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Ozonation of Erythromycin and the Effects of pH, Carbonate and Phosphate Buffers, and Initial Ozone Dose

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Ozonation of Erythromycin and the Effects of pH, Carbonate and Phosphate Buffers, and Initial Ozone Dose

by

Ling Huang, B.E.

Thesis

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science in Engineering

The University of Texas at Austin

August 2011

Dedication

To my family

Acknowledgements

First of all, I want to express my heartfelt gratitude and thanks to my advisors, Dr. Desmond Lawler and Dr. Lynn Katz, for guiding, helping and supporting me through my master studies at University of Texas at Austin. I want to thank Dr. Lawler for always believing in me and supporting me whenever I need help; I will always remember Lawler's No.1 Rule—when in doubt, do a mass balance. I also want to thank Dr. Katz for your enthusiasm and passion for teaching, which has always been my motivation; I will always remember the cool things I learned in your water chemistry class and surface chemistry class.

Second, I want to thank people in Lawler, Katz and Reible research group for your assistance and encouragement in the laboratory. I want to thank:

- Lee Blaney to his work with me all the way along;
- Ijung Kim for your kind help and encouragement;
- Fei Yan for all the helpful suggestions and discussion you offered;
- Hector Garcia for LC/MS help and developing the detection method;
- Patrick Dunlap for staying late at school so that I could find you whenever I need you help;
- Yeonjoeng Ha, Daigo Shimizu and Matt Dobbertien for helping me with my experiments—I couldn't finish any experiments without your help
- Katie Alfredo and Dr. Xiaoxia Lu for helping me with the lab equipments;
- Corin Marron for provide precious experience from previous research.

To my fellow Environmental and Water Resources Engineering students, thank you for making my last two year memorable.

I would also like to thank my parents, Haixiao Huang and Ying Lin for their support and understanding for all the twenty-two years. Finally, I want to thank my friend, Kai for everything you did—nobody other than you would stay with me doing experiments at Christmas Eve. I couldn't be what I am right now without your unwavering support and understanding.

August 2011

Abstract

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The University of Texas at Austin, 2011

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The ubiquitous presence and chronic effect of pharmaceuticals is one of the emerging issues in environmental field. As a result of incomplete removal by sewage treatment plants, pharmaceuticals are released into the environment and drinking water sources. On the other hand, conventional drinking water treatment processes such as coagulation, filtration and sedimentation are reported to be ineffective at removing pharmaceuticals. Therefore, the potential presence of pharmaceuticals in finished drinking water poses a threat on public health. Antibiotics, as an important group of pharmaceuticals, are given special concerns because the potential development of bacteria-resistance.

Ozonation and advanced oxidation processes are shown to be quite effective at removing pharmaceuticals. The oxidation of pharmaceuticals is caused by ozone itself and hydroxyl radicals that are generated from ozone decomposition. Whether ozone or hydroxyl radicals are the primary oxidant depends on the specific pharmaceutical of interest and the background water matrix.

In this research, erythromycin, a macrolide antibiotic, was chosen as the target compound because of its high detection frequency in the environment and its regulation status. The objective of this research was to investigate the removal performance of erythromycin by ozonation from the standpoint of kinetics. The effects of pH, carbonate and phosphate buffers, and initial ozone dose on ozonation of erythromycin were also studied. The second-order rate constant for the reaction between deprotonated erythromycin and ozone was determined to be 4.44×10^{9} $M^{-1}\cdot s^{-1}$ while protonated erythromycin did not react with ozone. Ozone was determined to be the primary oxidant for erythromycin removal by ozonation. pH was found to have great positive impact on the degradation of erythromycin by ozonation due to the deprotonation of erythromycin at high pH. Carbonate and phosphate buffers were found to have negligible effects on the degradation of erythromycin by ozonation. Initial ozone dose showed a positive impact on the total erythromycin removal rate by ozonation.

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Chapter 1 Introduction

1.1 Research Objective

The ubiquitous presence and the potential chronic effects on human health make the occurrence and fate of pharmaceuticals an emerging issue in the environmental field. Pathways for pharmaceuticals entering the environment mainly include the discharge of wastewater from sewage plants and runoff associated with veterinary use. The occurrence of pharmaceuticals in surface water and groundwater raises concerns about drinking water safety. The use of contaminated drinking water sources leads to the possibility of the presence of pharmaceuticals in finished drinking water.

Antibiotics, an important group of pharmaceuticals, are always of great concern because of their indispensable use and the possibility of developing bacterial resistance. Antibiotics may be present either as the original "parent" compound if complete removal is not achieved during treatment or as transformation intermediates with unknown toxicity that are produced during chemical processes such as oxidation. Erythromycin belongs to the macrolide subclass of antibiotics; it is widely used for control of bacteria in humans as well as in food-producing animals. Erythromycin is chosen as the target compound in this research for three primary reasons: a high frequency of detection, concerns regarding its persistence in the environment and potential for upcoming drinking water regulations.

Conventional drinking water treatment processes such as coagulation, sedimentation and filtration are found to be ineffective at removing pharmaceuticals. Ozonation and advanced oxidation processes have shown promise as a treatment alternative for a number of pharmaceuticals. Moreover, existing drinking water treatment plants could easily be upgraded or retrofitted for these technologies. However, the cost associated with advanced oxidation processes in aqueous media is likely to be high, especially when treatment cost per unit mass is considered because the initial

concentration of most pharmaceuticals is expected to be extremely low in drinking water sources.

The oxidation of pharmaceuticals by ozone may occur by ozone itself and/or by hydroxyl radicals that are generated during ozone decomposition. Ozone is a relatively selective oxidant which only reacts with certain functional groups; on the other hand, hydroxyl radicals are rather non-selective oxidant and may attack at any number of sites on a given pharmaceutical. The non-specificity of hydroxyl radicals is reflected by the similarity among rate constants for the degradation of several target compounds by hydroxyl radical (Broseus *et al*., 2009). Therefore, advanced oxidation processes have emerged in an effort to generate as much hydroxyl radical as possible by combining the use of chemical agents, irradiation and catalysts. However, whether ozone or hydroxyl radicals are the main contributor to the degradation of a particular pharmaceutical depends on the particular target compound and the background water chemistry. Common background water parameters, such as pH, alkalinity and natural organic matter affect the rate of ozone decomposition, i.e., hydroxyl radical exposure; therefore, they have an indirect impact on the removal of pharmaceuticals by ozonation. Initial ozone dose is also a key operating parameter that should be taken into consideration. The effect of pH is especially complex; on one hand, it affects the speciation of some target compounds and thus their reactivity with ozone; on the other hand, it determines the speciation of carbonate buffer and natural organic matter.

The degradation of two compounds that are similar to erythromycin, namely roxithromycin and clarithromycin, by ozonation has been studied and the second order rate constants between these compounds and ozone have been reported (Lange *et al*., 2006; Huber *et al*., 2003). However, no data yet is available for the degradation of erythromycin degradation by ozonation. Furthermore, the effect of natural water constituents on the ozonation of erythromycin has not been investigated.

1.2 Objectives and Approach

This research was designed to evaluate the kinetics of erythromycin degradation by ozonation. The objectives of this research are outlined below:

- Determine the rate constant for the reaction between erythromycin and ozone.
- Determine the rate constant for the reaction between erythromycin and hydroxyl radicals
- Identify the primary oxidant responsible for erythromycin degradation during ozonation
- Investigate the effect of pH, carbonate and phosphate buffers, and initial ozone dose on erythromycin degradation by ozonation

1.3 Research Approach

To achieve the objectives of this research, batch experiments were conducted to examine concentrations of erythromycin as a function of time under various background conditions of pH, carbonate and phosphate buffer concentrations, and initial ozone dose.

1.4 Thesis Structure

This thesis is divided into five chapters that provide an introduction, background knowledge, methodologies, results and conclusions. Chapter 1 serves as a brief introduction describing the motivation and objectives of this research. Chapter 2 presents a literature review including the occurrence of pharmaceuticals in the environment, treatment processes for pharmaceutical removal from water with emphasis on ozonation and advanced oxidation processes, and previous research results that have applied ozonation to macrolide antibiotics. Materials and methods employed in this research are described in Chapter 3. Chapter 4 presents the interpretation of the experimental data and discussion based on the results. The last chapter provides the conclusions and recommendations of future work.

Chapter 2 Literature Review

2.1 INTRODUCTION

The ultimate objective of this research is to delineate the effect of natural water parameters on the kinetics of degradation of erythromycin by ozonation. The achievement of this goal necessitates the understanding of the chemistry of erythromycin, the ozonation process and the impact of several natural water parameters on the ozonation processes. This literature review aims to provide basic background knowledge related to this research. First, the occurrence of pharmaceuticals in the environment and their potential effects are presented. Antibiotics, especially erythromycin, are described in greater details since erythromycin is the target compound of this research. A review of treatment processes to remove pharmaceuticals both in drinking water treatment and wastewater treatment follows the review of the impacts of pharmaceuticals. Ozone and advanced oxidation processes are described in a separate section dedicated to examining the effects of natural water characteristics on the ozonation process, which is closely related to the goal of this research. Finally, previous research results regarding ozonation of macrolide antibiotics are presented.

2.2. PHARMACEUTICALS IN THE ENVIRONMENT AND ERYTHROMYCIN

2.2.1 Pharmaceuticals in the environment

Interest in and concerns about the ubiquitous presence of pharmaceuticals in the environment have grown dramatically in the past few years, especially due to the application of advanced measurement technologies (Fatta *et al*., 2007). Pharmaceuticals are a class of chemical substances that are designed to have a physiological effect on humans and animals (Klavarioti *et al*., 2009). Compared with the persistent organic pollutants in soil or sediment, which can be present with concentrations in the range of mg/L, the concentrations of pharmaceuticals in the environment are usually found at levels in the range of μg/L (Kim & Aga 2007); as a result, their presence as

environmental pollutants has been generally overlooked in the past. Occurrences of pharmaceuticals in the water environment have been reported worldwide, including the United States (Kolpin *et al*., 2002), Germany (Ternes 1998), Italy (Lalumera *et al*., 2004), Spain (Rodríguez *et al*., 2003), and Taiwan (Lin *et al*., 2008). Pharmaceutical compounds detected include antibiotics, anticonvulsants, painkillers, cytostatic drugs, hormones, lipid regulators, beta-blockers, antihistamines, and X-ray contrast media (Ikehata *et al*., 2006). The presence of pharmaceuticals have been reported in groundwater (Eckel *et al*., 1993; Holm *et al*., 1995) , surface water (Watts *et al*., 1983; Aherne *et al*., 1985), drinking water (Halling-Sørensen *et al*., 1998; Ternes *et al*., 2002a; Doll & Frimmel 2003), sediment (Coyne *et al*., 1994; Kerry *et al*., 1995), ocean (Lee & Arnold 1983) and soil (Warman & Thomas 1981).

Several pathways are available for pharmaceuticals to enter the environment. The primary pathway is through discharges of treated wastewater from sewage plants. After being ingested and metabolized, pharmaceuticals are excreted via urine and feces and then sent to wastewater treatment plants (WWTP). However, WWTPs are not designed to remove polar and large micropollutants like pharmaceuticals. Depending on the nature of specific pharmaceuticals and the processes employed by different WWTPs, the removal efficiency of pharmaceuticals encompasses the entire range from 0% to 100% (Radjenovic *et al*., 2007; Drewes 2007). Due to the low concentrations of pharmaceuticals in wastewater, enzyme affinities might be limited, meaning that the degradation of pharmaceuticals is more likely to occur in receiving waters or sediments than in WWTPs (Daughton & Ternes 1999). Pharmaceuticals can also be introduced to landfills in direct (via domestic and industrial routes) and indirect (via sewage sludge) ways. Although few pharmaceuticals have been detected in domestic drinking water, the presence of pharmaceuticals is possible when polluted groundwater is employed as a water source and treatment technologies do not remove the pharmaceuticals from drinking water treatment plants.

The focus of research regarding pharmaceuticals has shifted from early analysis and detection of the compounds in various water sources to dose-response studies of the pharmaceuticals in the lab and in field trials (Kümmerer 2009). Although the concentration of pharmaceuticals detected in the aquatic environment is quite low (in the range of ng/L to μg/L), recent research suggests that even this low concentration of pharmaceuticals in the environment could yield adverse ecological and human health effects. For example, the synthetic hormone 17α-ethynil estradiol at ng/L concentrations typically found in sewage effluents has the potential to induce endocrine disruption (Desbrow *et al*., 1998; Routledge *et al*., 1998). Moreover, pharmaceuticals are not typically present as individual compounds, but rather as multi-component mixtures in the environment, which raises the concern for synergistic and variable effects compared to responses observed for isolated exposure to the pure substance (Kümmerer 2009). The potential interactions among individual pharmaceuticals present at trace concentrations are difficult to evaluate.

Another concern regarding the effect of pharmaceuticals is the potential risk of long-term exposure on aquatic and terrestrial organisms as a result of continuous pharmaceutical input and environmental persistence. Resistance to biodegradation is a key property of pharmaceuticals. Several commonly used pharmaceuticals such as erythromycin, cyclophosphamide, naproxen and sulphamethoxazole could persist for more than one year (Zuccato *et al*., 2000). The potential for persistence combined with proven ecological impacts has led to regulation of a number of pesticides such as DDT; however, it is unlikely that limitations will be placed on the use of pharmaceuticals due to their known health benefits to humans as well as their economic importance (Jones *et al*., 2005). The increasing use and environmental persistence of pharmaceuticals implies that there is significant potential for long-term exposure of pharmaceuticals to organisms in the environment.

Whether the concern for the presence of pharmaceuticals in drinking water is warranted is still under debate. Indeed, there are several studies suggesting that the effects of pharmaceuticals on human health are minimal. Schwab *et al*. (2005) evaluated the potential effect on human health of 26 active pharmaceutical ingredients and/or their metabolites in US surface waters. They reached the conclusion that the trace concentrations of pharmaceuticals in surface water and drinking water had no appreciable risk to human health. In contrast, a concern because of the possible development of antibiotic-resistant bacteria (Boreen *et al*., 2004; Qiang *et al*., 2004; Andreozzi *et al*., 2006).

2.2.2 Antibiotics

Antibiotics, an important group of pharmaceuticals, are widely used for treating bacterial infection in both human and veterinary medicine (Hirsch *et al*., 1999). The presence of antibiotics has been detected in surface water in many countries such as the United States, Canada, Germany and other European countries. Examples of common antibiotics detected include macrolide antibiotics (clarithromycin, dehydro-erythromycin), sulfonamides, fluoroquinolones and trimethoprim (Hirsch *et al*., 1999; Ternes 2001; Kolpin *et al*., 2002; Metcalfe *et al*., 2003; Bruchet *et al*., 2005).

Antibiotics enter the environment mainly through the discharge of wastewater treatment plants or land application of sewage sludge (Kim & Aga 2007). Unmetabolized antibiotics can leave an organism via urine or feces. The incomplete elimination of antibiotics by wastewater treatment plants would then result in the presence of antibiotic substances in the receiving surface waters (Ternes 1998). Runoff deriving from the dispersion of manure on fields as fertilizer is an alternative pathway into the aquatic environment (Hirsch *et al*., 1999).

Depending on the specific antibiotic property and different wastewater treatment processes employed, the removal efficiency of different antibiotics in wastewater

treatment plants varies. Kasprzyk-Hordern *et al*. (2009) launched a 5-month monitoring program in the UK to determine the fate of 55 pharmaceuticals in two different wastewater treatment plants using different treatment technologies. They found that the removal efficiencies of erythromycin-H_2O and sulfamethoxazole were 50% and 70% in one wastewater treatment plant, respectively, while no removal was observed in the other treatment plant.

The removal mechanisms of antibiotics in wastewater treatment plants include biodegradation, adsorption to activated sludge, volatilization due to aeration and photodegradation, with the first two identified as the primary mechanisms (Kim & Aga 2007). The octanol-water partitioning coefficient, K_{ow}, is a parameter that is often used to estimate the potential for adsorption of hydrophobic contaminants to sludge or sediments. However, in contrast to highly lipophilic contaminants such as polychlorinated biphenyls, the molecular structures of antibiotics have multiple functional groups that exhibit varying properties such as acidity and polarity. For example, a given antibiotic may contain both acidic and basic groups within the same molecule. Therefore, the sorption of antibiotics depends not only on the log K_{ow} value, but is also affected by pH, redox potential, stereo chemical structure and chemical nature of both the sorbent and the sorbed molecule (Kümmerer 2009).

Although the concentrations of antibiotics reported in the environment are generally in the μg/L or even ng/L range, two major concerns are associated with low-level antibiotic exposure. First, ecosystems and human health may be adversely affected by chronic exposure; second, such exposure can result in the development of antibiotic-resistant bacteria (Boreen *et al*., 2004; Qiang *et al*., 2004; Andreozzi *et al*., 2006).

Antibiotics are usually divided into four sub-classes, including β-lactam, macrolide, quinolone, and tetracycline, as well as several other types of antibiotics that do not belong to these subclasses (Ikehata *et al*., 2006). Azithromycin, clarithromycin, erythromycin and roxithromycin belong to macrolide antibiotics, which are characterized

by their ability to inhibit bacterial protein synthesis (Beers & Berkow 1999) and the possession of tertiary-amine groups, as shown in Figure 2.1. For this research, erythromycin was chosen as the target compound because of its high detection frequency in the environment, which will be described in details in Section 2.2.3.

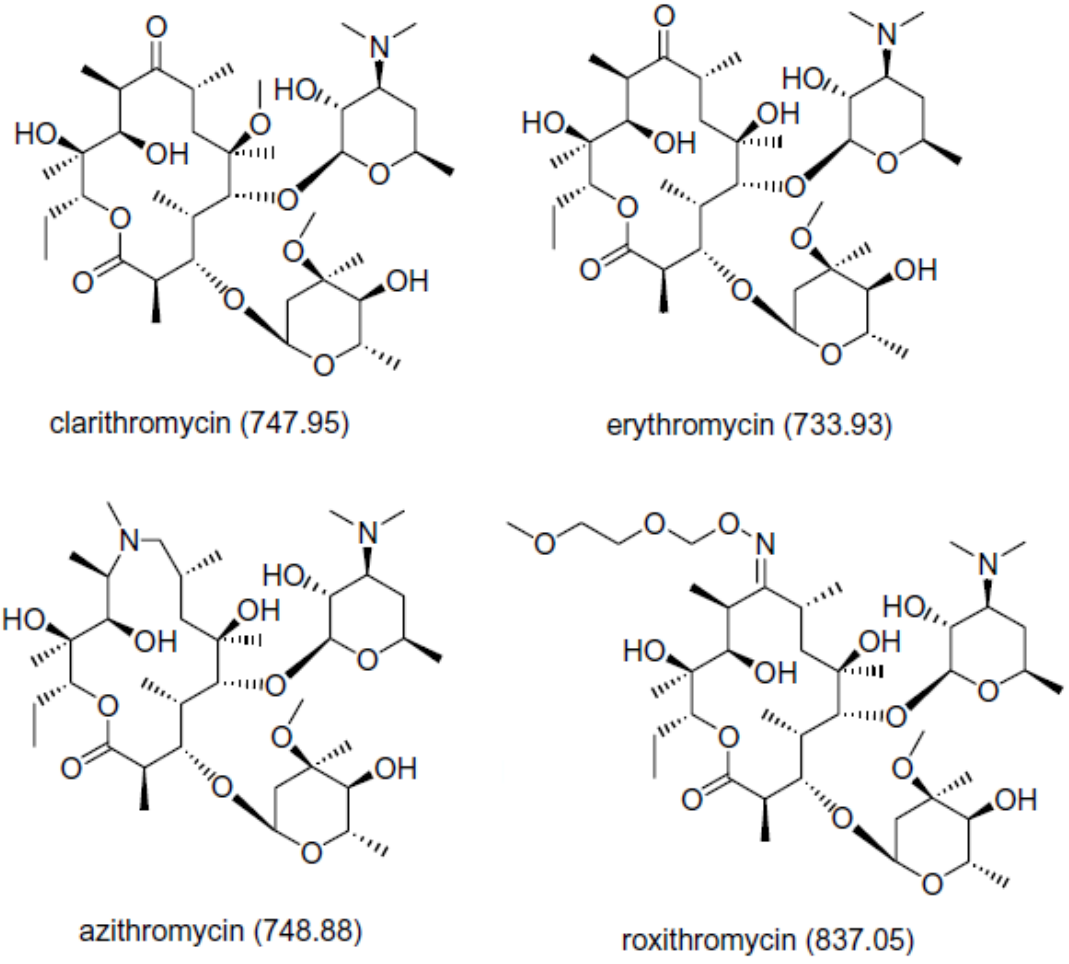

Figure 2.1: Molecular structure of common macrolide antibiotics (Ikehata *et al*., 2006)

2.2.3 Erythromycin

Erythromycin is a therapeutically useful, wide-range macrolide antibiotic produced by the actinomycete species, Streptomyces erythreous (McGuire *et al*., 1952); it is used in humans as well as in food-producing animals to control bacterial diseases and promote animal growth (Pothuluri *et al*., 1998). It is interesting to note that consumption of macrolide antibiotics varies dramatically in different countries. For example, the use of

erythromycin was approximately 1200 mg per person per year in the UK while it was only 24 mg per person per year in Switzerland (McArdell *et al*., 2003). The chemical structure of erythromycin is shown in Figure 2.1. Erythromycin contains a 14-membered lactone ring with ten asymmetric centers and two sugars ($_{L}$-cladinose and $_{D}$-desosamine). As the result of the tertiary amine of desosamine, erythromycin has a pK_a of 8.8 (Kanfer *et al*., 1998).

The presence of erythromycin in the environment has been reported by many researchers. Kolpin *et al*. (2002) analyzed 85 organic wastewater contaminants in 139 US stream sites that were considered susceptible to contamination. Among 21 detected veterinary and human antibiotics, dehydro-erythromycin (erythromycin-H_2O) was most frequently detected with a detection frequency of 21.5%; the maximum and median concentrations of dehydro-erythromycin were 1.7 μg/L and 0.1 μg/L, respectively. Lin *et al*. (2008) investigated eleven antibiotics in three rivers in Taiwan and found consistent presence of erythromycin-H_2O and a maximum concentration of 76 μg/L. Høverstad *et al*. (1986) determined several antibiotics in human feces during 6 days of regular application and found erythromycin concentrations ranging from 200 to 300 mg/kg.

In September 2009, EPA published a final list of contaminants which might require regulation under the Safe Drinking Water Act (SDWA). In this final Contaminant Candidate List 3[1] (CCL3), erythromycin is the only antibiotic that was added from the draft to the final CCL3 along with nine hormones under the category of pharmaceuticals, based on new health effects and occurrence data in ambient water. The high frequency of detection, the environmental persistence and the regulatory status are the main reasons that erythromycin was chosen as the target compound in this research.

As confirmed by experiments using the published analytical procedure, erythromycin was not detected in its original form but as a self-degradation product with

1 http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm#chemical

an apparent loss of one molecule of water: erythromycin-H_2O (Hirsch *et al*., 1999). Kim *et al*. (2004) conducted a kinetic study on the self-degradation of erythromycin A, the major component of erythromycin base, in aqueous solution. The degradation of erythromycin A can be described by a first-order reaction with respect to the concentration of erythromycin A:

$$\frac{\partial[EA]}{\partial t} = -k_{obs}[EA] \qquad \text{(Equation 2.1)}$$

where [EA] is the concentration of erythromycin A; k_{obs}, (min^{-1}) is the observed degradation rate constant.

They proposed an empirical model (

Figure 2.2) to interpret the observed rate constant k_{obs} as a function of pH at 25°C:

$$k_{obs} = 17.2 \times 10^{(-0.870 \times pH)} + (2.70 \times 10^{-8}) \times 10^{(0.387 \times pH)} \qquad \text{(Equation 2.2)}$$

According to the model, the slowest degradation of erythromycin occurred around pH 7.3; increasing or decreasing the pH from 7.3 resulted in more rapid self-degradation of erythromycin. In this research, the pH of the samples was lowered to approximately 2.5 prior to analysis as a consequence of adding indigo reagent for ozone measurement. At pH 3, the observed self-degradation rate constant, k_{obs}, was 0.042 min^{-1}; this rate constant would result in a half-life of approximately 16 min. In other words, over 90 percent of the erythromycin initially present in a sample would degrade into erythromycin-H_2O within one hour. Therefore, erythromycin-H_2O was measured in this research rather than erythromycin.

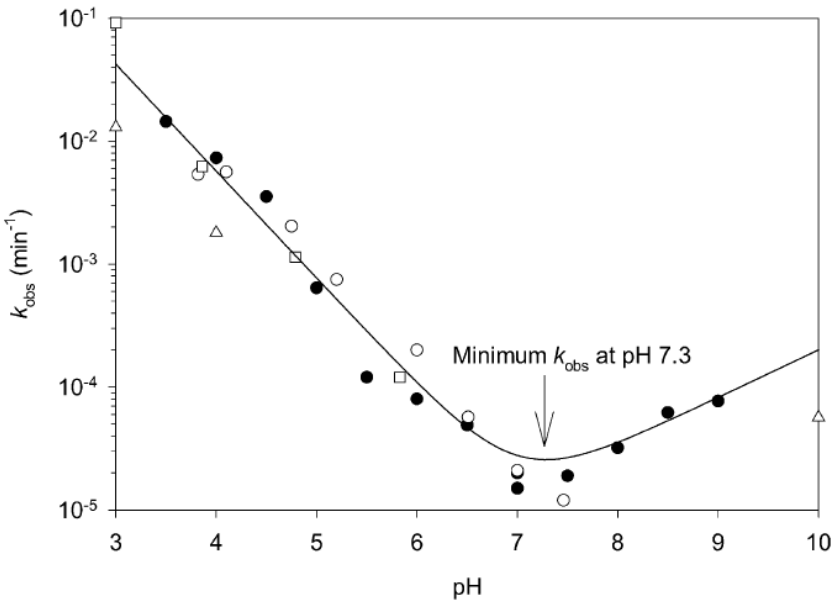

Figure 2.2: Prediction of erythromycin A degradation in terms of pH of aqueous solution (Kim *et al*., 2004). Measured data points were obtained from Kim *et al*. (2004, ●), Atkins *et al*. (1986, ○), Cachet *et al*. (1989, △), and Volmer & Hui (1998, □).

2.3 TREATMENT OF PHARMACEUTICALS

2.3.1 Drinking water treatment

The presence of pharmaceuticals in drinking water has rarely been reported, in part due to the limited number of studies and lack of regulatory reporting requirements. However, Daughton (2010) presented a comprehensive review of the literature reporting pharmaceuticals and related products in source waters and finished drinking water. Several pharmaceuticals, including erythromycin (the subject of this study) have been reported in the finished drinking water of more than one plant. The concentrations in finished drinking water, however, have rarely been above 1 ng/L.

Conventional treatment including coagulation, flocculation, sedimentation and filtration are reported to achieve minimal removal (Westerhoff *et al*., 2005). Boyd *et al*. (2003) showed that oxidation (e.g., chlorination and ozonation) and sorption (dual media) processes might be effective at removing naproxen. Ternes *et al*. (2002) investigated the elimination of selected pharmaceuticals during drinking water treatment at laboratory and

pilot scale as well as in actual treatment plants. The results showed that slow sand filtration and flocculation by iron (III) chloride were inefficient at removing pharmaceuticals while activated carbon filtration and ozonation were very effective removal processes. Huerta-Fontela *et al*. (2010) reached a similar conclusion. They studied the removal of 55 pharmaceuticals, hormones and metabolites through a drinking water treatment plant and they reported that coagulation, flocculation and sand filtration were ineffective while chlorination and ozonation processes accounted for higher efficiencies. However, even if processes such as ozonation, chlorination or photodegradation are efficient at removing pharmaceuticals, degradation by-products may be potentially toxic (Mompelat *et al*., 2009).

2.3.2 Wastewater treatment

The main mechanisms of pharmaceutical removal in wastewater treatment process are transformation in secondary (biological) treatment and sorption. Conventional activated sludge treatment was reported to degrade pharmaceuticals to varying extents that ranged from very poor to complete degradation (Gobel *et al*., 2007). Nakada *et al*. (2007) showed that the combination of conventional activated sludge treatment, sand filtration and ozonation was effective at removing pharmaceuticals in a sewage treatment plant, although the individual removal efficiency of the first two steps were moderate or poor.

However, even when complete removal of pharmaceuticals from an activated sludge process has been observed, partial or complete degradation may not have occurred; sorption from wastewater onto activated sludge is possible without transformation or complete mineralization (Kasprzyk-Hordern *et al*., 2009). It is also worth mentioning that antibiotics have the potential to inhibit the growth of microorganisms which is another important area of research that needs to be addressed in the future

2.4 OZONATION AND ADVANCED OXIDATION PROCESS

Various studies have shown that ozonation is a promising technology for the oxidation of pharmaceuticals in drinking water (Huber *et al*., 2003; Ternes *et al*., 2002) and wastewater *(*Ternes *et al*., 2003; Huber *et al*., 2005). Kim and Tanaka (2010) investigated the removal of 40 pharmaceuticals by ozone-based processes in wastewater and found that an ozone dose of more than 6 mg/L plus a contact time of more than 10 minutes yielded removals of greater than 90% for all pharmaceuticals investigated. For drinking water treatment, ozone has traditionally been employed for odor and taste control and disinfection. Jasim *et al*. (2006) evaluated the occurrence of pharmaceuticals and endocrine disruption chemicals in the Detroit River water, which is the source of drinking water for approximately 4.5 million residents. They concluded that ozonation showed great promise and should be considered as the focus of treatment studies for the removal of pharmaceuticals in the Great Lakes Region since other treatment processes showed minimal removal of pharmaceuticals.

2.4.1 Ozone properties

Ozone is a light blue gas with pungent odor which can be detected at concentrations as low as 0.01 ppm. It is 1.5 times as dense as oxygen and 12.5 times as soluble in water. Due to its unique structure, ozone can behave as a dipole, an electrophilic or nucleophilic agent (Kasprzyk-Hordern *et al*., 2003).

Ozone is currently used as a strong oxidant/disinfectant in water treatment throughout Europe and in numerous drinking water treatment plants in the United States. The application of ozone has been used for disinfection, taste and odor control, oxidation of iron, manganese and sulfides, and micropollutant removal (Crittenden *et al*., 2005).

2.4.2 Ozone decomposition

One of the reasons that ozone has not been as popular as other disinfectants in the United States is due to its rapid decay. The decay of ozone in aqueous solution has been studied

for decades. According to kinetic studies, the decomposition of ozone is usually described by a two stage process. The first stage is characterized by a rapid depletion of ozone during the initial phase ($t < \sim 20s$) of ozone introduction, which is considered to be caused by constituents that are readily oxidized by ozone; this stage is also defined as instantaneous ozone demand (Buffle *et al*. 2006a).The second stage of ozone decay is described by a first-order radical chain process (see

Figure 2.3); once hydroxyl radicals are produced, they will react with ozone to generate more hydroxyl radicals in a chain reaction process. According to a model, proposed by Hoigne, Staehelin and Bader (HSP), the radical chain reaction process can be initiated, promoted and inhibited by different compounds in natural water. The initiator of the free-chain reaction can be any compound that can induce the formation of superoxide (O_2^-), for example, hydroxide ion (OH^-), H_2O_2, UV at 253.7nm (Langlais *et al*.,1991). Promoters and inhibitors are considered hydroxyl radical scavengers. The difference between the two groups of compounds is that promoters can produce O_2^- superoxide anion or other species that is capable of accelerating the chain reaction; on the other hand, inhibitors consume hydroxyl radicals without generating the superoxide anion O_2^-. Common promoters include humic acid, formic acid, primary alcohols; common inhibitors are bicarbonate and carbonate ions, alkyl groups, tertiary alcohols. Regarding phosphate, there are different opinions about whether it is regarded as an inhibitor or just as an inert compound (Mizuno *et al*., 2007; Morozov & Ershov 2010). Natural organic matter can serve as an inhibitor or promoter depending on the specific functional groups.

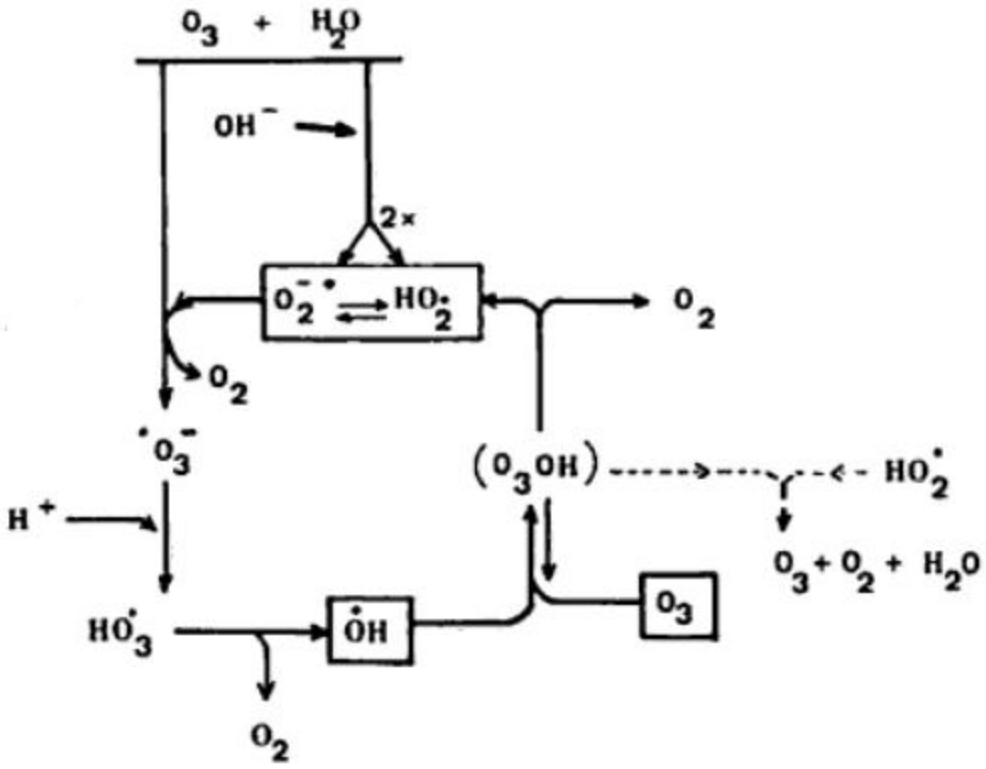

Figure 2.3: Global cycle of ozone decomposition in water (adopted from Masschelein, 1992)

2.4.3 Advanced oxidation process

The oxidation of pharmaceuticals can be caused by O_3 directly or indirectly by hydroxyl radicals ($^{\circ}OH$), a product of ozone decomposition (Hoigne & Bader 1976). Sufficient hydroxyl radicals would be produced only when ozone doses exceed the instantaneous ozone demand (Wert *et al*., 2009). Compared with hydroxyl radicals, ozone is a relatively selective oxidant which only attacks certain functional groups of the target compound; on the other hand, the hydroxyl radical is a nonselective oxidant and can react with a large number of functional moieties.

Since the hydroxyl radical is a much less selective oxidant compared with ozone, an advanced oxidation process (AOP) is defined as an oxidation process that generates sufficient hydroxyl radicals to affect water treatment (Kasprzyk-Hordern *et al*., 2003).

AOPs utilize a combination of chemical agents (e.g., ozone, hydrogen peroxide), irradiation (e.g., UV, ultrasound) and catalysts (e.g., transition metals, metal oxides) to generate as much hydroxyl radicals as possible. Examples of AOPs include O_3/H_2O_2, O_3/UV, O_3/H_2O_2/UV, Fe^{2+}/H_2O_2. The diversity of technologies involved and the potential areas of application have resulted in immense research and development regarding AOPs (Klavarioti *et al*., 2009).

In addition to the production of higher concentrations of hydroxyl radicals during advanced oxidation, another advantage of an O_3/H_2O_2 AOP over the ozone process is that the former one would result in less residual ozone concentration and therefore reduce the formation of bromate (Kim & Tanaka 2010).

The cost regarding treatment of pharmaceuticals by AOPs in aqueous media is likely to be relatively high because high conversion is required since even at minute concentrations these compounds retain their adverse effect; additionally, initial concentration are usually very low resulting excessive treatment cost per unit mass (Klavarioti *et al*., 2009).

2.4.4 Kinetics of oxidation with ozone

Reactions between ozone and pharmaceuticals, P, are typically described by an overall second-order reaction, i.e., first-order with respect to each oxidant (ozone and hydroxyl radicals) and first order with respect to the contaminant as shown by Equation 2.3 and Equation 2.4 (Von Gunten 2003):

$$-r_P = k_{O_3}[P][O_3] + k_{^\circ OH}[P][^\circ OH] \qquad \text{(Equation 2.3)}$$

In a batch system, $r_P = \frac{d[P]}{dt}$, so upon integration, we find:

$$\ln\frac{[P]}{[P]_0} = -(k_{O_3}\int[O_3]dt + k_{^\circ OH}\int[^\circ OH]dt) \qquad \text{(Equation 2.4)}$$

with $[O_3] = f(t)$ and $[^{\circ}OH] = f(t)$. [P], [O_3] and [°OH] are concentrations of pharmaceutical, ozone and hydroxyl radicals respectively.

k_{O_3}, $k_{^{\circ}OH}$ are physical-chemical constants and oxidant exposures ($\int [O_3]dt$ and $\int [^{\circ}OH]dt$) are dependent on a number of operating and environmental conditions such as pH, alkalinity, initial ozone dose and scavenging capacity of water matrix (Buffle *et al*., 2006a). Usually, 10 mM of tert-butanol (t-butanol) was added in order to determine the k_{O_3} value (Marron, 2010). The calculation of these parameters is shown in Chapter 4.

For different compounds, hydroxyl radical rate constants i.e., $k_{^{\circ}OH}$ generally only differ by one order of magnitude due to the low selectivity of $^{\circ}$OH radicals. The values are typically on the order of 10^9 $M^{-1} \cdot s^{-1}$ (Broseus *et al*., 2009). In contrast, ozone rate constants k_{O_3} differ substantially depending on the compound.

The relative importance of $^{\circ}$OH oxidation versus O_3 oxidation can be quantified using the molar ratio of hydroxyl exposure to ozone exposure, R_{ct} (Elovitz & von Gunten 1999):

$$R_{ct} = \int [^{\circ}OH]dt / \int [O_3]dt \qquad \text{(Equation 2.5)}$$

Employing the R_{ct} concept, Equation 2.4 can be rewritten as Equation 2.6:

$$\ln \frac{[P]}{[P]_0} = -(k_{O_3} + R_{ct} k_{^{\circ}OH}) \int [O_3]dt \qquad \text{(Equation 2.6)}$$

R_{ct} was observed to remain constant during ozonation processes in natural water (Elovitz & von Gunten 1999), which allows for simplification of the mathematical solution. However, in wastewater ozonation, R_{ct} was shown to vary; as a result the R_{ct} concept is not recommended for modeling compound degradation in wastewaters (Buffle *et al*., 2006b). Another limitation of the R_{ct} concept occurs in systems in which 99% removal is achieved prior to measuring ozone exposure (Wert *et al*., 2009).

2.4.5 Impact of natural water characters on ozonation process

Natural water characteristics affect the performance of ozonation process mainly through their impact on ozone decomposition, i.e., indirect reaction of ozone. The parameters considered here are temperature, pH, carbonate and phosphate buffers, natural organic matter and initial ozone dose.

2.4.5.1 Temperature

Temperature is negatively correlated with ozone solubility while it is positively correlated with ozone decomposition rate. Higher temperatures reduce the solubility of ozone, and therefore less ozone is available for direct reaction. On the other hand, the decomposition of ozone based on a second-order reaction model was calculated to be 2.2 times faster for every 5°C increase in temperature within the range of 15 ~ 30°C (Mizuno *et al*., 2007), suggesting that more hydroxyl radicals can be generated at higher temperature. Furthermore, the reaction kinetics between pharmaceuticals and ozone also increase with increasing temperature.

2.4.5.2 pH

pH has a more complex impact on the removal of pharmaceuticals by ozone processes. First of all, ozone decomposition is directly affected by pH through the initiation reaction between ozone and hydroxide anions (OH^-). Therefore, a higher pH leads to faster ozone decomposition and less ozone stability, while lowering the pH stabilizes aqueous ozone. Elovitz *et al*. (2000) showed that the second-order ozone decomposition rate increased by a factor of 3.55 per unit pH increase. On the other hand, pH also affects the speciation of deprotonated and protonated moieties. As shown by Dodd *et al*. (2006), the apparent second-order rate constant of macrolides was strongly dependent on pH because ozone was more reactive with deprotonated tertiary amines. Moreover, pH also affects the properties of other common natural water parameters, such as speciation of the carbonate buffer and natural organic matter, which indirectly affects

ozone decomposition. Models have been developed to address the impacts of these parameters on ozone decomposition and these models should be modified to address the impacts on degradation of pharmaceuticals by ozonation.

2.4.5.3 Carbonate buffer

As mentioned above, chemical speciation of the carbonate buffer is determined by pH. However, for the pH range prevalent in most natural waters, the dominant species is bicarbonate. Bicarbonate and carbonate are known to be ozone decomposition inhibitors by scavenging hydroxyl radicals without generating superoxide (O_2^-) or other species that can accelerate the decomposition of ozone (Staehelin & Hoigne 1985). The reactions between hydroxyl radicals and HCO_3^-/CO_3^{2-}[2] are shown by Equation 2.7 and Equation 2.8 (Buxton *et al*., 1988).

$$HCO_3^- + {}^\circ OH \rightarrow HCO_3^{\circ -} + OH^- \quad k = 8.5\times10^6 M^{-1}\cdot s^{-1} \qquad \text{(Equation 2.7)}$$

$$CO_3^{2-} + {}^\circ OH \rightarrow CO_3^{\circ -} + OH^- \quad k = 3.9\times10^8 M^{-1}\cdot s^{-1} \qquad \text{(Equation 2.8)}$$

As either the HCO_3^- or CO_3^{2-} concentration increases, the ozone decomposition rate decreases resulting in higher ozone exposure and lower hydroxyl radical exposure. It has also been observed that there is an apparent leveling off effect of ozone stabilization with increasing HCO_3^-/CO_3^{2-} concentration (Elovitz *et al*., 2000). However, the impact of carbonate buffer on the removal of a specific pharmaceutical by ozonation depends on whether ozone itself or hydroxyl radicals govern the oxidation. If the oxidation is primary governed by indirect hydroxyl radical oxidation, then increasing the HCO_3^-/CO_3^{2-} concentration would lead to a higher oxidation efficiency; on the other hand, if direct ozone oxidation is the primary mechanism, then increasing the HCO_3^-/CO_3^{2-} concentration would have less of a positive effect or, perhaps, even negative effect on the removal efficiency.

2 HCO_3^-/CO_3^{2-} is not the ratio of HCO_3^- to CO_3^{2-}; it refers to both HCO_3^- and CO_3^{2-}.

2.4.5.4 Phosphate buffer

Phosphate is used extensively as a buffer in studies of ozone self-decomposition in "pure" water in which it is assumed that it does not react with ozone (Morozov & Ershov 2010). However, there are some reports suggesting that phosphate species can affect ozone decomposition. Sotelo *et al*. (1987) showed that the rate constant for ozone decomposition decreased by a factor of 30 when the concentration of phosphate increased from 0.1 to 1.0 M. Morozov & Ershov (2010) also found that, at all pH values, the rate constant of ozone decomposition decreased as phosphate concentration increased. On the other hand, according to Mizuno *et al*. (2007), phosphate buffer did not influence the second-order rate constant for ozone decomposition. As a whole, the argument that phosphate does affect ozone decomposition seems to be more persuasive.

2.4.5.5 Natural organic matter

Natural organic matter (NOM) is ubiquitous in the environment and contains a heterogeneous mixture of organic compounds that makes the quantification and identification of its structural characteristics very difficult. It is measured most commonly by using total organic carbon (TOC) as a surrogate.

NOM can directly react with ozone (Equation 2.9 and Equation 2.10) or affect ozone stability by scavenging $^{\circ}OH$ radicals (Equation 2.11 and Equation 2.12), either as a promoter with the formation of O_2^- or as an inhibitor. The effect of NOM on ozone stability not only depends on the concentration of NOM, but also its origin and composition (Buffle *et al*., 2006a). Elovitz *et al*. (2000) found a three-fold difference of ozone decomposition rate in six waters with similar DOC concentration and alkalinity. Pi *et al*. (2005) found that aromatic compounds present at micromolar concentrations, could accelerate ozone decomposition considerably. Reactions of NOM with ozone have been described by the following equations (Westerhoff *et al*., 1999):

$$O_3 + NOM \rightarrow products \quad \text{(Equation 2.9)}$$

$$O_3 + NOM \rightarrow (O_2^- + H_2O_2) \rightarrow {}^\circ OH + products \quad \text{(Equation 2.10)}$$

$${}^\circ OH + NOM \rightarrow O_2^- + products \quad \text{(Equation 2.11)}$$

$${}^\circ OH + NOM \rightarrow products \quad \text{(Equation 2.12)}$$

For some pharmaceuticals, like carbamazepine and diclofenac, natural organic matter could absorb contaminants and therefore decrease their ability to be oxidized from advanced treatment processes (Jasim *et al*., 2006).

2.4.5.6 Ozone dose

Ozone dose is a key operating parameter when ozonation processes are applied. Buffle *et al*. (2006a) found that the rate of ozone decomposition decreased as ozone dose was increased. Various researchers have studied the impact of ozone dose on the removal performance of particular compounds. Ternes *et al*. (2003) applied a 5 mg/L ozone dose to sewage treatment plant effluent and five antibiotics that were studied including erythromycin (0.62±0.24 μg/L), clarithromycin (0.21±0.02 μg/L) and roxithromycin (0.54±0.04 μg/L), were no longer detected. However, it is difficult to determine the optimal dosage of ozone to achieve a given level of degradation in the complex background water matrices typically encountered and the range of pharmaceuticals that may be present in a system. And they concluded that long ozonation time coupled with large ozone dose did not have a significant impact on the level of degradation.

2.5 OZONATION OF MACROLIDE ANTIBIOTICS

Common macrolide antibiotics include azithromycin, clarithromycin, erythromycin and roxithromycin; lincomycin is a lincosamide antibiotic, which has a similar antibacterial spectrum with macrolides Ikehata *et al*. (2006). The primary ozone attack site of macrolides is the tertiary nitrogen (Huber *et al*., 2005). It is reported that

ozonation of azithromycin, erythromycin and clarithromycin could yield removal rates of 90% to 99%with an ozone dose of more than 2 mg/L (Huber *et al*., 2005).

Since the chemical nature of the reactive moieties is very similar for the macrolide antibiotics, the rate constants for the degradation of these moieties by O_3 are expected to be very similar. Indeed, roxithromycin has been reported to have an apparent rate constant of 6.3×10^4 $M^{-1}\cdot s^{-1}$ at pH 7 (Dodd *et al*., 2006); clarithromycin is reported to have a very similar rate constant of 7×10^4 $M^{-1}\cdot s^{-1}$ at pH 7 (Lange *et al*., 2006). The rate constant for reaction of azithromycin with ozone is expected to be higher because ozone can attack both the exocyclic tertiary amine and the heterocyclic tertiary amine. To date, there are no kinetic studies for erythromycin degradation by ozone reported in the literature but it is expected that the rate constant should be similar to roxithromycin and clarithromycin. One of the objectives of this research is to determine the rate constant of the reaction between erythromycin and ozone because it is the only macrolide that is on the contaminant candidate list.

Detailed kinetic studies and intermediate product identification were conducted on tertiary amines by Muñoz *et al*. (2001), Muñoz & von Sonntag (2000) indicating that the formation of N-oxide dominated (90%) the oxidation process. Lange *et al*. (2006) studied the degradation of clarithromycin by ozonation and they concluded that the oxidation product data for clarithromycin would be similar for all the other macrolide antibiotics. They identified the N-oxide as the major product for clarithromycin oxidation by ozone and suggested that this would also be the primary product for other macrolides. They also proposed that the formation of N-oxide inactivated the ribosomal antibiotic activity of clarithromycin

Kim & Tanaka (2010) found that for erythromycin and clarithromycin, removal efficiency was only slightly increased when O_3/H_2O_2 was applied instead of ozone only. This result indicates that for macrolides which already reacts very quickly with ozone itself, ozone is the main contributor to the degradation during O_3/H_2O_2 process.

2.6 SUMMARY

Ozonation of pharmaceuticals has been studied by many researchers and most of the results demonstrate that ozonation and/or advanced oxidation processes are very effective at removing pharmaceuticals in aqueous phase. The effects of natural water characteristics on ozonation of pharmaceuticals have also been investigated.

Erythromycin, a very common antibiotic, is has been identified in source waters with high detection frequency and appears to be relatively persistent in the environment. The ozonation of erythromycin has not been investigated yet; although, ozonation kinetics and intermediate product identification has been reported for similar compounds. In this research, batch experiments were carried out to quantify the kinetics of ozonation of erythromycin and to evaluate the effects of natural water characteristics on ozonation of erythromycin.

Chapter 3 Materials and Methods

3.1 INTRODUCTION

This chapter describes the experimental methods and analytical techniques employed to achieve the goal of this research. The research design is presented first to provide an overview of the range of experimental conditions conducted. Then, experimental procedures for each set of experiment are described in detail. Finally, analytical methods for all the parameters involved are presented.

3.2 RESEARCH DESIGN

The high detection frequency and the anticipated regulation requirement led to the study of erythromycin removal by ozonation. This research was designed to determine the rate constant between erythromycin and ozone in a range of background waters to delineate the effects of pH, carbonate and phosphate buffers, and initial ozone dose on the removal rate of erythromycin by ozonation.

Many existing drinking water treatment plants use ozonation both as a pretreatment process for primary disinfection, reduction of color and odor compounds and as an indispensable disinfection process after filtration. These plants could simply improve trace organic contaminant removal performance by modifying operational conditions. pH and carbonate buffer were selected as the focus parameters because of their importance and ubiquity in drinking water treatment processes. Additionally, the effect of initial ozone dose was investigated since it is a direct operational parameter. The effect of phosphate buffer on the rate constant was also evaluated since it behaves as a hydroxyl radical scavenger just like carbonate, it is regarded as “inert” and is always neglected in studies of kinetics of ozone decomposition in “pure” water. Batch experiments were conducted to understand the effect of these parameters on the reaction kinetics of erythromycin degradation by ozonation. The matrix of the experiments is shown in Table 3.1.

Table 3.1: Matrix of experiments

Exp. Set No.	Exp. No.	pH	t-BuOH (mM)	pCBA (μM)	Total Carbonate (mM)	Buffer Conc. (mM)	Ozone dose (mg/L)	Objective
1	1	5.35	10					Determine the rate constant of erythromycin degradation by ozone only
	2	5.66	10					
	3	6.00	10					
	4	6.40	10					
	5	6.81	10					
2	6	6.00	10		4			Confirm that Erythromycin degradation by ozone only is not affected by the presence of carbonate
3	7	6.00		1	0	5	0.58	Understand the effect of carbonate buffer on ozone exposure and hydroxyl radical exposure
	8	6.00		1	0.5	5	0.68	
	9	6.00		1	1	5	0.62	
4	10	6.00		1	0	1	0.16	Understand the effect of carbonate buffer on erythromycin degradation by ozonation
	11	6.00		1	1	1	0.16	
	12	6.00		1	1	1	0.18	
	13	6.00		1	2	1	0.16	
	14	6.00		1	4	1	0.15	
5	15	6.00		1	0	1	0.12	Understand the effect of buffer effect on erythromycin degradation by ozonation
	16	6.00		0.5	0	5	0.11	
	17	6.00		0.5	0	10	0.10	
6	18	6.00		0.5	0	5	0.27	Understand the effect of initial ozone dose on erythromycin degradation by ozonation
	19	6.00		1	0	5	0.17	
	20	6.00		1	0	5	0.11	

3.3 EXPERIMENTAL PROCEDURES

3.3.1 Ozone reactor setup

The setup of the ozone reactor was described in detail by Marron (2010). Briefly, a concentrated aqueous ozone stock solution (typically between 50 mg/L and 65 mg/L after running ozone generator several hours) was prepared by bubbling ozone-containing O_2 gas through a gas-washing bottle containing deionized water, which was cooled in ice to increase the solubility of ozone. A diagram of the ozone reactor is shown in Figure 3.1.

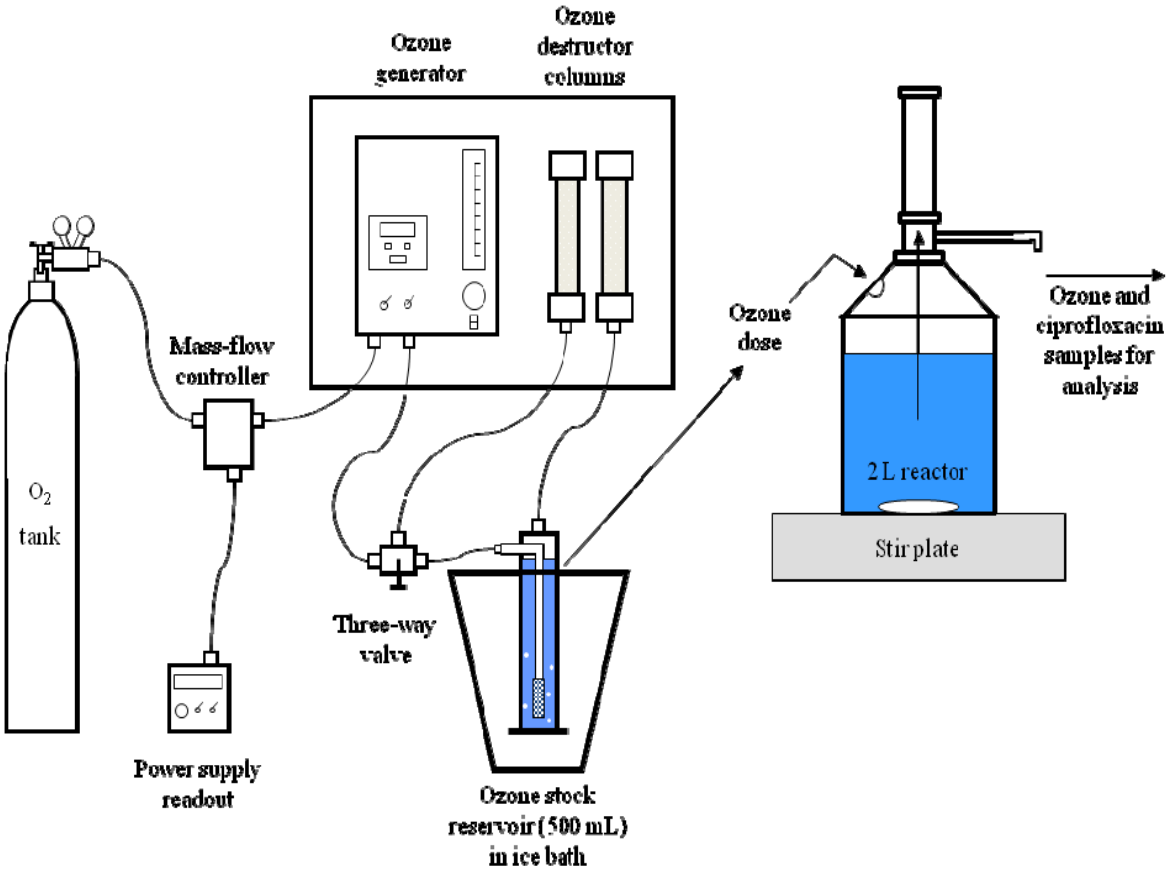

Figure 3.1: Experiment setup (adopted from Marron, 2010)

3.3.2 Experiment setup

All the experiments were conducted in a 2 L, well-mixed amber glass reactor. A small hole was drilled near the top of the reactor for injecting aqueous ozone stock

solution. A bottle top dispenser (VWR LabMax) was utilized for taking samples during experiment; all wetted parts of the dispenser were made of Teflon. The range of the dispenser was from 0 mL to 25 mL; sample volume was typically 8 mL or 9 mL. During experiments, two additional extractions of samples were wasted before each sample was actually collected for analysis. Samples were also taken before the experiment to determine the initial erythromycin concentration. A picture of the experimental setup is shown in Figure 3.1.

Reactions were initiated by injecting a certain amount of the aqueous ozone stock solution at time zero into the well-mixed reactor containing the reaction matrix of interest specified in Table 3.1. After each sample was taken with the dispenser, it was quenched immediately using an indigo solution and analyzed for O_3 and other compounds as needed.

3.3.3 Pre-experiment preparation

All the glassware involved in the experiments was prepared as organic-free according to the following procedure: wash with Liqui-Nox soap, rinse six times with deionized water, soak at least for 30 minutes in a 10% nitric acid solution, rinse six times with deionized water and bake at 550°C for one hour (for amber glassware, bake at 350°C overnight).

3.3.4 pH experiment (Experiment Set No.1 and 2)

The first set of experiments was designed to evaluate the effect of pH on the rate constant for degradation of erythromycin by ozone only. Experiments were conducted at five different pH values: 5.35, 5.66. 6.00, 6.41 and 6.81. pH was adjusted using concentrated HCl and NaOH; all other conditions were maintained at the same level as shown in Table 3.1. 10 mM t-butanol was added to isolate the reaction between ozone and erythromycin; the total carbonate concentration was negligible. The initial

erythromycin concentration was 1 mg/L, and the phosphate buffer concentration was 1 mM.

A second set of experiments was conducted at pH 6.00 with 4 mM total carbonate to confirm that the carbonate buffer did not affect the rates of degradation of erythromycin by ozone only.

3.3.5 Carbonate experiment (Experiment Set No.3 and 4)

Total carbonate concentration was the only variable for these two sets of experiments. The first set of experiments was conducted without erythromycin and was aimed to study the effect of carbonate concentration on ozone decomposition at pH 6.0. The total carbonate concentrations tested were 0 mM, 0.5 mM and 1 mM; 1 μM *p*CBA was added to track hydroxyl radical exposure.

A second set of experiments were conducted with 1mg/L erythromycin at pH 6.0 and 0 mM, 1 mM, 2 mM and 4 mM total carbonate concentration to study the effect of the carbonate buffer on ozonation of erythromycin as well as to obtain the rate constant between erythromycin and hydroxyl radicals. At pH values less than 6.0, the bicarbonate concentration was 32% of total carbonate concentration and the concentration of CO_3^{2-} was negligible. *p*CBA was added to track hydroxyl radical exposure.

Carbonate-free water was prepared by bubbling deionized water with nitrogen gas to purge CO_2 for more than 30 minutes. Then a certain amount of 0.5 M Na_2CO_3 stock solution was added to achieve the required total carbonate concentration; pH was then adjusted by adding concentrated NaOH or HCl under a gentle stream of nitrogen to reach a final value of 6.0 (Elovitz *et al*., 2000).

3.3.6 Buffer experiment (Experiment Set No.5)

Phosphate buffer experiments were conducted at pH 6 with 0 mM carbonate to study the effect of phosphate buffer on ozonation of erythromycin. The concentrations of phosphate buffer were 1 mM, 5 mM and 10 mM. Note that even the initial *p*CBA

concentration was not identical for the three experiments, it will be shown that *p*CBA concentrations did not change during the experiment, meaning that different initial *p*CBA concentrations did not present any problem for comparison of the results from these experiments

3.3.7 Initial ozone dose experiment (Experiment Set No.6)

For this set of experiments, initial ozone dose was the only variable. Experiments were conducted at pH 6 with 0 mM carbonate buffer and 5 mM phosphate buffer. Initial ozone doses were 0.11, 0.17 and 0.27 mg/L.

3.4 Analytical Methods

3.4.1 Chemicals and stock solutions

Erythromycin was purchased from Teknova. t-butanol and para-chlorobenzoic acid (*p*CBA) came from Fisher Scientific and Acros Organics, respectively. Stock solutions of erythromycin and *p*CBA were prepared at 1.36 mM (1 g/L) and 200 μM (31.2 mg/L), respectively. Both solutions were stored in the dark at 4°C. Stock solutions of phosphate buffer and Na_2CO_3 were both prepared at 0.5 M.

3.4.2 Ozone

The measurement of ozone is described in detail by Marron (2010). Briefly, the concentration of ozone stock solution was measured by direct ozone absorbance at 258 nm based on the known molar absorptivity of ozone while the samples were measured by the Indigo colometric method (4500-O_3 Residual; AWWA, 2005).

For measuring stock solutions, 500 μL of stock solution were added to a 1 cm quartz cuvette containing 3.5 mL of 5 mM phosphate buffer. The solution was covered with a Teflon cap immediately. The sample was analyzed using a spectrophotometer (Agilent 8453) within 30 seconds to minimize ozone decomposition. Ozone concentration is calculated as

$$\text{mol } O_3 / L = \frac{A_{258nm}}{\varepsilon L} \times \frac{\text{total volume}}{\text{sample volume}} \quad \text{(Equation 3.1)}$$

where A_{258nm}, ε and L are the absorbance of sample at 258 nm, molar absorptivity (3000 $M^{-1} \cdot cm^{-1}$) and path length of the cuvette (1 cm). All experiments were doused with a specific concentration of aqueous ozone stock solution based on the measured concentration. Specific glass pipettes were used to dose ozone.

For measuring ozone in reaction solutions, the samples were quenched with 1 mL or 2 mL of indigo reagent II. Sample absorbance was analyzed in a 4 cm glass cuvette at 600 nm. A blank sample with respect to ozone was taken right before the experiment. The concentration is calculated per Equation 3.2:

$$\text{mg } O_3 / L = \frac{\Delta A_{600nm}}{0.42L} \times \frac{\text{total volume}}{\text{sample volume}} \quad \text{(Equation 3.2)}$$

where ΔA_{600nm} is the difference in absorbance at 600 nm between sample and blank; L is the path length of cuvette (4 cm).

As a result of rapid sampling (about 10 s per sample), the mark of the dispenser was easily displaced during the experiment; therefore, mass difference before and after sampling was used to get the exact sample volume. The concentration calculation based on mass difference is shown as Equation 3.3:

$$\text{mg } O_3 / L = \frac{(A_B \times 100) - (A_S \times V_T)}{f \times V_S \times b} \quad \text{(Equation 3.3)}$$

where A_B and A_S are the absorbance of blank and sample, respectively; V_S and V_T are the volume of sample and total volume of sample plus indigo, measured by weight difference before and after sampling. b is the path length of the cell and 0.42 is a reasonable for f.

3.4.3 LC/MS

Liquid Chromatography/Mass Spectrometry (LC/MS) was employed to detect erythromycin. The method was developed based on Calamari *et al*. (2003) and then modified in this research. The separation column used is Premier C18 (150 mm ×

4.6 mm, 5 μm) with a binary gradient eluent system (solvent A: LC/MS grade methanol; solvent B: 0.1% formic acid in water) and a flow rate of 700 μL/min. The eluent gradient is shown in Table 3.2. The parent ion mass is 716.4 and the product ion mass is 558.3. For the MS settings, the sheath gas pressure was 25 psi; auxiliary pressure was 5 psi; collision pressure was 1.5 mTorr; the spray voltage is 4000 V; the vaporizer temperature was 400°C. The injection volume was 20 μL and total run time was 18min. The calibration curve of erythromycin is shown in Appendix A.

Table 3.2 Eluent gradient for erythromycin detection by LC/MS

Time (min)	Methanol	0.1% Formic Acid
0	0%	100%
3	12%	88%
10	25%	75%
12.1	50%	50%
16.1	50%	50%
18	88%	12%

3.4.4 High Performance Liquid Chromatography

High performance liquid chromatography (HPLC; Water Corporation, Milford, MA) was used for *p*CBA detection. A Sonoma C18 column (3 μm particle size, 3.2 mm by 150 mm) was employed; flow rate was 0.7 mL/min and column temperature was 40°C; sample volume was 10 μL and sample time was 10 min for each sample. Analysis of *p*CBA was based on the method described by (De Witte *et al*., 2009): a mobile phase containing 75% water (0.1% phosphoric acid) and 25% ACN with quantification at 234 nm. *p*CBA was detected around 5.3 min. The calibration curve of *p*CBA is shown in Appendix B.

3.4.5 pH

pH were measured by a Thermo Electron Corporation pH meter (Orion 720 A+) at ambient temperature. The probe was calibrated before use with three standard buffers (pH 4.0, 7.0 and 10.0).

Chapter 4 Results

The ultimate purpose of this research was to delineate the effects of pH, carbonate and phosphate buffers, and initial ozone dose on the kinetics of removal of the antibiotic erythromycin by ozonation. Similar research was performed with another antibiotic, ciprofloxacin (Marron, 2010), which provided the initial design criteria and preliminary results verifying the expected performance of the reactor, including ozone reactor setup (see Chapter 3), *p*CBA detection method (see Chapter 3) and tracer studies (Section 4.2). Based on the results obtained from previous research, several sets of experiments were conducted to achieve the goals of this research. The first set of experiments was designed to determine the second-order rate constant for erythromycin degradation by ozone. Three additional sets of experiments examined the effect of carbonate and phosphate buffers, and initial ozone dose on erythromycin degradation by ozonation, respectively. Two separate sets of experiments were conducted to confirm the effect of carbonate buffer on ozone decomposition and its effect on erythromycin degradation by ozone only. All experiments were conducted at room temperature in a batch reactor with pH, carbonate and phosphate buffers, and/or initial ozone dose as variables.

4.1 Problem related to Erythromycin Stock Solution

An issue related to stock solution was discovered after all the experiments were finished. This issue will be described as follows and should be considered when the results presented by this thesis are being considered.

Per the discussion in Section 2.2.3, erythromycin undergoes self-degradation by losing one water molecule to form erythromycin-H_2O. This self-degradation is also expected to occur in the erythromycin stock solution, which resulted in an unstable erythromycin stock solution in which the speciation of erythromycin and erythromycin-

H_2O varied with time. Due to this problem with the stock solution, the initial solutions of all the experiments were not exactly identical. Fortunately, the rate of self-degradation of erythromycin is relatively slow and the experiments are conducted over short time periods. As a result, changes in the concentration of erythromycin in the stock solution during the experiments were minimal.

4.1.1 Self-degradation rate of erythromycin

The self-degradation of erythromycin can be described by a first-order reaction with respect to the concentration of erythromycin. Equation 2.2 described a proposed model to interpret the observed degradation rate constant as a function of pH at 25°C. However, the stock solution was always kept in 4°C room except during the short time periods in which it was removed to perform experiments. Therefore, experimental data was employed to determine the self-degradation rate of erythromycin at 4°C.

Three measurements of the speciation of erythromycin stock solution were carried out at day 62, 64 and 69. The speciation between erythromycin and erythromycin-H_2O was shown in Table 4.1.

Table 4.1 Speciation data for erythromycin stock solution

Day	Erythromycin (%)	Erythromycin-H_2O (%)	ln([Ery]/[Ery]$_0$)
0	100	0	0
62	76.0	24.0	-0.274
64	76.3	23.7	-0.271
69	75.5	24.5	-0.281

Equation 2.1 can be rewritten as:

$$\ln \frac{[Ery]}{[Ery]_0} = -k_{obs}t \qquad \text{(Equation 4.1)}$$

By plotting ln[Ery]/[Ery]$_0$ against time, the linear regression slope is the observed self-degradation rate constant for erythromycin at 4°C. As shown by Figure 4.1, the self-degradation rate constant k_{obs} of erythromycin at 4°C is 0.0042 d^{-1}.

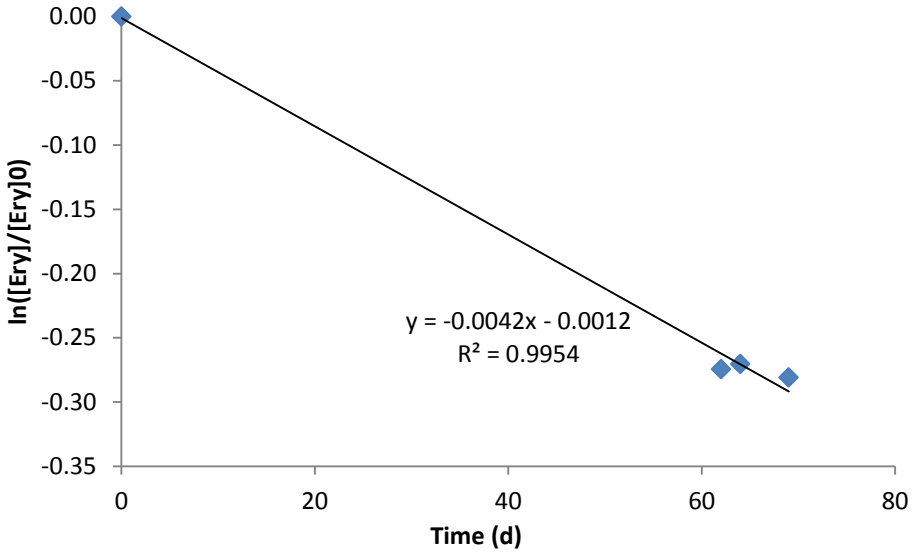

Figure 4.1 Verification of first-order model for self-degradation erythromycin at 4°C

4.1.2 The change of erythromycin stock solution

Once the self-degradation rate constant was obtained, the change in erythromycin concentration in the stock solution was tracked for to estimate the potential impact on the experimental results. The relative change in the erythromycin stock solution during a certain period of time (Δt) can be calculated as:

$$\frac{\Delta[Ery]}{[Ery]_1} = \frac{[Ery]_0 e^{-k_{obs}t_1} - [Ery]_0 e^{-k_{obs}(t_1+\Delta t)}}{[Ery]_0 e^{-k_{obs}t_1}} = 1 - e^{-k_{obs}\Delta t} \qquad \text{(Equation 4.2)}$$

By inserting the observed degradation rate constant and the time periods considered relative change in concentration of erythromycin in the stock solution was calculated to be 2.89%, 5.71% and 11.8% for time periods of one week, two weeks and 30 days, respectively. For the six sets of experiments completed in this thesis, each set of

experiments was completed within two weeks; therefore, for each set of the experiments, the stock solution remained relatively constant. However, the exact speciation of the stock solution was not determined. As a result, the data presented by this thesis is only applicable under certain conditions. Nevertheless, as evident from the above discussion, the relatively constant stock solution during the experimental period suggests that comparisons within each set of experiments valid.

4.2 SUMMARY OF PREVIOUS EXPERIMENTAL RESULTS

Tracer studies were performed on the experimental reactor in previous research conducted by Marron (2010). The results showed that the reactor did not achieve complete mixing until approximately 15 s after the addition of ozone, meaning that there would be a potential systematic bias if samples were taken within the first 15 s and this bias might be reflected in the estimation of rate constants based on the experimental data. Nevertheless, the same research showed that the incomplete mixing during the initial period did not significantly impact the values of rate constants determined using data collected during this time period .

As noted in the literature review, data from previous research by Marron (2010) and others (Buffle *et al*. 2006a; Wert *et al*., 2009) exhibited an instantaneous ozone demand. This initial rapid reduction in ozone concentration at the beginning of the experiment was also observed in this research. Marron (2010) did not consider the instantaneous ozone demand for determination of the kinetics of ciprofloxacin in her research, and a similar approach was used for this research.

4.3 DETERMINATION OF ERYTHROMYCIN OXIDATION RATE CONSTANT BY OZONE AND THE EFFECT OF PH

The objectives of this set of experiments were to determine the second-order rate constant for the reactions of erythromycin and ozone and to quantify the effect of pH on the apparent second-order rate constant.[3] Tert-butanol (t-butanol) was added to eliminate oxidation by hydroxyl radicals. Since the carbonate buffer and natural organic matter only affect the ozone decomposition rate, i.e., hydroxyl radical exposure, they were not included in this set of experiments. Results from the experiment conducted at pH 6 are used here to illustrate the approach for determining the apparent second-order rate constant between erythromycin and ozone.

4.3.1 Analysis of a single experiment (pH 6)

A single experiment was performed at pH 6 in deionized water with 1 mM phosphate buffer, 10 mM t-butanol and negligible carbonate. The reaction was initiated by the addition of ozone stock solution at time zero; samples were taken at recorded times during the experiment and concentrations of ozone and erythromycin were monitored.

Ozone concentration was determined by the indigo method described in Section 3.4.2. Ozone exposure was calculated by numerically integrating the ozone concentration over the reaction time using the trapezoidal rule:

$$\text{Ozone Exposure (i)} = \int_i^N [O_3]dt = \sum_{i=1}^{N} \frac{[O_3]_{t_{i+1}} + [O_3]_{t_i}}{2}(t_{i+1} - t_i) \qquad \text{(Equation 4.3)}$$

where i and N represent the first and last data point of the experiment, respectively.

3 Note that "apparent second-order rate constant" refers to the observed rate constant under a specific pH while "second-order rate constant" without "apparent" refers to the rate constant between ozone and deprotonated erythromycin, which is independent of pH as explained in Section 4.3.3 .

Figure 4.2 shows the ozone concentration profile and ozone exposure for this experiment. Instantaneous ozone demand appeared for the first sample (second data point). For the following samples, ozone concentration remained almost unchanged (standard deviation less than 7%) for two reasons: first, the decomposition of ozone in this experiment was relatively slow because the concentration of hydroxyl anions which initiate ozone decomposition was small at pH 6; second, according to the rate constant calculated in the following section, ozone reacted very fast with erythromycin so that even a small amount of ozone was able to cause significant degradation of erythromycin. The small fluctuation of ozone concentration might be due to the sampling and measuring error.

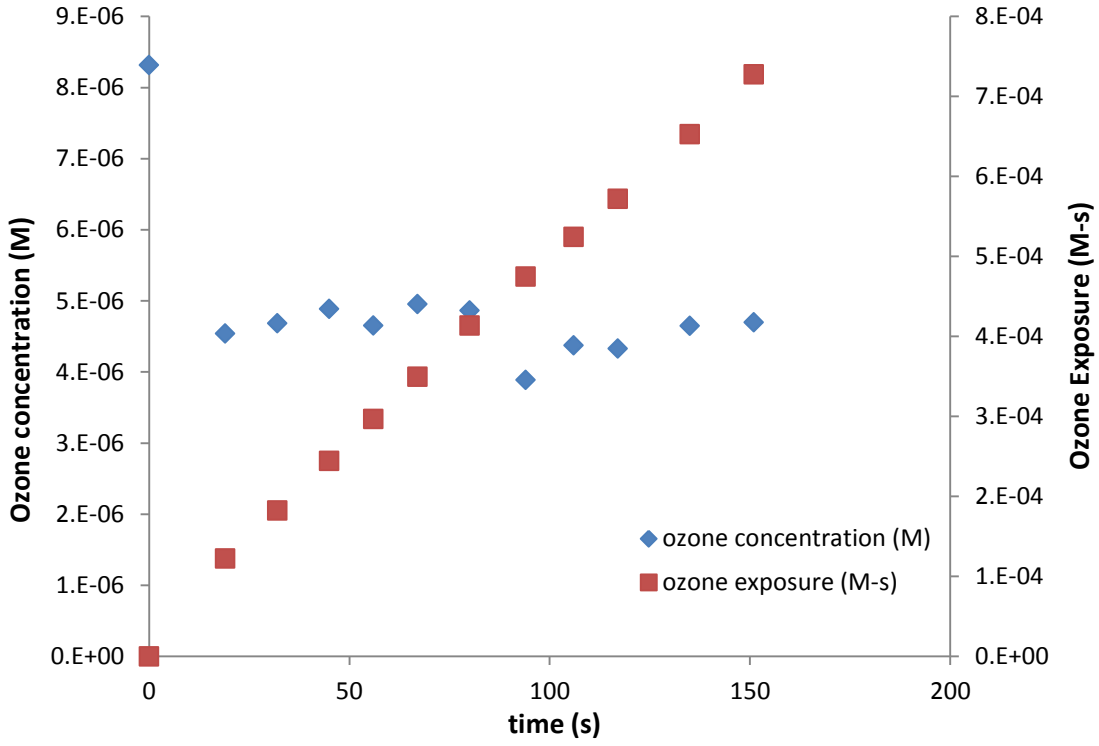

Figure 4.2: Ozone concentration and ozone exposure profiles from experiment at pH 6 (1 mg/L erythromycin, 1 mM phosphate buffer, 10 mM t-butanol, negligible total carbonate)

Since t-butanol was added to isolate the reaction between erythromycin and ozone, the degradation of erythromycin was only caused by the presence of ozone, which can be described as a second-order reaction, first order with respect to ozone and erythromycin as shown in Equation 4.4:

$$\frac{d[Ery]_{tot}}{dt} = -k_{O_3,app,Ery}[Ery]_{tot}[O_3] \qquad \text{(Equation 4.4)}$$

Ery represents erythromycin and $[Ery]_{tot}$ is the concentration of erythromycin within the reactor[4]; $k_{O_3,app,Ery}$ is the apparent second-order rate constant for erythromycin with ozone. By separating the variables and integrating, Equation 4.4 can be rewritten as:

$$\ln\frac{[Ery]}{[Ery]_0} = -k_{O_3,app,Ery}\int[O_3]dt \qquad \text{(Equation 4.5)}$$

where $[Ery]_0$ is the initial concentration of erythromycin that was measured directly.

Based on Equation 4.5, the apparent second-order rate constant $k_{O_3,app,Ery}$ can be obtained using ozone exposure data and erythromycin concentration profiles. By plotting the log transformed normalized erythromycin concentration against ozone exposure, the linearly regressed slope is the apparent second-order rate constant $k_{O_3,app,Ery}$ for the reaction between erythromycin and ozone. Figure 4.3 shows that at pH 6, the apparent second-order rate constant was 7.10×10^3 $M^{-1}\cdot s^{-1}$ (R^2=0.9969).

4 $[Ery]_{tot}$ refers to the total concentration of erythromycin, including deprotonated form and protonated form. In the rest of this thesis, [Ery] is used as total erythromycin concentration instead of $[Ery]_{tot}$ if not pointed particularly.

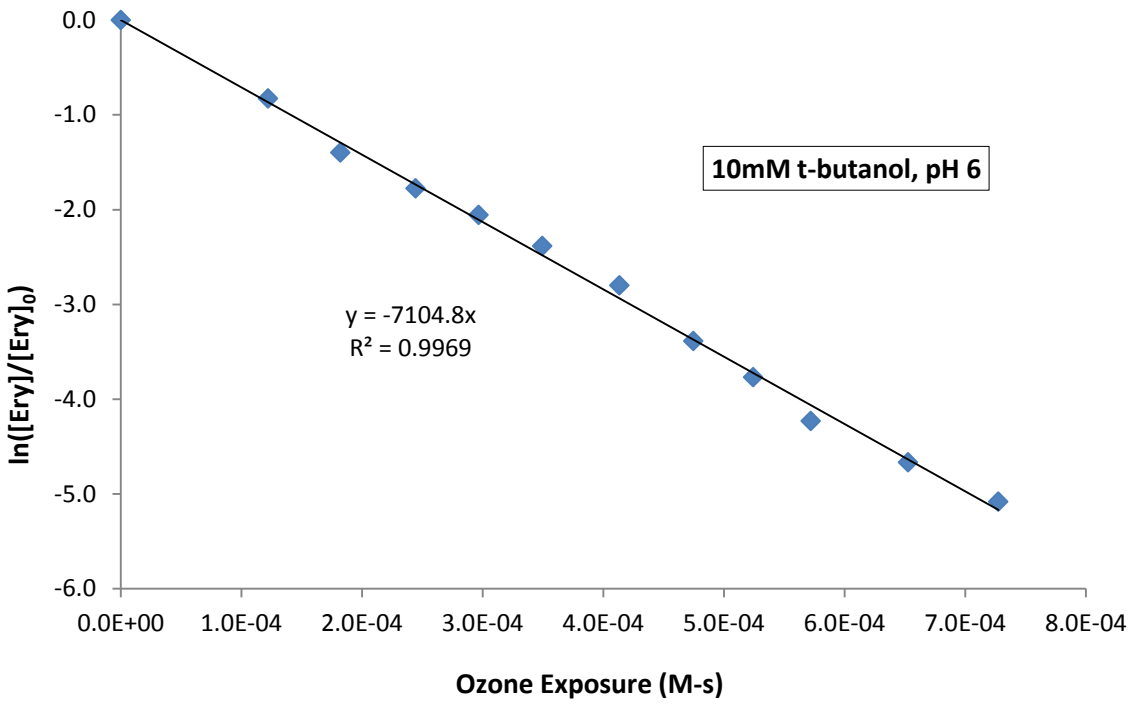

Figure 4.3: Illustration of approach to determine the apparent second-order rate constant of erythromycin oxidation by ozone (pH 6, 1 mg/L erythromycin, 1 mM phosphate buffer, 10 mM t-butanol, negligible carbonate)

4.3.2 Effect of pH on erythromycin degradation rate constant by ozone

Five parallel experiments were conducted at five different pH values (pH 5.35, 5.66, 6.00, 6.40, 6.81) to determine the effect of pH on the apparent second-order rate constant for reaction between erythromycin and ozone.

Experimental results showed that even within the narrow pH range from 5.35 to 6.81, the apparent second-order rate constants differed substantially, i.e., the slope of the $\ln([Ery]/[Ery]_0)$ vs. ozone exposure differed considerably (see Figure 4.4). As the pH increased from 5.35 to 6.81, the apparent second-order rate constant increased by a factor of approximately 30. This strong pH dependency of the apparent second-order rate constant was also observed in studies on a similar compound, roxithromycin, which also

belongs to the macrolide antibiotics category and is also characterized by a tertiary amine group (Huber *et al*., 2003; Dodd *et al*., 2006).

Erythromycin has a pK_a of 8.8; the strong pH dependence of $k_{O_3,app,Ery}$ suggests that ozone reacts primarily with the deprotonated amine group while the reaction between ozone and the protonated amine group could be neglected (Dodd *et al*., 2006). Therefore, at acidic pH values, when protonated erythromycin predominates, a lower reaction rate is expected; on the contrary, at alkaline pHs, when deprotonated erythromycin predominates, a higher reaction rate is expected. Moreover, the continuous increase in $k_{O_3,app,Ery}$ with increasing pH due to deprotonation of erythromycin's tertiary amine, suggests that ozone reactivity with the remainder of the erythromycin structure is very slow.

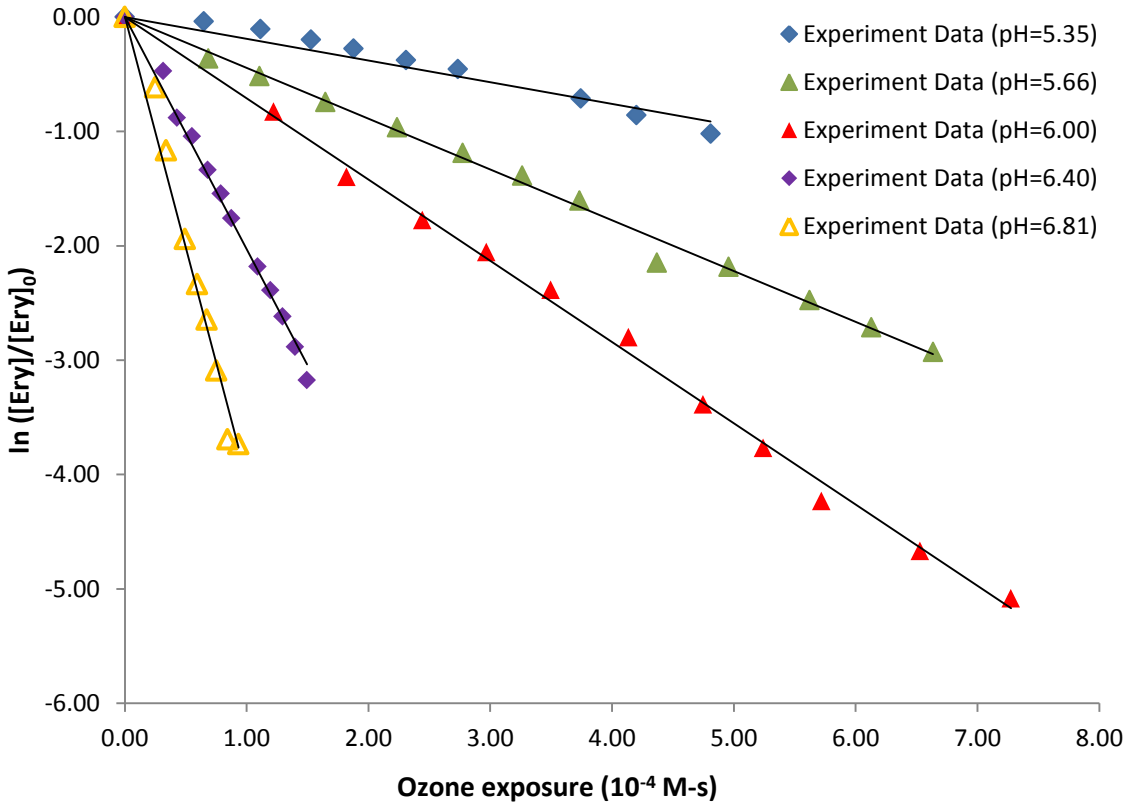

Figure 4.4 Experimental results of erythromycin oxidation by ozone at different pHs. (1 mg/L erythromycin, 1 mM phosphate buffer, 10 mM t-butanol, negligible carbonate) Points are experimental data and lines are linear regression results.

4.3.3 Rate constant for deprotonated erythromycin degradation by ozone

Since erythromycin only undergoes one proton dissociation within the pH range considered, indicated by its unique pK_a, a model initially proposed by (Hoigné & Bader 1983) and later applied by other researchers (Javier Benitez *et al*., 2003) is employed here to obtain the second-order rate constant between deprotonated erythromycin[5] and ozone. The overall ozonation rate of erythromycin can be expressed as:

5 Since only deprotonated erythromycin reacts with ozone at appreciable rate, protonated erythromycin is assumed to be negligible.

$$r_{O_3,app,Ery} = k_{O_3,app,Ery}[O_3][Ery]_{tot} = k_{O_3,Ery}[O_3][Ery] + k_{O_3,Ery^+}[O_3][Ery^+] \approx k_{O_3,Ery}[O_3][Ery]$$

(Equation 4.6)

where k_{O_3,Ery^+} is the rate constant between ozone and protonated erythromycin (Ery^+).

The ratio of the deprotonated erythromycin to protonated form is given by the degree of dissociation (α_1):

$$\alpha_1 = \frac{[Ery]}{[Ery]+[Ery^+]} = \frac{[Ery]}{[Ery]_{tot}} = \frac{K_a}{K_a + [H^+]} \qquad \text{(Equation 4.7)}$$

Therefore, Equation 4.6 can be rewritten as:

$$k_{O_3,app,Ery} = k_{O_3,Ery} \times \alpha_1 = k_{O_3,Ery} \times \frac{K_a}{K_a + [H^+]} \qquad \text{(Equation 4.8)}$$

Replacing $k_{O_3,app,Ery}$, Equation 4.5 can be rewritten as:

$$\ln \frac{[Ery]}{[Ery]_0} = -k_{O_3,app,Ery} \int [O_3]dt = -k_{O_3,Ery} \times \frac{K_a}{K_a + [H^+]} \times \int [O_3]dt \qquad \text{(Equation 4.9)}$$

For each data point in Figure 4.3, there would be a data pair of $\ln([Ery]/[Ery]_0)$ and $\int [O_3]dt$ under a specific pH. By plotting $\ln([Ery]/[Ery]_0)$ vs. $\frac{K_a}{K_a + [H^+]} \int [O_3]dt$, the slope yields the value of $k_{O_3,Ery}$, which is shown in Figure 4.5. The second-order rate constant $k_{O_3,Ery}$ for the reaction of deprotonated erythromycin with ozone was 4.44×10^6 $M^{-1} \cdot s^{-1}$. Huber *et al*., (2003) reported the second-order rate constant for roxithromycin with ozone as $(4.5 \pm 0.5) \times 10^6$ $M^{-1} \cdot s^{-1}$, which is very close to the value obtained in this study. This confirms that for macrolide antibiotics, ozone reacts with the common tertiary

amine group. A similar value (4×10^6 $M^{-1}\cdot s^{-1}$) was also reported for reactions between free tertiary amine and ozone by (Lange *et al*., 2006).

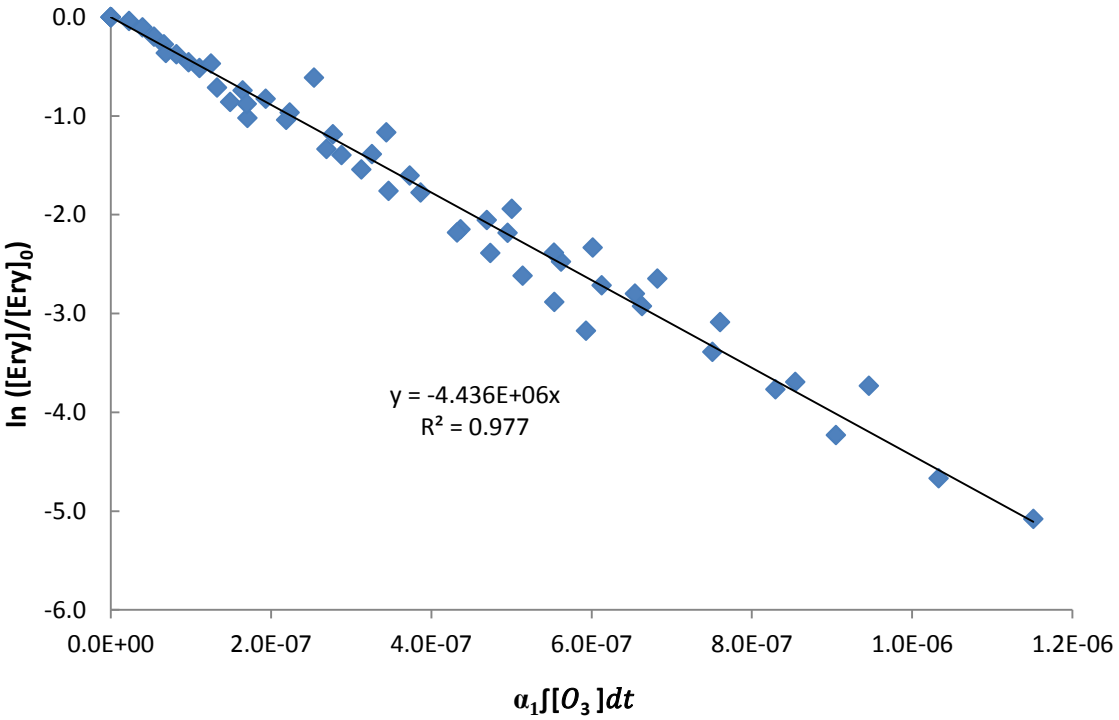

Figure 4.5: Illustration of approach to determine the second-order rate constant of erythromycin oxidation by ozone

Once $k_{O_3,Ery}$ is known, the apparent second-order rate constant $k_{O_3,app,Ery}$ could be back calculated for any pH (See Table 4.2). The experimental data points can also be modeled based on the value of $k_{O_3,app,Ery}$ for each pH value. Figure 4.6 shows the results of modeling this data using different ordinates. As can be seen from the two figures, the model provides a better fit around pH 6; at lower or higher pH, the model does not match the data perfectly. At high pH, the reaction between erythromycin and ozone was too rapid and there was likely more sampling error; at low pH, the reaction was so slow that

ozone depletion caused by processes other than the reaction with the tertiary amine group cannot be fully neglected (Lange *et al*., 2006).

Table 4.2: Apparent second-order rate constant of erythromycin oxidation by ozone at five experiment pH based on k_{O_3,Ery^-} and speciation coefficient α_1

pH	α_1	$k_{o_3,app,Ery}$($M^{-1}\cdot s^{-1}$)
5.35	3.55E-04	1.57E+03
5.66	9.99E-04	4.43E+03
6.00	1.58E-03	7.02E+03
6.40	3.97E-03	1.76E+04
6.81	1.01E-02	4.49E+04

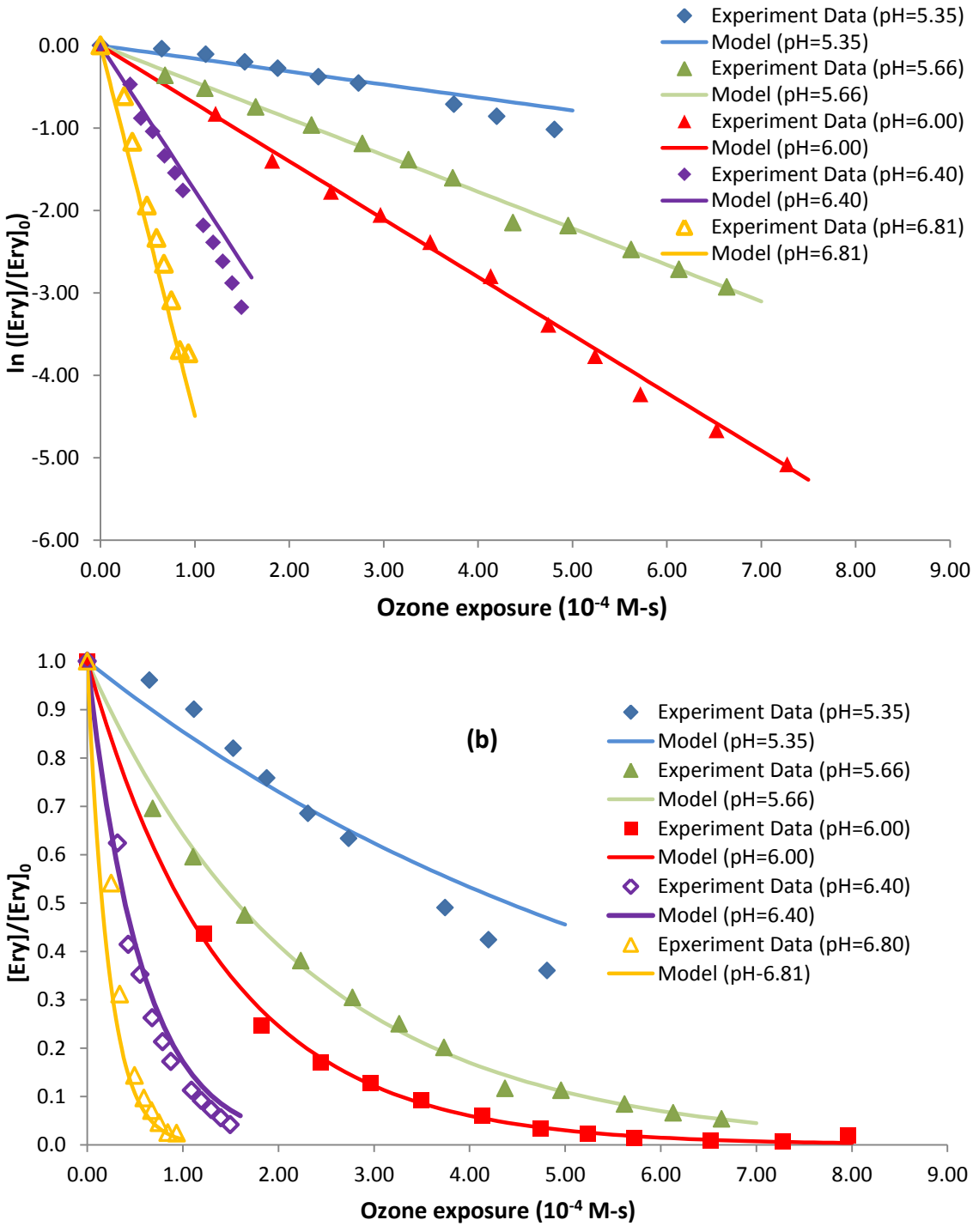

Figure 4.6: Modeling results of erythromycin oxidation by ozone at different pHs (a) y-axis being ln([Ery]/[Ery]$_0$); (b) y-axis being [Ery]/[Ery]$_0$. Data are plotted as symbols and models are presented as lines.

The half-life concept can also be applied to evaluate the performance of erythromycin degradation by ozonation. When a constant ozone concentration of 1 mg/L (20 μM) is applied, the half-life of erythromycin can be calculated as:

$$t_{\frac{1}{2}} = \frac{\ln([Ery]/[Ery]_0)}{k_{O_3,app,Ery}[O_3]} = \frac{0.5}{k_{O_3,app,Ery} \times 20\mu M} \qquad \text{(Equation 4.10)}$$

Figure 4.7 shows the half-life of erythromycin (for $[O_3]$ =1mg/L) and apparent second-order rate constant $k_{O_3,app,Ery}$ as a function of pH. By comparing Figure 4.7 and Figure 1 from (Huber et al. 2003), the similarity between the data collected for erythromycin and roxithromycin is evident. At pH 7, the rate constant for erythromycin was determined in this research as $k_{O_3,app,Ery} = 6.93 \times 10^4$ $M^{-1} \cdot s^{-1}$. Huber *et al.* (2003) estimated a rate constant at pH 7 for macrolide antibiotics with ozone as high as 10^5 $M^{-1} \cdot s^{-1}$, which is consistent with the value determined in this research.

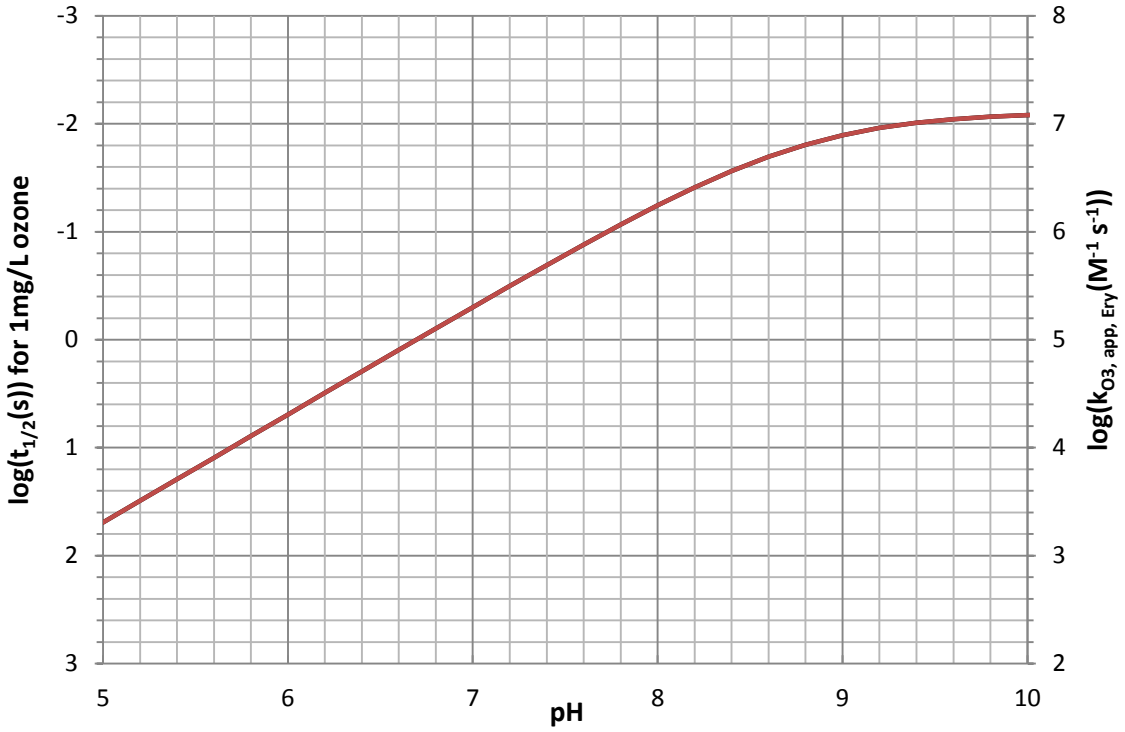

Figure 4.7: Half-life and apparent second-order rate constant for erythromycin degradation by ozone as a function of pH. (The half-life is calculated for an ozone concentration of 1 mg/L; reactions with $^{\circ}$OH neglected)

4.3.4 Effect of carbonate buffer on erythromycin degradation by ozone only

As pointed out at the beginning of Section 4.3, the effect of carbonate buffer was not taken into consideration for this set of pH experiments since carbonate buffer only affects ozone decomposition, i.e., the hydroxyl radical exposure. To confirm that carbonate had no effect under these conditions, a separate experiment was conducted to verify that the rate constant of degradation of erythromycin, or deprotonated erythromycin, by ozone was the same regardless of carbonate buffer concentration.

The experiment was carried out under exactly the same conditions as the pH 6 experiment, which was used as an illustration in Section 4.3.1, except that a total carbonate concentration of 4 mM (bicarbonate concentration = 1.28 mM) was added to

the reactor. The same analysis method was used to determine the observed rate constant for this experiment. The results from the two experiments are compared in Figure 4.8. The data from the two experiments followed the same trend with apparent second-order rate constant of 7.10×10^4 $M^{-1}\cdot s^{-1}$ with negligible carbonate[6] and 7.56×10^4 $M^{-1}\cdot s^{-1}$ with 4mM total carbonate. The difference between the two rate constants was only 6.25%, which was within the level of experimental error for this research. Therefore, the assumption that carbonate buffer would not affect the reactions between erythromycin and ozone has been verified.

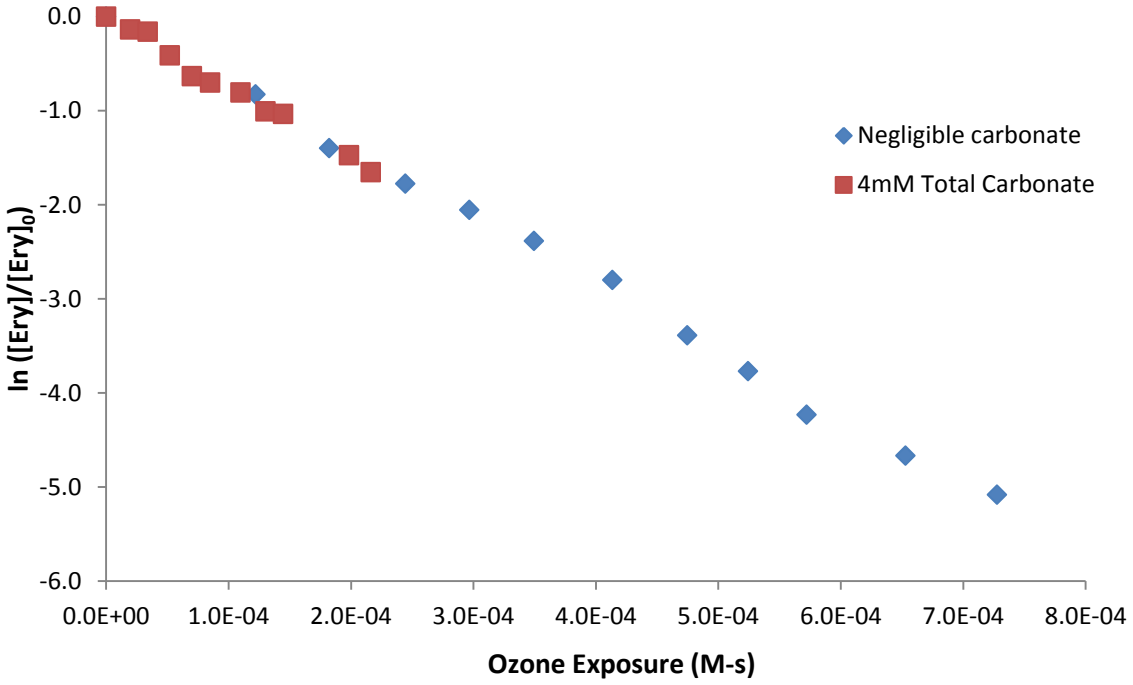

Figure 4.8: Degradation of erythromycin by ozone only at negligible carbonate and 4 mM total carbonate (pH=6, 10 mM t-butanol, 1 mM phosphate buffer)

[6] The carbonate concentration in deionized water is assumed to be negligible.

4.4 EFFECT OF CARBONATE BUFFER

The degradation of erythromycin is caused by both ozone and hydroxyl radicals that form during ozone decomposition (when t-butanol is not added as a hydroxyl radical scavenger). In conventional ozonation processes, carbonate, mainly HCO_3^- and CO_3^{2-}, are known to be ozone decomposition inhibitors since they consume hydroxyl radicals and do not accelerate the chain reaction as described in Section 2.4.2 (Staehelin & Hoigne 1985). Therefore, carbonate buffer would theoretically affect the performance of erythromycin degradation by ozonation or AOP indirectly since ozone decomposition is directly affected by carbonate buffer. Two sets of experiments were conducted to confirm the effect of carbonate buffer on ozone decomposition and then to investigate the effect of carbonate buffer on erythromycin degradation by ozonation (ozone and hydroxyl radicals).

4.4.1 Effects of carbonate buffer on ozone decomposition

The goal of the first set of experiments was to confirm the effect of carbonate buffer on aqueous ozone decomposition through the quantification of hydroxyl radical exposure and by obtaining an R_{ct} value. The total carbonate concentration was the only variable in this set of experiments conducted at pH 6; bicarbonate concentration was 32% of the total carbonate concentration at this pH and the CO_3^{2-} concentration was negligible. Erythromycin was not added in this set of experiment; other experimental conditions are shown in Table 3.1.

It is known that initial ozone dose would also affect the ozone decomposition rate (Buffle *et al*., 2006a); therefore, the initial ozone doses for these three experiments were maintained within a 7% difference (see Table 4.3).

Table 4.3: R_{ct}, initial ozone dose, ozone exposure and hydroxyl radical exposure at 1min of experiments with 0 mM, 0.5 mM and 1 mM total carbonate (pH=6, no erythromycin)

Total carbonate (mM)	R_{ct}	Initial ozone dose (mg/L)	Ozone exposure at 1min (10^{-4} M-s)	Hydroxyl radical exposure at 1min (10^{-10} M-s)
0	9.91E-07	0.579	5.274	5.226
0.5	1.75E-07	0.685	6.612	1.160
1	5.73E-08	0.624	7.170	0.411

The concentration of hydroxyl radicals cannot be measured directly; therefore, a probe compound, *para*-chlorobenzoic acid (*p*CBA) was used to quantify hydroxyl radical concentration. The reaction of *p*CBA with hydroxyl radicals follows a second-order rate law. The second-order rate constant of 5.2×10^{9} $M^{-1}\cdot s^{-1}$ is significantly higher than the a second-order rate constant of $0.15M^{-1}\cdot s^{-1}$ that has been reported for ozone; therefore, the disappearance of *p*CBA can be used to track hydroxyl radical concentrations since the disappearance of *p*CBA only occurs through reaction with hydroxyl radicals. The reaction between *p*CBA and hydroxyl radicals can be described by Equation 4.11:

$$\frac{d[pCBA]}{dt} = -k_{^\circ OH,pCBA}[pCBA][^\circ OH] \qquad \text{(Equation 4.11)}$$

where [*p*CBA] and [°OH] are the concentration of *p*CBA and hydroxyl radicals, respectively; $k_{^\circ OH,pCBA}$ is the second-order rate constant between °OH and *p*CBA, i.e., 5.2×10^{9} $M^{-1}\cdot s^{-1}$.

By separating the variables and integrating, Equation 4.11 can be rewritten as:

$$\ln\frac{[pCBA]}{[pCBA]_0} = -k_{^\circ OH,pCBA}\int[^\circ OH]dt \qquad \text{(Equation 4.12)}$$

R_{ct} is used to describe ozone decomposition and is defined as the ratio of hydroxyl radical exposure to ozone exposure, i.e.,

$$R_{ct} = \frac{\int [^{\circ}OH]dt}{\int [O_3]dt} \qquad \text{(Equation 4.13)}$$

A high R_{ct} value means rapid ozone decomposition. Typical R_{ct} values range from 10^{-7} to 10^{-9} depending on the background water matrix (Elovitz & von Gunten 1999; Elovitz *et al*., 2000).

By employing the R_{ct} concept, Equation 4.12 can be rewritten as:

$$\ln \frac{[pCBA]}{[pCBA]_0} = -k_{^{\circ}OH,pCBA} \cdot R_{ct} \cdot \int [O_3]dt \qquad \text{(Equation 4.14)}$$

By plotting $\ln[pCBA]/[pCBA]_0$ against ozone exposure, R_{ct} is the linear regression slope divided by $k_{^{\circ}OH,pCBA}$. The results are shown in Figure 4.9 with 0 mM, 0.5 mM and 1 mM total carbonate concentration, respectively. The R_{ct} value decreased by almost an order of magnitude as the total carbonate concentration increased from 0 mM to 1 mM.

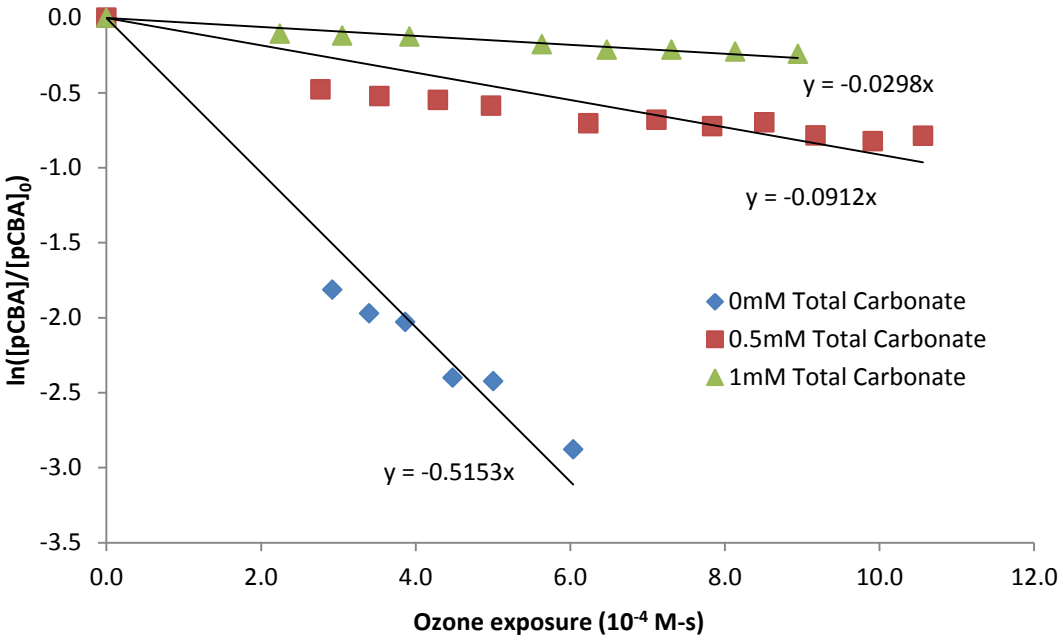

Figure 4.9: $\ln([pCBA]/[pCBA]_0)$ vs. Ozone exposure at 0 mM, 0.5 mM, 1 mM total carbonate concentration (pH=6, 1 mM buffer, 1 μM initial *p*CBA, no erythromycin)

Once the R_{ct} value was obtained, the hydroxyl radical exposure could be back calculated using Equation 4.13. Note that the calculation of ozone exposure and hydroxyl radical exposure presented in Table 4.3 and

Figure 4.10 were based on a one minute experimental time frame. Again, by comparing ozone exposure and hydroxyl radical exposure from the three experiments, under the same initial ozone concentration (the solid line in

Figure 4.10) and same time frame (1 min), ozone decomposition was greatly inhibited by the presence of carbonate; ozone exposure increased by 36% and hydroxyl radical exposure decreased by a factor of approximately 12 when the total carbonate concentration increased from 0 mM to 1 mM. The higher ozone exposure and lower hydroxyl radical exposure resulted in a small R_{ct} value at high carbonate concentration. The decrease in R_{ct} value and hydroxyl radical exposure with increasing carbonate concentration demonstrated impact of carbonate buffer as an inhibitor to ozone decomposition. These results are as expected and consistent with other research results (Elovitz *et al*., 2000).

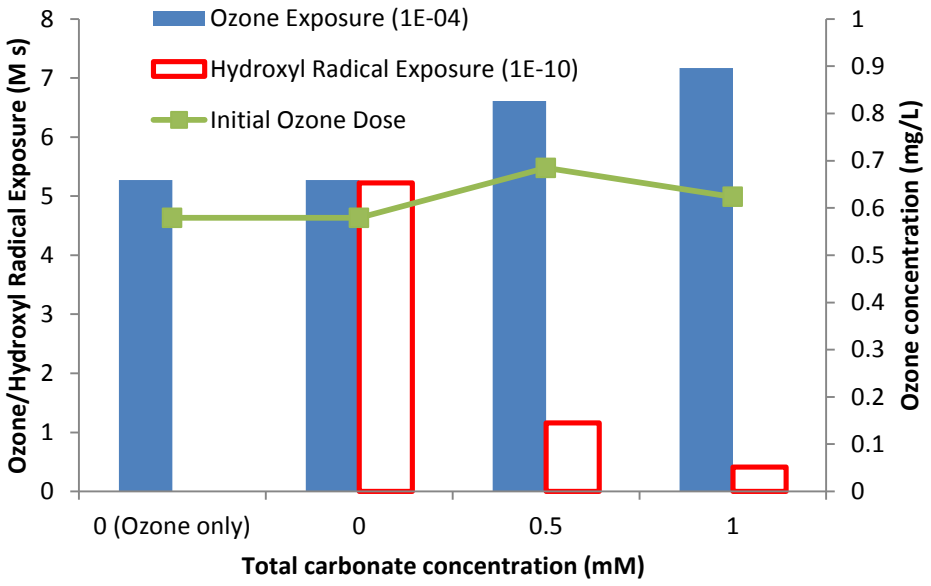

Figure 4.10: Ozone exposure, hydroxyl radical exposure and initial ozone dose at 0 mM, 0.5 mM and 1 mM total carbonate concentration (Exposures are calculated based on 1min with initial ozone dose of 0.58, 0.69, 0.62 mg/L, respectively; pH=6, 1 mM phosphate buffer, 1 μM initial pCBA, no erythromycin).

4.4.2 Effects of carbonate buffer on erythromycin degradation by ozonation

The purpose of this set of experiments was to investigate the effect of carbonate buffer on erythromycin degradation by ozonation when both ozone and hydroxyl radicals were present. Total carbonate concentration was the only variable in this set of experiments and the initial ozone dose was kept constant (around a 7% difference). At the experimental pH (pH=6), the bicarbonate concentration was 32% of the total carbonate concentration and the CO_3^{2-} concentration was negligible; other experimental conditions are shown in Table 3.1.

As noted in Section 2.4.4, the disappearance of erythromycin can be described by the following equation:

$$\frac{d[Ery]}{dt} = -(k_{O_3,app,Ery}[Ery][O_3]dt + k_{^\circ OH,Ery}[Ery][^\circ OH]dt) \qquad \text{(Equation 4.15)}$$

where [$^{\circ}$OH] is the concentration of hydroxyl radicals and $k_{^{\circ}OH,Ery}$ is the apparent second-order rate constant between erythromycin and hydroxyl radicals.

The first term on the left side of Equation 4.15 represents the direct oxidation of erythromycin caused by ozone and the second term represents the oxidation caused by hydroxyl radicals.

By separating variables and integrating, Equation 4.15 can be rewritten as:

$$\ln \frac{[Ery]}{[Ery]_0} = -(k_{O_3,app,Ery} \int [O_3]dt + k_{^{\circ}OH,Ery} \int [^{\circ}OH]dt) \qquad \text{(Equation 4.16)}$$

Substituting R_{ct} into Equation 4.16 yields:

$$\ln \frac{[Ery]}{[Ery]_0} = -(k_{O_3,app,Ery} + k_{^{\circ}OH,Ery} R_{ct}) \int [O_3]dt = -k_T \int [O_3]dt \qquad \text{(Equation 4.17)}$$

$$k_T = k_{O_3,app,Ery} + k_{^{\circ}OH,Ery} R_{ct} \qquad \text{(Equation 4.18)}$$

k_T is the overall rate constant for oxidation of erythromycin caused by both ozone and hydroxyl radicals. By plotting $\ln([Ery]/[Ery]_0)$ against ozone exposure, the overall rate constant k_T can be determined from the linear regression slope.

Figure 4.11 shows the results from experiments with 0 mM, 1 mM, 2 mM and 4 mM total carbonate concentration. Although the total carbonate concentration increased from 0 mM to 4 mM, the overall rate constant k_T only varied by 2.72% with an average value of 9.54×10^4 $M^{-1}\cdot s^{-1}$; all of the results are considered to be identical. As confirmed by Section 4.3.4, the carbonate buffer did not affect the rate constant for the reaction of ozone with erythromycin, i.e., the first term of k_T—$k_{O_3,app,Ery}$. Therefore, it is reasonable to assume that the second term in the expression for k_T, i.e., the hydroxyl radical degradation term, imparted either a negligible contribution or remained constant regardless of total carbonate concentration. However, Section 4.4.1 did show that the

carbonate buffer had a large impact on ozone decomposition as indicated by changes in the magnitude of R_{ct} for different total carbonate concentrations. Consequently, it is inferred that hydroxyl radicals were not the primary oxidant for degradation of erythromycin; hence, even though the carbonate buffer showed a significant impact on ozone decomposition itself, it had no appreciable effect on the degradation of erythromycin during ozonation. Kim & Tanaka (2010) studied the removal efficiency of 13 pharmaceuticals including erythromycin and the results showed slightly higher removal efficiency with an ozone process compared with the O_3/H_2O_2 process; they concluded that ozone was the primary oxidant responsible for degradation of the 13 pharmaceuticals during O_3/H_2O_2 process. This conclusion coincides with the results of this research.

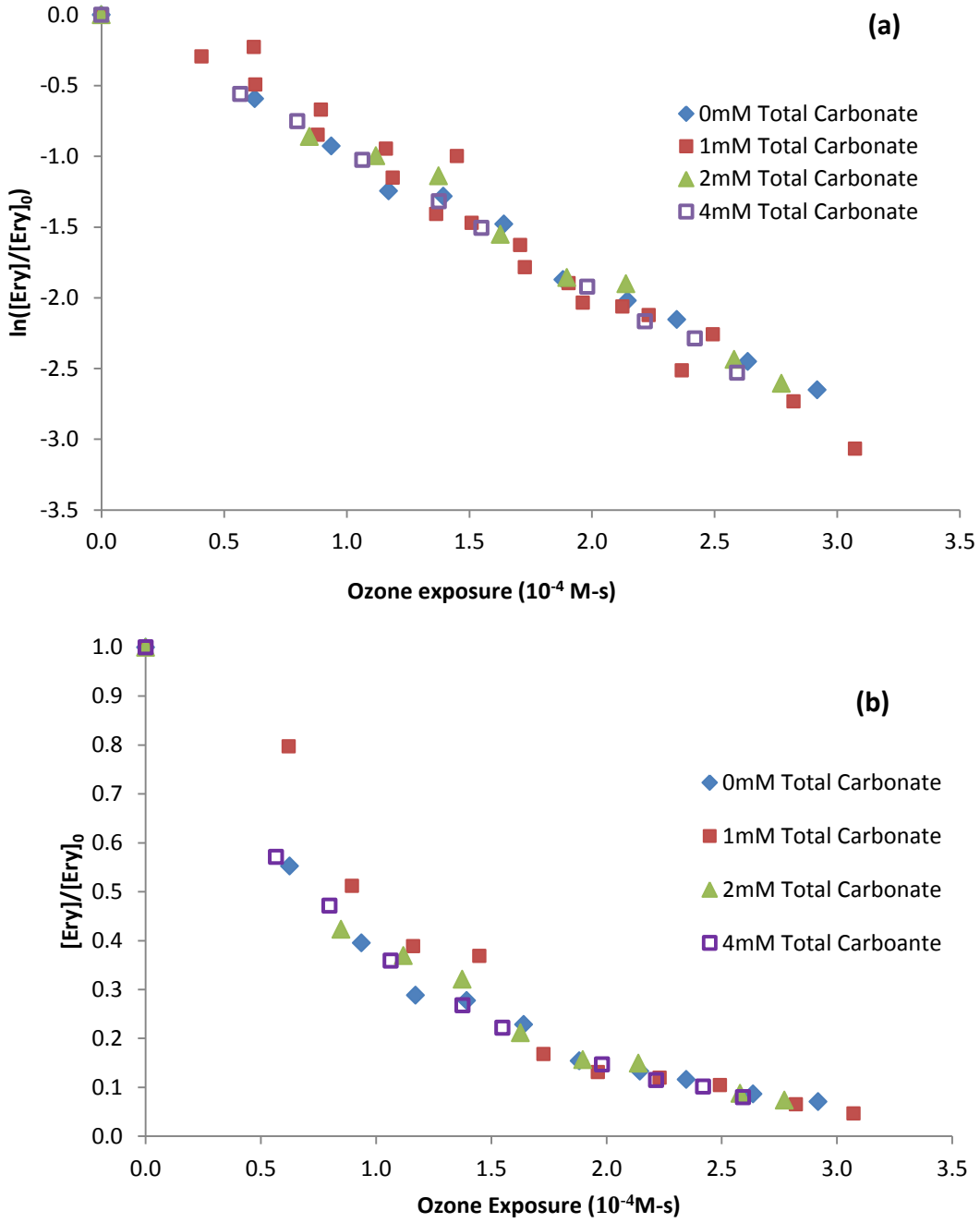

Figure 4.11: Effect of carbonate concentration on erythromycin degradation by ozonation (a) y-axis being ln([Ery]/[Ery]$_0$); (b) y-axis being [Ery]/[Ery]$_0$.(pH=6, 1 mM phosphate buffer, 1 mg/L erythromycin, 1 µM *p*CBA).

The average overall rate constant k_T was determined to be 9.54×10^3 $M^{-1}\cdot s^{-1}$, which was 32% larger than $k_{O_3,app,Ery}$ (7.02×10^3 $M^{-1}\cdot s^{-1}$ at pH 6, see Table 4.2). The slightly higher rate constant was expected since, in the absence of t-butanol, hydroxyl radicals were not scavenged and participated in the degradation of erythromycin. Even though hydroxyl radicals are not the primary oxidant for erythromycin, there was a measurable contribution of hydroxyl radicals to the overall removal of erythromycin.

On the other hand, *p*CBA added into the reactor at the beginning of the experiment for the purpose of tracking hydroxyl radicals did not change within the time frame of all of the experiments, suggesting that no significant hydroxyl radical exposure was available. As a result, the R_{ct} concept was not appropriate for determining hydroxyl radical exposure in this case.

That *p*CBA concentration did not change during the experiment could also be interpreted based on the relative rates of removal of erythromycin and pCBA. From Section 4.3.2, at pH 6, erythromycin reacted with ozone at an apparent rate constant of approximately 7000 $M^{-1}\cdot s^{-1}$, which was considered a very fast reaction. Based on the results from Section 4.4.1 and previous research (Elovitz & von Gunten 1999; Elovitz *et al*., 2000) , R_{ct} values typically range from 10^{-7} to 10^{-9}. Consider an R_{ct} value of 10^{-8} for demonstration. The direct reaction between *p*CBA and ozone is negligible (rate constant being 0.15 $M^{-1}\cdot s^{-1}$); however, the reaction between *p*CBA and hydroxyl radicals can be transformed into the indirect reaction of *p*CBA and ozone using the R_{ct} value as shown::

$$\ln\frac{[pCBA]}{[pCBA]_0} = -k_{^\circ OH,pCBA}\int[^\circ OH]dt = -k_{^\circ OH,pCBA}R_{ct}\int[O_3]dt = -k_{O_3,app,pCBA}\int[O_3]dt$$

(Equation 4.19)

where $k_{O_3,app,pCBA}$ represents the indirect rate constant between ozone and *p*CBA.

Inserting the value of R_{ct} and $k_{^{\circ}OH,pCBA}$ at pH 6, the rate constant for the indirect reaction between ozone and *p*CBA yields:

$$k_{O_3,app,pCBA} = k_{^{\circ}OH,pCBA} R_{ct} = 5.2\times10^{9} M^{-1}s^{-1} \times 10^{-8} = 52 M^{-1}s^{-1} << 7000 M^{-1}s^{-1}$$

Therefore, comparing the rate constant of erythromycin and ozone (~7000 $M^{-1}\cdot s^{-1}$) with the indirect rate constant of *p*CBA and ozone (~52 $M^{-1}\cdot s^{-1}$) demonstrates that erythromycin reacts much faster than *p*CBA with ozone. When erythromycin is degraded to less than 10%, *p*CBA remains at 98% of the initial concentration (see Appendix C). In other words, before there is a noticable *p*CBA concentration change, erythromycin is already gone, i.e., erythromycin and *p*CBA change cannot be tracked at the same time. Even if an R_{ct} value of 10^{-7} is applied, erythromycin still reacts 10 times faster than *p*CBA. Therefore, it is reasonable that *p*CBA did not change during the experiments in which erythromycin degraded appreciably. This demonstrates a new scenario for which the R_{ct} concept is not valid; namely, when degradation of the compound of interest is degraded before hydroxyl radical exposure can be determined.

That *p*CBA did not change during the experiments and identical overall rate constants were obtained from four different experiments confirms that degradation of erythromycin is primarily caused by ozone, whereas hydroxyl radicals have a relatively minor contribution to the degradation of erythromycin during ozonation. Moreover, hydroxyl radicals could not be quantified using *p*CBA as a probe molecule because the reaction between ozone and erythromycin dominated the loss of ozone in the system. Although the presence of carbonate buffer does greatly influence ozone decomposition, it had a negligible effect on the overall removal of erythromycin by ozonation.

4.5 EFFECTS OF PHOSPHATE BUFFER

Phosphate has been reported to be an efficient ozone decomposition inhibitor at typical buffer concentrations (Staehelin & Hoigne 1985; Morozov & Ershov 2010). At all pH values, ozone decomposition rate constants decreased as phosphate concentration increased (Morozov & Ershov 2010).

This set of experiments evaluated the effect of phosphate buffer on erythromycin degradation by ozonation. Similar to the carbonate buffer experiments, phosphate buffer concentration was the only variable; all experiments were conducted with 0 mM total carbonate. The initial concentration of ozone was maintained within 10% difference. Other experimental conditions are shown in Table 3.1.

Figure 4.12 shows the results of experiments conducted at 1 mM, 5 mM and 10 mM phosphate buffer. It can be seen that the three experiments roughly followed the same trend, which was similar to the results found in carbonate buffer experiments. The overall rate constant k_T from the three experiments differed by 9.8% which is thought to be caused by the 10% difference in initial ozone dose. Meanwhile, *p*CBA concentration didn't change in this set of experiments. These two results lead to the conclusion that, although phosphate buffer was reported to have an impact on ozone decomposition, it had negligible effect on erythromycin degradation by ozonation.

The average overall rate constant k_T for these three experiments was 9.85×10^3 $M^{-1}\cdot s^{-1}$; this value was again greater than $k_{O_3,app,Ery}$ for the same reason explained in Section 4.4.2. Moreover, this value of 9.85×10^3 $M^{-1}\cdot s^{-1}$ was very close to the average overall rate constant obtained in the carbonate experiments (9.54×10^3 $M^{-1}\cdot s^{-1}$); this again indicated that ozone was the primary oxidant for erythromycin degradation.

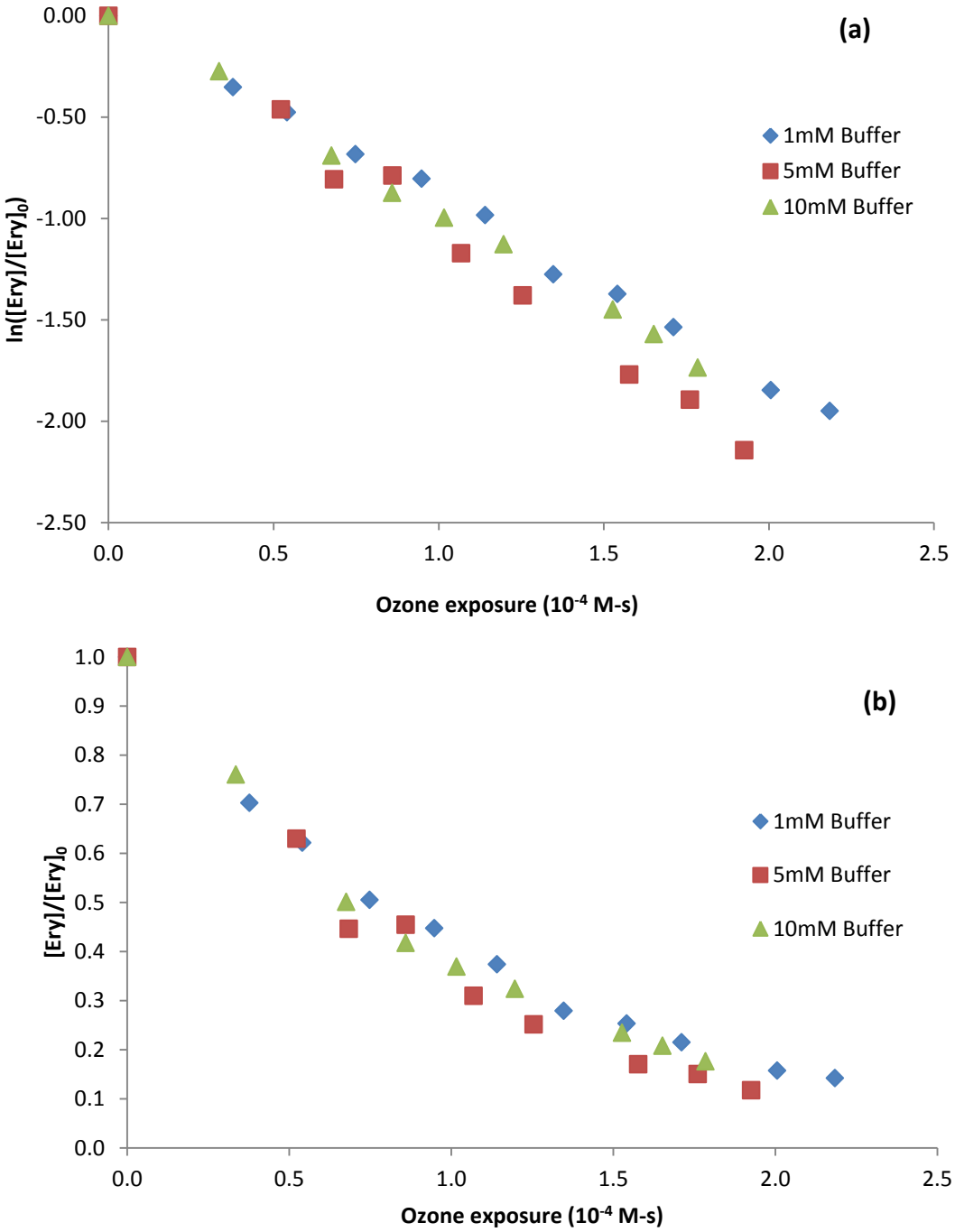

Figure 4.12: Effect of phosphate buffer on erythromycin degradation by ozonation (a) y-axis being ln([Ery]/[Ery]$_0$); (b) y-axis being [Ery]/[Ery]$_0$.(pH=6, 0 mM total carbonate, 1 mg/L erythromycin)

4.6 EFFECTS OF INITIAL OZONE DOSE

Initial ozone dose is a key control parameter in full-scale ozonation plants and it is reported to have a great impact on ozone decomposition (Buffle *et al*., 2006a). However, as demonstrated by Section 4.4 and Section 4.5, ozone was the primary oxidant responsible for the degradation of erythromycin. Therefore, it was expected that initial ozone dose would have little effect on erythromycin degradation by ozonation, based on the conclusions from the carbonate and phosphate buffer experiments as well as the "fact" that the reaction between ozone and erythromycin was modeled by a second-order reaction, first order with respect to both ozone and erythromycin. This set of experiments was designed to verify whether this assumption was valid. Initial ozone dose was the only variable and ranged from 0.11 mg/L to 0.27 mg/L. Other conditions were maintained at values shown in Table 3.1.

Figure 4.13 shows the results from the experiments. It is evident from the figure that there is a clear positive relationship between initial ozone dose and the overall rate constant for erythromycin degradation. As the initial ozone dose increased from 0.11 mg/L to 0.27 mg/L, the overall rate constant k_T also increased from 1.09×10^4 $M^{-1}\cdot s^{-1}$ to 2.82×10^4 $M^{-1}\cdot s^{-1}$ (see Figure 4.14). Clearly, this result disagrees with the expectation that initial ozone dose would have negligible impact on the degradation rate of erythromycin by ozonation.

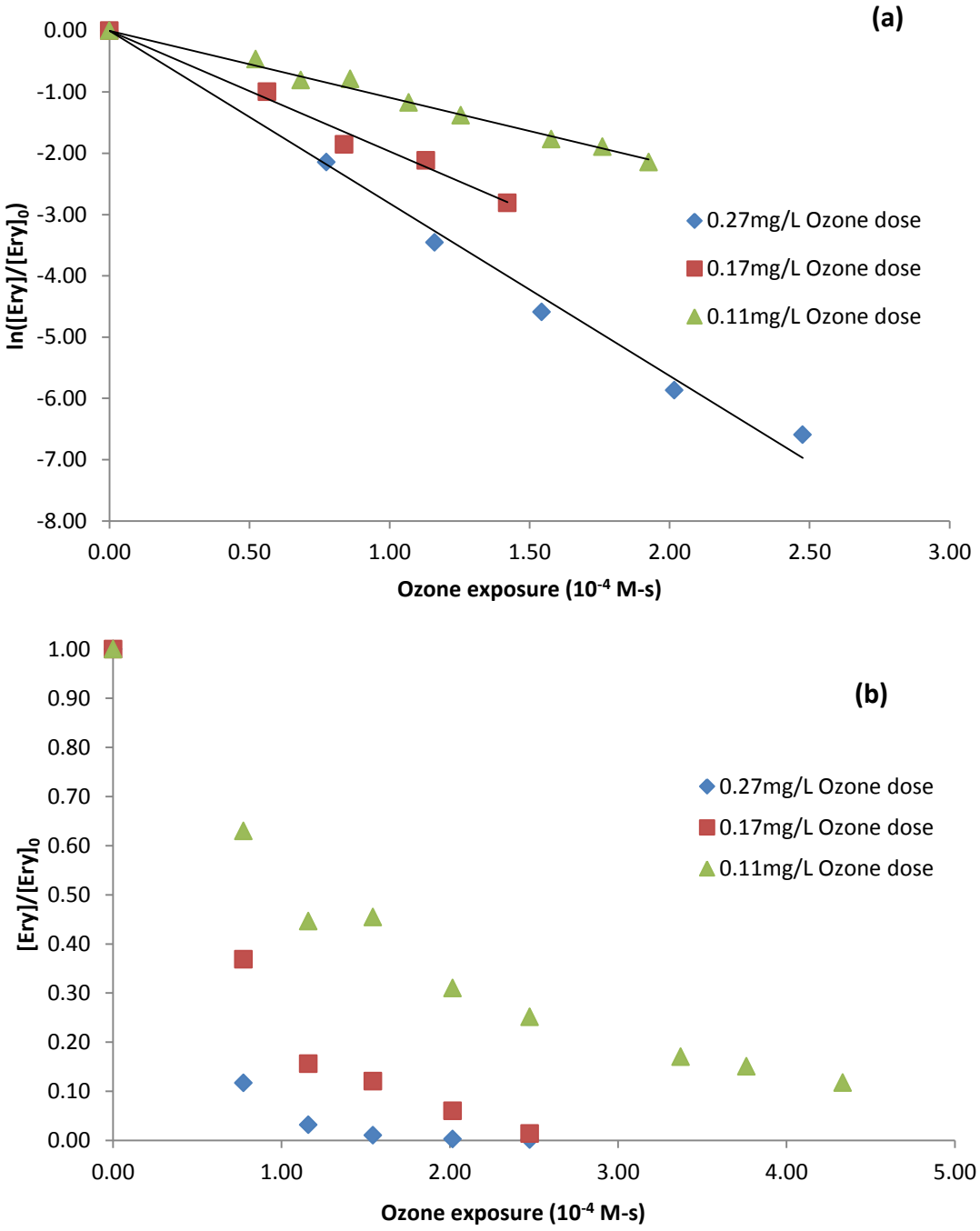

Figure 4.13: Effect of initial ozone dose on erythromycin degradation by ozonation. (a) y-axis being ln([Ery]/[Ery]$_0$); (b) y-axis being [Ery]/[Ery]$_0$.(pH=6, 5 mM phosphate buffer, 1 mg/L erythromycin, 0 mM total carbonate).

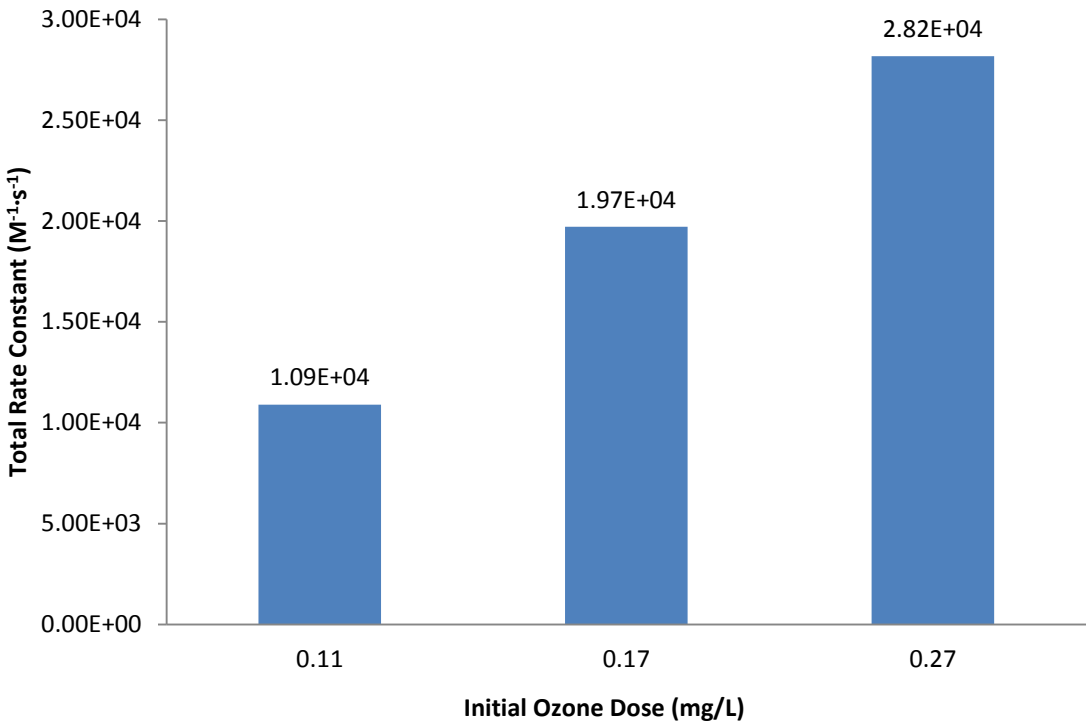

Figure 4.14: Total rate constant at different initial ozone dose (pH=6, 5 mM phosphate buffer, 1 mg/L erythromycin, 0 mM total carbonate)

On the other hand, the pCBA concentration did not change during the experiments, which excluded the possibility that it hydroxyl radicals caused the increase in the overall rate constant as initial ozone concentration increased.

The apparent linear close relationship between initial ozone dose and overall rate constant (Figure 4.14) leads to the possibility that the reaction between erythromycin and ozone was actually second-order with respect to ozone instead of first-order, which is described by Equation 4.20 (hydroxyl radical term neglected):

$$\frac{d[Ery]}{dt} = -k''_{O_3,app,Ery}[Ery][O_3]^2 \qquad \text{(Equation 4.20)}$$

where $k''_{O_3,app,Ery}$ represents the new apparent third-order rate constant with second-order respect to ozone concentration[7].

By separating variables and integrating, Equation 4.20 can be rewritten as:

$$\ln \frac{[Ery]}{[Ery]_0} = -k''_{O_3,app,Ery} \int [O_3]^2 dt \qquad \text{(Equation 4.21)}$$

Instead of the "ozone exposure" as in the case of first-order reaction to ozone, a "second-order ozone exposure" $\int [O_3]^2 dt$ appears on the right side of the equation.

The calculation of second-order ozone exposure still follows trapezoidal rule[8]:

$$\int [O_3]^2 dt = \sum_{i=1}^{N} \left(\frac{[O_3]_i + [O_3]_{i+1}}{2}\right)^2 (t_{i+1} - t_i) \qquad \text{(Equation 4.22)}$$

By plotting ln([Ery]/[Ery]$_0$) against second-order ozone exposure, $k''_{O_3,app,Ery}$ is the slope of linear regression. Figure 4.15 shows the results when second-order ozone exposure was applied to the experiments data.

7 To differentiate the two rate constants, double prime was used in the expression of the third-order rate constant.

8 There are two ways to calculate second-order ozone exposure and is explained in Appendix D in detail.

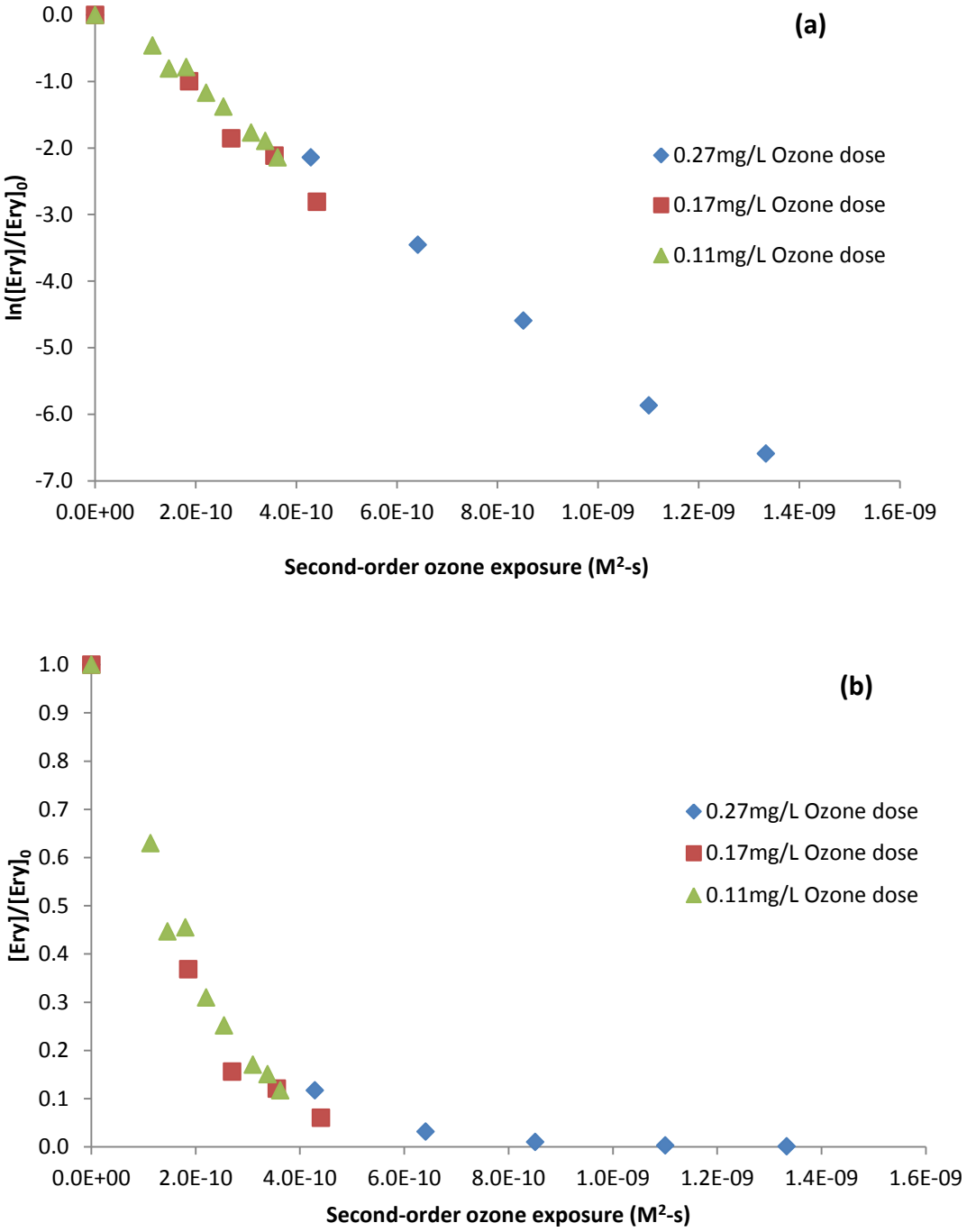

Figure 4.15: Effect of ozone initial dose on erythromycin degradation when second-order ozone exposure applied (a) y-axis being ln([Ery]/[Ery]$_0$); (b) y-axis being [Ery]/[Ery]$_0$. (pH=6, 0 mM total carbonate, 1 mM buffer, 1 mg/L erythromycin).

When second-order ozone exposure was applied, results from the three experiments at different initial ozone concentrations were consistent; that is the rate constant between erythromycin and second order ozone exposure was constant. The apparent third-order rate constants between erythromycin and ozone at 0.11, 0.17, and 0.27 mg/L ozone dose was 5.17×10^9, 5.53×10^9 and 6.26×10^9 $M^2\cdot s^{-1}$ with a difference of less than 10%. This indicates that second-order ozone exposure is a reasonable possibility to explain the effect of initial ozone dose on erythromycin degradation—that is the reaction between ozone and erythromycin is actually a second-order reaction with respect to ozone.

In order to test the validity of this hypothesis, further experiments are needed. It is recommended that several parallel experiments be conducted with the addition of t-butanol at the same pH (i.e., pH=6) but with different initial ozone doses to determine whether the rate constants differ significantly. If the results show that the rate constants are similar, then the results support the hypothesis that in the presence of hydroxyl radicals, reaction between erythromycin and ozone follow a second-order kinetic model with respect to ozone while in the absence of hydroxyl radicals, the reaction between erythromycin and ozone is first-order with respect to ozone.

4.7 SUMMARY

This research studied the kinetics of degradation of erythromycin by ozonation and the impacts of pH, carbonate and phosphate buffers, and initial ozone dose on the performance of erythromycin degradation.

By conducting experiments at varying pH, protonated erythromycin was found to be inert to ozone and the second-order rate constant between deprotonated erythromycin and ozone was calculated to be 4.44×10^6 $M^{-1}\cdot s^{-1}$. Based on this value, the apparent

second-order rate constant between erythromycin and ozone at any pH can be estimated with a value of 6.93×10^4 $M^{-1}\cdot s^{-1}$ at pH 7. Additional experiments verified the assumption that carbonate buffer would not affect the degradation of erythromycin by ozone.

Carbonate and phosphate buffers are reported to be ozone decomposition inhibitors. The effect of carbonate buffer on ozone decomposition was also studied using the R_{ct} concept and hydroxyl radical exposure. The results verified that the impacts of carbonate and phosphate buffers on the overall oxidation of erythromycin by ozonation were negligible, as the total rate constant differed by only 10% or less under a variety of conditions for these acid/base systems. Moreover, the fact that *p*CBA concentration did not change during the experiment led to the conclusion that ozone was the primary oxidant at erythromycin oxidation.

Initial ozone dose is also reported to affect ozone decomposition rate and the results from ozone dose experiments did show a significant impact of ozone dose on the removal rate of erythromycin by ozonation. An overall third-order reaction model, with second-order with respect to ozone, was proposed to successfully explain the effect of ozone dose on erythromycin oxidation by ozonation. This second-order ozone exposure also successfully described data from the carbonate and phosphate buffers experiments but did not adequately describe the impact of pH. The failure of applying this model to the data from the set of experiments conducted at different pH suggests that further research is required to evaluate the impact of ozone dose.

Chapter 5 Conclusion

5.1 Restatement of Objectives and Approach

This research was dedicated to investigate the oxidation of erythromycin by ozonation and the effects of various natural water parameters as well as initial ozone dose—a key operating parameter. One objective of this study was to obtain the rate constant for the reaction between erythromycin and ozone, which has not previously been reported. pH, carbonate and phosphate buffers, and initial ozone dose were the variables examined in this study because of their importance and ubiquitous presence.

All experiments were conducted in a batch reactor initiated by the addition of a certain amount of the aqueous ozone stock solution at time zero to a solution containing erythromycin, the selected buffers and *p*CBA or t-butanol per the experimental matrix. For each set of experiments, only one parameter was chosen as the variable while all the other parameters were maintained at identical values. Samples were taken at recorded times and the concentrations of ozone, erythromycin, and *p*CBA were measured. Self-degradation of erythromycin was shown to be an insignificant issue during the course of individual experiments; however, self-degradation in the stock solution employed during the experiments should be accounted for when the results presented in this thesis are being considered.

The data from the experiments were modeled using a second-order reaction model, first order with respect to oxidant (ozone and hydroxyl radicals) and erythromycin. The majority of conclusions drawn from these results are derived from comparisons among the second-order rate constants obtained by fitting the model to individual data sets.

5.2 CONCLUSIONS

Major results and conclusions from this research are outlined as below:

1. Ozone only reacts with deprotonated erythromycin; the reaction between ozone and protonated erythromycin can be neglected.
2. The apparent rate constant for the reaction between ozone and erythromycin increased by a factor of 10 per unit pH increase at pH values well below the pK_a of erythromycin due to the deprotonation of erythromycin at higher pH.
3. For the oxidation of erythromycin by ozonation, ozone is the primary oxidant; the oxidation caused by hydroxyl radicals is minor. Therefore, there is no need to apply advanced oxidation processes to remove erythromycin since ozone oxidation itself is very effective.
4. Although carbonate and phosphate buffers are known to impact ozone decomposition, their effects on the overall erythromycin degradation by ozonation are negligible.
5. The application of the R_{ct} concept (the ratio of hydroxyl radical exposure to ozone exposure) was limited in this research because R_{ct} could not be quantified before erythromycin was completely degraded by ozone.
6. Initial ozone dose has a positive impact on erythromycin degradation by ozonation. The effect was modeled empirically by second-order ozone exposure, although the underlying cause of these results is not understood at this time.

5.3 FUTURE WORK

Recommendations for future work are outlined as below:

1. Additional experiments should be done to test the proposed hypothesis that the reaction between erythromycin and ozone is first-order with respect to

ozone in the absence of hydroxyl radicals and is second-order with respect to ozone in the presence of hydroxyl radicals. It is recommended to conduct several experiments at constant pH in the presence of t-butanol but with varied initial ozone doses to determine whether a single rate constant can adequately describe the data collected at different ozone doses.

2. The method employed in this research failed to obtain the value of the rate constant between erythromycin and hydroxyl radical because the R_{ct} concept was not applicable. Therefore, in order to obtain the rate constant between erythromycin and hydroxyl radicals, alternative oxidation processes are recommended for generation of hydroxyl radicals, for example, H_2O_2/UV or H_2O_2 photolysis.
3. Although ozonation is very effective at removing erythromycin in the original form, oxidation by-products were not investigated in this research. Therefore, it would be valuable to track the formation of oxidation by-products and assess the potential for these intermediates to exhibit anti-microbial activity.

Appendix A Calibration Curve of erythromycin-H_2O

As mentioned in Section 2.2.3, erythromycin-H_2O was measured instead of erythromycin. The total length of detection was 18 min. The peak of erythromycin-H_2O occurred around 15.5 min and there was some noise afterwards (see Figure A.1). The area ratio of noise to response increased as concentration increased.

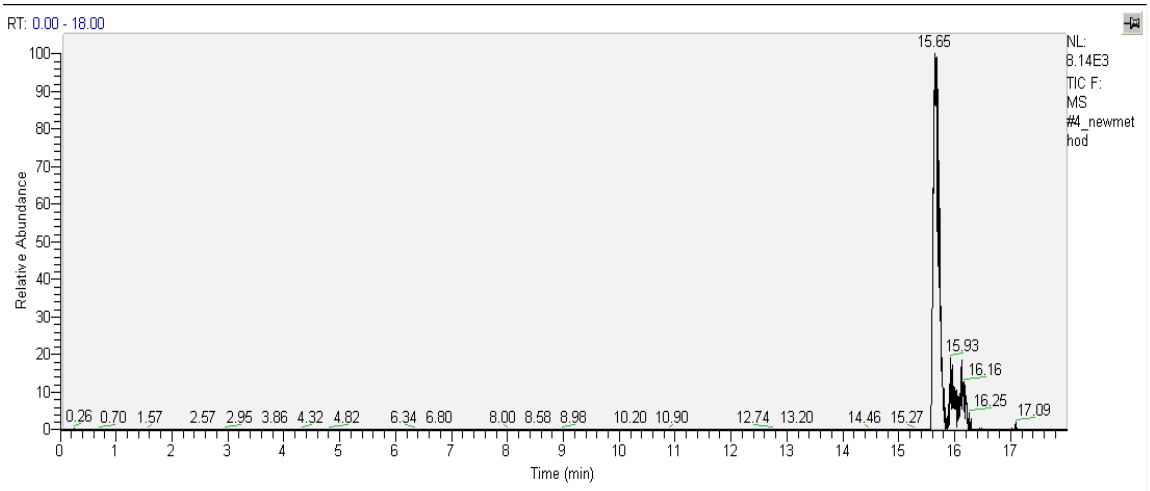

Figure A.1: Illustration of LC/MS response for erythromycin-H_2O detection

Standard solution was prepared under exactly same condition as samples. The data for standard solution is shown in Table A.1 and calibration curve is shown in Figure A.2.

Table A.1: LC/MS response for erythromycin-H_2O standard solution

Concentration (ppb)	Response	Noise	Noise/Response
0	17266	1172	6.79%
0.05	79659	8755	10.99%
0.1	153520	20303	13.22%
0.5	668738	106558	15.93%
0.9	1309921	198585	15.16%

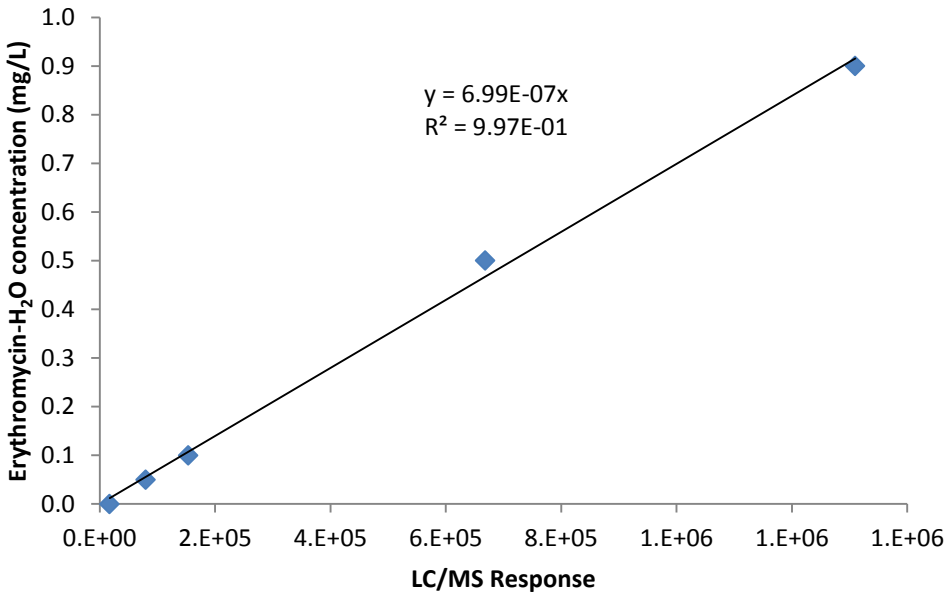

Figure A.2: Calibration curve of erythryomycin-H_2O by LC/MS

Appendix B Calibration Curve of *p*CBA

The data for standard pCBA solution is shown in Table B.1 and calibration curve is shown in Figure B.1.

Table B.1: HPLC response for *p*CBA standard solution

Concentration (μM)	HPLC Response
0	0
0.05	4255
0.1	7769
0.5	42048
0.9	77460

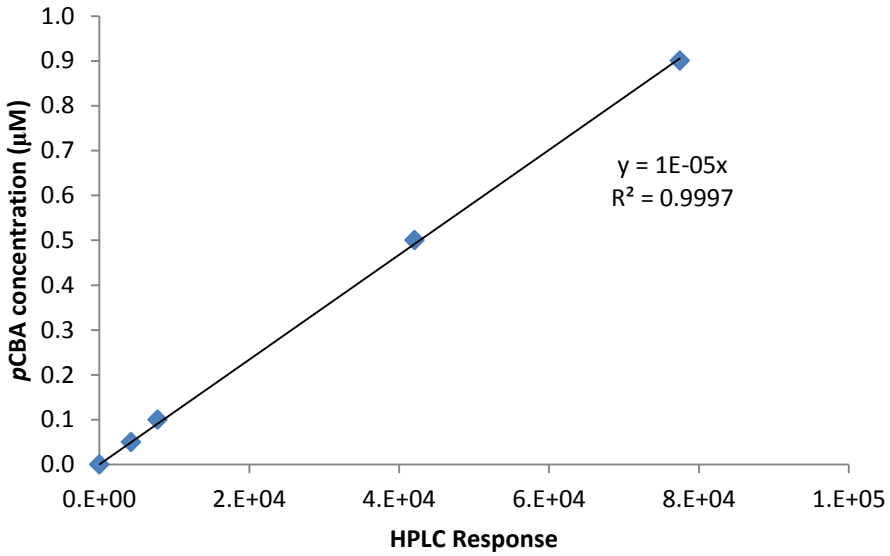

Figure B.1: Calibration curve of *p*CBA

Appendix C Comparison of reaction between erythromycin and ozone and reaction between *p*CBA and ozone

At pH 6, erythromycin reacts with ozone at a second-order rate constant of about 7000 $M^{-1}\cdot s^{-1}$. Meanwhile, it is estimated that *p*CBA reacts with ozone indirectly at a second-order rate constant of about 52 $M^{-1}\cdot s^{-1}$.

Therefore, the normalized remaining erythromycin and *p*CBA can be calculated as:

$$\frac{[Ery]}{[Ery]_0} = \exp(-7000 \times \int [O_3]dt) \quad \text{(Equation C.1)}$$

$$\frac{[pCBA]}{[pCBA]_0} = \exp(-52 \times \int [O_3]dt) \quad \text{(Equation C. 2)}$$

Figure C.1 and Figure C.2 show the relative reaction of erythromycin and *p*CBA with ozone with no noticeable *p*CBA change in first case and with noticeable *p*CBA change in second case.

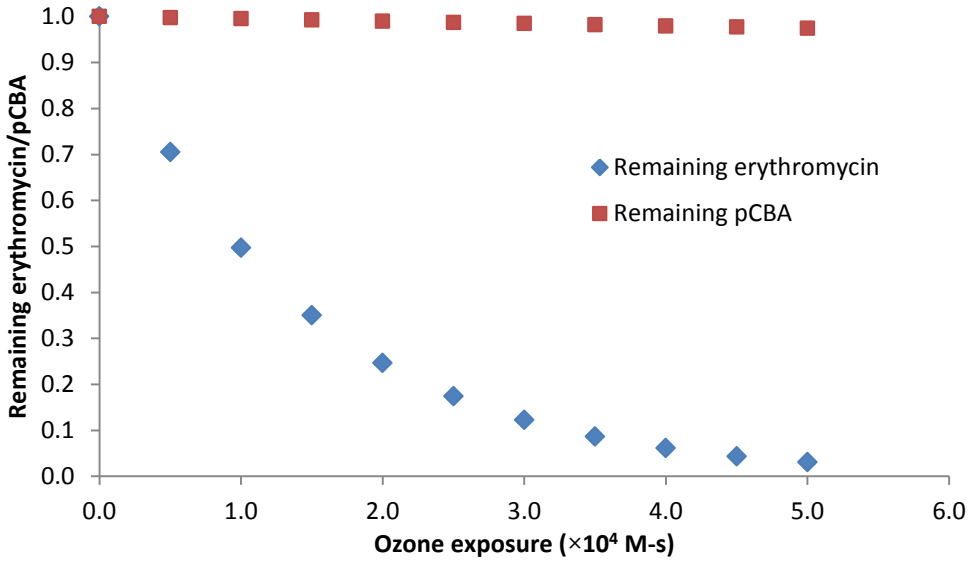

Figure C.1: Relative reaction of erythromycin and pCBA with ozone under no noticeable pCBA change

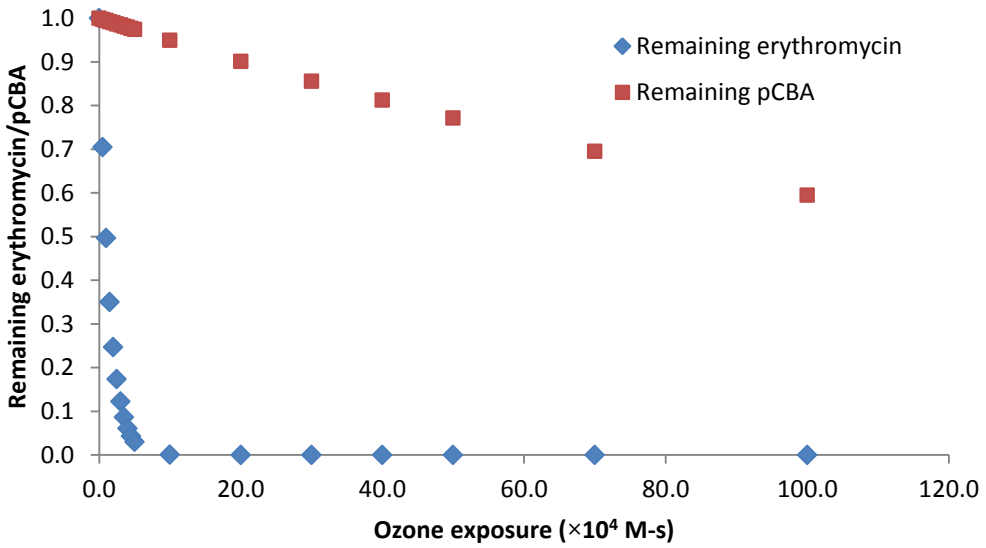

Figure C.2: Relative reaction of erythromycin and *p*CBA with ozone under no noticeable *p*CBA change

From the above two figures, it is clear that if erythromycin concentration is tracked till less than 5%, pCBA remains unchanged; if noticeable pCBA change is required, erythromycin is gone immediately. In other words, it is impossible to track the change of erythromycin and pCBA at the same time.

Appendix D Second-order ozone exposure

Second-order ozone exposure $\int [O_3]^2 dt$ can be calculated in two ways. The first way is shown by Equation 4.22:

$$\int [O_3]^2 dt = \sum_{i=1}^{N} \left(\frac{[O_3]_i + [O_3]_{i+1}}{2}\right)^2 (t_{i+1} - t_i) \qquad \text{(Equation 4.20)}$$

The other way to calculate second-order ozone exposure is:

$$\int [O_3]^2 dt = \sum_{i=1}^{N} \frac{[O_3]_i^2 + [O_3]_{i+1}^2}{2} (t_{i+1} - t_i) \qquad \text{(Equation D.1)}$$

Comparing this two second-order ozone exposure, the first way is always less than the second way by a value of $\frac{([O_3]_i - [O_3]_{i+1})^2}{4}(t_{i+1} - t_i)$.

However, this difference is fully negligible since ozone concentration during the experiments was very small (less than 10^{-5} M); the difference between two ozone concentration is even smaller. Therefore, the second-order ozone exposure calculated by two ways is identical (shown by Figure D.1).

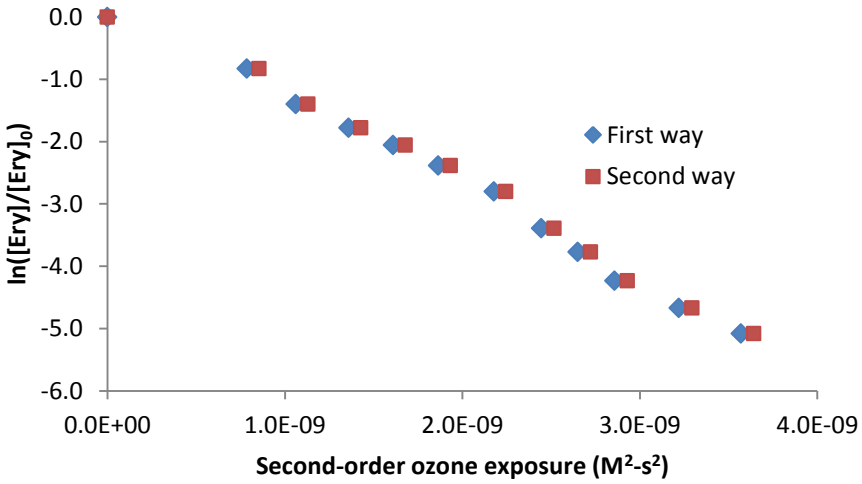

Figure D.1: Two ways of calculating second-order ozone exposure (pH=6, 1 mM buffer, 1 mg/L erythromycin, negligible carbonate, 10 mM t-butanol)

References

Aherne, G., English, J. & Marks, V. 1985, "The role of immunoassay in the analysis of microcontaminants in water samples", *Ecotoxicology and environmental safety,* vol. 9, no. 1, pp. 79-83.

Andreozzi, R., Canterino, M., Giudice, R.L., Marotta, R., Pinto, G. & Pollio, A. 2006, "Lincomycin solar photodegradation, algal toxicity and removal from wastewaters by means of ozonation", *Water research,* vol. 40, no. 3, pp. 630-638.

Atkins, P.J., Herbert, T.O. & Jones, N.B. 1986, "Kinetic studies on the decomposition of erythromycin A in aqueous acidic and neutral buffers", *International journal of pharmaceutics,* vol. 30, no. 2-3, pp. 199-207.

Beers, M.H. & Berkow, R. 1999, *The Merck manual of diagnosis and therapy.* Merck and Co. Inc.

Boreen, A.L., Arnold, W.A. & McNeill, K. 2004, "Photochemical fate of sulfa drugs in the aquatic environment: Sulfa drugs containing five-membered heterocyclic groups", *Environ.Sci.Technol,* vol. 38, no. 14, pp. 3933-3940.

Boyd, G.R., Reemtsma, H., Grimm, D.A. & Mitra, S. 2003, "Pharmaceuticals and personal care products (PPCPs) in surface and treated waters of Louisiana, USA and Ontario, Canada", *The Science of the total environment,* vol. 311, no. 1-3, pp. 135-149.

Broseus, R., Vincent, S., Aboulfadl, K., Daneshvar, A., Sauve, S., Barbeau, B. & Prevost, M. 2009, "Ozone oxidation of pharmaceuticals, endocrine disruptors and pesticides during drinking water treatment", *Water research,* vol. 43, no. 18, pp. 4707-4717.

Bruchet, A., Hochereau, C., Picard, C., Decottignies, V., Rodrigues, J. & Janex-Habibi, M. 2005, "Analysis of drugs and personal care products in French source and drinking waters: the analytical challenge and examples of application", *Water science and technology,* , pp. 53-61.

Buffle, M.O., Schumacher, J., Meylan, S., Jekel, M. & von Gunten, U. 2006a, "Ozonation and Advanced Oxidation of Wastewater: Effect of O 3 Dose, pH, DOM and HO•-Scavengers on Ozone Decomposition and HO• Generation", *Ozone: Science & Engineering,* vol. 28, no. 4, pp. 247-259.

Buffle, M.O., Schumacher, J., Salhi, E., Jekel, M. & Von Gunten, U. 2006b, "Measurement of the initial phase of ozone decomposition in water and wastewater

by means of a continuous quench-flow system: Application to disinfection and pharmaceutical oxidation", *Water research,* vol. 40, no. 9, pp. 1884-1894.

Buffle, M.O. & von Gunten, U. 2006, "Phenols and Amine Induced HO• Generation During the Initial Phase of Natural Water Ozonation", *Environ.Sci.Technol,* vol. 40, no. 9, pp. 3057-3063.

Buxton, G.V., Greenstock, C.L., Helman, W.P. & Ross, A.B. 1988, "Critical review of rate constants for reactions of hydrated electrons, hydrogen atoms and hydroxyl radicals", *Phys.Chem.Ref.Data,* vol. 17, pp. 513-886.

Cachet, T., Van den Mooter, G., Hauchecorne, R., Vinckier, C. & Hoogmartens, J. 1989, "Decomposition kinetics of erythromycin A in acidic aqueous solutions", *International journal of pharmaceutics,* vol. 55, no. 1, pp. 59-65.

Coyne, R., Hiney, M., O'Connor, B., Kerry, J., Cazabon, D. & Smith, P. 1994, "Concentration and persistence of oxytetracycline in sediments under a marine salmon farm", *Aquaculture,* vol. 123, no. 1-2, pp. 31-42.

Crittenden, J.C., Harza, M.W. & Knovel (Firm) 2005, *Water treatment: principles and design,* J. Wiley.

Daughton, C.G., 2010, "Pharmaceutical ingredients in drinking water: Overview of occurrence and significance of human exposure", Chapter 2 in Contaminants of Emerging Concern in Environment: Ecological and Human Health Considerations, ACS Symposium Series 1048, pp. 9-68.

Daughton, C.G. & Ternes, T.A. 1999, "Pharmaceuticals and personal care products in the environment: Agents of subtle change?", *Environmental health perspectives,* vol. 107, pp. 907-938.

De Witte, B., Dewulf, J., Demeestere, K. & Van Langenhove, H. 2009, "Ozonation and advanced oxidation by the peroxone process of ciprofloxacin in water", *Journal of hazardous materials,* vol. 161, no. 2-3, pp. 701-708.

Desbrow, C., Routledge, E., Brighty, G., Sumpter, J. & Waldock, M. 1998, "Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening", *Environ.Sci.Technol,* vol. 32, no. 11, pp. 1549-1558.

Dodd, M.C., Buffle, M.O. & von Gunten, U. 2006, "Oxidation of antibacterial molecules by aqueous ozone: moiety-specific reaction kinetics and application to ozone-based

wastewater treatment", *Environmental science & technology,* vol. 40, no. 6, pp. 1969-1977.

Doll, T.E. & Frimmel, F.H. 2003, "Fate of pharmaceuticals--photodegradation by simulated solar UV-light", *Chemosphere,* vol. 52, no. 10, pp. 1757-1769.

Drewes, J.E. 2007, "Removal of pharmaceutical residues during wastewater treatment", *Comprehensive Analytical Chemistry,* vol. 50, pp. 427-449.

Eckel, W.P., Ross, B. & Isensee, R.K. 1993, "Pentobarbital found in ground water", *Ground Water,* vol. 31, no. 5, pp. 801-804.

Elovitz, M.S. & von Gunten, U. 1999, "Hydroxyl radical/ozone ratios during ozonation processes. I. The Rct concept", *Ozone: Science & Engineering,* vol. 21, no. 3, pp. 239-260.

Elovitz, M.S., von Gunten, U. & Kaiser, H.P. 2000, "Hydroxyl radical/ozone ratios during ozonation processes. II. The effect of temperature, pH, alkalinity, and DOM properties", *Ozone: Science & Engineering,* vol. 22, no. 2, pp. 123-150.

Fatta, D., Achilleos, A., Nikolaou, A. & Meriē, S. 2007, "Analytical methods for tracing pharmaceutical residues in water and wastewater", *TrAC Trends in Analytical Chemistry,* vol. 26, no. 6, pp. 515-533.

Gobel, A., McArdell, C.S., Joss, A., Siegrist, H. & Giger, W. 2007, "Fate of sulfonamides, macrolides, and trimethoprim in different wastewater treatment technologies", *Science of the total environment,* vol. 372, no. 2-3, pp. 361-371.

Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P., Ingerslev, F., Holten Lützhøft, H. & Jørgensen, S. 1998, "Occurrence, fate and effects of pharmaceutical substances in the environment-a review", *Chemosphere,* vol. 36, no. 2, pp. 357-393.

Hirsch, R., Ternes, T., Haberer, K. & Kratz, K.L. 1999, "Occurrence of antibiotics in the aquatic environment", *The Science of the total environment,* vol. 225, no. 1-2, pp. 109-118.

Hoigne, J. & Bader, H. 1976, "The role of hydroxyl radical reactions in ozonation processes in aqueous solutions", *Water research,* vol. 10, no. 5, pp. 377-386.

Hoigné, J. & Bader, H. 1983, "Rate constants of reactions of ozone with organic and inorganic compounds in water—I: Non-dissociating organic compounds", *Water research,* vol. 17, no. 2, pp. 173-183.

Holm, J.V., Ruegge, K., Bjerg, P.L. & Christensen, T.H. 1995, "Occurrence and distribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill (Grindsted, Denmark)", *Environmental science & technology,* vol. 29, no. 5, pp. 1415-1420.

Høverstad, T., Carlstedt-Duke, B., Lingaas, E., Norin, E., Saxerholt, H., Steinbakk, M. & Midtvedt, T. 1986, "Influence of oral intake of seven different antibiotics on faecal short-chain fatty acid excretion in healthy subjects", *Scandinavian journal of gastroenterology,* vol. 21, no. 8, pp. 997-1003.

Huber, M.M., Canonica, S., Park, G.Y. & Von Gunten, U. 2003, "Oxidation of pharmaceuticals during ozonation and advanced oxidation processes", *Environmental science & technology,* vol. 37, no. 5, pp. 1016-1024.

Huber, M.M., GÖbel, A., Joss, A., Hermann, N., LÖffler, D., McArdell, C.S., Ried, A., Siegrist, H., Ternes, T.A. & von Gunten, U. 2005, "Oxidation of pharmaceuticals during ozonation of municipal wastewater effluents: a pilot study", *Environmental science & technology,* vol. 39, no. 11, pp. 4290-4299.

Huerta-Fontela, M., Galceran, M.T. & Ventura, F. 2010, "Occurrence and removal of pharmaceuticals and hormones through drinking water treatment", *Water research,* .

Ikehata, K., Naghashkar, N.J. & El-Din, M.G. 2006, "Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: A review", *Ozone: Science & Engineering,* vol. 28, no. 6, pp. 353-414.

Jasim, S.Y., Irabelli, A., Yang, P., Ahmed, S. & Schweitzer, L. 2006, "Presence of pharmaceuticals and pesticides in Detroit river water and the effect of ozone on removal", *Ozone: Science & Engineering,* vol. 28, no. 6, pp. 415-423.

Javier Benitez, F., Acero, J.L., Real, F.J. & García, J. 2003, "Kinetics of photodegradation and ozonation of pentachlorophenol", *Chemosphere,* vol. 51, no. 8, pp. 651-662.

Jones, O., Voulvoulis, N. & Lester, J. 2005, "Human pharmaceuticals in wastewater treatment processes", *Critical Reviews in Environmental Science and Technology,* vol. 35, no. 4, pp. 401-427.

Kanfer, I., Skinner, M.F. & Walker, R.B. 1998, "Analysis of macrolide antibiotics", *Journal of Chromatography A,* vol. 812, no. 1-2, pp. 255-286.

Kasprzyk-Hordern, B., Dinsdale, R.M. & Guwy, A.J. 2009, "The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during

wastewater treatment and its impact on the quality of receiving waters", *Water research,* vol. 43, no. 2, pp. 363-380.

Kasprzyk-Hordern, B., Ziķek, M. & Nawrocki, J. 2003, "Catalytic ozonation and methods of enhancing molecular ozone reactions in water treatment", *Applied Catalysis B: Environmental,* vol. 46, no. 4, pp. 639-669.

Kerry, J., Hiney, M., Coyne, R., NicGabhainn, S., Gilroy, D., Cazabon, D. & Smith, P. 1995, "Fish feed as a source of oxytetracycline-resistant bacteria in the sediments under fish farms", *Aquaculture,* vol. 131, no. 1-2, pp. 101-113.

Kim, I. & Tanaka, H. 2010, "Use of Ozone-Based Processes for the Removal of Pharmaceuticals Detected in a Wastewater Treatment Plant", *Water Environment Research,* vol. 82, no. 4, pp. 294-301.

Kim, S. & Aga, D.S. 2007, "Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants", *Journal of Toxicology and Environmental Health, Part B,* vol. 10, no. 8, pp. 559-573.

Kim, Y.H., Heinze, T.M., Beger, R., Pothuluri, J.V. & Cerniglia, C.E. 2004, "A kinetic study on the degradation of erythromycin A in aqueous solution", *International journal of pharmaceutics,* vol. 271, no. 1-2, pp. 63-76.

Klavarioti, M., Mantzavinos, D. & Kassinos, D. 2009, "Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes", *Environment international,* vol. 35, no. 2, pp. 402-417.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B. & Buxton, H.T. 2002, "Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999− 2000: A national reconnaissance", *Environ.Sci.Technol,* vol. 36, no. 6, pp. 1202-1211.

Kümmerer, K. 2009, "The presence of pharmaceuticals in the environment due to human use-present knowledge and future challenges", *Journal of environmental management,* vol. 90, no. 8, pp. 2354-2366.

Lalumera, G.M., Calamari, D., Galli, P., Castiglioni, S., Crosa, G. & Fanelli, R. 2004, "Preliminary investigation on the environmental occurrence and effects of antibiotics used in aquaculture in Italy", *Chemosphere,* vol. 54, no. 5, pp. 661-668.

Lange, F., Cornelissen, S., Kubac, D., Sein, M.M., Von Sonntag, J., Hannich, C.B., Golloch, A., Heipieper, H.J., Moeder, M. & Von Sonntag, C. 2006, "Degradation of

macrolide antibiotics by ozone: A mechanistic case study with clarithromycin", *Chemosphere,* vol. 65, no. 1, pp. 17-23.

Langlais, B., Reckhow, D.A. & Brink, D.R. 1991, *Ozone in water treatment: Application and engineering: Cooperative research report,* CRC.

Lee, W.Y. & Arnold, C. 1983, "Chronic toxicity of ocean-dumped pharmaceutical wastes to the marine amphipod Amphithoe valida", *Marine pollution bulletin,* vol. 14, no. 4, pp. 150-153.

Lin, A.Y.C., Yu, T.H. & Lin, C.F. 2008, "Pharmaceutical contamination in residential, industrial, and agricultural waste streams: Risk to aqueous environments in Taiwan", *Chemosphere,* vol. 74, no. 1, pp. 131-141.

Marron, C.A., 2010, "Kinetics of ciprofloxacin degradation by ozonation: effects of natural organic matter, the carbonate system, and pH", unpublished thesis, University of Texas at Austin

Masschelein, W. 1992, *Unit processes in drinking water treatment,* CRC.

McArdell, C.S., Molnar, E., Marc, J.F.S. & Giger, W. 2003, "Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley Watershed, Switzerland", *Environ.Sci.Technol,* vol. 37, no. 24, pp. 5479-5486.

McGuire, J., Bunch, R., Anderson, R., Boaz, H., Flynn, E., Powell, H. & Smith, J. 1952, "Blotycin,'a new antibiotic", *Antibiotics and Chemotherapy,* vol. 2, pp. 281.

Metcalfe, C.D., Miao, X.S., Koenig, B.G. & Struger, J. 2003, "Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower Great Lakes, Canada", *Environmental toxicology and chemistry,* vol. 22, no. 12, pp. 2881-2889.

Mizuno, T., Tsuno, H. & Yamada, H. 2007, "Development of ozone self-decomposition model for engineering design", *Ozone: Science and Engineering,* vol. 29, no. 1, pp. 55-63.

Mompelat, S., Le Bot, B. & Thomas, O. 2009, "Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water", *Environment international,* vol. 35, no. 5, pp. 803-814.

Morozov, P. & Ershov, B. 2010, "The influence of phosphates on the decomposition of ozone in water: Chain process inhibition", *Russian Journal of Physical Chemistry A, Focus on Chemistry,* vol. 84, no. 7, pp. 1136-1140.

Muñoz, F., Mvula, E., Braslavsky, S.E. & von Sonntag, C. 2001, "Singlet dioxygen formation in ozone reactions in aqueous solution", *Journal of the Chemical Society, Perkin Transactions 2,* , no. 7, pp. 1109-1116.

Muñoz, F. & von Sonntag, C. 2000, "The reactions of ozone with tertiary amines including the complexing agents nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) in aqueous solution", *J.Chem.Soc., Perkin Trans.2,* , no. 10, pp. 2029-2033.

Nakada, N., Shinohara, H., Murata, A., Kiri, K., Managaki, S., Sato, N. & Takada, H. 2007, "Removal of selected pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) during sand filtration and ozonation at a municipal sewage treatment plant", *Water research,* vol. 41, no. 19, pp. 4373-4382.

Pi, Y., Schumacher, J. & Jekel, M. 2005, "Decomposition of aqueous ozone in the presence of aromatic organic solutes", *Water research,* vol. 39, no. 1, pp. 83-88.

Pothuluri, J., Nawaz, M. & Cerniglia, C. 1998, "Environmental fate of antibiotics used in aquaculture", *Bioremediation: Principles and practice,* vol. 2, pp. 221-248.

Qiang, Z., Adams, C. & Surampalli, R. 2004, "Determination of ozonation rate constants for lincomycin and spectinomycin", *Ozone: Science & Engineering,* vol. 26, no. 6, pp. 525-537.

Radjenovic, J., Petrovic, M. & Barceló, D. 2007, "Advanced mass spectrometric methods applied to the study of fate and removal of pharmaceuticals in wastewater treatment", *TrAC Trends in Analytical Chemistry,* vol. 26, no. 11, pp. 1132-1144.

Rodríguez, R., Alvarez-Lorenzo, C. & Concheiro, A. 2003, "Interactions of ibuprofen with cationic polysaccharides in aqueous dispersions and hydrogels:: Rheological and diffusional implications", *European Journal of Pharmaceutical Sciences,* vol. 20, no. 4-5, pp. 429-438.

Routledge, E., Sheahan, D., Desbrow, C., Brighty, G., Waldock, M. & Sumpter, J. 1998, "Identification of estrogenic chemicals in STW effluent. 2. In vivo responses in trout and roach", *Environ.Sci.Technol,* vol. 32, no. 11, pp. 1559-1565.

Schwab, B.W., Hayes, E.P., Fiori, J.M., Mastrocco, F.J., Roden, N.M., Cragin, D., Meyerhoff, R.D., D'Aco, V.J. & Anderson, P.D. 2005, "Human pharmaceuticals in US surface waters: A human health risk assessment", *Regulatory Toxicology and Pharmacology,* vol. 42, no. 3, pp. 296-312.

Sotelo, J.L., Beltran, F.J., Benitez, F.J. & Beltran-Heredia, J. 1987, "Ozone decomposition in water: kinetic study", *Industrial & Engineering Chemistry Research,* vol. 26, no. 1, pp. 39-43.

Staehelin, J. & Hoigne, J. 1985, "Decomposition of ozone in water in the presence of organic solutes acting as promoters and inhibitors of radical chain reactions", *Environmental science & technology,* vol. 19, no. 12, pp. 1206-1213.

Ternes, T.A. 1998, "Occurrence of drugs in German sewage treatment plants and rivers", *Water research,* vol. 32, no. 11, pp. 3245-3260.

Ternes, T.A. 2001, "Analytical methods for the determination of pharmaceuticals in aqueous environmental samples", *TrAC Trends in Analytical Chemistry,* vol. 20, no. 8, pp. 419-434.

Ternes, T.A. & Hirsch, R. 2000, "Occurrence and behavior of X-ray contrast media in sewage facilities and the aquatic environment", *Environmental science & technology,* vol. 34, no. 13, pp. 2741-2748.

Ternes, T.A., Meisenheimer, M., McDowell, D., Sacher, F., Brauch, H.J., Haist-Gulde, B., Preuss, G., Wilme, U. & Zulei-Seibert, N. 2002, "Removal of pharmaceuticals during drinking water treatment", *Environmental science & technology,* vol. 36, no. 17, pp. 3855-3863.

Ternes, T.A., Stuber, J., Herrmann, N., McDowell, D., Ried, A., Kampmann, M. & Teiser, B. 2003, "Ozonation: a tool for removal of pharmaceuticals, contrast media and musk fragrances from wastewater?", *Water research,* vol. 37, no. 8, pp. 1976-1982.

Volmer, D.A. & Hui, J.P.M. 1998, "Study of erythromycin A decomposition products in aqueous solution by solid-phase microextraction/liquid chromatography/tandem mass spectrometry", *Rapid Communications in Mass Spectrometry,* vol. 12, no. 3, pp. 123-129.

Von Gunten, U. 2003, "Ozonation of drinking water: Part I. Oxidation kinetics and product formation", *Water research,* vol. 37, no. 7, pp. 1443-1467.

Warman, P. & Thomas, R. 1981, "Chlortetracycline in soil amended with poultry manure", *Canadian Journal of Soil Science,* vol. 61, no. 1, pp. 161-163.

Watts, C., Crathorne, B., Fielding, M. & Steel, C. 1983, "Identification of non-volatile organics in water using field desorption mass spectrometry and high performance

liquid chromatography", *Analysis of Organic Micropollutants in Water (Angeletti G, Bjorseth A, eds).Dordrecht, the Netherlands: D.Reidel Publishing Company,* .

Wert, E.C., Rosario-Ortiz, F.L. & Snyder, S.A. 2009, "Effect of ozone exposure on the oxidation of trace organic contaminants in wastewater", *Water research,* vol. 43, no. 4, pp. 1005-1014.

Westerhoff, P., Aiken, G., Amy, G. & Debroux, J. 1999, "Relationships between the structure of natural organic matter and its reactivity towards molecular ozone and hydroxyl radicals", *Water research,* vol. 33, no. 10, pp. 2265-2276.

Westerhoff, P., Yoon, Y., Snyder, S. & Wert, E. 2005, "Fate of endocrine-disruptor, pharmaceutical, and personal care product chemicals during simulated drinking water treatment processes", *Environmental science & technology,* vol. 39, no. 17, pp. 6649-6663.

Zuccato, E., Calamari, D., Natangelo, M. & Fanelli, R. 2000, *The Lancet,* vol. 355, no. 9217, pp. 1789-1790.