	Estimated Rate Constants at pH 7, T = 20 °C ($M^{-1} s^{-1}$)
β-blockers	β-blocker	amine	$(1\text{-}10) \times 10^3$
fluorochinolones	antibiotic	amine	$(1\text{-}10) \times 10^3$
macrolides	antibiotic	amine	$> 10^5$
sulfonamides	antibiotic	amine	$> 10^5$
tetracyclines	antibiotic	phenol	$(1\text{-}10) \times 10^6$
triclosan	antimicrobial disinfectant	phenol	$> 10^6$
oxybenzone	sunscreen agent	phenol	$(1\text{-}10) \times 10^6$
estradiol	reproductive hormone	phenol	10^{6} [a]
testosterone	reproductive hormone	double bond	10^5
4-nonylphenol	nonionic surfactant metabolite	phenol	$(1\text{-}10) \times 10^6$
bisphenol A	plasticizer	phenol	$(1\text{-}10) \times 10^6$

[a] measured

2.3.2 Expected Reactivity of Other Pharmaceuticals

Table 2-4 lists different classes of pharmaceuticals as well as some important personal care products and endocrine disrupters together with the ozone-reactive moieties. Based on these characteristics and our results, expected second-order rate constants can be estimated. Generally, many pharmaceuticals, personal care products and endocrine disrupters contain phenol or amino groups in their structures. Sulfamethoxazole and roxithromycin belong to the antibiotic classes of sulfonamides and macrolides, respectively. The reactive groups (aromatic amine and tertiary amine, respectively) are characteristic for all the compounds

in these two groups. Therefore, rate constants for all sulfonamides and macrolides will be very similar to the rate constants for sulfamethoxazole and roxithromycin, respectively. The endocrine disrupter 17α-ethinylestradiol is a synthetic steroid hormone. Many steroid hormones have a phenolic group (e.g., 17α-estradiol, 17β-estradiol, estrone, and equilin) and, therefore, will exhibit about the same reactivity as 17α-ethinylestradiol. For 17β-estradiol, this was confirmed by measurements. Other steroid hormones lack the phenolic group, but have a double bond (progesterone, testosterone). These compounds will react about one order of magnitude slower than phenolic steroid hormones.

2.3.3 Rate Constants for the Reaction of Pharmaceuticals with Hydroxyl Radicals

Besides the direct reaction with O_3, also reactions with hydroxyl radicals ($^{\bullet}OH$) contribute to the oxidation of micropollutants during ozonation (*26*). Moreover, $^{\bullet}OH$ is the main oxidant in AOPs. Therefore, the rate constants for the reaction of 8 selected pharmaceuticals with $^{\bullet}OH$ were determined. Results are summarized in Table 2-5.

Most rate constants were measured with the UV/H_2O_2 method. Some pharmaceuticals, however, were photolyzed by UV-radiation. Direct photolysis accounted for 75%, 13% and 7% of the observed rate constant for diclofenac, iopromide and sulfamethoxazole, respectively. Since direct photolysis followed first-order kinetics, as did the oxidation by $^{\bullet}OH$, the rate constant for sulfamethoxazole was corrected for the percentage mentioned above. Rate constants for diclofenac and iopromide were determined using γ-radiolysis.

The rate constants for the reactions of pharmaceuticals with $^{\bullet}OH$ range from 3 to 10×10^9 M^{-1} s^{-1}. For half of the investigated compounds, rate constants lie between 7 and 9×10^9 M^{-1} s^{-1} demonstrating the relatively non-selective nature of $^{\bullet}OH$ reactions in aqueous solution. The X-ray contrast medium iopromide has

the lowest reactivity. Compared to other important micropollutants (atrazine, MTBE, perchloroethylene and trichloroethylene (*20*)) the selected pharmaceuticals react about two to three times faster with •OH. This indicates that AOPs, even if not ozone-based, would oxidize the selected pharmaceuticals more efficiently than many other relevant micropollutants. Also, pharmaceuticals that do not react with O_3 directly will be partly removed during conventional ozonation through reactions with •OH.

TABLE 2-5. Rate Constants for the Reaction of Hydroxyl Radicals with the Investigated Pharmaceuticals and Some Other Important Micropollutants

Compound	**Method**	k_{OH} **(10^9 M^{-1} s^{-1})[a]**
Bezafibrate	UV/H_2O_2	7.4 ± 1.2
Carbamazepine	UV/H_2O_2	8.8 ± 1.2
Diazepam	UV/H_2O_2, γ-radiolysis	7.2 ± 1.0
Diclofenac	γ-radiolysis	7.5 ± 1.5
17α-Ethinylestradiol	UV/H_2O_2	9.8 ± 1.2
Ibuprofen	UV/H_2O_2	7.4 ± 1.2
Iopromide	O_3/H_2O_2, γ-radiolysis	3.3 ± 0.6
Sulfamethoxazole	UV/H_2O_2	5.5 ± 0.7
Other Micropollutants	**Function**	k_{OH} **(10^9 M^{-1} s^{-1})[b]**
Atrazine	pesticide	2.4
MTBE	fuel additive	1.6
Perchloroethylene	solvent	2-3
Trichloroethylene	solvent	3-4

[a] experimental conditions pH = 7, T = 25 °C, errors = 95% confidence intervals

[b] reference: (*20*)

2.3.4 Product Formation

The reactions with O_3 and $^{\bullet}OH$ during an ozonation process will not result in the complete mineralization of pharmaceuticals. However, since pharmaceuticals generally react with specific receptors in the target organisms, transformation of the parent molecules by the above oxidants may be sufficient to reduce the intended pharmaceutical effects. In ongoing research the degradation pathways of the selected pharmaceuticals are investigated. From the literature, the transformation pathways for certain functional groups are known. An overview is given in (*26*). Reactions of O_3 with phenolic compounds results in the cleavage of the aromatic ring (*27*). Ozone attack at double bonds leads to bond cleavage and formation of carbonyl compounds (*19,28*). Hydroxylamines and amine oxides are formed in O_3 reactions with secondary (*29*) and tertiary amines (*30*), respectively. Hydroxylamines undergo further reactions with O_3. For the pharmaceuticals investigated, a major part of the reactions with $^{\bullet}OH$ will take place at benzene rings, resulting in the formation of phenolic compounds or ring cleavage. In ozone-based processes phenolic compounds will quickly react with O_3. Based on this information, it can be concluded that modifications caused by ozonation or AOPs should be sufficient to eliminate the intended pharmaceutical effects of most of the investigated compounds. However, it cannot be ruled out that for some compounds modifications may not be important or even lead to the formation of toxic by-products. For instance, the formation of hydroxylamines could be problematic from a toxicological point of view as in the case of sulfonamides, the hydroxylamine of these compounds is associated with hypersensitivity reactions to this class of antibiotics (*31*).

2.3.5 Oxidation of Fast-Reacting Pharmaceuticals in Natural Waters and Bromate Formation

Experiments have been performed in different natural waters to confirm the determined rate constants and to apply them to real treatment conditions. For pharmaceuticals with O_3 rate constants >100 $M^{-1}s^{-1}$, batch experiments were carried out with RS and LF waters, which exhibit high alkalinity and low DOC, and low alkalinity and high DOC, respectively. Ozone half-lives ($[O_3]_0$ = 2 mg L^{-1}, pH = 8, T = 10 °C) were 75 min for RS and 4 min for LF water. Figure 2-2 shows the transformation of the selected pharmaceuticals in these two waters as a function of different O_3 doses. In RS water, an O_3 dose of 0.2 mg L^{-1} was sufficient to achieve a transformation >97% with the exception of bezafibrate. LF water has a higher O_3 demand and an O_3 dose of 0.5 mg L^{-1} was necessary for the same transformation. The O_3 rate constant of bezafibrate is at least 100 times lower than the rate constants of the other compounds and bezafibrate can obviously not compete with the initial O_3 demand of these waters. The generally low transformation for the O_3 dose of 0.1 mg L^{-1} in LF and RS water may partly be caused by the fact that the O_3 concentration exceeds the pharmaceutical concentration only by a factor of 4. At more realistic pharmaceutical concentrations a better transformation can be expected. Results for O_3 doses of >0.2 mg L^{-1} can be directly extrapolated to pharmaceutical concentrations in the ng/L-range. Overall the results demonstrate that relatively low O_3 doses are sufficient to achieve a complete transformation of pharmaceuticals exhibiting rate constants of >10^5 $M^{-1}s^{-1}$.

Besides the transformation of pharmaceuticals, the formation of bromate, the major by-product of concern during ozonation processes, was measured as well. The concentration of the bromate precursor bromide was 60 µg L^{-1} in RS (natural level) and LF water (fortified). This represents a medium bromide concentration. In RS water the highest O_3 dose led to a bromate concentration of

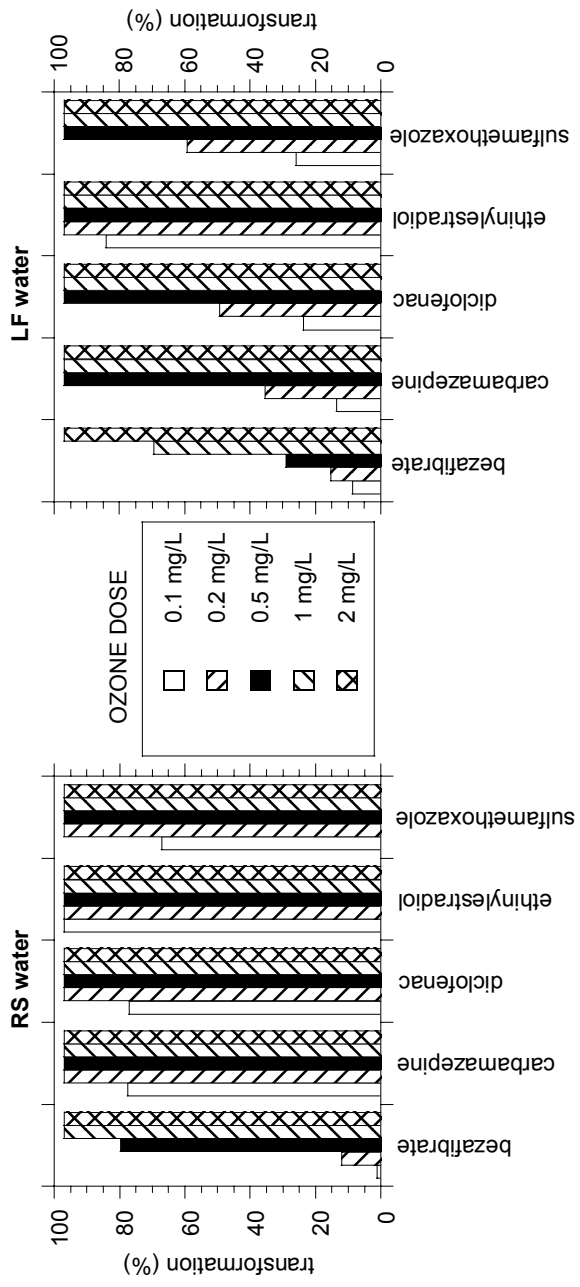


FIGURE 2-2. Oxidation of fast-reacting pharmaceuticals in RS water (DOC = 1.3 mg L^{-1}, alk = 4.1 mM) and LF water water (DOC = 3.7 mg L^{-1}, alk = 0.7 mM) as a function of the O_3 dose. Experimental conditions: pH = 8, T = 10 °C, $[pharmaceuticals]_0$ = 0.5 μM. Samples were measured after all O_3 was consumed. Transformations up to 97-99% could be measured with the existing detection limits.

12 μg L^{-1}, which is slightly higher than the drinking water standard of 10 μg L^{-1} set by the EU and the US. Since samples were only measured after all O_3 was consumed, the high O_3 stability in RS water led to a large O_3 exposure and, as a consequence, to a high bromate formation. For lower O_3 doses, bromate formation was <2 μg L^{-1}. In LF water bromate formation was <2 μg L^{-1} for all O_3 doses. Generally, the results show that bromate formation is not a problem for O_3 doses that are necessary to oxidize fast-reacting pharmaceuticals.

2.3.6 Oxidation of Slow-Reacting Pharmaceuticals during Ozonation of Natural Waters

Compared to fast reacting pharmaceuticals, which are completely transformed for typical O_3 doses applied in drinking water treatment, it is more difficult to predict the oxidation of pharmaceuticals exhibiting lower O_3 rate constants. In this case, reactions with O_3 and $^{\bullet}OH$ have to be considered and it is essential to know their concentrations during the ozonation process, respectively their exposures (i.e., concentration integrated over the reaction time). The R_{ct}-concept (*10,26*) is an experimental approach to calibrate ozonation processes and ozone-based AOPs with respect to O_3 and $^{\bullet}OH$ exposure. This calibration is done by determining the ratio of the $^{\bullet}OH$ exposure to the O_3 exposure in the investigated water (R_{ct}-value). After an initial phase, the R_{ct}-value remains constant for the rest of the ozonation process and therefore, also represents the ratio of $^{\bullet}OH$ concentration to O_3 concentration (R_c-value). The $^{\bullet}OH$ exposure is obtained by measuring the degradation of a probe compound (*p*CBA) that does not react with O_3. Simultaneously, the O_3 decrease is followed in order to determine the O_3 exposure. With eq 3 it is then possible to predict the oxidation of a micropollutant (M) as a function of O_3 exposure ($\int[O_3]dt$), R_c ($[^{\bullet}OH]/[O_3]$), k_{OH} and k_{O3}:

$$\ln\left(\frac{[M]}{[M]_0}\right) = -\left(\int [O_3]dt\right)\left(k_{OH}R_c + k_{O_3}\right) \quad (3)$$

where k_{OH} and k_{O3} are the second-order rate constants for the reactions of a micropollutant (M) with $^{\bullet}OH$ and O_3, respectively.

Experiments with four natural waters were performed to apply the rate constants determined to real treatment conditions. The corresponding water quality parameters are given in Table 2-2. The selected waters differed in DOC and alkalinity, two parameters controlling O_3 stability as well as $^{\bullet}OH$ formation and scavenging in natural waters. Based on the concept presented above, R_c-values were determined for the natural waters and the oxidation of the selected pharmaceuticals was predicted using eq 3. Ozone rate constants were adjusted to the experimental conditions using the measured activation energies for bezafibrate (39 kJ mol^{-1}) and ibuprofen (57 kJ mol^{-1}) and an average activation energy of 40 kJ mol^{-1} (*15*) for diazepam and iopromide. In the same experiments, the oxidation of the pharmaceuticals was measured to verify the predictions. Table 2-6 summarizes the results.

The measured oxidation of bezafibrate was >95% in all ozonation experiments. This is mainly due to the relatively high second-order rate constant for its reaction with O_3. The measured oxidation of diazepam, iopromide and ibuprofen ranged from 24% (diazepam and iopromide in WP water) to 77% (ibuprofen in LF water). The oxidation of these compounds is largely controlled by reactions with $^{\bullet}OH$. The oxidation efficiencies increased with increasing DOC and decreased with increasing alkalinity, respectively. An increased DOC leads to an enhanced rate of O_3 transformation into $^{\bullet}OH$, whereas alkalinity stabilizes O_3. The effect of DOC and alkalinity was less pronounced for ibuprofen due to a higher O_3 rate constant. In WP and RS water with a high O_3 stability, the higher O_3 rate constant resulted in a better oxidation of ibuprofen compared to diazepam, while in LZ and LF water, where O_3 reaction are less

relevant, the oxidation efficiencies were about the same. For bezafibrate, diazepam and ibuprofen predictions were in reasonable agreement with the measured data. However, the oxidation of iopromide could no be predicted accurately. No explanation has yet been found for this discrepancy.

TABLE 2-6. Predicted and Measured Oxidation of Slow-Reacting Pharmaceuticals during Conventional Ozonation and Advanced Oxidation of Natural Waters[a]

	Oxidation of Pharmaceuticals							
	WP water		**RS water**		**LZ water**		**LF water**	
Conv. Ozonation	pred.	meas.	pred.	meas.	pred.	meas.	pred.	meas.
Bezafibrate	>99%	nd	>99%	>99%	>99%	nd	97%	98%
Diazepam	23%	24%	29%	nd	57%	65%	74%	nd
Iopromide	6%	24%	14%	27%	31%	58%	46%	68%
Ibuprofen	31%	41%	37%	40%	56%	62%	69%	77%
AOP (O_3/H_2O_2)								
Ibuprofen	80%	84%	80%	78%	90%	90%	nd	nd
Bezafibrate	98%	nd	92%	nd	95%	97%	nd	nd
Hydroxyl Radical Scavenging Capacity								
Total [s^{-1}]	7.6×10^4		7.2×10^4		5.5×10^4		9.9×10^4	
DOC [s^{-1}] [(b)]	2.0×10^4		3.2×10^4		3.0×10^4		9.2×10^4	
HCO_3^-/CO_3^{2-} [s^{-1}] [(c)]	5.6×10^4		4×10^4		2.5×10^4		0.7×10^4	

[a] Conditions: O_3 dose = 2 mg L^{-1}, contact time = 10 min, pH = 8, T = 10 °C; for AOP, H_2O_2 dose = 0.7 mg L^{-1}, for water quality parameters see Table 2-2

[b] Scavanging Capacity (DOC) = $k_{OH}(DOC) \times [DOC]$

[c] Scavanging Capacity (HCO_3^-/CO_3^{2-}) = $k_{OH}(HCO_3^-) \times [HCO_3^-] + k_{OH}(CO_3^{2-}) \times [CO_3^{2-}]$

For RS water, Figure 2-3 illustrates the oxidation of three pharmaceuticals during ozonation (symbols: experimental data; lines: predictions). Bezafibrate, which has an intermediate rate constant with O_3, was oxidized within 5 minutes

to the detection limit, mainly through the direct reaction with O_3. Ibuprofen exhibits a much lower k_{O3} and its removal is to a large extent caused by reactions with •OH. For iopromide, it is expected that reactions with •OH are the major oxidation pathway. However, k_{OH} is rather small and the predicted removal underestimates the measured oxidation with •OH. Due to high alkalinity and low DOC the O_3 stability is rather high in RS water. In LZ and LF water, lower O_3 stability leads to an accelerated •OH formation. As a result, compounds that react mainly with •OH are oxidized significantly faster in these waters.

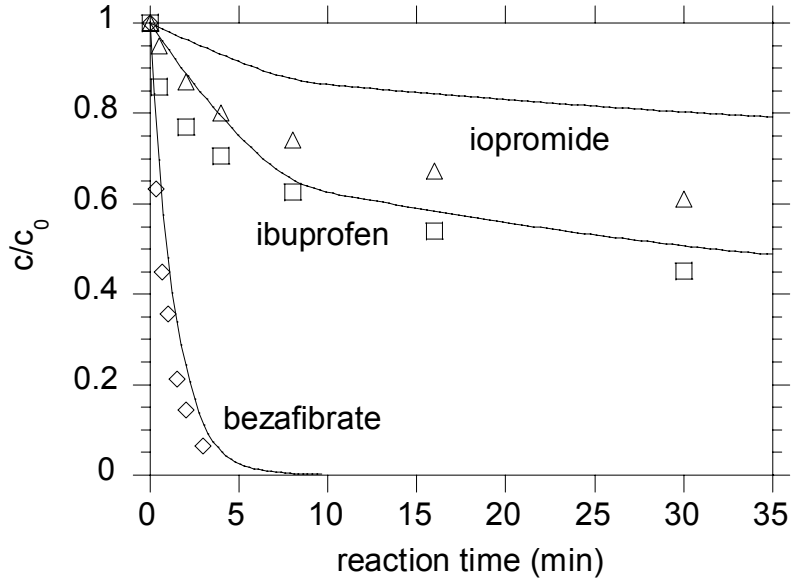


FIGURE 2-3. Oxidation of three pharmaceuticals during ozonation of RS water (DOC = 1.3 mg L^{-1}, alk = 4.1 mM). Experimental conditions: pH = 8, T = 10 °C, O_3 dose = 2 mg L^{-1}, [pharmaceuticals]$_0$ = 0.5 μM. Symbols represent measured data, and lines represent model calculations.

For waters with high O_3 stability, the oxidation of micropollutants can be considerably accelerated by adding H_2O_2 to the ozonation process. In the O_3/H_2O_2 AOP, O_3 is converted into •OH within a few minutes. It has to be emphasized that the overall •OH formation does not change significantly

compared to conventional ozonation in which the ozonation process is not stopped before all O_3 is consumed. Table 2-6 presents data for the predicted and measured oxidation in the O_3/H_2O_2 AOP. Oxidation of bezafibrate is slightly lower in this AOP than during conventional ozonation (10 min contact time). This is due to a reduced O_3 exposure, which cannot be fully compensated by $^{\bullet}OH$ reactions. However, the oxidation of ibuprofen could be considerably increased. The efficiency of an AOP strongly depends on the $^{\bullet}OH$ scavenging capacity of the selected natural water. Therefore, the highest oxidation was achieved in LZ water, which has the lowest scavenging capacity. Transformation in WP water was similar to the one in RS water. This can be explained by the comparable $^{\bullet}OH$ scavenging capacity. In LF water, it would not be possible to increase the oxidation efficiency of the ozonation process by adding H_2O_2, because the O_3 half-life is already quite short and the scavenging capacity of the water rather high.

Figure 2-4 compares the ibuprofen oxidation in conventional ozonation and in the O_3/H_2O_2 AOP. The oxidation of ibuprofen could be increased from 40% to over 80% for a hypothetical contact time of 10 min. These results can also be applied to diazepam, which has a similar k_{OH} and barely reacts with O_3. Other important micropollutants such as atrazine and MTBE showed a substantially lower transformation in the O_3/H_2O_2 AOP under similar conditions. In River Seine water, atrazine oxidation was about 50% (*11*). MTBE oxidation in different natural waters ranged from 30% to 50% (*12*).

For the investigation of oxidation products formed during the ozonation of pharmaceuticals, it will be important to know the fraction of a pharmaceutical compound reacting with O_3 and $^{\bullet}OH$, respectively. Figure 2-5 shows which fraction of the slow-reacting pharmaceuticals reacts with either of the two oxidants as a function of the R_c-value ($R_c = ([^{\bullet}OH]/[O_3])$, see eq 3). For LZ, RS and WP water, conventional ozonation yielded R_c-values ranging from 10^{-10} to 5

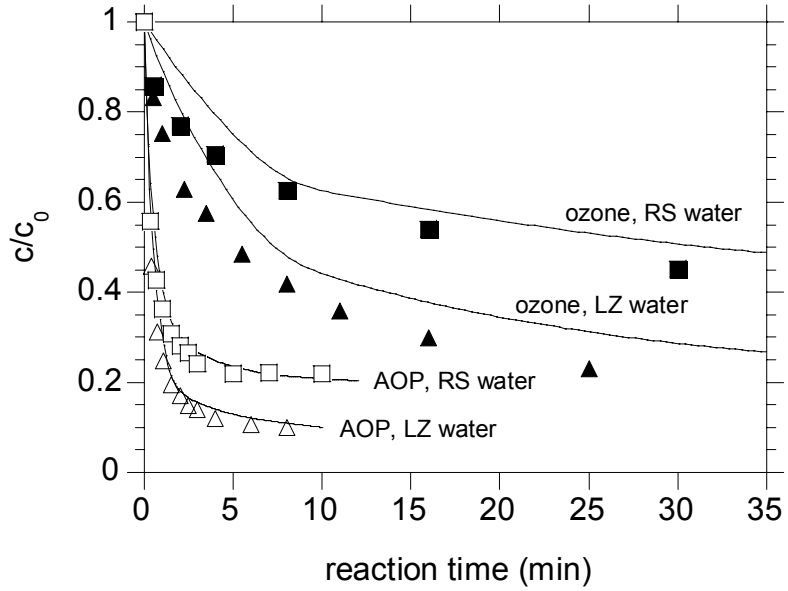


FIGURE 2-4. Oxidation of ibuprofen during ozonation and advanced oxidation of RS water (DOC = 1.3, alk = 4.1 mM) and LZ water (DOC = 1.2, alk = 2.6 mM). Experimental conditions: pH = 8, T = 10 °C, O_3 dose = 2 mg L^{-1}, [pharmaceuticals]$_0$ = 0.5 μM, ratio of H_2O_2/O_3 = 0.34 w/w (AOP). Symbols represent measured data, and lines represent model calculations.

$\times 10^{-9}$. Under these conditions both O_3 and $^{\bullet}OH$ contribute to the oxidation of ibuprofen, iopromide and diazepam, whereas bezafibrate is oxidized by O_3 alone. Ozonation of LF-water and AOPs resulted in R_c-values ranging from 10^{-8} to 10^{-7}. In this case, only $^{\bullet}OH$ reactions are relevant for the oxidation of ibuprofen, iopromide and diazepam. However, both O_3 and $^{\bullet}OH$ are involved in the oxidation of bezafibrate under these conditions. In contrast to the slow-reacting pharmaceuticals, fast-reacting pharmaceuticals are almost exclusively oxidized by O_3 for all treatment conditions.

The batch experiments with natural waters represent ozonation processes or AOPs in ideal plug-flow reactors. To make exact predictions for real water treatment, it is necessary to account for reactor hydraulics. However, only small

deviations are expected between different reactor types because the transformation of slow-reaction pharmaceuticals is lower than one log unit.

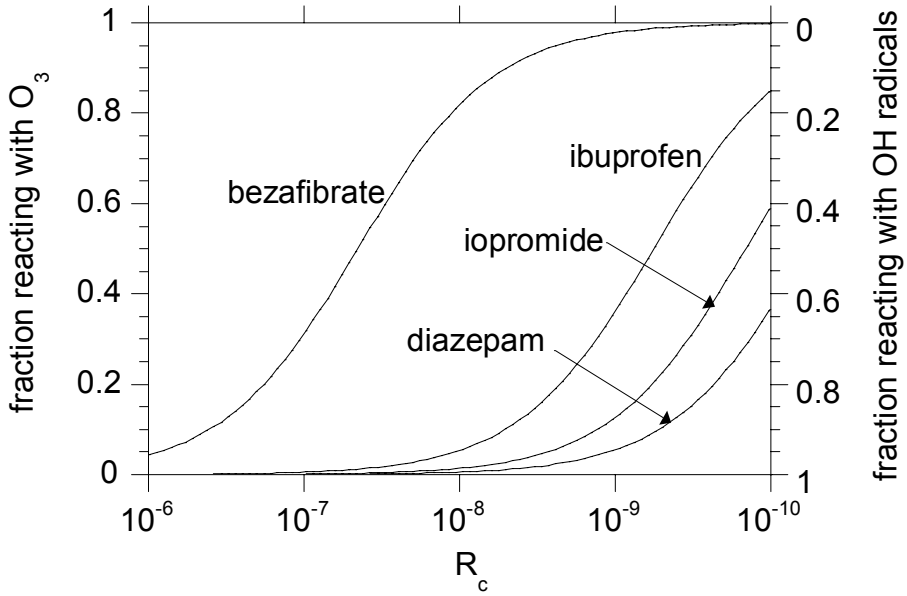


FIGURE 2-5. Comparison of the contribution of O_3 and $^{\bullet}OH$ to the overall oxidation of slow-reacting pharmaceuticals at T = 10 °C. The fraction of pharmaceuticals reacting with O_3 and $^{\bullet}OH$, respectively, is plotted as a function of the R_c-value (R_c= ([$^{\bullet}OH$]/[O_3]). Ozone rate constants were corrected for T = 10 °C.

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3

Oxidation of Pharmaceuticals during Water Treatment with Chlorine Dioxide

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Abstract

The potential of chlorine dioxide (ClO_2) for the oxidation of pharmaceuticals during water treatment was assessed by determining second-order rate constants for the reaction with selected environmentally relevant pharmaceuticals. Out of 9 pharmaceuticals only the 4 following compounds showed an appreciable reactivity with ClO_2 (in brackets apparent second-order rate constants at pH 7 and T = 20 °C): the sulfonamide antibiotic sulfamethoxazole (6700 ± 700 M^{-1} s^{-1}), the macrolide antibiotic roxithromycin (220 ± 40 M^{-1} s^{-1}), the estrogen 17α-ethinylestradiol (~2 × 10^5 M^{-1} s^{-1}), and the antiphlogistic diclofenac (10500 ± 1000 M^{-1} s^{-1}). Experiments performed using natural water showed that further sulfonamides and macrolides, the natural hormones estrone and 17β-estradiol as well as 3 pyrazole derivatives (phenazone, propylphenazone, and dimethylaminophenazone) also react fast with ClO_2. However, a significant share of the studied compounds was ClO_2 refractive. Experiments with lake water and groundwater that were partly performed at microgram/L to nanogram/L levels proved that the rate constants determined in pure water could be applied to predict oxidation of pharmaceuticals in natural waters. Compared to ozone, ClO_2 reacted more slowly and with fewer compounds. However, it reacted faster with the investigated compounds than chlorine. Overall, the results indicate that ClO_2 will only be effective to oxidize certain compound classes like the investigated classes of sulfonamide and macrolide antibiotics and estrogens.

3.1 Introduction

3.1.1 Pharmaceuticals in the Environment

The widespread occurrence of a large number of pharmaceuticals in the aquatic environment (*1,2*) may threaten the purity of drinking water. Because surface water is the most affected, pharmaceuticals may first pose a problem to water utilities that use surface water as a water resource for drinking water production. However, studies on different treatment processes including ozonation (*3,4*), activated carbon (*4*,*5*), and nanofiltration / reverse osmosis membranes (*5*) have lately demonstrated that these processes can efficiently remove a relatively large number of pharmaceuticals. In Europe, typical treatment trains for surface water include at least one of the above mentioned processes. Therefore, these findings suggest that at least in Europe only few pharmaceuticals will be present in treated surface water. The relatively few cases, where pharmaceuticals were detected in finished water up to now, provide further support for this conclusion (*4,6-8*). However, pharmaceuticals were not only detected in surface waters, but also in groundwaters (*9-11*). Sources for groundwater contamination can be land application of sludge or manure contaminated with human or veterinary pharmaceuticals, river bank filtration of contaminated surface water into groundwater, and artificial groundwater recharge with contaminated water (*12*). Groundwater does usually not require multiple treatment steps, because of its good chemical and biological quality. Often, it is only subjected to a disinfection step (e.g., treatment with chlorine, UV, or chlorine dioxide) or is not treated at all. Consequently, there is a risk that pharmaceuticals could be present in drinking water derived from groundwater.

3.1.2 Application of Chlorine Dioxide for Water Treatment

Chlorine dioxide (ClO_2) is an oxidant used for the disinfection of relatively high quality water, such as groundwater or treated surface water. Dosing of chlorine dioxide to the treated water in amounts providing residual concentrations will protect the drinking water distribution system from microbiological recontamination and fouling. In Europe, these ClO_2 residuals are often kept < 0.05 – 0.1 mg L^{-1} (*13*). In the USA, ClO_2 is rather used for the preoxidation of surface water. ClO_2 dosages ranging from 1 to 1.4 mg L^{-1} (*13,14*) are common in this case because higher doses are likely to result in an exceeding of the USEPA chlorite standard of 1 mg/L (*15*). Chlorite is the major reduction product of chlorine dioxide. It is considered to be a blood poison (*16*). A further application of ClO_2 in water treatment is disinfection of wastewater. Compared to chlorine, the major advantages of ClO_2 are the more effective inactivation of protozoa (e.g., *Cryptosporidium* and *Giardia*) and the fact that halogenated disinfection by-products are not formed under proper generation conditions (*17*).

3.1.3 Chemical Aspects of Chlorine Dioxide

Chemically, ClO_2 is a stable free radical that reacts with other water matrix components and micropollutants mainly through a one electron transfer reaction. A comprehensive study on the kinetics of ClO_2 reactions in water has been published by Hoigné and Bader (*18*). They have shown that ClO_2 is a highly selective oxidant with respect to organic compounds. Only compounds exhibiting specific functional groups like phenolic moieties or tertiary amino groups have a high reactivity toward ClO_2. The reactivity of these moieties is governed by speciation because the reactivity of the phenoxide ion and the neutral form of the amine is many orders of magnitude higher than the reactivity of the neutral phenol and the protonated amine.

Many pharmaceuticals exhibit phenolic moieties and/or amino groups in their structure. Therefore, ClO_2 is expected to oxidize a relatively large number of pharmaceuticals, despite its lower oxidation potential compared to ozone and hypochlorous acid. Considering the current use of ClO_2, it is important to know to what extent ClO_2 can act as barrier for pharmaceuticals when disinfection is the only treatment step. Furthermore, ClO_2 should be compared to other oxidants such as ozone and chlorine to check whether it is as effective for the oxidation of pharmaceuticals when used for preoxidation of surface water or for the oxidation of wastewater.

3.1.4 Objectives of the Present Study

The aim of the present study was to assess the potential of ClO_2 to oxidize pharmaceuticals during water treatment. For this purpose, second-order rate constant of selected environmentally relevant pharmaceuticals were determined in bench-scale experiments using pure aqueous solutions. In addition, rate constants of further pharmaceuticals were estimated based on their oxidation in lab-scale experiments performed using drinking water. With experiments in surface water and groundwater at concentrations in the nanogram/L to microgram/L range the validity of the rate constants was verified. Finally, the rate constants were compared to rate constants available for ozone and chlorine.

3.2 Experimental Methods

3.2.1 Chemicals

Chlorine dioxide was produced by mixing 50 mL of 4% potassium peroxodisulfate ($K_2S_2O_8$, 2 g in 50 mL Milli-Q water) with 50 mL of 8% sodium chlorite ($NaClO_2$, 4 g in 50 mL Milli-Q water) according to a method described by Gates (*13*). Caution: ClO_2 present in the gas-phase equilibrated with an

aqueous solution containing > 8 g L^{-1} of ClO_2 (>20 °C) is explosive. Pharmaceuticals were purchased from commercial suppliers at the purest grade available. All chemicals used for solutions (buffer, eluents, etc.) were reagent grade and were used without further purification. Stock solutions of pharmaceuticals were prepared either directly in Milli-Q water or in methanol.

3.2.2 Analytical Methods

ClO_2 concentrations were determined using three different methods as a function of experimental setup and conditions: (1) The direct spectrophotometrical determination at 359 nm using an absorption coefficient of ε = 1200 M^{-1} cm^{-1} (*18*), (2) the ABTS method (using 2,2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid-diammonium salt), a colorimetric method adapted from Pinkernell et al. (*19*), and (3) the LGB method, a colorimetric method using lissamine green B (*20*). The latter method had to be applied because certain target compounds produced interferences with ABTS.

For the determination of rate constants and experiments with water from Lake Zurich, sulfamethoxazole and diclofenac concentrations were measured with a Hewlett-Packard 1050 series HPLC equipped with an Ultra Aqueous C18 column from Restek (3.2 × 100 mm, 5 μm) and a variable wavelength detector. The eluents consisted of 10 mM phosphoric acid (A) and pure acetonitrile (B). Isocratic elution at a flow rate of 0.5 mL min^{-1} was carried out using A:B = 60%:40% and 25%:75% for diclofenac and sulfamethoxazole, respectively. With detection of diclofenac at 275 nm and sulfamethoxazole at 267 nm, a limit of quantification (LOQ) of approximately 20 nM (5 μg L^{-1}) was achieved. The 95% confidence intervals for a single measurement were typically ± 3 – 5%. With a similar method, 17α-ethinylestradiol (EE2) was detected using fluorescence detection as described in ref (*21*). The LOQ was 5 nM (1.5 μg L^{-1}) for this method.

For the analysis of trace concentrations of antibiotics in groundwater (for a list of compounds see Table 3-1), aliquots of 500 mL were adjusted to pH 4, and enriched unfiltered using solid phase extraction on Oasis HLB polymeric cartridges (*22*). Measurement was conducted using reversed-phase liquid chromatography coupled to electrospray positive tandem mass spectrometry (TSQ Quantum Discovery, Thermo Finnigan, San Jose, CA, USA). Quantification was performed using a standard addition method. The selected estrogens (Table 3-1) were extracted from 500 mL samples at pH 3 with pre-packed Lichrolut EN/PR-C18 cartridges followed by a silica clean-up (*23*). Separation and detection were performed with reversed phase chromatography coupled to tandem mass spectrometry (API 4000, Applied Biosystems, Foster City, CA, USA). Acidic and neutral pharmaceuticals (Table 3-1) spiked to drinking water were detected according to the methods described by Löffler and Ternes (*24*) and Ternes et al. (*25*).

3.2.3 Determination of Rate Constants

The kinetic concept, on which the present study is based, has been described in detail by Hoigné and Bader (*18*). Because they have demonstrated that reactions of ClO_2 with inorganic and organic compounds are largely first order with respect to ClO_2 and target compound concentration, the reaction order was not investigated explicitly in the present study. To determine the rate constants for the selected pharmaceuticals, kinetic runs were performed under pseudo-first order conditions with either the target compound or ClO_2 in excess.

Kinetic runs with excess of pharmaceuticals were conducted for the following compounds (concentrations in mM): bezafibrate (0.8), carbamazepine (0.2), diazepam (0.08), ibuprofen (0.8), iopromide (0.8), and roxithromycin (0.1). Except for roxithromycin, all solutions were buffered to pH 7 with 5 mM phosphate buffer. Solutions for roxithromycin were adjusted in the pH range of

TABLE 3-1. Investigated Pharmaceuticals.

Name	CAS RN	Use/Origin
antibiotics		
azithromycin	83905-01-5	macrolide antibiotic
clarithromycin	81103-11-9	macrolide antibiotic
dehydro-erythromycin		metabolite erythromycin
roxithromycin	80214-83-1	macrolide antibiotic
sulfamethazine	57-68-1	veterinary sulfonamide antibiotic
sulfamethoxazole	723-46-6	sulfonamide antibiotic
sulfapyridine	144-83-2	sulfonamide antibiotic
sulfathiazole	72-14-0	veterinary sulfonamide antibiotic
estrogens		
17α-ethinylestradiol (EE2)	57-63-6	synthetic steroid hormone
17β-estradiol	50-28-2	natural steroid hormone
estrone	53-16-7	natural steroid hormone
acidic pharmaceuticals		
bezafibrate	41859-67-0	lipid regulator
clofibric acid	882-09-7	metabolite of several lipid regulators
diclofenac	15307-86-5	antiphlogistic
fenoprofen	53746-45-5	antiphlogistic
gemifibrozil	25812-30-0	lipid regulator
ibuprofen	15687-27-1	antiphlogistic
ketoprofen	22071-15-4	antiphlogistic
naproxen	22204-53-1	antiphlogistic
neutral pharmaceuticals		
caffeine	58-08-2	psychostimulant
carbamazepine	298-46-4	antiepileptic drug
cyclophosphamide	6055-19-2	antineoplastic
diazepam	439-14-5	psychiatric drug
dimethylaminophenazone	58-15-1	antiinflammatory
glibenclamide	10238-21-8	antidiabetic
ifosfamide	3778-73-2	antineoplastic
pentoxifylline	6493-05-6	vasodilator
phenazone	60-80-0	antiinflammatory
propylphenazone	479-92-5	antiinflammatory
X-ray contrast media		
iopromide	73334-07-3	contrast medium

6.2 to 7.1 to investigate the pH dependency of its reactivity. The kinetic runs were started by the addition of ClO_2 doses (8 to 80 μM) that were at least 10 times lower than the target compound concentrations. The experiments were performed in spectrophotometric cells of 35-mL volume with Suprasil quartz window and 10-cm optical path length. The cell was mounted in a temperature-stabilized metal block (20 °C) inside the spectrophotometer (Kontron Instruments, Uvikon 940). ClO_2 decrease was monitored directly in the spectrophotometer. All runs were conducted in triplicates. For slow-reacting or nonreactive compounds, the decay was monitored during 1 hour. For roxithromycin, half-lives of ClO_2 ranged only from 20 s to 2 min under the investigated conditions. Consequently, monitoring time did not exceed 4 min.

Rate constants for diclofenac, sulfamethoxazole, and EE2 were determined by monitoring their decrease in the presence of an at least 10 fold excess of ClO_2. Initial concentrations for diclofenac, sulfamethoxazole, and EE2 were 0.2, 0.15, and 0.1 μM, respectively. Duplicate experiments were performed at pH 5, 6, 7, 8, 9, and 10 for diclofenac and sulfamethoxazole. Due to its increased reactivity at higher pH, the 6 experiments performed with EE2 were conducted in duplicate between pH 4 and 6. As a reaction vessel, 500-mL amber glass bottles with a dispenser system mounted onto the screwtop were used. Kinetic runs were started by the addition of the target compound. After 10 seconds, the first sample (5 mL) was withdrawn with the dispenser system. Subsequently, 5 further samples were withdrawn in suitable time intervals ranging from 10 s to 2 min depending on the apparent oxidation rate of the target compounds. Simultaneously, 2 to 5 samples were collected for ClO_2 analysis. For target compound analysis, ClO_2 residuals were immediately quenched by mixing the sample with 0.1 mL sodium thiosulfate (25 mM, for diclofenac and sulfamethoxazole) or ascorbic acid (25 mM, for EE2). Pseudo first-order rate constant were obtained by plotting the natural logarithm of the target compound

concentration versus the time. Since ClO_2 concentration typically decreased 10 to 20% during the course of the experiments, averaged concentrations were used to calculate the second-order rate constants.

3.2.4 Oxidation in Drinking Water

Water samples were taken at a German drinking water treatment plant (water source: bank filtrate from River Rhine) after sand filtration and immediately before the disinfectant dosing (ClO_2, for water quality parameter see Table 3-2). The samples were spiked with acidic and neutral pharmaceuticals (Table 3-1) up to the concentration of 1 μg L^{-1}. The bench-scale experiments were performed at room temperature in batch reactors (500 mL). The ClO_2 doses were 0.95 and 11.5 mg L^{-1}. After a reaction time of 30 min, the oxidation reaction was stopped by adding $Na_2S_2O_3$ solution to the reactor. The samples were then analyzed as described above.

TABLE 3-2. Water Quality Parameters

	pH	DOC (mg L^{-1})	alkalinity (mM HCO_3^-)
drinking water, Wiesbaden , Germany	7.4	1.0	4.4
water source: bank filtrate River Rhine			
lake water, Zurich, Switzerland	7.9	1.6	2.5
groundwater, Duebendorf, Switzerland	7.2	1.3	6.6

3.2.5 Oxidation in Lake Water

Water from Lake Zurich, Switzerland (for water quality parameters see Table 3-2) was buffered to pH 8 with borate buffer (10 mM). To assess the competition for ClO_2 between the water matrix and pharmaceuticals, 0.3 μM of iopromide, sulfamethoxazole, diclofenac, or EE2 was spiked to the lake water

that was subsequently treated with ClO_2 doses of 0.1, 0.2 0.5, and 1 mg L^{-1}. After 10 minutes, the reaction was quenched with sodium thiosulfate (for sulfamethoxazole and diclofenac) or ascorbic acid (for iopromide and EE2). Experiments were performed in duplicate and for every experiment only one compound was spiked to the Lake Zurich water. Sample analysis was carried out within a few hours using the HPLC methods described above.

Experiments investigating the oxidation kinetics of sulfamethoxazole and diclofenac in lake water were conducted under the same conditions except that these experiments were only carried out for one ClO_2 dose of 0.5 mg L^{-1}. As a reaction vessel, 500-mL amber glass bottles with a dispenser system mounted onto the screwtop were used. Kinetic runs were started by the addition of ClO_2. After 10, 20, 30, 50, and 70 s, samples (5 mL) were withdrawn with the dispenser system and immediately mixed with the sodium thiosulfate. Simultaneously, ClO_2 concentrations were measured with the LGB method.

3.2.6 Oxidation in Groundwater

In the first series of experiments, groundwater from Duebendorf, Switzerland (for water quality parameters see Table 3-2) was spiked with 1 μg L^{-1} of sulfapyridine, sulfathiazole, sulfamethazine, and sulfamethoxazole and 100 ng L^{-1} of azithromycin, clarithromycin, dehydro-erythromycin, and roxithromycin. Aliquots of 4 L of the spiked water were distributed into 3 amber bottles. A ClO_2 dose of 0.1 mg L^{-1} was added to each bottle. From each bottle, a few samples were taken to follow the ClO_2 concentration with the ABTS method. The reaction was then stopped by the addition of sodium thiosulfate after 5 min for the first bottle, after 30 min for the second bottle, and after 180 min for the third bottle. In the second series of experiments, the same groundwater was spiked with 1 μg L^{-1} of the estrogens EE2, 17β-estradiol, and estrone. The estrogens were treated with ClO_2 doses identical to those in the first series and

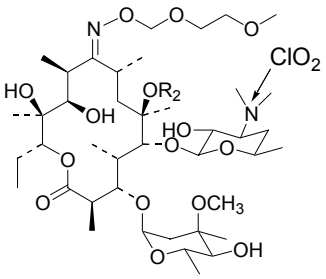
the samples were obtained by a similar procedure. Analysis of the samples was performed as described above.

3.3 Results and Discussion

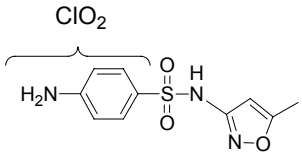
Table 3-3 reports the second-order rate constants of selected pharmaceuticals together with theoretical half-lives at a ClO_2 concentration of 0.1 mg L^{-1}. Out of the 9 investigated compounds only diclofencac, 17α-ethinylestradiol (EE2), roxithromycin, and sulfamethoxazole showed an appreciable reactivity (see Figure 3-1 for structures). The pH dependency of the respective rate constants is illustrated in Figure 3-2. EE2, roxithromycin, and sulfamethoxazole exhibit all strongly pH-dependent rate constants, which result in relative high reactivities at pH ≥ 7. These findings can be explained by the fact that ClO_2 reacts very selectively with certain functional groups with high electron densities such as phenoxide ions and neutral tertiary amines (*18*). The reactivity of the protonated forms of these functional groups is usually many orders of magnitude lower. Therefore, the pH-dependent reactivity of EE2 and roxithromycin, which exhibit a phenolic and a tertiary amino group, respectively, can be explained by the protonation state of their reactive functional groups. . The relatively high rate constant of the aniline derivative diclofenac indicates that also the aniline group is reactive to ClO_2. Surprisingly, the rate constant of neutral sulfamethoxazole (pK_a = 5.7), which also exhibits an aniline group, is <100 $M^{-1}s^{-1}$. However, because the deprotonation of the acidic nitrogen of the sulfonamide moiety enhances the reactivity of sulfomethoxazole considerably, the apparent rate constant at neutral pH is similar to that of diclofenac. Fitting the measured apparent rate constants for sulfamethoxazole to a kinetic model taking into account the speciation yielded the best fit when a pK_a of 6.1 instead of 5.7 was used. Due to the uncertainties associated with pK_a determination and kinetic

TABLE 3-3. Second-order Rate Constants for the Reaction of Chlorine Dioxide with Selected PPCPs

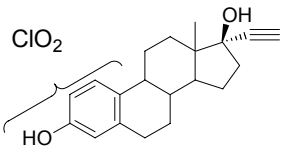
Compound (Species)	pK_a	k_{ClO2} [M^{-1} s^{-1}]	$t_{1/2}$ for 0.1 mg ClO_2 at pH 7	Studied pH Range
bezafibrate (anion)	3.6	<0.01		7
carbamazepine (neutral)		<0.015		7
diazepam (neutral)		<0.025		7
diclofenac (anion)	4.2	$(1.05 \pm 0.1) \times 10^4$	45 s	5 to 10
EE2 (neutral)	10.4	<200		2 to 3
EE2 (anion)		$(4.6 \pm 0.8) \times 10^8$	2.5 s	4 to 6
ibuprofen (anion)	4.9	<0.01		7
iopromide (neutral)		<0.01		7
roxithromycin (neutral)	8.8	$(1.4 \pm 0.3) \times 10^4$	35 min	6 to 7
sulfamethoxazole (neutral)	5.7	<100		4
sulfamethoxazole (anion)		$(7.9 \pm 0.9) \times 10^3$	70 s	5 to 10



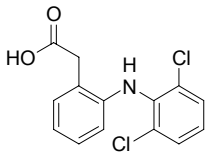
roxithromycin



sulfamethoxazole



EE2



diclofenac

FIGURE 3-1. Chemical structures of reactive pharmaceuticals and site of proposed ClO_2 attack.

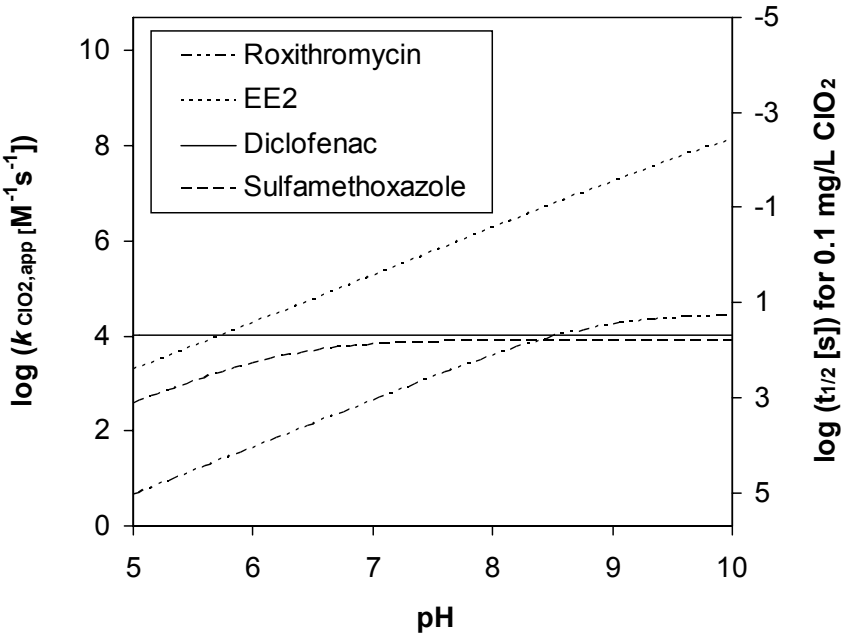


FIGURE 3-2. Apparent second-order rate constants and half-lives of the 4 reactive pharmaceuticals as a function of pH. The half-lives are calculated for a ClO_2 concentration of 0.1 mg L^{-1} (1.5 μM).

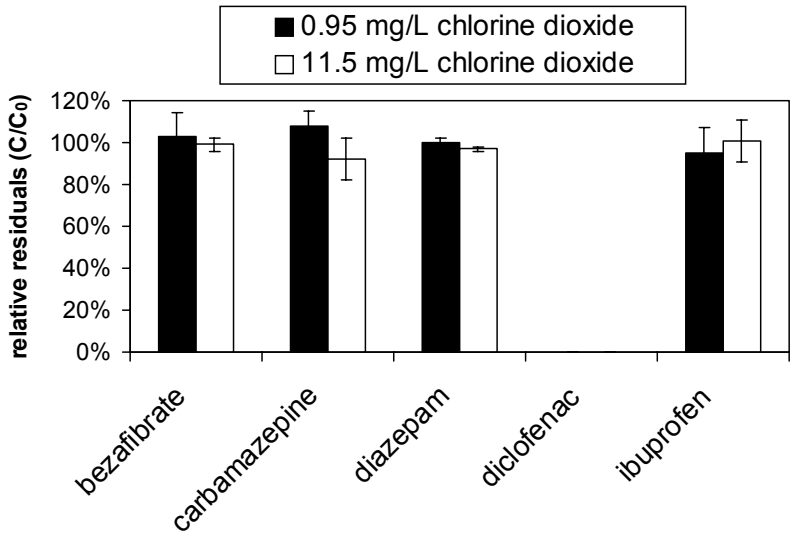


FIGURE 3-3. Relative residual concentrations of pharmaceuticals in drinking water after 30 min of treatment at ClO_2 doses of 0.95 and 11.5 mg L^{-1}. The pharmaceuticals were spiked to the drinking water at a level of 1 μg L^{-1} before the experiments.

measurements, this deviation may not be significant and was not investigated further.

3.3.1 Oxidation in Drinking Water

Drinking water spiked with selected pharmaceuticals (1 μg L^{-1}) was treated for 30 min at a dose of 0.95 and 11.5 mg L^{-1} ClO_2. Figure 3-3 shows the relative residuals of pharmaceuticals for which second-order rate constants were determined in pure water (Table 3-3). As predicted by the rate constants, bezafibrate, carbamazepine, diazepam, and ibuprofen showed no reactivity, whereas diclofenac was readily oxidized. Table 3-4 reports the residual concentrations of further pharmaceuticals investigated in the same experiments. Based on this data, the second-order rate constants given in the last column of Table 3-4 were estimated. Among these pharmaceuticals, only phenazone derivatives and naproxen exhibited an appreciable reactivity. Due to the relatively high reactivity of phenazone derivatives, only a lower limit of the rate constants could be estimated for these compounds.

3.3.2 Oxidation in Lake Water

In water treatment, ClO_2 is also applied for preoxidation of surface water. To find out whether the rate constants determined in pure water can be applied to predict the oxidation kinetics of pharmaceuticals in natural surface waters, water from Lake Zurich was spiked with selected pharmaceuticals and treated with ClO_2. To predict the extent of parent compound oxidation of a pharmaceutical compound (P), the following equation can be used:

$$\frac{[P]_\tau}{[P]_0} = e^{-k_{ClO2}\int_0^\tau [ClO_2]dt} \qquad (1)$$

TABLE 3-4. Relative Residual Concentrations (C/C_0) of Selected Pharmaceuticals after 30 min Treatment of Drinking Water Spiked with 0.95 and 11.5 mg L^{-1} ClO_2 and Estimated Second-order Rate Constants.

Compound (Species)	0.95 mg L^{-1} ClO_2 C/C_0 [%]	11.5 mg L^{-1} ClO_2 C/C_0 [%]	Estimated k_{ClO2} [M^{-1} s^{-1}]
caffeine	100 (±12)	99 (±7)	<1
clofibric acid	100 (±9)	100 (±5)	<1
cyclophosphamide	97 (±3)	88 (±13)	<1
fenoprofen	103 (±23)	103 (±30)	<1
gemifibrozil	93 (±10)	59 (±6)	<10
glibenclamide	86 (±14)	71 (±8)	<10
ifosfamide	98 (±1)	97 (±6)	<1
ketoprofen	105 (±12)	99 (±3)	<1
naproxen	53 (±9)	-	10-100
pentoxifylline	98 (±4)	102 (±12)	<1
phenazone	< LOQ	< LOQ	>100
dimethylaminophenazone	< LOQ	< LOQ	>100
propylphenazone	< LOQ	< LOQ	>100

[(a)] experimental conditions: pH = 7.4, T = 20 °C, contact time = 30 min.

where k_{ClO2} is the second-order rate constant for the reaction with ClO_2 and $\int_0^{\tau}[ClO_2]dt$ the chlorine dioxide exposure (ClO_2 concentration integrated over time) from t = 0 to t = τ. Consequently, predictions according to eq 1 can be made, if k_{ClO2} and the respective chlorine dioxide exposure are known. Figure 3-4 depicts the oxidation of sulfamethoxazole and diclofenac for a ClO_2 dose of 0.5 mg L^{-1} (symbols represent experimental data; lines represent predictions). As predicted, both compounds were oxidized by more than 90% within 60 s. This

demonstrates clearly that the determined rate constants can be applied for the description of ClO_2 oxidation processes in natural waters.

When ClO_2 is applied to a surface water that has a substantial ClO_2 demand, pharmaceuticals and the water matrix will compete for ClO_2 and even highly reactive pharmaceuticals may not be completely transformed below a certain ClO_2 dose. Figure 3-5 illustrates this competition for Lake Zurich water for various ClO_2 doses between 0.1 and 1 mg L^{-1}. As expected, the ClO_2 dose required to oxidize a pharmaceutical compound to more than 95% decreased with increasing rate constants. For sulfamethoxazole, a residual could still be detected after treatment with 0.2 mg L^{-1} ClO_2, whereas for EE2, which has the highest rate constant, a dose of 0.1 mg L^{-1} was sufficient to oxidize it to a residual accounting for 3% of its initial concentration.

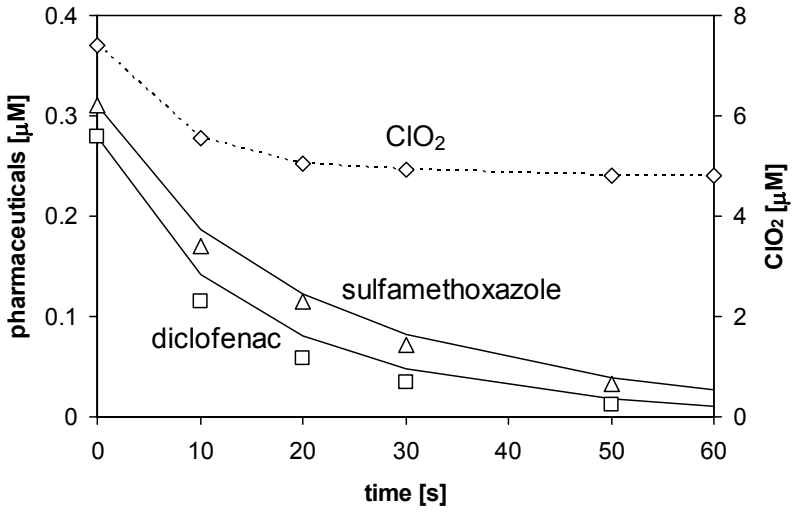


Fig. 4. Oxidation of sulfamethoxazole and diclofenac during treatment of Lake Zurich water with ClO_2. Experimental conditions: pH = 8, T= 20 °C, ClO_2 dose = 0.5 mg L^{-1}. Symbols represent measured data, and solid lines represent model calculations.

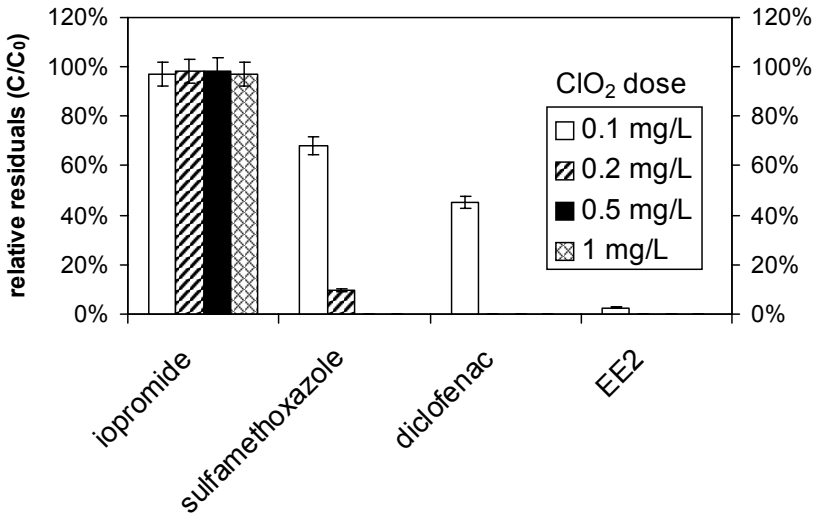


FIGURE 3-5. Relative residual concentrations of selected pharmaceuticals after treatment of lake water with different ClO_2 doses. Experimental conditions: pH = 8, T = 20 °C, $[pharmaceuticals]_0$ = 0.3 μM, contact time = 10 min.

3.3.3 Oxidation in Groundwater

ClO_2 is often dosed to finished drinking water as a final disinfectant. In this case, very low ClO_2 doses can be applied because this water does usually not exhibit a high ClO_2 demand (good quality groundwater or treated surface water). To simulate such conditions, groundwater was spiked with 4 sulfonamide (1 μg L^{-1} each) and 4 macrolide antibiotics (100 ng L^{-1} each) as well as 3 estrogens (1 μg L^{-1} each) and subsequently treated with 0.1 mg L^{-1} ClO_2. The half-life of ClO_2 was approximately 15 min under these conditions. Figure 3-6 shows the relative residual concentrations of the investigated compounds measured after 5, 30 und 180 min.

The investigated sulfonamides sulfamethoxazole, sulfamethazine, sulfapyridine, and sulfathiazole were oxidized to more than 95% after 30 min of contact time. Despite structural similarities, in particular the common aniline group that is expected to be reactive toward ClO_2, the reactivity of the different sulfonamides varied somewhat more than expected. These variations can be

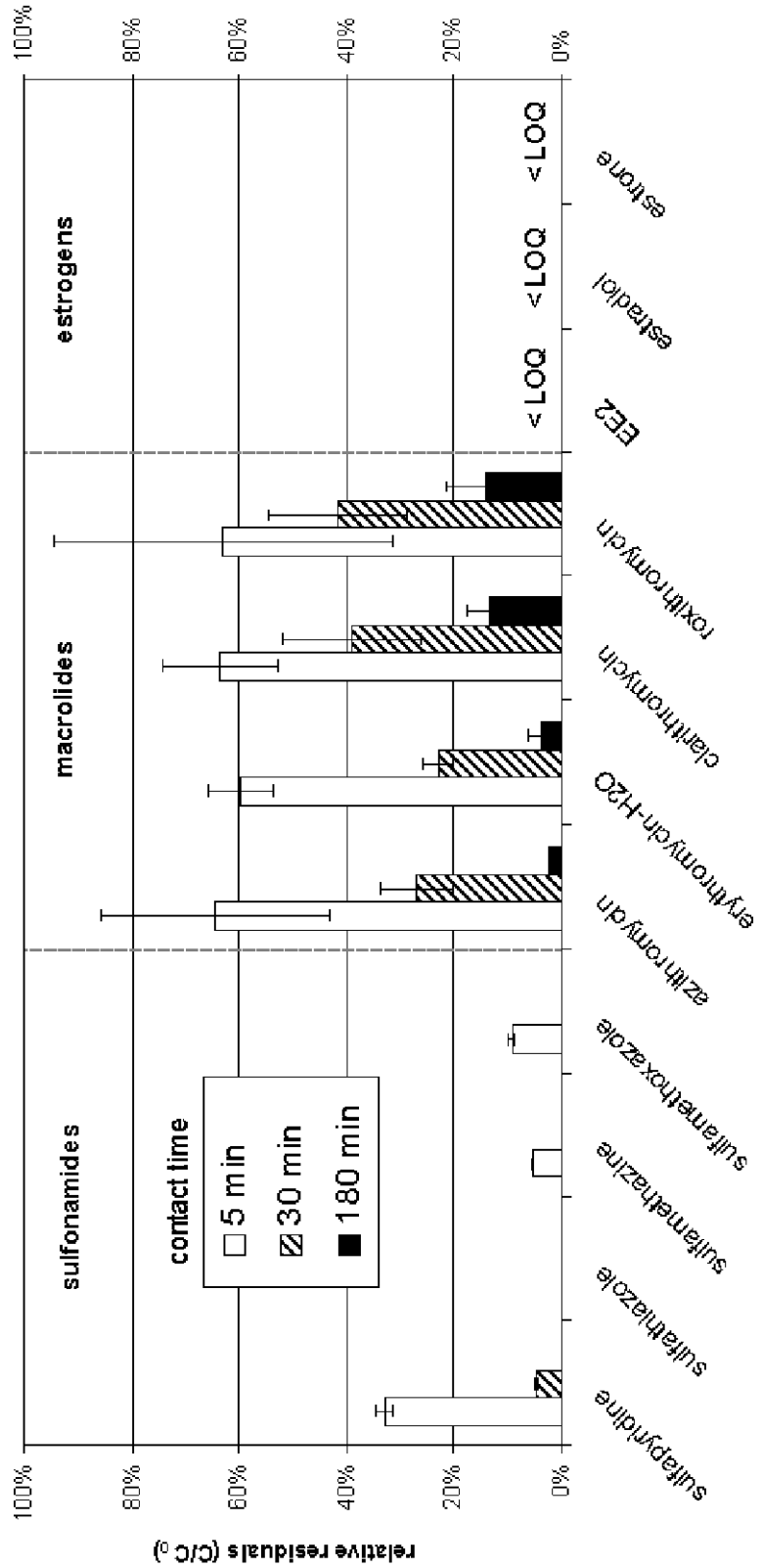


FIGURE 3-6. Relative residual concentrations of selected sulfonamides, macrolides and estrogens in groundwater (Dübendorf, Switzerland) as a function of contact time with ClO_2. Experimental conditions: ClO_2 dose = 0.1 mg L^{-1}, pH = 7.5, T = 20 °C, $[sulfonamides]_0$ and $[estrogens]_0$ = 1 µg L^{-1}, $[macrolides]_0$ = 100 ng L^{-1}.

explained to some extent by the differences in speciation. Based on the results for sulfamethoxazole, the reactivity of the sulfonamides seems strongly influenced by the protonation state of the acidic nitrogen of the sulfonamide group. The pK_a value of this nitrogen ranges from 5.7 for sulfamethoxazole to 8.4 for sulfapyridine. Accordingly, sulfapyridine was predominantly present in its neutral form in the groundwater and therefore less reactive. However, it cannot be excluded that some of the differences are also caused by the fact that ClO_2 attack does not occur primarily on the aniline moiety but on the sulfur bearing thiazole group of sulfathiazole or on the isoxazole moiety of sulfamethoxazole.

The investigated macrolides azithromycin, clarithromycin, dehydro-erythromycin, roxithromycin were oxidized more slowly than the sulfonamides and the extent of parent compound oxidation reached > 80% only after 180 min. This can be expected from their lower apparent rate constants for the reaction with ClO_2. According to their common reactive moiety (tertiary amino group), the oxidation pattern for all compounds is very similar. However, it has to be taken into account that standard deviations for some of the macrolides were unusually large. The large errors could be the result of a suboptimal solid phase extraction. Due to the use of better surrogate standards (structurally identical isotop-labeled compounds instead of structurally similar compounds), the precision of sulfonamide measurements was much better.

The estrogens EE2, 17β-estradiol, and estrone reacted too fast to be detected after 5 min contact time. As expected on the basis of their similar structures, 17β-estradiol, and estrone must exhibit a similar rate constant as EE2. Overall, the results for the groundwater experiments show that the rate constants for sulfamethoxazole, roxithromycin, and EE2 can be taken as rough estimates for the rate constants of other pharmaceuticals (hormones) belonging to the same chemical class.

In Figure 3-7, the relative residuals predicted on the basis of the ClO_2 exposure (eq 1) and the measured residuals of roxithromycin and sulfamethoxazole are plotted as a function of time. To give an estimate for the uncertainty associated with the used model, the minimal and maximal residuals, predicted on the basis of minimal and maximal values for the rate constant of roxithromycin and the chlorine dioxide concentrations, are also indicated (dashed lines). Unfortunately, the measurements for roxithromycin were not very precise. Nevertheless, it can be concluded that predictions and measured data agreed reasonably well for both compounds. This demonstrated that the rate constants determined in pure water at high concentrations of target compounds can be applied to natural waters containing realistic concentrations of pharmaceuticals.

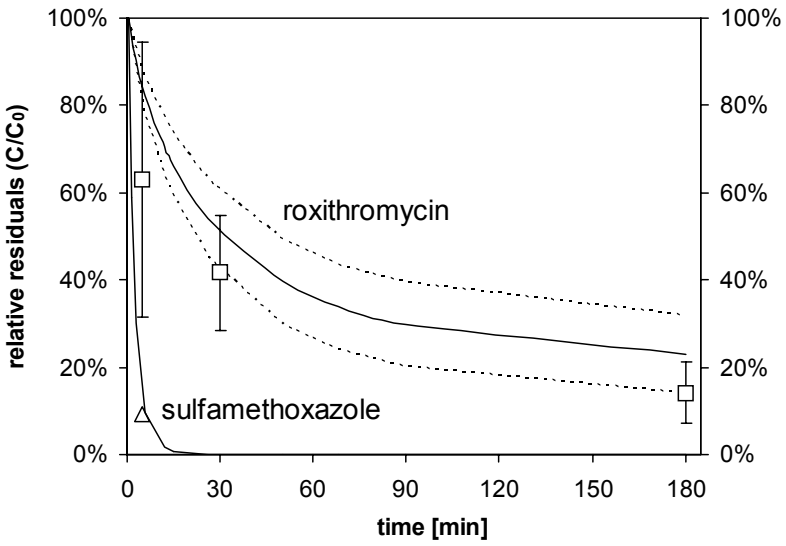


FIGURE 3-7. Relative residual concentrations of roxithromycin for treatment of groundwater with a 0.1 mg L^{-1} ClO_2 dose. Experimental conditions: pH = 7.5, T = 20 °C, $[roxithromycin]_0$ = 100 ng L^{-1} (0.3 μM), $[sulfamethoxazole]_0$ = 1 μg L^{-1} (0.3 μM). Symbols represent measured data, solid lines represent model calculations, and dashed lines indicate the estimated uncertainty of the model calculations.

3.3.4 Oxidation Products

Oxidative treatment should not only lead to the disappearance of parent compounds, but also destroy pharmacological or biological effects of pharmaceuticals. Because full mineralization is generally not achievable with oxidant doses commonly used in water treatment, oxidative treatment can only deactivate pharmaceuticals by selectively oxidizing functional groups that are crucial for their effects. To date, little is known about the oxidation products resulting from the reaction of ClO_2 with pharmaceuticals. However, the work of Rosenblatt et al. (*26*) provides strong evidence that for tertiary amines ClO_2 attack leads to the cleavage of one of the N-C bonds. In the case of the macrolide antibiotics, this reaction will result in the loss of a methyl group or the loss of the whole amino group. Li et al. (*27*) have shown that the antibiotic activity of demethylated roxithromycin is much lower than that of the parent compound.

For ozone, it could be shown that most probably small modifications of the phenolic moiety of EE2 (caused by substoichiometric ozone doses) already resulted in a significant decrease of its estrogenicity (*21*). It is expected that oxidation of EE2 by ClO_2 yields similar quinone type products as the initial oxidation by ozone (*28*). Therefore, treatment with ClO_2 could decrease the estrogenic activity of EE2, even though the phenolic moiety will probably not be cleaved to the same extent as under typical treatment conditions for ozonation. On the basis of these considerations, it can be assumed that treatment with ClO_2 does not only result in the disappearance of macrolides and estrogens, but also potentially reduces their pharmacological effects.

3.3.5 Comparison of Chlorine Dioxide with Ozone and Chlorine

It is of great interest to compare the rate constants determined for ClO_2 with rate constants for other oxidants such as chlorine and ozone used in water

treatment. Recent publications on ozonation (*3*) and chlorination (*29,30*) of pharmaceuticals as well as some experiments with chlorine performed within the framework of this study provided a set of rate constants which can be compared to that of chlorine dioxide. In Figure 3-8, the rate constants of these compounds are presented as apparent rate constant at pH 7. Furthermore, theoretical half-lives are given for an oxidant concentration of 1 mg L^{-1}. Reactions with ozone are clearly fastest and result in the shortest half-lives. Additionally, ozone reacts with the largest number of compounds. The apparent rate constants for the reaction with ClO_2 are roughly 2 orders of magnitude lower than ozone rate constants, but higher (to varying extents) than chlorine rate constants. However, the latter observation can not be generalized, because functional groups like aliphatic primary and secondary amines are known to react significantly faster with chlorine than chlorine dioxide (*18,31,32*). For the limited number of pharmaceuticals presented here, it was shown that ClO_2 and chlorine react with the same compounds. Both oxidants react first of all with electron rich functional groups like amines and phenols. Ozone reacts with the same functional groups, however additionally with C=C double bonds and activated benzene rings. With respect to ozone, it has also to be taken into account that hydroxyl radicals, which are formed due to ozone decay, can lead to a substantial oxidation of ozone refractive pharmaceuticals (*3*).

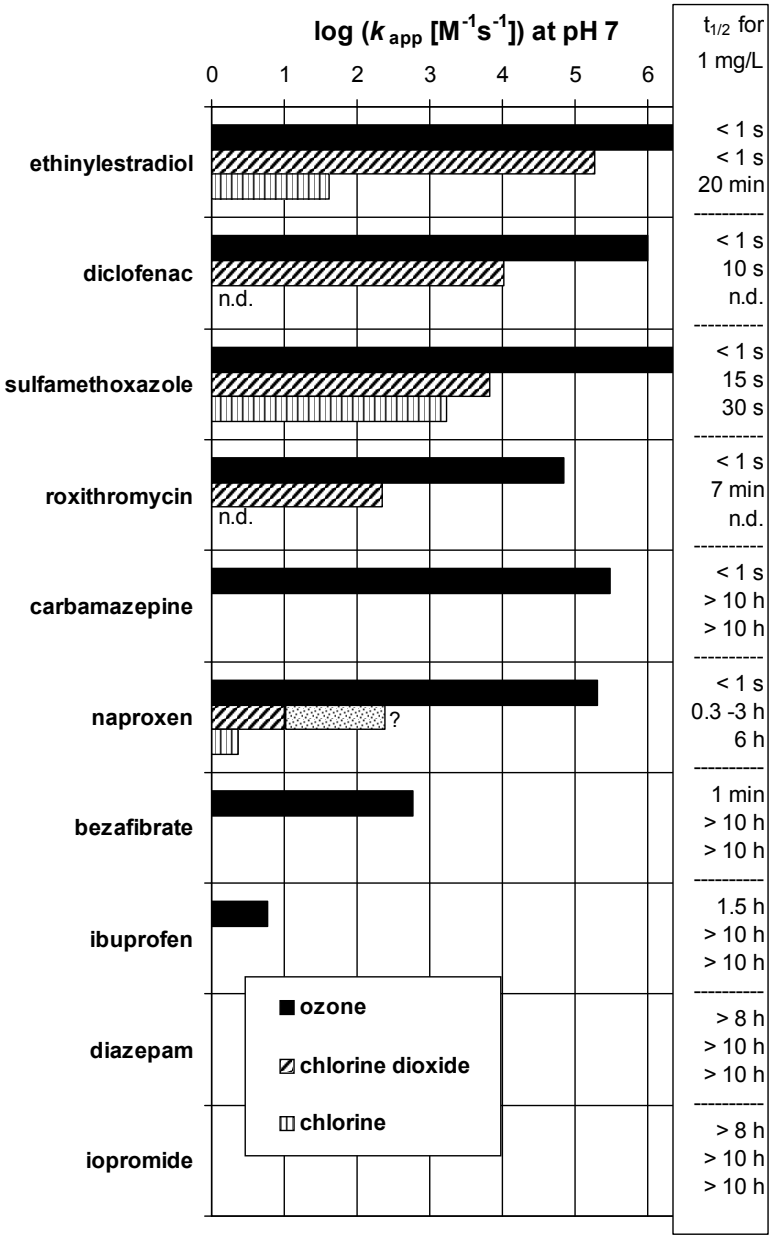


FIGURE 3-8. Comparison of apparent second-order rate constants for the reaction of selected pharmaceuticals with ozone, chlorine dioxide and chlorine at pH 7. The column on the right hand side presents theoretical half-lives calculated for an oxidant concentration of 1 mg L^{-1}. For ozone, only the direct reactions with O_3 and no hydroxyl radical reactions were considered. Unavailable rate constants are designated with n.d. for not determined. For the reaction of naproxen with ClO_2 only a range for k_{app} can be given. Sources for rate constants ($k_{oxidant}$) not determined in the present study: for k_{O3} (*3*), k_{Cl2} with EE2 (*33*), k_{Cl2} with sulfamethoxazole (*30*), and k_{Cl2} with naproxen and ibuprofen (*29*).

3.4 Conclusions

Macrolide and sulfonamide antibiotics as well as estrogens and phenazones are readily oxidized by ClO_2. However, many of the investigated compounds did not react at an appreciable rate with ClO_2. Therefore, it can be concluded that ClO_2 applied in water treatment does only partly act as barrier for pharmaceuticals, even though it is relatively effective in oxidizing antibiotics and estrogens, two compound classes that merit special concern due to their high biological activity. Ozone, which exhibits higher rate constants and reacts with a larger number of pharmaceuticals, seems to be considerably more efficient for pharmaceutical control than ClO_2. However, ClO_2 appears slightly more powerful for the oxidation of pharmaceuticals than chlorine. For a more comprehensive comparison of these oxidants, additional knowledge about the formation of oxidation product and their pharmacological or biological effects would be necessary.

Acknowledgment

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4

Removal of Estrogenic Activity and Formation of Oxidation Products during Ozonation of 17α-Ethinyl-estradiol

Huber, M. M.; Ternes, T. A.; von Gunten, U. *Environ. Sci. Technol.* **2004**, 38, 5177-5186.

Abstract

This study investigated the oxidation of the oral contraceptive 17α-ethinylestradiol (EE2) during ozonation. First, the effect of ozone (O_3) on the estrogenic activity of aqueous solutions of EE2 was studied using a yeast estrogen screen (YES). It could be shown that O_3 doses typically applied for the disinfection of drinking waters were sufficient to reduce estrogenicity by a factor of more than 200. However, it proved impossible to completely remove estrogenic activity due to the slow reappearance of 0.1-0.2% of the initial EE2 concentration after ozonation. Second, oxidation products formed during ozonation of EE2 were identified with LC-MS/MS and GC/MS and the help of the model compounds 5,6,7,8-tetrahydro-2-naphthol (THN) and 1-ethinyl-1-cyclohexanol (ECH), which represent the reactive phenolic moiety and the ethinyl group of EE2. Additionally, oxidation products of the natural steroid hormones 17β-estradiol (E2) and estrone (E1) were identified. The chemical structures of the oxidation products were significantly altered as compared to the parent compounds, explaining the diminished estrogenic activity after ozonation. Overall, the results demonstrate that ozonation is a promising tool for the control of EE2, E2, and E1 in drinking water and wastewater.

4.1 Introduction

In recent years, several studies have reported the occurrence of a large number of pharmaceuticals in the aquatic environment (*1-3*). The presence of pharmaceutical compounds in water resources makes it necessary to assess water-treatment processes with respect to their removal efficiency for such compounds. Several studies have recently shown that ozonation is a very promising technology for the oxidation of pharmaceuticals during water treatment (Chapter 2, (*4-6*)). The results of these studies are based on the disappearance of the parent compounds. At O_3 doses typically used in water treatment, full mineralization of pharmaceuticals is not achievable, and consequently ozonation results in the formation of oxidation products. Depending on the functional group that is attacked by O_3 and its location in the molecule, changes in the molecular structure might be significant enough to destroy the pharmacological effects of the parent compound. However, it cannot be excluded that in some cases oxidation products still produce the original pharmacological effects. To confirm that ozonation is suitable for the removal of pharmaceuticals, it should be at least shown that there is high evidence that pharmacological effects are significantly reduced. So far, the formation of ozonation products has been investigated for very few pharmaceutical compounds (*7,8*), and the decrease in pharmacological effects never has been checked in a comprehensive manner.

In the present study, the oral contraceptive 17α-ethinylestradiol (EE2) was selected on the basis of its LOEC (lowest observed effect concentration) of 0.1 ng L^{-1} for vitellogenin induction in rainbow trout (*9*). In effluents of sewage treatment plants (STPs), its concentration typically ranges from < 0.5 ng L^{-1} to 10 ng L^{-1} (*10*). In vivo tests showed that EE2 is approximately 11-27 times more potent than the female sex hormone 17β-estradiol (E2) (*11*). On the basis of in

vivo estrogenic potency, EE2 might be the most important endocrine disruptor in STP effluents together with the octyl- and nonylphenols (*12*).

The estrogenic activity can be considered the primary pharmacological effect of EE2. With the yeast estrogen screen (YES) described by Routledge and Sumpter (*13*), a robust and easy to handle test was available to quantify the effect of ozonation on the estrogenic activity of pure aqueous solutions of EE2. In detail, the effects of substoichiometric O_3 doses, resulting in a partial transformation of EE2, as well as the effects of higher O_3 doses typical for drinking water treatment have been investigated in bench-scale experiments. In the second part of this study, a number of oxidation products formed during the ozonation of EE2, E2, and E1 were identified.

During ozonation, micropollutants such as EE2 can be oxidized either by ozone (O_3) directly or by hydroxyl radicals ($^{\bullet}OH$), which are formed as a consequence of O_3 decay. The two oxidants vary strongly in their reactivity. O_3 attacks selectively certain functional groups whereas $^{\bullet}OH$ is a nonselective oxidant that reacts very fast with a large number of moieties. Consequently, most $^{\bullet}OH$ are scavenged by the water matrix in natural waters. EE2 reacts fast with O_3 and $^{\bullet}OH$ (Chapter 2). However, due to the higher selectivity of O_3, oxidation by O_3 is normally the predominant process. Therefore, the present study focused on direct O_3 reactions and reactions with $^{\bullet}OH$ were suppressed by the use of scavenger compounds such as *tert*-butyl alcohol (TBA). The performance of ozonation processes with respect to the oxidation of micropollutants can be assessed with O_3 and $^{\bullet}OH$ exposure (i.e., concentration of oxidant integrated over the reaction time) (*14*). When $^{\bullet}OH$ is scavenged, the oxidation of micropollutants is only a function of O_3 exposure. This parameter is also used to assess the disinfection efficiency of ozonation processes. Therefore, O_3 exposure allows us to compare the removal of estrogenic activity to the inactivation of microorganisms.

4.2 Experimental Section

4.2.1 Standards and Reagents

Estrone (E1), 17β-estradiol (E2), 1-ethinyl-1-cyclohexanol (ECH), 5,6,7,8-tetrahydro-2-naphthol (THN), adipic acid, cyclohexanone, 2-methyl-3-butyn-2-ol, α-hydroxyisobutyric acid, and the methylester of 1-hydroxycyclohexane-1-carboxylic acid were purchased from Sigma-Aldrich (see Figure 4-1 for chemical structures). The chemicals were of the highest purity available. 17α-Ethinylestradiol (EE2) was provided by Schering/Berlin, Germany. Depending on the experiments, stock solutions of EE2 and the model compounds ECH and THN were prepared either in Milli-Q purified water (Millipore), in *tert*-butyl alcohol (TBA), or in acetone. Stock solutions of E1 and E2 were prepared in TBA or in acetone. All chemicals used for solutions (buffer, eluents, etc.) were reagent grade and were used without further purification. O_3 was produced with a Fischer 500 and a Fischer 502 ozone generator by using pure oxygen as feed gas. O_3 stock solutions (~1 mM) were produced by sparging O_3-containing oxygen through Milli-Q water that was cooled in an ice bath (*15*).

4.2.2 Determination of EE2

EE2 was determined with a Hewlett-Packard 1050 series HPLC system equipped with a Nucleosil-100 C18 column (4 × 125 mm, 5 μm) and a fluorescence detector (HP 1064A). The mobile phase consisted of 50% 10 mM phosphoric acid and 50% acetonitrile at a flow rate of 0.6 mL/min. An excitation wavelength of 229 nm and emission wavelength of 309 nm were used for fluorescence detection. Under the experimental conditions, the quantification limit of EE2 was 5 nM (1.5 μg L^{-1}) for an injection volume of 100 μL. The error of a single measurement was approximately ±5%. Enrichment by freeze-drying lowered the quantification limit to 0.2 nM (60 ng L^{-1}). The EE2 recovery for freeze-dried samples was 70-90%.

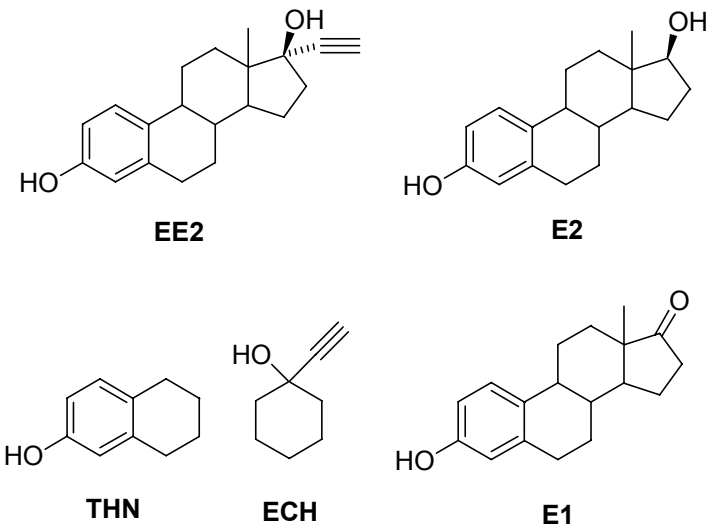


FIGURE 4-1. Structures of 17α-ethinylestradiol (EE2), 17β-estradiol (E2), estrone (E1), and the model compounds 5,6,7,8-tetrahydro-2-naphthol (THN) and 1-ethinyl-1-cyclochexanol (ECH).

4.2.3 Determination of Ozone, Hydroperoxides, and Formic Acid

Dissolved O_3 was determined with the indigo method (*15*) or spectrophotometrically by measuring the absorbance at 258 nm (ε = 3000 M^{-1} cm^{-1}). Hydroperoxides and hydrogen peroxide (H_2O_2) were measured using Allen's reagent as described by Flyunt et al. (*16*). Catalase was applied to selectively quench H_2O_2. Formic acid was determined with ion chromatography using a method adapted from ref (*17*).

4.2.4 LC-MS/MS Analysis

The HPLC system consisted of a Merck-Hitachi pump with an Inertsil ODS-3 column (4.6 × 100 mm, 3 μm). Elution was performed with 0.1% acetic acid (A) and acetonitrile (B) at a flow rate of 0.4 mL min^{-1}. The gradient was as follows: B started at 20% and was increased with a linear gradient to 80% after 15 min. After 2 min with B at 80%, the system was reequilibrated for 15 min. Mass spectrometry was performed using an API 365 (PE Sciex) triple quadrupole

mass spectrometer with turbo-electrospray ionization (ESI). Nitrogen was used as curtain gas, and synthetic air was used as nebulizer gas. Curtain and nebulizer gas flows were both operated with a flow rate of 1.0 L min^{-1}. The ESI interface was heated to 400 °C. The HPLC flux was split 1:10 to diminish the flow rate in the ESI.

Full scans as well as precursor and product ion scans were conducted to determine the quasi-molecular ions and the structure of major oxidation products. Before analysis, aliquots of the ozonated solutions (100-250 mL) were freeze-dried. The residual components were resuspended in approximately 2 mL of a water/acetonitrile (20/80, v/v) mixture and filtered with a 0.2 μm filter. The samples were analyzed in the positive as well as the negative mode. Adipic acid, cyclohexanone, 1,2-cyclohexanedione, and α-hydroxyisobutyric acid were quantified using the MRM (multiple reaction monitoring) mode after optimizing the LC-MS/MS for each compound. For quantification, aqueous solutions were analyzed directly without any concentration steps.

4.2.5 GC/MS Analysis

After freeze-drying 100-250 mL aliquots of ozonated model-compound solutions, the residual components were resuspended in approximately 2 mL of acetone and filtered with a 0.2 μm filter to remove insoluble components. Aliquots of 0.2 mL were derivatized by adding 0.2 mL of a diazomethane/diethyl ether solution at -20 °C. The samples were incubated for 1 h at -20 °C before diazomethane was quenched with 2-3 drops of acetic acid/acetone solution (1:10, v/v). Subsequently, the volume of the samples was reduced to 200 μL by a gentle nitrogen stream. Diazomethane is highly toxic and carcinogenic. All work has to be conducted in a fume hood and skin contact has to be strongly avoided.

Separation and detection were accomplished with a Varian GC 3400 coupled to a Varian Saturn 4D mass spectrometer. The gas chromatograph was equipped with a PTV injector and a Restek XTI-5 column (30 m × 0.25 mm × 0.25 μm). GC injection parameters: 5 μL, splitless; 50 °C; 100 °C min^{-1} to 300 °C, 300 °C isothermal 10 min. Oven temperatures: 50 °C isothermal for 2 min, 2.8 °C min^{-1} to 120 °C, 15.4 °C min^{-1} to 290 °C, and 290 °C isothermal for 10 min.

4.2.6 Recombinant Yeast Estrogen Screen (YES)

The YES was conducted as described by Routledge and Sumpter (*13*). Briefly, the recombinant yeast strain used in the screen expresses the human estrogen receptor (hER), which can interact with estrogenic compounds. Upon binding an active compound, the hER triggers the expression of the reporter gene lac-Z, which promotes the production of the enzyme β-galactosidase. The enzyme metabolizes the yellow dye chlorophenol red-β-D-galactopyranoside (CPRG) into a red product that can be measured spectrophotometrically.

Depending on the expected EE2 concentrations, aqueous samples were freeze-dried and resuspended in ethanol or directly diluted in ethanol to obtain appropriate concentrations for the YES. Based on these solutions, series of 6 or 12 dilutions (1:1) were prepared in ethanol. Of each dilution, 20 μL was added to a 96-well microtiter plate. Besides the samples, each plate contained a standard curve with E2 in ethanol (final concentration dissolved in assay medium: 2×10^{-8} to 1×10^{-11} M) and a row of blanks. After ethanol was evaporated to dryness, the yeast cells were added together with the assay medium. The color development was measured after incubating the microtiter plate for 72 h at 30 °C.

To evaluate the data, the sigmoidal concentration-response curve of the E2 standard was fitted to a symmetric logistic function (eq 1) using the software Prism (GraphPad, San Diego, CA).

$$response = a + \frac{b - a}{1 + 10^{(\log EC50 - \log c)\cdot m}} \quad (1)$$

a is the baseline response (bottom), b is the maximum response (top), c is the concentration, m is the Hill slope, and EC50 is the concentration provoking the half-maximal response.

With the values received for b (top) and a (bottom), the response of the standard and the samples was expressed as the percent of maximum response evoked by E2. To determine the EC50 and the Hill slope m of the E2 standard curve, the concentration-response curve was fitted again to eq 1 with the response expressed in percent whereby a and b were held constant at 0% and 100%, respectively. To calculate the EC50 values for the samples, the response in percent was fitted to eq 1 whereby a (0%), b (100%), and m were held constant. The concentration c in eq 1 was replaced by the concentration factor. The estrogenic activity of a sample expressed in 17β-estradiol equivalents (EEQs) was then calculated as the ratio of the EC50 for E2 to the EC50 for the sample.

4.2.7 Ozonation Experiments for YES

If not stated otherwise, the reaction solutions consisted of Milli-Q purified water spiked with 1 or 10 µM EE2 and were buffered to pH 8 with 5 mM phosphate buffer. Furthermore, the solutions contained 5 mM TBA as an $^{\bullet}OH$ scavenger.

Substoichiometric O_3 Doses. Experiments with substoichiometric O_3 doses were carried out as follows: To a series of identical reaction solutions (20 mL) with 10 µM EE2, O_3 doses ranging from 5 to 24 µM were added under vigorous stirring. Immediately after the addition of O_3, 10 mL of the solution was removed from the reaction vessel and stored for 2-3 days at room temperature. Of the 10 mL, 0.5 mL was taken for HPLC analysis and 9.5 mL was freeze-

dried. After freeze-drying, the residual components were redissolved with the help of sonication in 0.95 mL of ethanol. Insoluble phosphate salts were allowed to settle before the liquid phase was removed and stored at -20 °C until the YES was conducted. Samples with EE2 > 0.5 μM were diluted directly with ethanol without freeze-drying.

Reduction of Estrogenicity as a Function of O_3 Exposure. To quantify the reduction in estrogenicity as a function of O_3 exposure**,** a reaction solution (500 mL) spiked with 1 μM EE2 was thermostated at 10 °C. After adding 20 μM (1 mg L^{-1}) O_3, a series of 10 mL samples was taken over the course of the reaction. Thiosulfate was used to quench O_3 in the samples. The corresponding O_3 exposures of the samples were calculated on the basis of an O_3 decay curve, which was determined in a preliminary experiment under conditions identical to those described in ref (*18*). In an experiment with a slightly different setup, 20 μM (1 mg L^{-1}) O_3 was added to a series of 250 mL solutions. Samples of 100 mL were taken after the desired time intervals and immediately quenched with thiosulfate. These experiments allowed for the quantification of EE2 concentration < 1 nM with HPLC due to a higher enrichment during freeze-drying. After quenching O_3, samples of both experiments were stored for 3 days at room temperature before they were freeze-dried. With the help of sonication, the residual components were redissolved in 1 and 2 mL of ethanol, respectively.

4.2.8 Kinetics of Reappearance of EE2 after Ozonation

The reappearance kinetics of EE2 was investigated at two different O_3 concentrations (50 and 100 μM), which were added to two reaction solutions spiked with 10 μM EE2. After 5 min, 2 mL of the solutions was withdrawn and transferred into an amber HPLC vial where O_3 was immediately quenched with thiosulfate. Each of the samples was repeatedly analyzed over the next 165 h.

To investigate the pH dependence of the EE2 reappearance, reaction solutions were buffered to pH 5, 6, 7, 8, and 9. The experiments were performed as described for the reappearance kinetics except that samples were only analyzed after 3 days.

4.2.9 Investigation of Product Formation

The oxygen stream containing 2% O_3 was continuously bubbled through a 1-L amber glass bottle, which contained the reaction solution. The solution was stirred with a magnetic stir bar at 80 rpm. Reaction solutions of the model compounds THN (0.2 mM) and ECH (5 mM) as well as of 2-methyl-3-butyn-2-ol (5 mM) were prepared by dissolving the pure chemicals in acetone or TBA and spiking them to Milli-Q water. The reaction solutions containing the model compounds were ozonated during 5-40 min. The experiments that produced the highest yield with respect to the quantified oxidation product were repeated at pH 7 (5 mM phosphate buffer) with the corresponding ozonation time. Reaction solutions containing the estrogens consisted of EE2 (0.02 mM), E2 (0.005 mM), or E1 (0.005 mM) dissolved in Milli-Q water and either acetone or TBA as $^{\bullet}OH$ scavenger. Due to the low solubility of the estrogens in aqueous solutions, only low starting concentrations could be selected. In a first step, estrogen solutions were ozonated for 0.5 min (E1, E2) to 2 min (EE2). To increase the product yield, the ozonated solution was spiked again with the corresponding estrogen and subjected to ozonation for an identical time interval. Overall, this procedure was repeated 10 times. The fact that oxidation products were more polar than the parent compound prevented the precipitation of oxidation products.

4.3 Results and Discussion

The second-order rate constant (k_{O3}) for the reaction of 17α-ethinylestradiol (EE2) with ozone (O_3) is extremely high (at pH 7, $k_{O3} = 3 \times 10^6$ M^{-1} s^{-1} (Chapter

2)), resulting in a half-life of approximately 10 ms for an O_3 concentration of 1 mg L^{-1}. O_3 attack must take place on the phenolic moiety of EE2, because phenols are well known to react fast with ozone at neutral or basic pH (*19*). In this pH range, the reaction of O_3 with the phenolate anion of EE2 is the predominant reaction whereas the reaction with neutral EE2 is negligible. Consequently, the reaction of EE2 (pK_a = 10.4) with O_3 is strongly pH-dependent (the apparent k_{O3} at pH 8 is 10 times higher than at pH 7). The second reactive group of EE2, the ethinyl group, has a considerably lower reactivity toward O_3. Its k_{O3} is estimated to be close to the k_{O3} of ECH, which was determined in this study to be 200 M^{-1} s^{-1} at 20 °C. The corresponding half-life is approximately 3 min for an O_3 concentration of 1 mg L^{-1}. On the basis of these kinetic considerations, it is clear that EE2 disappears very fast during ozonation processes. However, if ozonation is applied to treat an EE2-containing water, the removal of estrogenic activity has to be assessed in addition to the disappearance of EE2. To test whether ozonation reduces estrogenic activity, aqueous solutions of EE2 were treated with O_3 and subsequently tested for estrogenic activity using a yeast estrogen screen (YES).

4.3.1 Reduction of Estrogenicity with Substoichiometric Ozone Doses

The high k_{O3} for the oxidation of EE2 refers to the first transformation step. On the basis of a study on the ozonation of phenol (*20*), it can be assumed that some of the important subsequent oxidation steps of EE2 proceed as fast as the first transformation step. This sequence of fast transformation steps will lead to a modification and partly to a cleavage of the phenol ring in EE2. To test whether this results in a substantial removal of estrogenicity, experiments were performed with sub-stoichiometric O_3 doses. Under these conditions, only the fast initial transformation steps take place.

Figure 4-2a illustrates the correlation between decrease in estrogenicity and the concentration of EE2 as a function of O_3 dosages. The estrogenicity

expressed in 17β-estradiol equivalents (EEQs) and EE2 concentrations were normalized to the values of the solution without O_3 ($[EE2]_0$ = 10 μM). Approximately 1.8 mol of O_3 was consumed per mole of EE2. The stoichiometry is higher than 1 because oxidation products of the first oxidation step obviously underwent further reactions with O_3. Adding 19 μM O_3 reduced the estrogenicity of the solution to 1.5%. The reduction in estrogenicity was proportional to the decrease in EE2. This indicates that the estrogenicity of the intermediates of the first transformation step is much lower than that of EE2. In Figure 4-2b, the estrogenicity expressed in EEQ is plotted versus the EE2 concentration. The fact that the data forms a straight line with a slope of approximately 1 demonstrates that the parent compound EE2 is responsible for the observed estrogenicity. Even at the two lowest EE2 concentrations, accounting for 0.12% and 0.28% of the initial EE2 concentration, no significant deviation from the straight line occurs. This means that the sum of the estrogenic activity of the intermediates is at least 200 times lower than the estrogenicity of the original EE2 solution.

These findings were confirmed with an estrogen receptor competitive-binding assay adapted from Blair et al. (*21*). In this assay, the estrogen receptor can directly interact with the investigated compounds. Because no living organism is used, biological processes associated with test organisms do not influence the response of the assay. After ozonation, EE2-containing solutions

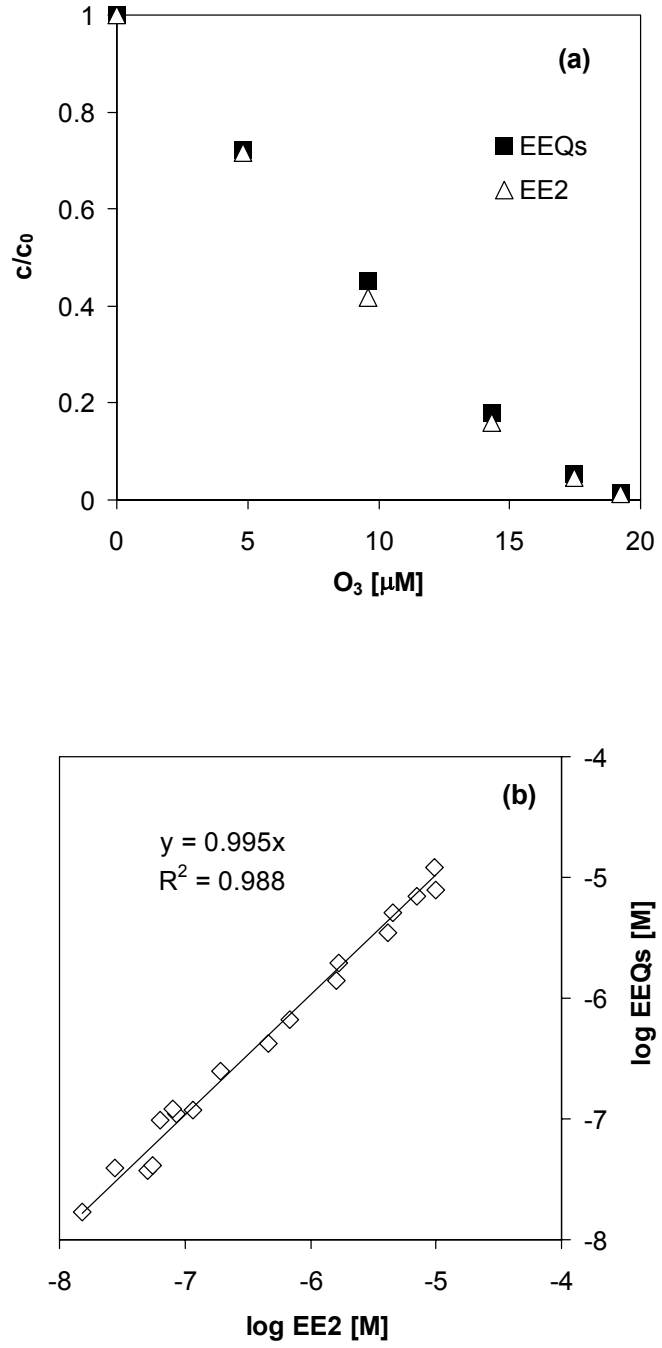


FIGURE 4-2. The effect of substoichiometric O_3 doses on the estrogenicity of aqueous solutions of EE2. Experimental conditions: $[EE2]_0$ = 10 μM, pH = 8, T = RT, [TBA] = 5 mM. (a) Relative decrease of estrogenicity and EE2 concentration as a function of O_3 dose. (b) Logarithmic plot of estrogenicity in 17β-estradiol equivalents (EEQs) versus EE2. Data are from Figure 4-2a and an additional experiment.

did not displace radiolabeled E2 from the rat estrogen receptor, whereas untreated solutions displaced it completely. This means that the oxidation products of EE2 do not bind efficiently to the estrogen receptor. The YES experiments described above were designed to yield a reduction of estrogenicity by a factor of 1000-10000. However, the observed reduction was lower due to a slow reappearance of EE2 after oxidation. The reappearance of EE2 will be discussed later. To achieve concentrations lower than 0.1 μM with nearly stoichiometric O_3 doses, the reaction solutions had to be spiked with a second O_3 dose after storing the solutions for 3 days at room temperature. The second O_3 dose was kept as low as possible to avoid significant changes in the product distribution of the fast initial transformation steps.

4.3.2 Reduction of Estrogenicity as a Function of Ozone Exposure

In drinking-water treatment, O_3 exposures are much higher than in the experiments with substoichiometric O_3 doses, in which O_3 was immediately consumed. To test whether higher O_3 exposures result in further removal of estrogenicity, aqueous solutions of EE2 at 10 °C and pH 8 were subjected to O_3 exposures ranging from 0.5 to 20 mg L^{-1} min. Figure 4-3 shows the decrease of the EE2 concentration together with the EEQ reduction. As expected, the results showed a drop of the estrogenicity by a factor of 200-500 for the lowest measured O_3 exposure, confirming the results of the experiments with substoichiometric O_3 doses. However, at higher O_3 exposures, the further decrease of estrogenicity was very slow. To reduce estrogenicity by a factor of 1000, an O_3 exposure of approximately 10 mg L^{-1} min was necessary.

The O_3 exposures reported above can be related to O_3 exposures applied for disinfection, which is often the primary objective of ozonation. O_3 exposures required to achieve certain levels of inactivation for specific microorganisms were calculated on the basis of inactivation rate constants and activation energies compiled by von Gunten (*22*). At an O_3 exposure of 0.5 mg L^{-1} min and

10 °C the inactivation of *Giardia muris* cysts is approximately 2 log units. Under the same conditions, inactivation for *E. coli* is more than 6 log units. A much larger O_3 exposure of approximately 9 mg L^{-1} min is required for a 2-log inactivation of *Cryptosporidium parvum* oocysts. On the basis of this inactivation data, it can be predicted that EE2-based estrogenicity is reduced at least by a factor of 200, if an ozonation processes achieves a 2-log inactivation of *Giardia muris* cysts or by a factor of 1000 if a 2-log inactivation of *Cryptosporidium parvum* oocysts is attained.

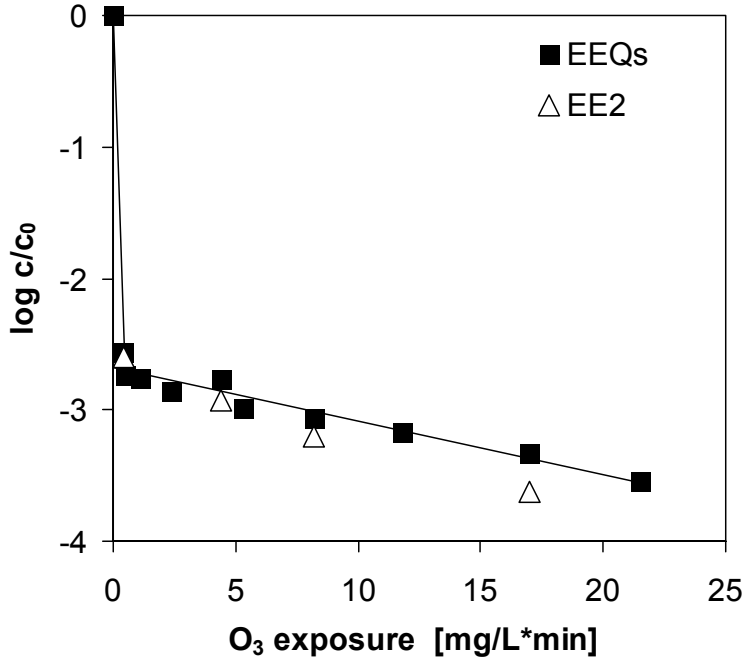


FIGURE 4-3. Reduction of estrogenicity and the EE2 concentration as a function of O_3 exposure. Experimental conditions: $[EE2]_0$ = 1 μM, $[O_3]_0$ = 1 mg L^{-1}, pH = 8, T = 10 °C, [TBA] = 5 mM.

To quantify how much of the residual estrogenicity was caused by EE2 itself, EE2 concentrations were measured with HPLC and fluorescence detection as well as LC-MS/MS for confirmation. The results show that up to O_3 exposures

of approximately 10 mg L^{-1} min, residual EE2 accounted for most of the estrogenicity. At higher O_3 exposures, EE2 concentrations were close to the quantification limit of the HPLC method (0.2 nM). Therefore, on the basis of the current data, it cannot be excluded that other compounds are responsible for a part of the estrogenicity observed at higher O_3 exposures. A possible explanation for the formation of estrogenic products will be given later.

4.3.3 Reappearance of EE2

The presence of EE2 in solutions that were treated with high O_3 exposures was unexpected. For an O_3 concentration of 1 mg L^{-1}, the half-life of EE2 at pH 8 is approximately 1 ms. Consequently, EE2 should disappear below the detection limit in less than 1 s. To assess the reappearance of EE2, the kinetics of this process was investigated. Figure 4-4 shows the reappearance of EE2 after ozonation as a function of time. The EE2 concentration rose quickly during the first hours after the experiments. After this initial step, the concentrations increased more slowly over several days. As compared to an O_3 dose of 50 μM, the reappearance at 100 μM O_3 was less pronounced but still significant. The same pattern of reappearance was also detected for THN and E2 (data not shown). This observation was not limited to the selected experimental conditions with pure water. Experiments with pretreated water from the River Seine in Paris, France, showed the same behavior of EE2. Furthermore, the dependence on EE2 concentration seemed to be small. Starting with 10, 1, and 0.1 μM EE2 resulted in comparable percentages of reappearance (0.05-0.2%) for similar O_3 doses.

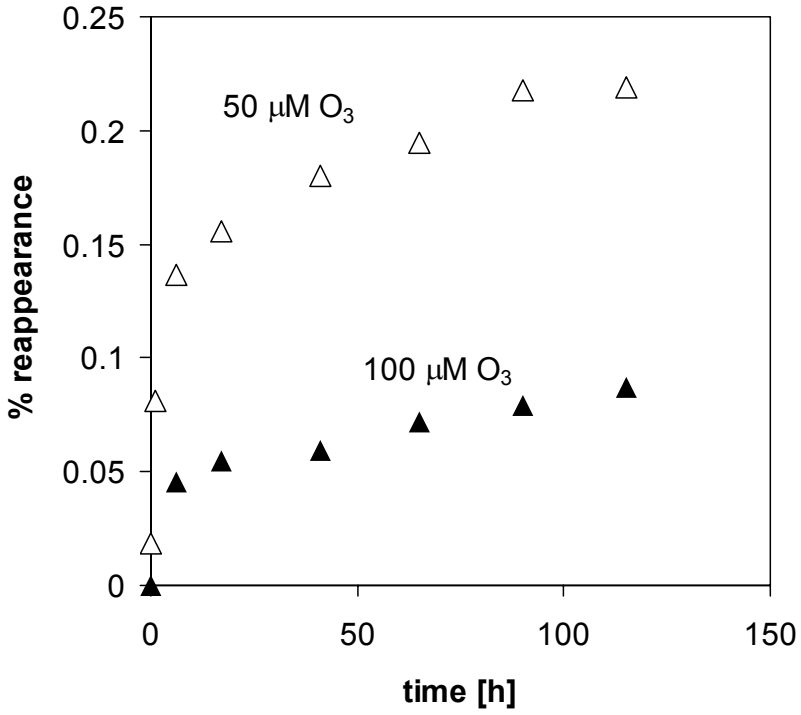


FIGURE 4-4. Reappearance of EE2 as a function of O_3 dose and time. Experimental conditions: $[EE2]_0$ = 10 μM, pH = 8, T = RT, [TBA] = 5 mM. O_3 was quenched with thiosulfate after 5 min. An O_3 dose of 50 μM resulted in an O_3 exposure of 4–5 mg L^{-1} min, whereas 100 μM O_3 yielded an approximately 4 times higher O_3 exposure of 15–20 mg L^{-1} min.

The reappearance of EE2 can only be explained if a small fraction of EE2 is "protected" from O_3 attack. We hypothesize that such "protected" forms of EE2 consist of hydroperoxides formed by the fast reaction of phenoxyl radicals with superoxide anion. This reaction was investigated in detail for radiolytically generated phenoxyl-type radicals by d'Alessandro et al. (*23*). It can be expected that such hydroperoxides would not be highly reactive toward O_3. Because they revert to phenols in a slow reaction by eliminating dioxygen, as shown in the study mentioned above, the formation of hydroperoxides provides a plausible explanation for the slow reappearance of EE2 observed in the present study.

These hydroperoxides would be "protected" from the fast oxidation of the phenolic moiety, but O_3 could still attack on the ethinyl group. On the basis of the reaction of O_3 with ECH, the second-order rate constant for the O_3 attack on the ethinyl group is estimated to be 160 $M^{-1}s^{-1}$ at 10 °C. The slow decrease of EE2 in Figure 4-3 corresponds to a second-order rate constant of approximately 150 $M^{-1}s^{-1}$ at 10 °C. Therefore, it seems to be plausible that the O_3 attack on the ethinyl group of "protected" EE2 contributes to the disappearance of EE2. This reaction would result in the formation of products for which the phenolic moiety of EE2 can be reformed after ozonation. It can be expected that these products still exhibit estrogenic activity. Therefore, we assume that they could be responsible for the remaining estrogenicity that seemed not to be caused by EE2.

Despite the fact that a reappearance of EE2 has been observed, it has to be emphasized that it accounts only for 0.1-0.5% of EE2 oxidized. We assume that this phenomenon will have little relevance in practice where a removal of 99.5% estrogenicity should be sufficient in most cases.

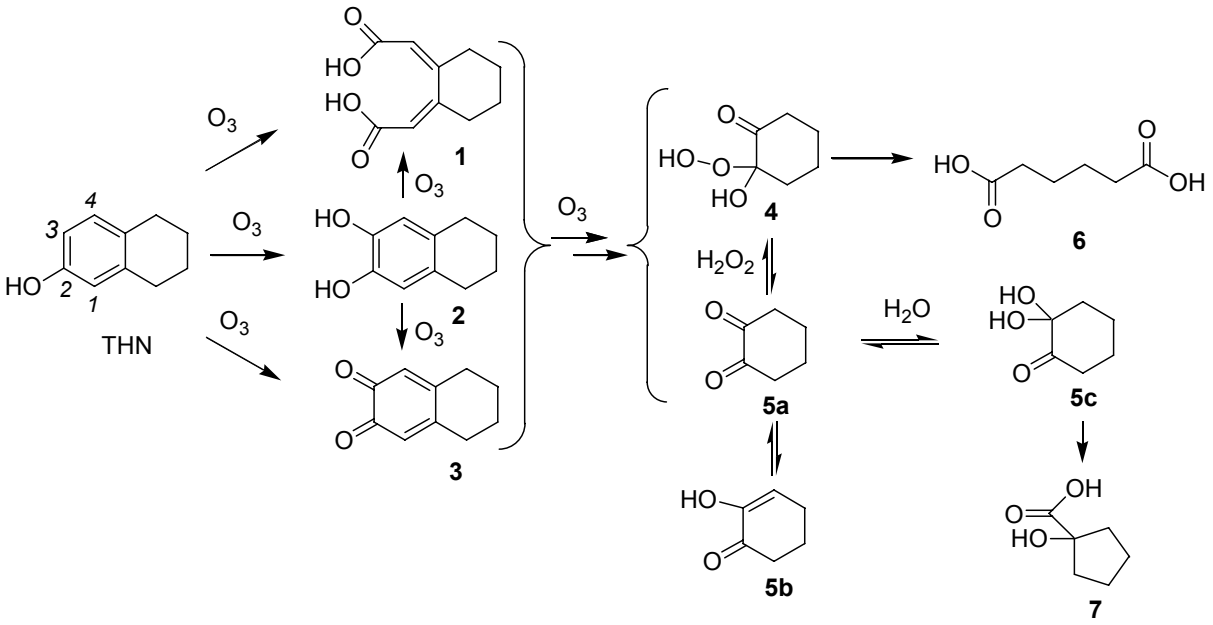
4.3.4 Identification of Oxidation Products

In the second part of this study, oxidation products formed during the ozonation of EE2 were identified. As mentioned earlier, EE2 has a highly ozone reactive phenolic moiety ($k_{O3} = 3 \times 10^6$ M^{-1} s^{-1} at pH 7, $t_{1/2} \approx 10$ ms for 1 mg L^{-1} O_3) and a significantly less reactive ethinyl group ($k_{O3} \approx 200$ M^{-1} s^{-1}, $t_{1/2} \approx 3$ min for 1 mg L^{-1} O_3). On the basis of these rate constants, it can be assumed that both reactive moieties will be attacked by O_3 during drinking water treatment. To facilitate the identification of oxidation products, two model compounds were chosen that represent the two different reactive moieties. The selected compounds were 5,6,7,8-tetrahydro-2-naphthol (THN) for the phenolic moiety and 1-ethinyl-1-cyclohexanol (ECH) for the ethinyl group (Figure 4-1).

4.3.5 Ozonation of the Model Compound THN

Aqueous solutions containing THN were ozonated and subsequently analyzed for oxidation products with LC-MS/MS. Scheme 4-1 shows the suggested reaction mechanism and the identified products. The chemical structure of two oxidation products could be determined with high certainty. The first product was identified as adipic acid (**6**) using an authentic standard for LC-MS/MS analysis. The second oxidation product was identified as 1-hydroxy-cyclopentanecarboxylic acid (**7**). No standard was available for this compound. After derivatization with diazomethane, the fragmentation in the GC/MS spectrum (Figure 4-5a) was very similar to the fragmentation found in the spectrum of the commercially available methylester of 1-hydroxy-cyclohexanecarboxylic acid (**11**) (Figure 4-5b). The spectrum of methylated **7** corresponded to the spectrum of methylated **11** shifted 14 Da (CH_2) to lower masses, suggesting that **7** consisted of a five- instead of a six-membered ring. The proposed structure of **7** is further supported by the loss of 46 (CH_2O_2) from the quasi molecular ion $[M-H]^-$ *m/z* = 129 in LC-MS/MS experiments performed in the negative mode (Table 4-2). Normally, carboxylic acids lose 44 (CO_2) in the negative mode (*24*), whereas the loss of 46 seems to be characteristic of α and β-hydroxy acids (*25*).

A third product with MW = 176 (not shown in Scheme 4-1) was only detected with LC-MS/MS. The MS/MS spectrum in the negative mode showed the loss of 18, 44, 46, and 62 from $[M-H]^-$. The compound was tentatively identified as 2-hydroxyheptanedioic acid ($C_7H_{12}O_5$) because the loss of 44 and 46 suggest that the compound contains a conventional and an α or β-hydroxy carboxylic acid. A forth major peak in the LC-MS chromatogram with MW = 154 could not be identified.



SCHEME 4-1. Reaction of O_3 with the Model Compound THN.

Scheme 4-1 shows the suggested reaction mechanism for O_3 attack at position 3 of the phenol ring. On the basis of a study on the ozonation of phenol (*20*), it can be assumed that the fast first reaction step leads to the muconic acid derivative **1**, 5,6,7,8-tetrahydro-2,3-naphthalendiol (**2**), and 2,3-nathphalendione (**3**) as major intermediates. These intermediates are still reactive to O_3 and consequently undergo further reactions, which might result in the formation of the hydroperoxide **4** and/or 1,2-cyclohexanedione (**5a**). Product **4** can be categorized as α-hydroxy or α-ketohydroperoxide. It can react through hydrogen peroxide elimination (*26*) leading to the formation of **5a**. A second reaction is a rearrangement by cleavage of the bond between the hydroperoxy and the keto group (*27*). The latter reaction results in the formation of adipic acid (**6**), which was identified as a major oxidation product. LC-MS/MS analysis indicated that if **5a** is formed during the reaction, it accounts for less than 10% of THN transformed. However, the fact that **5a** is almost entirely hydrated in aqueous solution (**5c**) and additionally slowly transformed into the enol form **5b** (*28*) makes analysis difficult, and it cannot be excluded that some of the species of **5** were missed in the analysis. The formation of **7** must be the

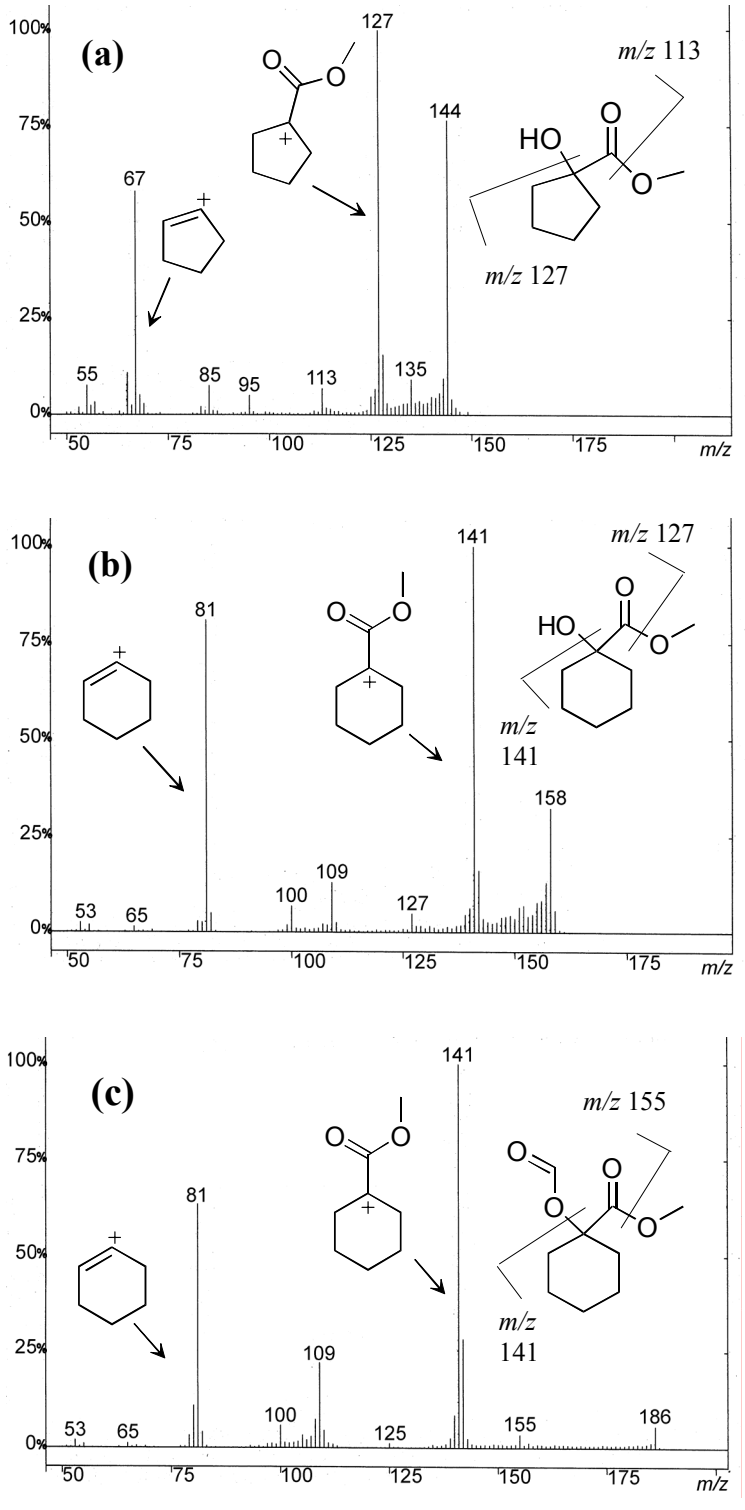


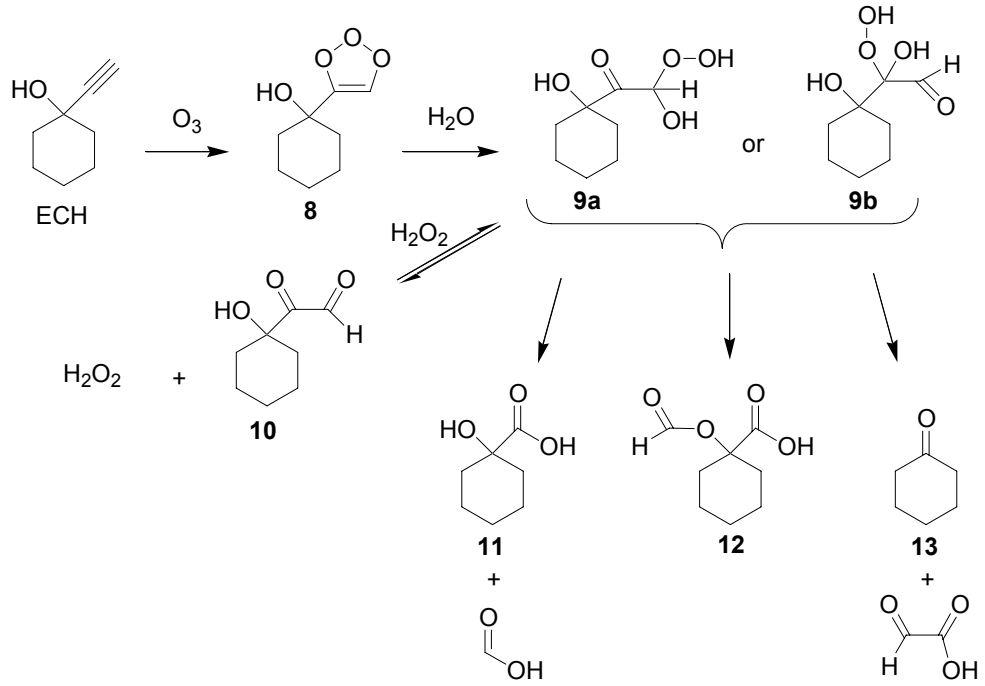
FIGURE 4-5. GC/MS spectra of derivatized oxidation products formed during the ozonation of THN (a) and ECH (b,c). Methylester of (a) 1-hydroxy-cyclopentane-1-carboxylic acid (7), (b) 1-hydroxycyclohexane-1-carboxylic acid (11), (c) 1-hydroxycyclohexane-1-carboxylic acid, formate (12).

product of a benzilic acid rearrangement (*29*) of **5a** via **5c**. Ring contractions of six-membered cyclic diketones to α-hydroxy acids have been reported by several studies dealing with steroid synthesis (e.g., ref (*30*)). Usually, these experiments were performed under alkaline conditions in solvent/water mixtures. In aqueous solution, a study on the enolization of **5a** (*31*) explained its slow irreversible disappearance and the formation of an acidic compound by the formation of **7** as well. On the basis of these considerations, we assume that **5** was at least partly transformed into **7** in a relatively slow reaction after ozonation.

Scheme 4-1 refers to the initial O_3 attack at position 3 of the phenol ring. O_3 can also attack at position 1. The corresponding first step intermediates will be formed. Further oxidation by O_3 might proceed in a different way and yield different products due to the different positions of the double bonds. However, it is possible that products **6** and **7** were partly formed by this way as well.

4.3.6 Ozonation of the Model Compound ECH

The ethinyl group represents the reactive moiety of the second model compound 1-ethinyl-1-cyclohexanol (ECH). Even if acetylenes react considerably more slowly with O_3 than olefins, it can be assumed that O_3 attack on the ethinyl group proceeds analogous to the well- established Crigee mechanism for double bonds (*32*). According to this mechanism, O_3 attack on the ethinyl group of ECH results in the formation of the primary ozonide **8,** which quickly decomposes into the hydroxyhydroperoxides **9a** and/or **9b** (Scheme 4-2). The fact that less than 2 min after the completion of the reaction no hydroperoxides except hydrogen peroxide could be determined demonstrates that **9a** and **9b** are only short-lived intermediates. The H_2O_2 yield was 65% relative to the added O_3 concentration. This indicates that the keto aldehyde **10** is the major product of this reaction (in aqueous solution **10** may be hydrated).



SCHEME 4-2. Oxidation Products Formed by the Reaction of O_3 with the Model Compound ECH.

With LC-MS/MS analysis, three further oxidation products were identified. The first product was identified as 1-hydroxy-cyclohexanecarboxylic acid (**11**). After derivatization with diazomethane, its GC/MS spectrum (Figure 4-5b) was identical to the spectrum of the commercially available methylester of the compound. The identification of **11** is in agreement with the detection of formic acid immediately after completion of the reaction of O_3 with ECH. For the second product **12,** no standard was available. The GC/MS spectrum of derivatized **12** (Figure 4-5c) was virtually identical to the spectrum of **11** except that the molecular ion was *m/z* 186 instead of *m/z* 158 and that the loss of 31 (OCH_3) from the molecular ion resulted consequently in a peak at *m/z* 155 instead of *m/z* 127. The difference of 28 Da between **12** and **11** and the associated derivatives can be explained best by the presence of an additional CO group in **12**. In both spectra, the base peak is at *m/z* 141, demonstrating that methylated **12** lost 45 (OCOH) instead of 17 (OH) as did **11**. The further fragmentation of the base peak (*m/z* 141) was identical for both molecules.

Obviously, the CO group has to be attached to the tertiary alcohol group. Additionally, it could be shown with LC/MS that **12** hydrolyzes at pH 12 to **11**. The third oxidation product was identified as cyclohexanone (**13**) using an authentic standard for LC-MS/MS analysis. Due to analytical problems, the formation of glyoxylic acid, which should be produced in an equivalent amount, could not be confirmed.

Product **10** was not detected with LC-MS/MS analysis. Because H_2O_2 was not destroyed in the samples and the formation of **10** from the hydroxyhydroperoxides **9a** and/or **9b** is a reversible process, **10** was slowly converted into **11, 12,** and **13** during sample storage. The half-life time for **10** is estimated to be < 40 h for 1 mM H_2O_2 at pH 7 ($k_{observed}$ = 5-7 M^{-1} s^{-1} at pH 10 and 20 °C). At pH 12 the base-catalyzed reaction of **10** with H_2O_2 yielded less than 30% formic acid, indicating that **12** and not **11** or **13** is the major product (glyoxylic acid quickly decomposes to formic acid under these conditions). The slow conversion of a very similar keto aldehyde into the corresponding formate by Baeyer-Villiger-type oxidation in aqueous solution has already been reported in (*33*). In the absence of H_2O_2, **10** seems to be fairly stable. A degradation product of hydrocortisone with a moiety analogous to **10** exhibited a half-life >40 d at pH 8 for base-catalyzed degradation (*34*). Therefore, **10** might be a more important product than **12** under realistic treatment conditions where H_2O_2 concentrations are significantly lower.

4.3.7 Quantification of Product Formation

The formation of adipic acid (**6**) could be quantified with LC-MS/MS, because an authentic standard was available. Product **6** accounted for up to 24% of THN transformed during ozonation. The second THN product **7** could not be quantified.

Based on measurements of H_2O_2 and formic acid, ozonation of ECH yielded 65% **10** and 20% **11**. Cyclohexanone (**13**) accounted for 15%. This compound was quantified with LC-MS/MS, and the result might have been influenced by the storage time. Ozonation of the similar ethinyl compound 2-methyl-3-butyn-2-ol yielded 25% α-hydroxyisobutyric acid at pH 7. This reaction is equivalent to the formation of **11** from ECH, and the 25% yield agrees with the yield determined for **11**. The mass balance for ECH seems complete whereas for THN the identified products probably do not account for more than 50% of THN transformed.

4.3.8 Oxidation Products of EE2

Table 4-1 lists the oxidation products of EE2 which were derived from the oxidation products of the model compounds detected with LC-MS/MS. Five out of six possible oxidation products of EE2 could also be detected with LC-MS/MS. Product **18** (Table 4-1) could not be unambiguously assigned to a peak of the LC/MS chromatogram due to interferences with fragment ions from other products. Table 4-2 reports the major mass fragments of the MS/MS spectra recorded in the negative mode. The suggested structures of the oxidation products are supported by the loss of characteristic masses depending on the functional groups present in the molecules. Molecule **14** (Table 4-1) primarily lost 44 Da (CO_2) as did the model-compound product adipic acid (**6**). In the negative mode, the loss of CO_2 is typical for carboxylic acids (*24*). In contrast, the α-hydroxy acid **17** lost 46 Da (CH_2O_2) instead of 44 Da. The same behavior was observed for the model-compound products **7** and **11.** As mentioned earlier, the loss of 46 Da seems to be characteristic for α or β-hydroxy acids (*25*). Product **15,** which has two conventional carboxyl groups and one α-hydroxy acid group, lost 46 Da but not 44 Da. However, the loss of 62 Da (CO_2+H_2O) indicated the presence of the two conventional carboxyl groups, because the

TABLE 4-1. Ozonation Products of EE2 Identified with the Model Compounds THN and ECH.

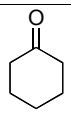
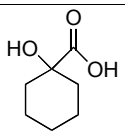
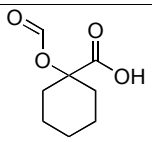
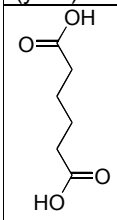
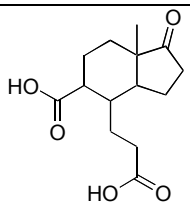
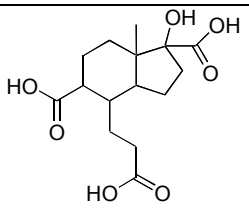
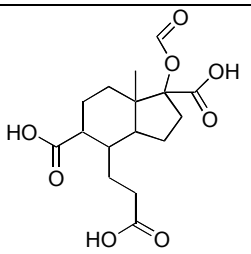
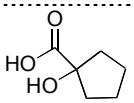
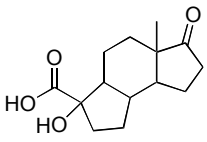
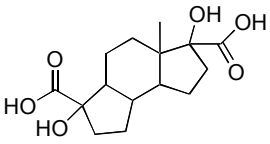
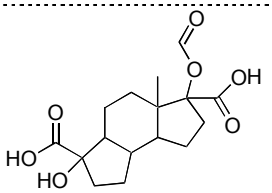
Products THN (yield) \ Products ECH (yield)	 (15%) MW=98 **13**	 (20%) MW=144 **11**	 (not quantified) MW=172 **12**
 (<24%) MW=146 **6**	 MW=268 **14**	 MW=314 **15**	 MW=342 **16**
 (not quantified) MW=130 **7**	 MW=252 **17**	 MW=298 **18**	 MW=326 **19**

TABLE 4-2. LC-MS/MS Spectra for Oxidation Products of Model Compounds and Steroid Hormones in the Negative Mode.[a]

	N°	MW	$[M-H]^-$ *m/z*	$-H_2O$ Δ18	-CO Δ28	$-CO_2$ Δ44	$-CH_2O_2$ Δ46	$-H_2O-CO_2$ Δ62
THN	**7**	130	**129**	111(13%)			83(84%)	
	6	146	145(32%)	127(25%)		**101**		83(95%)
	-	176	**175**	157(12%)		131(54%)	129(13%)	113(57%)
ECH	**11**	144	**143**				97(57%)	
	12	172	171(0%)		**143**			
EE2	**14**	268	267(7%)	249(1%)		223(44%)		**205**
	15	314	**313**	295(4%)			267(10%)	251(13%)
	16	342[b]	341(64%)		**313**			
	17	252	**251**	233(6%)			205 (12%)	
	19	326[b]	325(41%)		**297**		279(38%)	

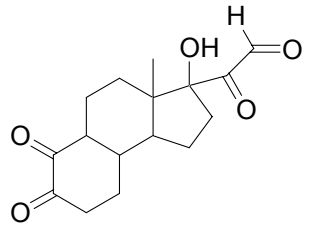
[a] For each spectrum, the *m/z* values for the quasi-molecular ion $[M-H]^-$ and the major fragment ions are given. Bold values correspond to the base peak. The intensity of the ions relative to the base peak is reported in brackets.

[b] full-scan spectra

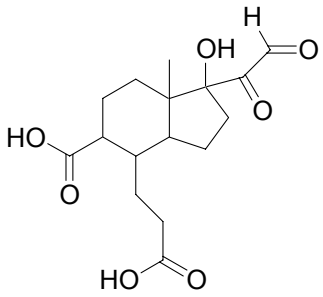
same fragmentation was also observed for **14** and **6**. The molecules **16** and **19** exhibiting a formate group primarily lost 28 Da (CO) and had the base peak at [M-1-28]. The same fragmentation was observed for the model-compound product **12**.

The oxidation products shown in Table 4-1 represent the stable oxidation products detected with LC-MS/MS. Experiments for the quantification of hydroperoxides and mechanistic considerations demonstrated that some of these oxidation products derive from less stable intermediates. Due to time-consuming sample preparation and the presence of H_2O_2 in the samples, these less stable intermediates decomposed and were not detected with LC-MS/MS. Figure 4-6 depicts two major transient oxidation products which must have formed but escaped detection. It is expected that these intermediates persist for a couple of hours to a few days in the water before they are transformed into the more stable products shown in Table 4-1.

The products listed in Table 4-1 and Figure 4-6 are formed at relatively high O_3 exposures as applied in ozonation of drinking water. At lower O_3 exposures, O_3 does not react with the ethinyl group. As a consequence, only the phenolic moiety will be transformed. Despite the lower O_3 exposure, it can be expected that the phenol moiety is, at least partly, transformed in the same way as for higher doses because the first reaction steps are fast.



20



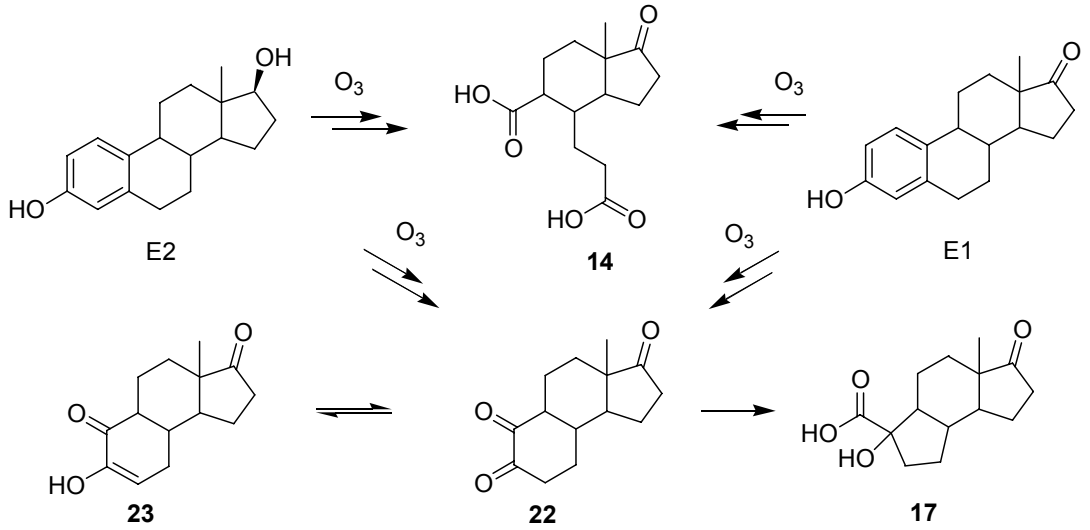
21

FIGURE 4-6. Major intermediates formed by the reaction of O_3 with EE2. These compounds were not detected with LC-MS/MS. However, mechanistic considerations provide strong evidence that the detected products in Table 4-1 were partly formed via 20 and 21. By superimposing the cyclohexanedione moiety of 20 on products 14, 15 and 16 (Table 4-1), three further products are obtained, which also may be formed. Product 20 can also be transformed into its enol forms as shown in Scheme 4-1.

4.3.9 Oxidation Products of 17β-Estradiol (E2) and Estrone (E1)

The oxidation products formed during the ozonation of the natural hormones E2 and E1 were investigated as well. Instead of an ethinyl group, E2 and E1 exhibit an alcohol and a keto group at the 17 position (Figure 4-1). As carbonyl and alcohol groups are much less reactive to O_3 than an ethinyl group, E2 and E1 were expected to react only at the phenolic moiety. Surprisingly, the experiments yielded the same two major products for E2 and E1. With LC-MS/MS, they were identified as **14** and **17,** which were also formed during the ozonation of EE2 (Scheme 4-3). This was expected for E1, because these oxidation products of EE2 have a carbonyl group at the 17 position identical to

that of E1. The corresponding alcohol group of E2 was unexpectedly oxidized to the carbonyl group under the applied conditions and yielded the same products as E1. Product **17** must be a decomposition product of the intermediate **22**. As shown in Scheme 4-1, the cyclohexanedione moiety can be hydrated and is partly present in the enol form **23**.



SCHEME 4-3. Oxidation Products Formed by the Reaction of O_3 with E2 and E1.

4.3.10 Relationship between the Structures and the Estrogenicity of Oxidation Products

The identified oxidation products of EE2 are expected to form at an O_3 exposure of 5-10 mg L^{-1} min at 10 °C. In the experiment shown in Figure 4-3, the corresponding O_3 exposure resulted in a reduction of estrogenicity by a factor of 5000 to 10000 if the estrogenicity caused by reappeared EE2 is subtracted. Most probably, the observed reduction of estrogenicity can be attributed to the cleavage of the phenolic moiety of EE2 because the 3-hydroxy group and the aromatic ring of the phenolic moiety is of particular importance for the binding of estrogens to the estrogen receptor (*35*). As demonstrated by our investigation, the phenol rings of EE2, E2, and E1 were cleaved in all of the identified products. However, the fact that the fast initial transformation steps, which result only in a partial cleavage of the phenol ring (Scheme 4-1), reduced

the estrogenicity by a factor of 200-500 indicates that only small modifications of the phenol ring may be required to reduce the estrogenicity of EE2 substantially. Overall, the present study provides direct evidence that the selective oxidation of the phenolic moiety efficiently reduces the estrogenicity of EE2-containing solutions.

For the first time, the oxidation of a pharmaceutical compound during ozonation has been investigated in a comprehensive manner, including oxidation kinetics, product formation, and the pharmacological effects of the oxidation products. The results demonstrate that O_3 doses applied for the disinfection of drinking water can efficiently remove EE2, E2, and E1 and the estrogenicity associated with their presence in the water. Due to the selective oxidation of estrogens by O_3, ozonation is also a promising tool for the control of estrogenicity in effluents of sewage treatment plants. This has already been confirmed by ozonation experiments with a pilot plant, which was operated with real wastewater (*36*). When the disinfection of wastewater is a legal requirement, ozonation, therefore, may be a viable alternative to other disinfection processes.

Acknowledgments

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5

Oxidation of Pharmaceuticals during Ozonation of Wastewater Effluents: A Pilot Study

Huber, M. M.; Göbel, A.; Joss, A.; et al. *Environ. Sci. Technol.* **2004**, submitted.

Abstract

To reduce the release of pharmaceuticals and endocrine disruptors into the aquatic environment or to remove them from wastewater intended for direct or indirect reuse, the application of advanced wastewater treatment may be required. In the present study, municipal wastewater effluents were treated with ozone (O_3) in a pilot-scale plant consisting of 2 bubble columns. The investigated effluents, which varied in suspended solids concentrations, comprised an effluent of conventional activated sludge treatment (CAS), the same effluent dosed with 15 mgTSS L^{-1} (CAS+SS), and the effluent of a membrane bioreactor pilot plant (MBR). Selected classes of pharmaceuticals were spiked to the wastewater at levels ranging from 0.5–5 μg L^{-1}. Samples taken at the inlet and the outlet of the pilot plant were analyzed with LC-electrospray tandem MS. Macrolide and sulfonamide antibiotics, estrogens, and the acidic pharmaceuticals diclofenac, naproxen and indomethacin were oxidized by more than 90-99% for O_3 doses ≥2 mg L^{-1} in all effluents. X-ray contrast media and a few acidic pharmaceuticals were only partly oxidized, but no significant differences were observed among the three effluents. These results show that many pharmaceuticals present in wastewater can be efficiently oxidized with O_3 and that suspended solids have only a minor influence on the oxidation efficiency of nonsorbing micropollutants.

5.1 Introduction

In recent years, various studies have reported the occurrence of a large number of pharmaceuticals in the aquatic environment (*1-3*). Even though the detected concentration levels are typically in the nanogram/L to microgram/L range, it cannot be excluded that molecules designed to be biologically active affect sensitive aquatic organisms even at such low concentrations. Furthermore, the large number of pharmaceuticals and other micropollutants that are particularly present in surface waters could produce additive effects. Immediate effects caused by pharmaceuticals may be subtle and difficult to detect, but nevertheless could lead to important long-term consequences in aquatic ecosystems (*4*).

Among the various classes of pharmaceuticals, three merit special concern: antibiotics, pharmaceuticals acting as endocrine disruptors, and antineoplastics. Primarily used for chemotherapy, antineoplastics are highly toxic agents which have a high potential to affect aquatic organisms. The release of antibiotics in the environment could promote the dissemination of antibiotic resistance, especially in human pathogens. Endocrine disruptors in general are thought to be responsible for feminizing and masculinizing effects observed in various animals that live in ecosystems affected by anthropogenic pollution (*5*). A prominent endocrine disrupting pharmaceutical is 17α-ethinylestradiol (EE2). Laboratory studies have already shown that environmentally relevant concentrations of EE2 and natural estrogens elicit estrogenic responses in fish (*6-8*). Among the estrogens and estrogenic chemicals associated with the induction of feminizing effects in fish exposed to effluents of wastewater treatment plants (WWTPs), EE2 is likely to be of considerable importance due to its high in vivo potency, its persistence in the environment, and its capacity to bioaccumulate (*9*).

Municipal wastewater is the major source of pharmaceuticals in the aquatic environment (*4*). In developed countries, wastewater is usually treated in WWTPs before it is discharged into receiving waters. Since it is highly unrealistic to reduce the consumption of pharmaceuticals, the improvement of wastewater treatment is one of the few options to diminish the release of these compounds into the aquatic environment. Conventional activated sludge treatment was shown to degrade pharmaceuticals to varying extents that ranged from complete to very poor degradation (*10,11*). Applying longer sludge retention times resulted generally in improved degradation, but most of the investigated compounds could not be completely degraded. Therefore, advanced treatment technologies have to be implemented to achieve further removal of pharmaceuticals.

Ozonation has been shown to have a high potential for the oxidation of pharmaceuticals in drinking water (*12,13*) and wastewater (*14*). In wastewater, O_3 doses ranging from 5 to 15 mg L^{-1} led to a complete disappearance of most of the pharmaceuticals except for iodinated X-ray contrast media. For O_3 doses typically applied in water treatment, ozonation only results in partial oxidation of pharmaceuticals and therefore could yield biologically still active oxidation products. However, recent studies on EE2 (*15*) and carbamazepine (*16*) have shown that partial oxidation was sufficient to significantly reduce pharmacological activity and toxicity, respectively.

In the present study, pilot experiments were conducted to get a better understanding of the oxidation of pharmaceuticals during ozonation of wastewater effluent. The pilot experiments were carried out using a wastewater with a DOC concentration representative for good quality secondary or tertiary effluent. DOC was substantially lower compared to the wastewater investigated in ref (*14*). The selected pharmaceutical classes (macrolide and sulfonamide antibiotics, iodinated X-ray contrast media, estrogens, and 3 acidic

pharmaceuticals) were spiked to the wastewater to be able to determine 95-99% removal. The major aims of the experiments were (i) to determine a minimal O_3 dose required for the oxidation of pharmaceuticals exhibiting a high reactivity toward O_3, (ii) to investigate the influence of suspended solids on the oxidation of pharmaceuticals in MBR, CAS, and CAS+SS effluents, and (iii) to assess the feasibility of the prediction of elimination by means of suitable probe compounds.

5.2 Experimental Section

5.2.1 Ozonation Pilot Plant

The pilot plant consisted of two columns operated in series with an active reactor volume of 140 liter each and a filling level of 4.8 m (0.193 m nominal inner diameter, 5.2 m total height; Figure 5-1). The first column is operated in the downstream mode, while the second upstream. Tracer experiments with a salt spike showed a slightly better plug flow behavior in the second column as compared to the first (the salinity profile at the outflow of column 1 and column 2 could best be simulated modeling the reactor volume as a series of 3 respectively 4 fully mixed compartments with comparable total volume). With a flow rate of 2 ± 0.1 m^3 h^{-1}, the total hydraulic retention time amounts to 4.2 ± 0.2 min in each column. O_3 was continuously supplied by an ozone generator (Ozomatic SWO 200) fed with oxygen and bubbled into column 1 at a gas flow rate of 200 ± 10 L h^{-1}. No O_3 was applied to column 2. Ozone concentrations in the feed and off gas were measured with a UV ozone monitor (BMT 936 Vent, 0.1-50 g m^{-3}). By adjusting the power input of the ozone generator, the desired O_3 concentrations were obtained. The respective concentrations yielded transferred O_3 doses ranging from 0.5-5 mg $L^{-1}_{wastewater}$. Transfer efficiencies were >98%.

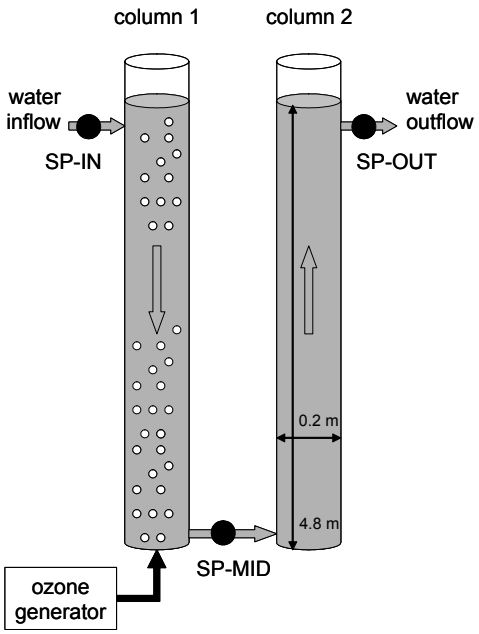


FIGURE 5-1. Scheme of the ozonation pilot plant. O_3 is being added only to column 1, which is operated in counter current mode. Black dots indicate the three sampling ports at the inlet (SP-IN) and the outlet (SP-MID) of column 1, and at the outlet of column 2 (SP-OUT).

5.2.2 Feed Wastewater

The pilot plant was operated on site at the municipal wastewater treatment plant (WWTP) in Kloten-Opfikon, Switzerland. Three types of WWTP effluents spiked with selected classes of pharmaceuticals were treated with O_3. The investigated effluents were: effluent of conventional activated sludge treatment (CAS), the same effluent dosed with 15 mgTSS L^{-1} of activated sludge (from the full scale plant) simulating an activated sludge treatment with suboptimal clarification (CAS+SS) and the effluent of a membrane bioreactor pilot plant (MBR; for water quality parameters see Table 5-1).

On the CAS plant, the combined sewage of 55'000 population equivalents (PE) is treated using a conventional activated sludge system equipped with grit, sand and oil trap, primary clarifier, nitrification and denitrification (11 ± 2 d sludge age). The MBR (100 PE) is operated in parallel with proportional inflow

of primary effluent (i.e. primary clarified wastewater) of the CAS. It is equipped with anaerobic, denitrifying and nitrifying compartments (sludge age >70 d; see (*10*) for a detailed description of both plants).

TABLE 5-1. Average Water Quality Parameters of the Effluents CAS, CAS+SS, and MBR[a]

Effluent	pH	T [°C]	DOC [mg L^{-1}]	COD [mg L^{-1}][b]	Alkalinity [mM]
CAS	7.0 ± 0.1	16 ± 1.0	7.7 ± 0.5	29 ± 3	3.1 ± 0.1
CAS+SS	6.95 ± 0.1	15 ± 0.5	7.0 ± 0.5	41 ± 1	3.2 ± 0.2
MBR	7.5 ± 0.1	17 ± 0.5	6.6 ± 0.2	22 ± 2	5.4 ± 0.2

[a] errors represent one standard deviation

[b] COD includes dissolved and particulate matter

5.2.3 Spiking of Analytes

The biologically treated wastewater of either plant was continuously pumped into a 300 L tub, where it was spiked continuously with an aqueous solution containing representative compounds from different classes of pharmaceuticals. It was taken care that acetone residuals from primary stock solutions were low enough that they did not influence the ozonation process (i.e., hydoxyl-radical ($^{\bullet}$OH) scavenging rate by acetone << $^{\bullet}$OH scavenging rate of the wastewater matrix). The tub was equipped with a stirrer to assure good mixing. A ~500 fold dilution of the spiking solution with wastewater effluent resulted in the approximate final concentrations given. Four iodinated contrast media, i.e. iopamidol (CASRN 60166-93-0), diatrizoate (CASRN 737-31-5), iopromide (CASRN 73334-07-3) and iomeprol, were spiked at a concentration of 5 µg L^{-1}. The concentration for the natural estrogens estrone (CASRN 53-16-7) and 17β-estradiol (CASRN 50-28-2) was 0.5 µg L^{-1}, while 1 µg L^{-1} of 17α-ethinylestradiol (CASRN 57-63-6) was added (for structures see Figure 5-2).

The group of acidic pharmaceuticals comprised ibuprofen (CASRN 15687-27-1), diclofenac (CASRN 15307-86-5), bezafibrate (CASRN 41859-67-0), naproxen (CASRN 22204-53-1), gemfibrozil (CASRN 25812-30-0), clofibric acid (CASRN 882-09-7) and indomethacin (CASRN 53-86-1). While only the first three were spiked at a concentration of 2 μg L^{-1}, the latter four were also included in the chemical analysis. Sulfadiazine (CASRN 68-35-9), sulfathiazole (CASRN 72-14-0), sulfapyridine (CASRN 144-83-2) and sulfamethoxazole (CASRN 723-46-6) were chosen from the group of sulfonamide antibiotics at a concentration of 2 μg L^{-1} (for structures see Table 5-2). Additionally N^4-acetylsulfamethazine (0.5 μg L^{-1}) was added. From the group of macrolide antibiotics 2 μg L^{-1} of roxithromycin (CASRN 80214-83-1), clarithromycin (CASRN 81103-11-9) and of the environmental metabolite dehydro-erythromycin were spiked (for structures see Figure 5-3). N^4-acetylsulfamethoxazole and azithromycin (CASRN 83905-01-5) were not spiked, but already present in the wastewater effluents investigated.

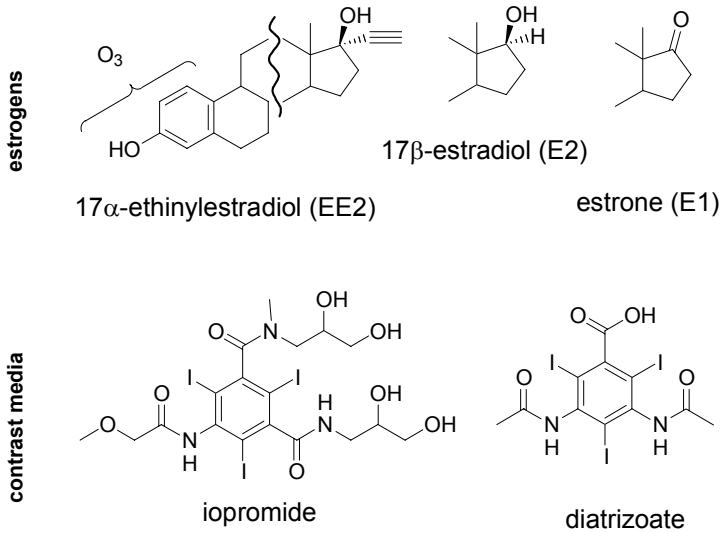


FIGURE 5-2. Chemical structure of estrogens and selected X-ray contrast media and proposed site of O_3 attack.

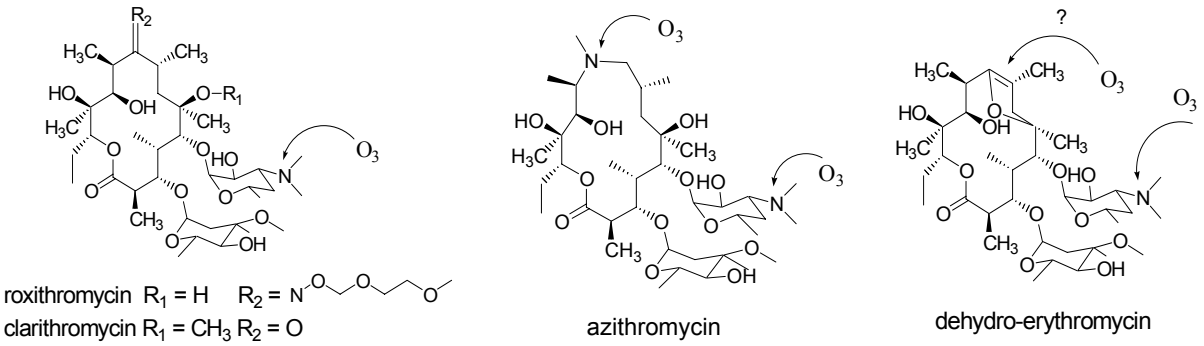
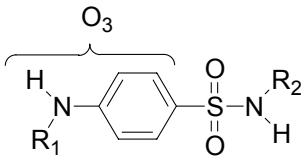
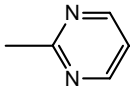
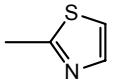
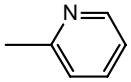
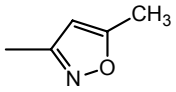
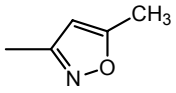
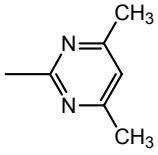


FIGURE 5-3. Chemical structure of macrolide antibiotics and proposed sites of O_3 attack.

TABLE 5-2. Chemical Structure and pK_a of Sulfonamide Antibiotics and Proposed Site of O_3 Attack



	pK_a[a]	R_1	R_2
sulfadiazine	6.4	H	
sulfathiazole	7.2	H	
sulfapyridine	8.4	H	
sulfamethoxazole	5.7	H	
N^4-acetylsulfamethoxazole (N^4AcSMX) [b]	5.0	$COCH_3$	
N^4-acetylsulfamethazine (N^4AcSMZ)	-	$COCH_3$	

[a] pK_a of sulfonamide group (*17*)

[b] not spiked

5.2.4 Sampling and Chemical Analysis

Samples were taken from the sample port at the inflow of column 1 (SP-IN), the sample port between the two columns (SP-MID), and at the outflow of column 2 (SP-OUT, Figure 5-1). Concentrations of dissolved O_3 were determined in the latter two samples, using the indigo method (*18*). The detection limit was 0.05 mg L^{-1}. For the analysis of pharmaceuticals, water samples of the inflow (SP-IN) and the outflow (SP-OUT) were enriched within 20 h after sampling using solid phase extraction. The dried cartridges were then frozen and transported to the laboratory, where they were eluted within one week. Filtration of the samples prior to enrichment was performed in the case of the acidic pharmaceuticals and the iodinated contrast media. The limits of quantification were in all cases sufficient to determine a reduction of ≥95% by ozonation. Details on the methods used have generally been published elsewhere, and therefore only a short description is given here (*19-22*).

For the iodinated contrast media 250 mL samples were adjusted to pH 2.8 and enriched on a copolymer material (ENV+, 200 mg). Detection was performed in the electrospray positive mode (*19*). In the case of the acidic pharmaceuticals the samples (250 mL inflow and 500 mL outflow) were adjusted to pH 2 and enriched on pre-packed Oasis MCX cartridges (60 mg) (*20*). Electrospray ionization in the negative ion mode was used for detection. The selected estrogens were extracted from 250 mL inflow and 500 mL outflow samples at pH 3 with pre-packed Isolute C18 cartridges (500mg) followed by a silica clean-up as described by Ternes et al. 1999 (*23*). Electrospray ionization in the negative ion mode was performed for the estrogens (*24*). For the quantification of iodinated contrast media, acidic pharmaceuticals, and estrogens a calibration (including SPE and further sample preparation) in local groundwater was used with desmethoxyiopromide (CASRN 76350-28-2), fenoprop (CASRN 93-72-1) and 17β-estradiol-17-acetate (CASRN 1743-60-8), respectively, as surrogate standards. Separation was achieved in all cases with

reversed phase chromatography coupled to tandem mass spectrometry. Instead of an API 365 tandem MS as described in (*19*) and (*20*) an API 4000 (Applied Biosystems, Foster City, CA, USA) was used for detection, maintaining most crucial method parameters such as the MRM transitions.

For the analysis of antibiotics, 100 mL of inflow and 250 mL of outflow were taken (n = 2), adjusted to pH 4, and enriched unfiltered using solid phase extraction on Oasis HLB polymeric cartridges (*22*). Measurement was performed using reversed-phase liquid chromatography coupled to electrospray positive tandem mass spectrometry (TSQ Quantum Discovery, Thermo Finnigan, San Jose, CA, USA). Quantification was performed using an external calibration curve in de-ionized water. Results were corrected by relative recovery rates determined in the same experiment and sample matrix (n = 2-4). The relative recovery for N^4-acetylsulfamethoxazole was set to 100%. The following substances were used as surrogate standards: sulfamethazine-phenyl-$^{13}C_6$, sulfamethoxazole-d^4, sulfadiazine-d^4, sulfathiazole-d^4, N^4-acetylsulfamethoxazole-d^5 and tylosin.

5.2.5 Calculation of Relative Residuals

In general, pharmaceutical concentrations measured in the inflow agreed reasonably well with the spiked amounts (e.g., < ±30% for sulfonamides and < ±50% for macrolides). Deviations may partly have occurred due to the presence of respective compounds in the nonspiked wastewater. To compensate differences in the input concentrations, the outflow concentrations are reported as relative concentrations which were calculated dividing the concentration measured in the outflow by the respective concentration measured in the inflow. The resulting error was calculated by linear error propagation under the assumption that the errors of both measurements are independent of each other.

5.3 Results and Discussion

An overview of the results of the pilot experiments is provided in Figure 5-4. Relative residual concentrations of iopromide, roxithromycin, sulfamethoxazole and 17α-ethinylestradiol (EE2) representing the classes of X-ray contrast media, macrolide antibiotics, sulfonamide antibiotics and estrogens, respectively, are plotted as a function of O_3 dosage for all three effluents. Additionally, O_3 concentrations determined directly at the outlet of column 1 (SP-MID) are given. The extent of parent compound oxidation increased with increasing O_3 dosage, but great differences were observed among the different compound classes. For iopromide and other contrast media, relative residual concentrations >40% were measured even at the highest O_3 dose. In contrast, roxithromycin, sulfamethoxazole and EE2 were efficiently oxidized in all three effluents (>90%) for O_3 doses ≥2 mg L^{-1}. These three compounds exhibit high second-order rate constants for the reaction with O_3 (Table 5-3). Therefore, a relatively low O_3 residual as present for O_3 doses ≥2 mg L^{-1} is sufficient to cause the observed loss of the parent compound. The same behavior was observed for the rest of the investigated compounds belonging to the respective classes and for the acidic pharmaceuticals diclofenac, naproxen and indomethacin (data not shown).

During ozonation, micropollutants can be oxidized either by O_3 directly or by hydroxyl radicals ($^{\bullet}OH$), which are formed as a consequence of O_3 decay. The two oxidants vary strongly in their reactivity. O_3 attacks very selectively certain functional group, whereas $^{\bullet}OH$ is a non-selective oxidant that reacts very fast with a large number of moieties. Consequently, most $^{\bullet}OH$ is scavenged by the water matrix in wastewater (*25*). Therefore, the oxidation of compounds that react slowly with O_3 is always less efficient than for compounds that react fast with O_3, even if the rate constants for the reaction with $^{\bullet}OH$ is almost diffusion controlled. Because the direct reaction of iopromide with O_3 is very slow (Table

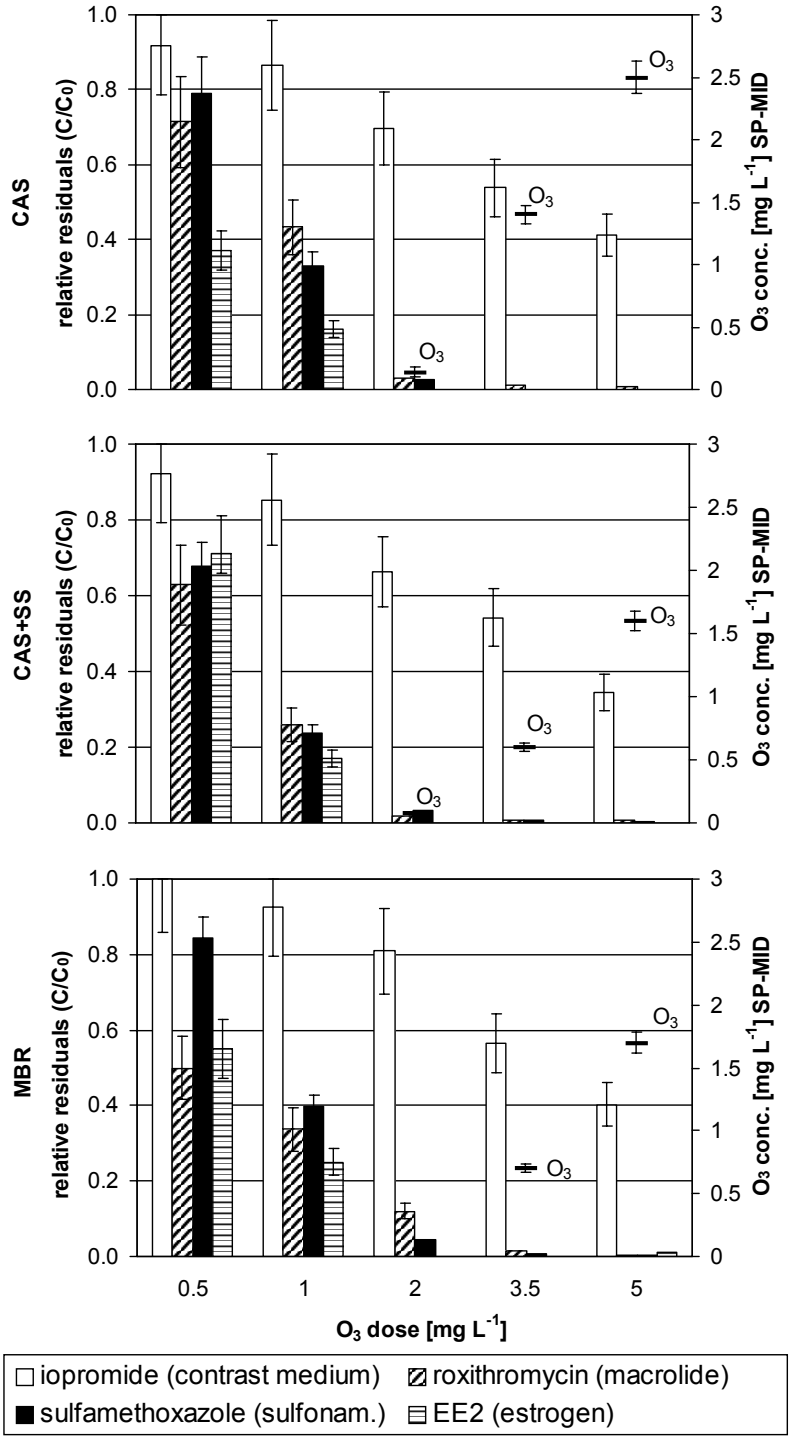


FIGURE 5-4. Relative residual concentrations of 4 compounds (iopromide, roxithromycin, sulfamethoxazole, and EE2) that represent the classes of contrast media, macrolides, sulfonamides, and estrogens, respectively. The residuals for the effluents CAS, CAS+SS and MBR are plotted versus O_3 dosages. Furthermore, absolute O_3 concentrations measured at the outlet of column 1 (SP-MID) are given.

5-3), the observed decrease can consequently be attributed to oxidation through $^{\bullet}$OH. Due to the lower efficiency of the oxidation by $^{\bullet}$OH, iopromide residuals up to 40% were detected for an O_3 dosage of 5 mg L^{-1}.Accordingly, relative high residuals of ibuprofen (20%) were detected under the same conditions (data not shown, for rate constants see Table 5-3).

TABLE 5-3. Second-order Rate Constants for the Reaction of O_3 and $^{\bullet}$OH with Selected Pharmaceuticals. [a]

Compound	pK_a	apparent k_{O3} (pH = 7,T = 20 °C) (M^{-1} s^{-1})	k_{OH} (pH = 7,T = 25 °C) (10^9 M^{-1} s^{-1})
bezafibrate	3.6	590 ± 50	7.4 ± 1.2
carbamazepine	-	~3 × 10^5	8.8 ± 1.2
clofibric acid	4.5	< 20 [b]	4.7 ± 0.3 [c]
diazepam	-	0.75 ± 0.15	7.2 ± 1.0
diclofenac	4.2	~1 × 10^6	7.5 ± 1.5
17α-ethinylestradiol	10.4	~3 × 10^6	9.8 ± 1.2
ibuprofen	4.9	9.1 ± 1	7.4 ± 1.2
iopromide	-	< 0.8	3.3 ± 0.6
naproxen	4.2	~2 × 10^5 [b]	9.6 ± 0.5 [c]
sulfamethoxazole	5.7	~2.5 × 10^6	5.5 ± 0.7
roxithromycin	8.8	~7 × 10^4	-

[a] Table adapted from ref (*12*)

[b] rate constants determined in the present study

[c] from ref (*26*)

5.3.1 Influence of the Water Matrix

To investigate the effect of suspended solids on micropollutant oxidation, the present study was performed with three effluents that varied in the concentration of suspended solids. MBR represented a wastewater practically free of suspended solids, CAS an average effluent quality and CAS+SS an activated-sludge process with suboptimal clarification. From studies that investigated the fate of the investigated pharmaceuticals during activated sludge treatment, it was clear that sorption onto suspended solids is not a relevant process for the selected compounds. However, suspended sludge particles could result in an increased O_3 demand, which would decrease the efficiency of the process for micropollutant oxidation. In Figure 5-5a, relative residual concentrations for diclofenac as well as O_3 residuals at SP-MID are compared for the three effluents. O_3 dosages of 0.5 and 1 mg L^{-1} did not yield measurable O_3 residuals in any of the three effluents. For 2 mg L^{-1}, low O_3 residuals were detected in CAS and CAS+SS. For 3.5 and 5 mg L^{-1}, residuals in CAS+SS (0.5 and 1.6 mg L^{-1}) and MBR (0.6 and 1.7 mg L^{-1}) were very similar, whereas residuals in CAS were significantly higher (1.4 and 2.5 mg L^{-1}). However, this difference has to be attributed to the fact that the latter experiments (CAS) were performed on a Monday when the wastewater was still diluted from the weekend. Similar experiments (CAS) performed with the same settings on a Tuesday, yielded a much lower O_3 residual of 1.8 mg L^{-1} at SP-MID for an O_3 dose of 5 mg L^{-1}.

The loss of diclofenac as a function of O_3 dosage was similar in CAS and CAS+SS, which can be expected from comparable O_3 residuals at SP-MID for different water matrices. Only in the case of the MBR effluent for an O_3 dosage of 1 and 2 mg L^{-1} significantly higher residuals were observed. This deviation seems to be related to a high turbidity caused by very fine particles that occurred for unknown reasons in the MBR permeate during these two

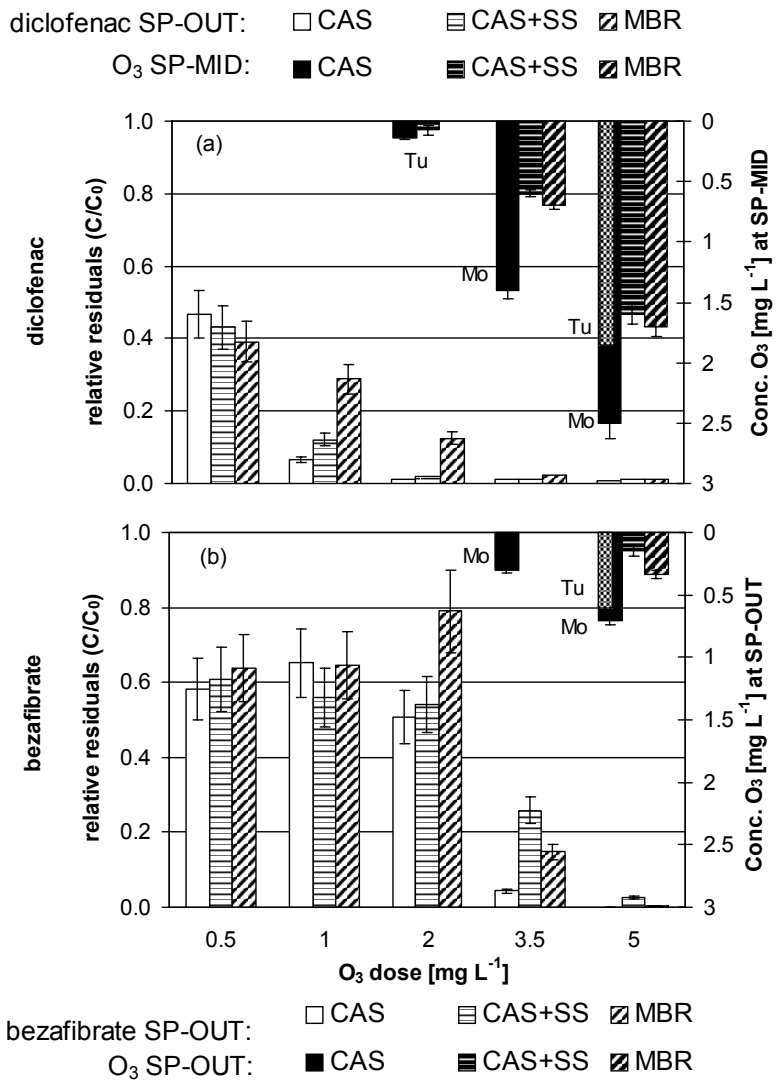


FIGURE 5-5. Residual concentrations of diclofenac, bezafibrate and O_3 as a function of O_3 dosages. The given O_3 residuals were measured (a) at the outlet of column 1 (SP-MID) and (b) at the outlet of column 2 (SP-OUT). For CAS, the week day on which the experiment was performed is indicated (Mo = Monday, Tu = Tuesday). Additionally, O_3 residuals for an O_3 dosage of 5 mg L^{-1} were determined in a second experiment conducted on a Tuesday (narrow bar).

experiments. Due to the large surface area created by these particles (probably much smaller particles than the sludge particles in CAS+SS), the turbidity may have had a significant influence on the ozonation process.

Figure 5-5b depicts relative residuals of the acidic pharmaceutical bezafibrate and O_3 residuals determined at SP-OUT. Bezafibrate exhibits an intermediate reactivity with O_3. Therefore, direct reactions with O_3 are not important at lower dosages and oxidation by $^{\bullet}OH$ is the predominant process. The results seem to indicate that under these conditions (0.5-2 mg L^{-1} O_3) the extent of parent compound oxidation is independent of the O_3 dosage. However, the deviation might be within the standard deviation of the analytical method and is therefore not visible. For iopromide, the extent of oxidation was slightly dosage-dependent as shown in Figure 5-4. At O_3 doses of 3.5 and 5 mg L^{-1}, for which significant O_3 residuals are present at SP-MID, oxidation by O_3 becomes relevant. Under these conditions, the pattern of O_3 residuals at SP-OUT is well reflected by the relative bezafibrate residuals, which are lowest for high O_3 residuals. The O_3 residuals detected at SP-OUT seem to be influenced to some extent by the water matrix. CAS+SS clearly yielded the lowest O_3 residual. But the fact, that the O_3 residual for CAS is higher than for the particle free MBR indicates that in this case the pH difference is more important than suspended solids. The pH of MBR effluent was approximately 7.5 as compared to 7 for CAS and CAS+SS. At higher pH, reactions of O_3 with the water matrix and the O_3 decay caused by radical-type chains reaction are accelerated.

Considering O_3 residuals at SP-MID and the results presented in Figure 5-4 and 5-5a for two antibiotics, EE2 and diclofenac, it can be concluded that suspended solids have only a minor influence on the oxidation of compounds that react fast with O_3. Furthermore, the oxidation by $^{\bullet}OH$ was not affected by suspended solids either, as shown for bezafibrate at low O_3 dosages or for iopromide (Figure 5-4). At higher O_3 dosages, however, the oxidation of compounds with intermediate reactivity seems to be influenced to some extent by the concentration of suspended solids and the pH due to significant differences in O_3 residuals and the associated O_3 exposures.

5.3.2 Estimation of the Ozone Absorption Rate of Sludge Particles

The minor effects of suspended solids on the ozonation process for low O_3 dosages demonstrate that O_3 is consumed by dissolved components of the wastewater before it reaches the sludge particles. O_3 transfer must be limited by either of the boundary layers surrounding the sludge floc and the gas bubble. To determine the relevant process during ozonation of CAS+SS effluent and to get a better understanding of the ozonation process in general, O_3 mass transfer through the boundary layers and the O_3 concentration in the bulk solution were estimated based on film theory (*27*).

The mass transfer of O_3 from the gas phase to the liquid phase is usually calculated according to:

$$N_{O3,bulk} = k_L a_b \left(C_{O3,eq} - C_{O3,bulk}\right) \text{ with } k_L = \frac{D_{O3}}{\delta_b} \quad (1)$$

where $N_{O3,bulk}$ is the O_3 absorption rate (flux per unit volume), k_L the mass transfer coefficient for O_3, a_b the specific interfacial area of the sum of the gas bubbles, $C_{O3,eq}$ the equilibrium concentrations of O_3 at the gas-liquid interface, and $C_{O3,bulk}$ the concentration in the bulk liquid. The value of k_L is related to the molecular diffusion coefficient of O_3 (D_{O3}) and the thickness of the liquid film (δ_b) surrounding the bubble (see Figure 5-6 for illustration). The parameters k_L and a_b were not determined in the present studies. Estimations of these parameters on the basis of a study of Roustan et al. (*28*) yielded $k_L \approx 3.4 \times 10^{-4}$ m s^{-1} and $a_b \approx 18$ m^2 m^{-3}. With $D_{O3} = 1.7 \times 10^{-9}$ m^2 s^{-1} (*29*) a film thickness of $\delta_b = 5$ μm is obtained. The study of Roustan et al. was performed using a comparable pilot plant and covered operating conditions (bubble diameter ≈ 3 mm, gas velocity ≈ 7 m h^{-1}, and liquid velocity ≈ 70 m h^{-1}) applied in the present study.

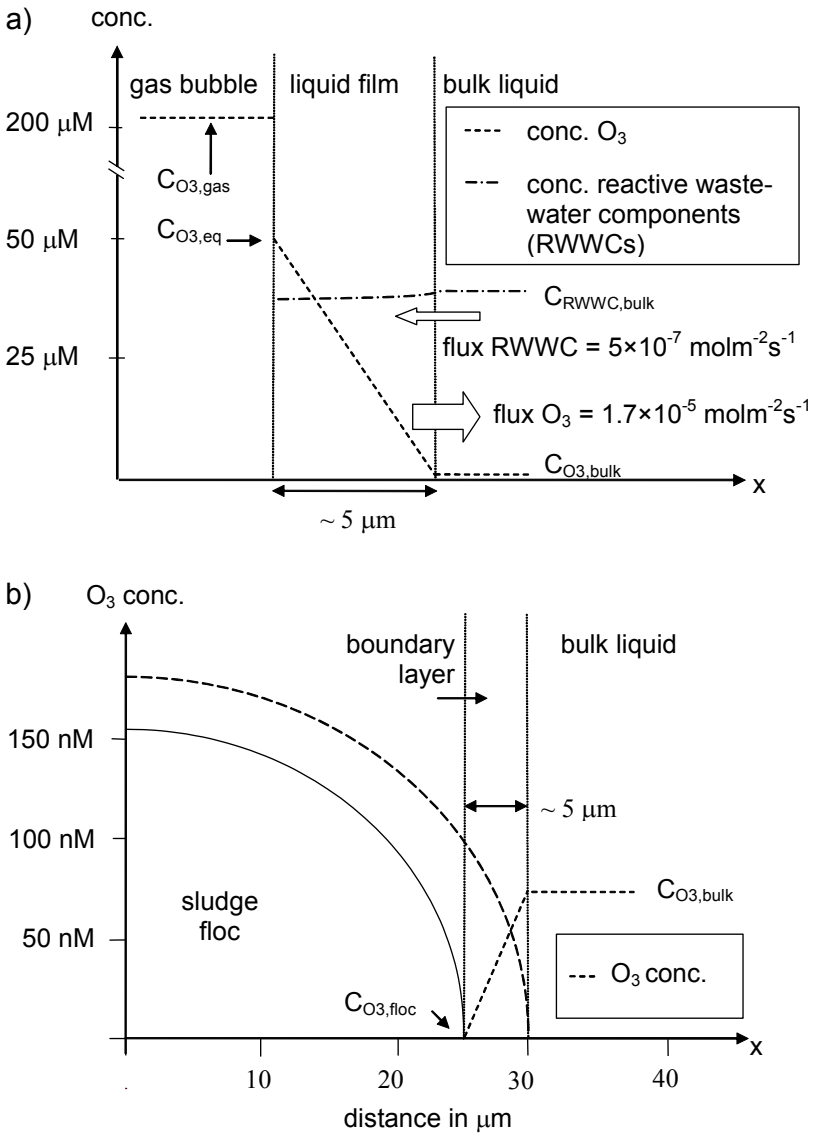


FIGURE 5-6. (a) Qualitative scheme of the diffusion of O_3 and reactive wastewater components (RWWC) through the liquid film according to film theory. The selected concentrations represent conditions at the bottom of column 1 for an O_3 dosage of 1 mg L^{-1}. (b) Qualitative diffusion profile of O_3 through the boundary layer of a sludge floc.

Conditions representing the bottom of column 1 for an O_3 dosage of 1 mg L^{-1} were selected for the estimation of the O_3 absorption rate of the bulk liquid and the sludge particles. Close to the ozone diffuser, the O_3 concentration in the gas phase is $C_{O3,gas}$ = 10 g Nm^{-3}. Henry's law relates $C_{O3,gas}$ to $C_{O3,eq}$:

$$H = \frac{C_{O3,eq}}{C_{O3,gas}} \quad (2)$$

where H is the dimensionless Henry constant. Using H = 0.24 (*30*), eq 2 yields $C_{O3,eq}$ = 2.4 g m^{-3} or 50 μM. The fact that no O_3 residuals could be measured at SP-MID for an O_3 dosage of 1 mg L^{-1} indicates that $C_{O3,bulk} << C_{O3,eq}$. Assuming that $C_{O3,bulk} \approx 0$, eq 1 yields $N_{O3,bulk} = 3 \times 10^{-7}$ mol s^{-1} L^{-1}.

In the presence of a high concentration of fast-reacting compounds, the O_3 mass transfer can be enhanced by reactions taking place in the liquid film. To check whether such an enhancement has to be considered in the present case, the concentration of reactive wastewater components in the bulk liquid ($C_{RWWC,bulk}$, e.g., O_3 reactive moieties of DOC and reactive inorganic compounds) and their rate constants with O_3 have to be estimated. Low O_3 residuals detected at SP-MID for O_3 dosages ≥2 mg L^{-1} indicated that the fast initial O_3 demand of the wastewater is equivalent to approximately 2 mgO_3 L^{-1} (40 μM) under the assumption that only direct O_3 reactions with a stoichiometry of 1:1 are involved. Therefore, $C_{RWWC,bulk}$ was set to 40 μM. The rate constant for the reaction of O_3 with RWWC was estimated to be $k_{O3} = 1 \times 10^5$ M^{-1} s^{-1}. This rate constant is representative for reactive moieties like phenols and amines. To assess the importance of the reaction of O_3 in the liquid film compared to O_3 mass transfer across the film, the Hatta number (Ha) was calculated assuming that in the film C_{RWWC} = const. = $C_{RWWC,bulk}$.

$$Ha = \frac{\sqrt{k_{O3} \cdot D_{O3} \cdot C_{RWWC,bulk}}}{k_L} \quad (3)$$

For the selected conditions, eq 3 yields 0.2. Ha < 0.3 means that gas absorption follows a so-called slow kinetic regime and that reactions take primarily place in the bulk liquid (*29*). Therefore, O_3 mass transfer is well described by eq 1 and no enhancement due to consumption of O_3 in the film has

to be considered. Figure 5-6a gives a qualitative representation of the diffusion profiles resulting from the above-described conditions. Solving the differential equations that describe diffusion and reaction of O_3 in the liquid film (*29*) yielded very similar results.

Knowing $N_{O3,bulk}$ and $C_{RWWC,bulk}$, the following equation can be used to estimate $C_{O3,bulk}$, which is needed for the calculation of the O_3 absorption rate of the sludge flocs:

$$\frac{dC_{O3,bulk}(t)}{dt} = N_{O3,bulk}(t) - k_{O3}C_{O3,bulk}(t)C_{RWWC,bulk}(t) \quad (4)$$

Under steady-state conditions the left side of eq 4 is zero and the variables become time-independent. Solving eq 4 for $C_{O3,bulk}$ results in a concentration of $C_{O3,bulk} = 7.5 \times 10^{-8}$ M. This value corroborates the assumption made for the calculation of the film diffusion and is in agreement with the fact that O_3 concentrations were < 0.05 mg L^{-1} (<1×10^{-6} M) at SP-MID for an O_3 dosage of 1 mg L^{-1}.

The diffusion of O_3 through the boundary layer of a sludge floc can be represented as shown in Figure 5-6b. The diffusive transport from the bulk liquid to the sludge flocs can be assessed using the following equation:

$$F_{O3} = \frac{D_{O3}}{\delta_f}\left(C_{O3,bulk} - C_{O3,floc}\right) \quad (5)$$

The thickness δ_f of the boundary layer surrounding the flocs must be in the same order of magnitude as δ_b. Because the flocs move more slowly than the bubbles, it is expected that δ_f is somewhat larger than δ_b. To make a conservative assumption, it was estimated that $\delta_f = \delta_b = 5$ μm. Assuming that the O_3 concentration at the liquid-floc interface ($C_{O3,floc}$) is zero, a flux of $F_{O3} = 2.6$ 10^{-8} mol s^{-1} m^{-2} is obtained using eq 5.

To estimate the O_3 absorption rate for 20 mgTSS L^{-1} (5 mgTSS L^{-1} of CAS + 15 mgTSS L^{-1} dosed), the specific interfacial area of the sludge flocs (a_f) has to be calculated. An area of $a_f = 48$ m^2 m^{-3} was obtained assuming a water content of the floc of 95%, sperical shape and a diameter of 50 μm. Laser diffraction analysis of a different activated sludge showed that 90% of the sludge volume is formed by flocs with a diameter > 50 μm (median = 200 μm) (*31*). The resulting O_3 absorption rate by the sludge flocs is $N_{O3,floc} = F_{O3} \times a_f / 1000 = 1.2 \times 10^{-9}$ mol s^{-1} L^{-1} compared to the absorption rate of the bulk phase of $N_{O3,bulk} = 3 \times 10^{-7}$ mol s^{-1} L^{-1}. According to this estimate, only 0.4 % of the O_3 transferred into the bulk solution is consumed by sludge particles. Changing the estimated parameters by a factor of two in any direction will not significantly increase the absorption rate of the sludge particles. Due to the unambiguity of the result, the rough estimate of mass transfer demonstrates clearly that the O_3 absorption rate must be limited by O_3 diffusion across the boundary layer surrounding the sludge particles. The fact that O_3 absorption by sludge particles is relatively low, explains also why oxidation by $^{\bullet}OH$ is relatively unaffected by suspended solids. Because the highest share of O_3 reacts in the bulk liquid, $^{\bullet}OH$ is formed in the bulk liquid as well and does not come into contact with sludge particles due to its extremely low life time.

The considerations presented above also imply that micropollutants sorbed to sludge particles will not be oxidized efficiently. Furthermore, the inactivation of microorganisms present in the floc will be difficult to achieve, because these microorganisms will only experience a relatively low O_3 exposure. In Table 5-4, concentrations of *E. coli* before and after ozonation of CAS and CAS+SS effluent demonstrate clearly the negative impact of suspended solids on disinfection efficiency.

TABLE 5-4. *E. coli* Concentrations in CFU/100mL before and after Ozonation of CAS and CAS+SS Effluent[a]

	CAS	CAS+SS
inlet pilot plant	~5 × 10^5	~4 × 10^5
2 mg L^{-1} O_3	~2 × 10^2	~3 × 10^3
5 mg L^{-1} O_3	<10^2	~6 × 10^2

[a] source: ref (*32*)

5.3.3 Oxidation Patterns

For macrolides, sulfonamides, estrogens, and contrast media several compounds of each class have been analyzed. Within these classes, compounds are structurally very similar and it can be assumed that O_3 attack takes place on the same functional groups. The reactive functional group in macrolides, estrogens and sulfonamides are the tertiary amino groups, the phenol moiety, and the aniline moiety, respectively (Figures 5-3 and 5-2, Table 5-2). As mentioned above, contrast media do not react with O_3 directly. Since the chemical environment of these reactive moieties is in most cases quite similar within one class, it can also be assumed that the rate constants for the reaction with O_3 must be very similar. Consequently, for a given O_3 exposure, the extent of parent compound oxidation should be similar for all compounds of a class. The oxidation patterns of macrolides, sulfonamides and estrogens in CAS are shown in Figure 5-7. As expected, parent compound oxidation for 4 macrolide antibiotics was very similar. Also the relative residuals measured for three estrogens were comparable except for unaccountable residuals of E1 for high O_3 dosages. In case of sulfonamides, somewhat greater variations were detected, which might be caused by one of the following reasons: On one hand, it cannot

be excluded that in case of sulfathiazole the thiazole moiety is more reactive to O_3 than the aniline moiety. On the other hand, the pK_a values of the investigated sulfonamides range from 5.7 (sulfamethoxazole) to 8.4 (sulfapyridine, Table 5-2). Consequently, sulfamethoxazole is present in its anionic and sulfapyridine in its neutral form, whereas the remaining sulfonamides are present as a mixture of both species. Anionic species can be many times more reactive toward O_3 than their neutral equivalents. It is therefore rather surprising that the differences between the investigated sulfonamides are not greater. A possible explanation is that the higher electron density on the acidic nitrogen reflected by a higher pK_a extents to the adjacent moieties, making them significantly more reactive toward O_3. The reactivity of the neutral form of sulfapyridine seems, therefore, to be as high as that of the anion of sulfamethoxazole. Consequently, the reasonable agreement in the oxidation pattern seems to be rather coincidence in case of the sulfonamides. In general, significant differences in the extent of parent compound oxidation have to be expected when the compared compounds exhibit different speciations under the investigated conditions.

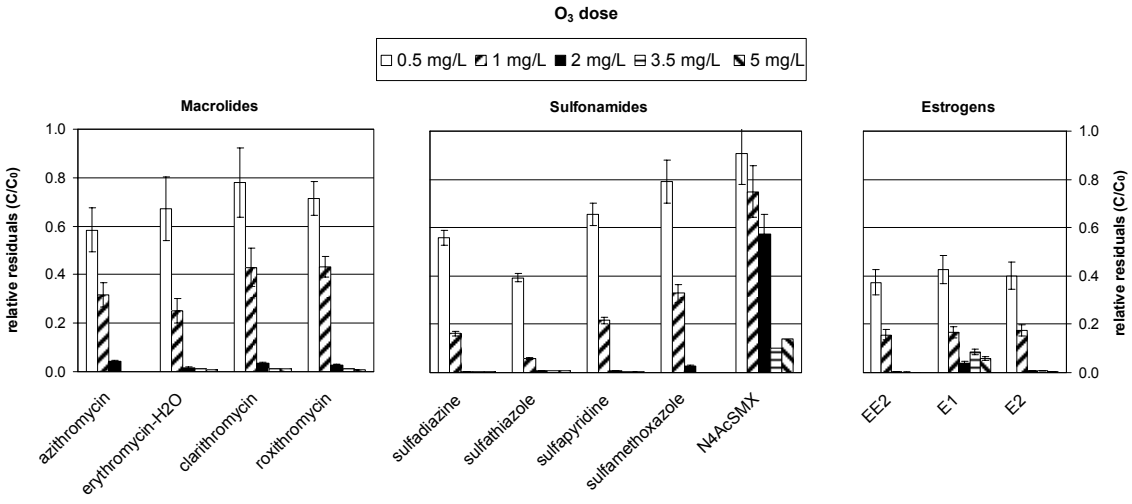


FIGURE 5-7. Relative residual concentrations of macrolides, sulfonamides and estrogens in CAS effluent for O_3 doses ranging from 0.5 to 5 mg L^{-1}.

A large share of sulfamethoxazole enters WWTPs in its acetylated form as N^4-acetylsulfamethoxazole (N^4AcSMX, Table 5-2) (*22*). This metabolite was also present in the investigated effluents. In Figure 5-7, the oxidation pattern of N^4AcSMX is given as well. The O_3 reactive aniline moiety of N^4AcSMX is protected with an acetyl group that changes the electron density of this moiety. Therefore, its reactivity to O_3 is considerably reduced and the extent of oxidation is much lower as compared to sulfamethoxazole. Accordingly, N^4-acetylsulfamethazine, which was spiked to the wastewater, showed a very similar behavior (data not shown).

Figure 5-8 illustrates the oxidation of contrast media in CAS. Contrast media do not react with O_3, but due to similarities in size and structure they all exhibit similar reactivities to $^{\bullet}$OH that result in similar extent of parent compound oxidation. Only the anionic contrast media diatrizoate showed a different pattern suggesting a substantially lower reactivity to $^{\bullet}$OH. Overall, these results show that within certain limits it is possible to predict the extent of parent compound oxidation of structurally similar molecules with the same reactive moiety based on a suitable probe compound present in the wastewater.

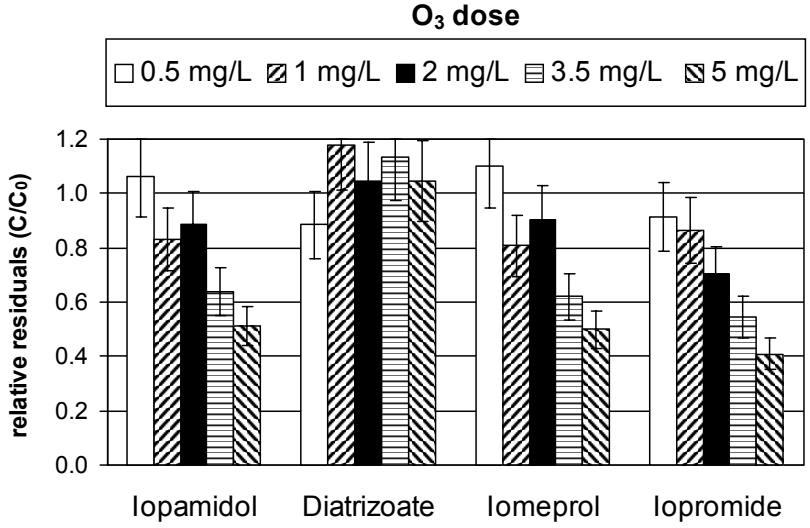


FIGURE 5-8. Relative residual concentrations of iodinated X-ray contrast media in CAS effluent for O_3 doses ranging from 0.5 to 5 mg L^{-1}.

5.3.4 Prediction of Parent Compound Oxidation

More important than the prediction of the oxidation of compounds within the same class of pharmaceuticals (such compounds can usually be measured with a single analytical method) would be a prediction for compounds of various classes on the basis of second-order rate constants for their reaction with O_3 and $^{\bullet}OH$ and appropriate probe compounds. The rate of oxidation of a pollutant during ozonation is given by the rate law:

$$\frac{dC_P(t)}{dt} = -k_{O3}C_{O3}(t)C_p(t) - k_{OH}C_{OH}(t)C_p(t) \qquad (6)$$

where C_P, C_{O3}, and C_{OH} are the concentrations of the pollutant, O_3, and $^{\bullet}OH$, respectively and k_{O3} and k_{OH} are the second-order rate constants for the reaction of the pollutant with the respective oxidants. To predict residual concentrations of a pollutant, the integrated form of eq 6 has to be used:

$$\frac{C_p(\tau)}{C_p(0)} = e^{-k_{O3}\int_0^\tau C_{O3}(t)dt - k_{OH}\int_0^\tau C_{OH}(t)dt} \qquad (7)$$

If k_{O3} and k_{OH} are known, only the O_3 exposure ($\int C_{O3}(t)dt$) and the $^{\bullet}OH$ exposure ($\int C_{OH}(t)dt$) have to be determined to make a prediction. On the basis of eq 7, Elovitz and von Gunten (*33*) developed the R_{ct} concept, with which the oxidation of pharmaceuticals that exhibit an intermediate or low k_{O3} was successfully predicted in bench-scale experiments performed under drinking water treatment conditions (*12*). In the cited study, O_3 exposures were determined by integrating the measured O_3 concentrations over time. $^{\bullet}OH$ exposures were calculated with help of a probe compound and the R_{ct} value. However, $^{\bullet}OH$ exposures can also be determined without the R_{ct} value by simply using a probe compound that has a known k_{OH} and that does not react with O_3. If $k_{O3} = 0$, eq 7 can be rearranged and the $^{\bullet}OH$ exposure can be calculated based on the relative residual concentration of the probe compound (C_{PC}) and its rate constant with $^{\bullet}OH$ ($k_{OH,PC}$):

$$\int_0^{\tau} C_{OH}(t)dt = -\frac{1}{k_{OH,PC}} \ln\left(\frac{C_{PC}(\tau)}{C_{PC}(0)}\right) \quad (8)$$

If it is not possible to measure O_3 concentrations during an ozonation process, it should be possible to determine an O_3 exposure with an appropriate probe compound in the same way as for $^{\bullet}OH$ exposures. However, since all organic compounds react with $^{\bullet}OH$ at appreciable rates, residuals of O_3 probes have always to be corrected for oxidation by $^{\bullet}OH$.

In the present study it was tested, whether predictions for fast-reacting pharmaceuticals can be made on the basis of this concept. Ibuprofen and naproxen were used as the $^{\bullet}OH$ and O_3 probes, respectively. To account for the relatively high uncertainties associated with the high rate constants and the residual concentrations of the probe compounds, Monte Carlo simulations were performed using a Matlab script (MathWorks, Inc.) to calculate 90% confidence intervals for the predictions.

As for drinking water, the prediction of the oxidation by $^{\bullet}OH$ radicals worked reasonably well for compounds like clofibric acid and iopromide that exhibit a low reactivity to O_3 (data not shown). To assess the quality of the predictions for fast-reacting pharmaceuticals, only data for an O_3 dosage of 1 mg L^{-1} could be used among the O_3 dosages considered (1, 2, and 3.5 mg L^{-1}), because the low residuals of fast reacting compounds cannot be properly measured for higher dosages. Out of the four considered compounds (EE2, sulfamethoxazole, diclofenac, and roxithormycin) the predictions for EE2 and sulfamethoxazole deviated strongly from the measured value. Taking into account the model uncertainty, maximal residuals of <1-2% were calculated compared to measured residuals of 15-30%. Also, naproxen, diclofenac and roxithromycin were oxidized to a higher extent than EE2 and sulfamethoxazole despite their lower rate constants. Obviously, the ozonation process under these conditions is too

complex as that predictions for fast-reacting compounds could be made with this relatively simple concept.

Reasons for the poor predictions might be the sorption of some compounds to sludge particles which prevented oxidation or the interaction of pharmaceuticals with colloids (*34*) which also might offer some protection against O_3 attack. On the basis of film theory, it can be concluded that diffusion limitations as a consequence of the relatively high rate constants for the reaction of O_3 with RWWCs and pharmaceuticals were most probably not the cause for the poor predictions, because O_3 reactions take predominantly place in the bulk liquid and not in the film as shown in Figure 5-6.

5.3.5 Oxidation by Ozone versus Oxidation by Hydroxyl Radicals

Using ibuprofen as a probe compound, the oxidation of O_3 refractive compounds by $^{\bullet}OH$ could be well predicted with the following equation:

$$\ln\left(\frac{C_P(\tau)}{C_P(0)}\right) = \frac{1}{k_{OH,IBU}} \cdot \ln\left(\frac{C_{IBU}(\tau)}{C_{IBU}(0)}\right) \cdot k_{OH} \qquad (9)$$

where C_{IBU} is the concentration of ibuprofen and $k_{OH,IBU}$ is the rate constant for the reaction of ibuprofen with $^{\bullet}OH$. In the same way, the oxidation by $^{\bullet}OH$ can be calculated for compounds that react fast with O_3, even if the prediction of oxidation by O_3 failed. The comparison of the predicted oxidation by $^{\bullet}OH$ with the measured residuals allows us to assess the relevance of the two oxidation pathways for a selected compound according to the following equation (*25*):

$$f(^{\bullet}OH) = \frac{\frac{1}{k_{OH,IBU}} \cdot \ln\left(\frac{C_{IBU}(\tau)}{C_{IBU}(0)}\right) \cdot k_{OH,P}}{\ln\left(\frac{C_P(\tau)}{C_P(0)}\right)} \qquad (10)$$

where $f(^{\bullet}OH)$ designates the importance of oxidation by $^{\bullet}OH$ and $1\text{-}f(^{\bullet}OH)$ the importance of oxidation by O_3. The knowledge of these values is important

because different products will be formed depending on the oxidation pathway. In Figure 5-9, the ratio between oxidation pathways of 4 fast reacting compounds is plotted for an O_3 dose of 1 mg L^{-1}. Despite the high reactivity of these compounds toward O_3, $^{\bullet}OH$ accounts for 20-50% of the parent compound oxidation. This demonstrates clearly that in wastewater $^{\bullet}OH$ radicals cannot be neglected in product studies, even if O_3 reactions would clearly predominate in a pure system. Because $^{\bullet}OH$ reaches the highest concentration at first contact of wastewater with O_3, it can be assumed that higher O_3 dosages do not diminish the role of the oxidation pathway by $^{\bullet}OH$.

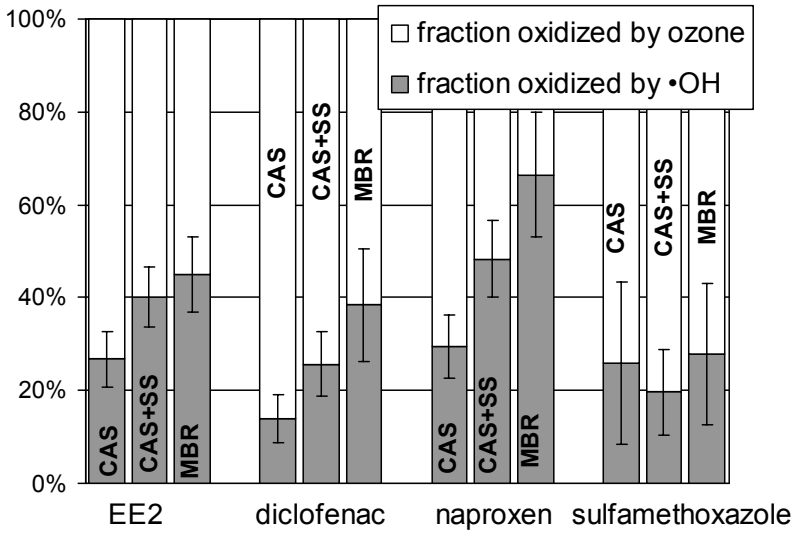


FIGURE 5-9. Calculated fractions oxidized by $^{\bullet}OH$ and O_3 for fast-reacting pharmaceuticals for an O_3 dose of 1 mg L^{-1}.

5.3.6 Practical Implications

The results of the present study have shown that important classes of pharmaceuticals present in wastewater effluents like macrolide and sulfonamide antibiotics as well as synthetic and natural estrogens can be selectively oxidized using relatively low O_3 doses. The water quality parameter that has the strongest influence on the efficiency of the ozonation process seems to be DOC. In

another study, O_3 doses >5 mg L^{-1} had to be applied to achieve a comparable result for wastewater with a higher DOC (*14*). Results of the present study show that suspended solids have only a minor effect on the oxidation of pharmaceuticals. Ozonation of wastewater effluents will mainly be a viable solution, when the treatment objectives include micropollutant oxidation and disinfection. Therefore, it has to be pointed out that suspended solids had a negative impact on disinfection. In the regular CAS effluent, an O_3 dosage of 5 mg L^{-1} would be sufficient to achieve the guideline values set by the EU bathing water quality directive. In contrast, this standard could not be achieved with the higher suspended solids concentration in CAS+SS effluent.

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6

General Discussion and Conclusions

The results presented in Chapter 2 and 3 have shown that from a kinetic point of view ozonation is considerably more effective in oxidizing pharmaceuticals than chlorine dioxide or chlorine. This finding is linked to the fact that in comparison with chlorine dioxide and chlorine, the rate constants for the reaction of ozone with reactive functional groups are generally higher. Consequently, in addition to amines and phenols, a larger number of moieties react with ozone at an appreciable rate (e.g., double bonds, alkoxy- and alkyl-substituted benzenes). Ranking the investigated pharmaceuticals in their descending order of reactivity toward O_3 yields: 17α-ethinylestradiol (EE2) > sulfamethoxazole ≈ diclofenac > carbamezepine > roxithromycin >> bezafibrate > ibuprofen > iopromide ≈ diazepam. Among these compounds, only the latter three pharmaceuticals are relatively O_3-refractive. However, due to the formation of $^{\bullet}OH$ even such O_3-refractive compounds are oxidized to a substantial extent during ozonation.

In addition to the disappearance of the parent compounds, the elimination of the pharmacological effects associated with the target compounds has also to be taken into account to assess oxidative treatment processes in a comprehensive manner. Taking EE2 as an example, the elimination of the estrogenicity during ozonation was studied in Chapter 4. The estrogenicity of EE2 can be considered as the primary pharmacological effect of EE2. The results showed that relatively low ozone doses were sufficient to oxidize EE2 and to produce a significant decrease in estrogenicity. The identification of oxidation products of EE2 proved that ozone attack primarily took place on the phenolic moiety, which is also crucial for the binding of estrogens to the estrogen receptor. In the light of this, EE2 is an excellent example how pharmaceuticals can be deactivated through selective oxidation of functional groups. Ideally, oxidation products should also be tested for toxic effects. However, EE2 concentrations in surface waters are extremely low and it is unlikely that oxidation products exhibit a

similarly strong biological activity as the extremely potent parent compound with respect to estrogenicity. Therefore, it seems to be acceptable to neglect other biological effects in the case of EE2.

This study on EE2 is the first study that investigated the oxidation of pharmaceuticals during ozonation in a comprehensive manner, including oxidation kinetics, product formation, and pharmacological effects of the oxidation products. To reach a conclusive assessment of ozonation it is indispensable to investigate further pharmaceuticals with regard to the elimination of their pharmacological effects and the formation of potentially toxic oxidation products. However, for many pharmaceuticals easy test systems which allow for the investigation of their pharmacological effect are not available. Also, testing oxidation products for toxic effects is a very time-consuming task. The product identification of EE2 has shown that ozonation can produce a large number of different oxidation products, which are usually not commercially available. Consequently, it may often not be feasible to isolate single oxidation products and to perform toxicity tests with single, pure compounds. The testing of mixtures of oxidation products may be a more efficient solution in such cases. If such a screening indicates that toxic oxidation products are formed for a certain pharmaceutical, the respective compound can be investigated more extensively to link the observed toxicity to the responsible oxidation products.

Bench-scale experiments with different natural waters have shown that rate constants for ozone, hydroxyl radical, and chlorine dioxide, which were determined in pure aqueous solution, can be applied to predict the oxidation of pharmaceuticals in natural waters. For chlorine dioxide, it could additionally be demonstrated that results obtained at relatively high target compound concentrations are also valid for 3 orders of magnitude lower, realistic concentrations of pharmaceuticals. The pilot-scale ozonation experiments

presented in Chapter 5 further demonstrated that the determined rate constants can at least be qualitatively applied to predict the oxidation of pharmaceuticals in wastewater. Pharmaceuticals with high rate constant were oxidized to an extent >90-99% for O_3 doses ≥2 mg L^{-1}, whereas slow-reacting pharmaceuticals were still present after treatment with 5 mg L^{-1} O_3. However, smaller differences in the extent of parent compound oxidation of highly reactive pharmaceuticals could only partly be explained by differences in rate constants. Probably, interactions of pharmaceuticals with particles and colloids present in the wastewater have influenced their oxidation behaviour. Overall the pilot-experiments confirmed that ozonation works also well at larger scale and at relatively realistic pharmaceutical concentrations.

To assess the role of $^{\bullet}OH$ during ozonation processes, the fraction of parent compounds oxidized by $^{\bullet}OH$ was calculated for the investigated pharmaceuticals under drinking water and wastewater treatment conditions on the basis of $^{\bullet}OH$ and O_3 exposures. Under drinking water treatment conditions, O_3-refractive compounds were oxidized to a substantial extent by $^{\bullet}OH$, whereas for fast-reaction pharmaceuticals the oxidation of the parent compound by $^{\bullet}OH$ was insignificant. In contrast, in wastewater the first oxidation step for fast-reacting pharmaceuticals proceeded up to 30-50% through oxidation by $^{\bullet}OH$. These results imply that in wastewater oxidation by $^{\bullet}OH$ can not be neglected and that the oxidation products expected from the direct reaction with O_3 will only account for 50-70% of the products.

Within the framework of the POSEIDON project, further treatment processes applied in drinking water treatment were investigated. Results show that most of the investigated pharmaceuticals adsorb well to activated carbon. An exception is the ionic X-ray contrast media diatrizoate. This compound was retained by fresh granular activated carbon (GAC), but broke through when GAC had been subjected to higher throughputs. Besides filtration with GAC, also the Cristal®-

process, a combination of ultrafiltration with powdered activated carbon proved to be efficient in removing pharmaceuticals. Ultrafiltration alone did not significantly remove pharmaceuticals. As expected, membranes with a lower molecular cut-off (nanofiltration, reverse osmosis) also removed the investigated pharmaceuticals efficiently. Coagulation and sand filtration had no significant impact on most of the investigated compounds.

On the basis of the result of this thesis and the POSEIDON project, the following conclusions can be drawn for the elimination of pharmaceuticals during treatment of surface water and groundwater:

- In Western Europe, state of the art treatment trains for surface water include the application of activated carbon and/or ozonation. Often, these processes are operated in conjunction with bank filtration and biological treatment (biological activated carbon filtration or slow sand filtration), which can act as further barriers. Consequently, most of the pharmaceuticals will be eliminated during treatment of surface waters.
- In the USA, conventional surface water treatment is usually limited to coagulation, filtration and disinfection with chlorine. In this case, the only barrier for pharmaceuticals is an oxidation with chlorine. Above all, compounds exhibiting phenol or amino groups like the estrogens and various antibiotics will be oxidized during chlorination. However, many compounds could pass the treatment unhindered.
- For groundwater in Europe and the USA, disinfection is often the only treatment. The use of chlorine or chlorine dioxide as disinfectant will result in the oxidation of specific pharmaceuticals (e.g, phenols and amines), but many compounds will not be affected by the treatment. Disinfection with UV eliminates specific compounds to some extent

(<20%), but UV doses are generally too small for a substantial photochemical transformation of pharmaceuticals.

- Groundwater derived from bank filtrate is most likely contaminated with specific pharmaceuticals such as contrast media and carbamezepine, which are typically not degraded during bank filtration even if the hydraulic residence time amounts to several days. For shorter residence times, also other pharmaceuticals are likely to pass bank filtration. If such water is not treated with either activated carbon, ozonation or membranes, the risk of the presence of pharmaceuticals in the finished drinking water is relatively high.

A part of the conclusion presented above could be confirmed in several full-scale treatment plants in Germany and France. Overall, it can be concluded that a contamination of drinking water with pharmaceuticals is unlikely, if advanced treatment processes are used. Only few pharmaceuticals (e.g., contrast media) have the potential to pass such treatment. To assure that drinking water derived from ground water remains free of pharmaceuticals, it is necessary to implement an efficient water resources protection scheme.

Curriculum Vitae

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