The Mechanisms, Products, and Kinetic of Carbamazepine-Free Chlorine Reactions

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(ABSTRACT)

Carbamazepine (CBZ) is an antiepileptic drug widely detected in drinking water supplies and wastewater effluent. It has been studied that CBZ is recalcitrant to biological removal processes. Therefore; active CBZ will expose into disinfection process which most treatment plants are using chlorination in United States. However; the chlorination mechanisms of CBZ have not been fully investigated and understood. Our experimental studies were conducted to examine the chlorination of CBZ under controlled conditions. The kinetics, products, and reactivity of CBZ/free chlorine reactions were investigated for the pH range 5.5-10. Results show that free chlorine reacts to CBZ and the reactivity is pH dependent. The simple kinetic models were developed for both free chlorine and CBZ loss. Furthermore; results indicate that temperature affects to the reactivity between CBZ and free chlorine. The results were applied to the Arrhenius equation. The calculated E_a and A values are 48.8 KJ/mol and $1.41 \times 10^4 s^{-1}$, respectively. Four common intermediates were detected based on both UV and mass spectral analysis and proposed structures were developed based on m/z from mass spectra.

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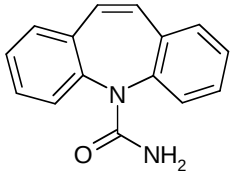
Chapter 1: Literature review

1.1: Background

Carbamazepine (5*H*-dibenzo [*b,f*]azepine-5-carboxamide) is a highly polar pharmaceutical compound that is commonly used as an anticonvulsant and mood stabilizing drug [1-4]. This compound was first manufactured in 1953 by Walter Schindler and its anti-epileptic properties were discovered in 1960 [5]. By the early 1970s, carbamazepine (CBZ) was being used to treat epilepsy via oral ingestion [5]. Other maladies such as personality disorder were found to be treatable by CBZ in later years and CBZ has become widely used in many countries. Studies have shown that CBZ use has expanded dramatically over the last decade [5-8] with the dosage (200-1600 mg per day) dependent on the age of the patient [9]. Reports also indicate that newer anti-epileptic drugs (AED), such as lamotrigine, are less effective than traditional medicines, such as CBZ [10], suggesting that CBZ usage will remain high. In 1997, the consumed quantities for CBZ were 6334 kg per year in Austria, [9] while for 2003 the amount of CBZ used in South Korea was estimated to be 9155 kg [11].

Once administered, the absorption of CBZ in the body is slow and unpredictable with approximately 2-3% of the applied dose discharged via urine [9]. Studies indicate the CBZ concentration in the blood plasma peaks about 4 to 8 hours after ingestion, but it may take up to 26 hours for the CBZ to take effect [12]. CBZ is metabolized by the cytochrome P450 system in the liver, producing several metabolites [12]. These metabolites can act to inhibit the pharmaceutically active form of CBZ. Other drugs can also interact with CBZ by affecting cytochrome P450 3A4 (CYP3A4) activity [12]. Some of these interactions, for example the usage of simethicone with CBZ, may result in toxicity to patients [12-14].

Table 1: Structure and properties of Carbamazepine.

Compound	Chemical Formula; CAS name	Structure	Log K_{ow}[1]	K_H (atm-m^3/mole)[1]
Carbamazepine	$C_{15}H_{12}N_2O$; 5H-Dibenz[b,f]azepine-5-carboxamide		2.45	1.08E-10

[1]Values obtained from ChemIDplus Database

1.2: Occurrence CBZ in the Environment

The occurrence of pharmaceutical and personal care products (PPCP) in the environment has been widely investigated across the world [15]. Through the use of advanced analytical techniques, CBZ has been detected in surface (1 μg/L) and drinking (30 ng/L) water in Berlin [16]. Although there are no publications that clearly indicate that CBZ has a direct negative impact on the environment, its recalcitrance is worrisome especially as its usage has seen an upward trend in recent years [5-8].

Pharmaceuticals may be discharged to the aquatic environment through human and animal usage [15]. CBZ is one type of PPCP that has been documented to be released into the environment primarily due to human usage. The presence of CBZ in wastewater can be attributed to excretion via urine/feces or from the indiscriminate disposal of pharmaceuticals into sewers. Once present in sewage, CBZ undergoes very little removal, with a maximum removal efficiency of about 10% in activated sludge treatment [15]. As a result, CBZ is typically discharged to surface water and may leach into groundwater aquifers. As surface and ground waters are used as the primary sources for drinking water treatment plants, CBZ is exposed to

drinking water treatment processes and, depending on the extent of treatment, may end up in finished drinking water.

CBZ has indeed been detected in various water and wastewater systems around the world. In Germany, CBZ was found in sewage at concentrations was up to 625 ng/L in both influent and effluent [15]. In South Korea, CBZ was detected in surface waters from more than 80% of the sources tested [17]. Within the United States, CBZ has been noted to have a 20% chance of being detected in groundwater [17]. Such studies in different water environments suggest that CBZ is ubiquitous in aquatic environments impacted by humans.

1.3: Effect of CBZ on environment

The effect of CBZ on the environment is still a mystery as results vary greatly in the few studies that have addressed its toxicological impact. Bioassays have typically been used to evaluate CBZ toxicity by exposing test organisms to specific concentrations of this compound. These bioassays are then used to calculate the predicted no-effect concentrations (PNEC) and the results are then compared to the measured environmental concentrations [11, 18]. *Vibro fischeri* (a marine bacterium), *Daphnia magna* (a freshwater invertebrate), and *Oryzias latipes* (the medaka fish) have all been used to evalate the acute aquatic toxicity of CBZ via half maximum effective concentration (EC_{50}) analysis. The EC_{50} values for *V. fischeri* and *D. magna* were 52.5 mg/L (5 min exposure) and 76.3 mg/L (96 hr exposure), respectively [11]. Lethal doses (LD_{50}) for CBZ were determined for medaka fish and ranged from 15-35 mg/L [11].

As CBZ has been noted to be a hazardous compound and is an expected water contaminant [18], its complete removal from the environment is desired. In a previous study, six different ecotoxicological model systems with eighteen endpoints were used to determine the level of PNEC toxicity associated with CBZ. Results suggested that CBZ would not produce acute toxicity to the aquatic environment under the studied conditions [19]. However, chronic and synergistic effects were not quantified [19]. Another study found that CBZ threatens the survival of midge, *Chironomus riparius,* which suggests that CBZ may threaten other aquatic

insects [20]. As can be seen, the effect of CBZ on the aquatic environment is highly uncertain and needs additional study. To be conservative, this xenobiotic compound should be eliminated from aquatic environments to avoid potential detrimental effects to wildlife.

1.4: Fate of CBZ in the environment and during engineered water/wastewater systems.

CBZ is has been shown to be very stable in the environment and is negligibly degraded via biotic mechanisms [8]. It is poorly soluble in water (17.7 mg/L) and its bioavailability is roughly 80% of its aqueous concentration [21]. CBZ has a relatively small log octanol-water partition coefficient (**Table 1**), which suggests that sorption to sediments and particles in aquatic environments is insignificant. The polar nature of CBZ can be attributed to the amine group present on its structure. Despite years of use, there is significant discrepancy in the reported pK_a values found in research studies and medical literature with pK_a values ranging from 7 to 13.9 [21]. Due to its prevalence in water bodies, researchers have begun to study removal process that can applied to CBZ, including biological processes such as activated sludge treatment and chemical processes such as ozonation and UV irradiation.

1.5: Removal of CBZ during water and wastewater treatment.

There are many studies on the removal of CBZ in both drinking water and wastewater applications. Four different removal processes, biological, chemical, photodegradation, and sorption are considered in this review.

1.5.1: Biological Processes

Biodegradation of CBZ in different biological processes have been studied at lab-scale, field–scale, and full-scale facilities. Sequencing batch reactor experiments (SBR), conducted by K. Stamatelatou et al. revealed that CBZ was not degraded during SBR operation over 10 days [16]. Lab-scale activated sludge treatment also indicated insignificant removal of CBZ [22].

Several studies at full-scale indicated small reductions of CBZ [23] of less than 30% [24]. Investigations using membrane bioreactors, which have been shown to be effective for the treatment of other PPCPs, also showed no biodegradation of CBZ [22, 25-27]. Lab-scale and field-scale studies using anaerobic digestion also indicated little to no removal of CBZ [16, 28]. To date, only one study showed the biodegradation of CBZ in a membrane reactor supplemented with powered activated carbon (PAC) [29]. In this study, the biological removal efficiency of CBZ was calculated to be 30-40% with the addition of 500 mg PAC in a 520 L reactor. These calculations suggest that in addition to the adsorption of CBZ by PAC, biodegradation may also have occurred [29].

1.5.2: Sorption Studies

Sorption is a common method that is used to eliminate contaminants from waste streams. There have been multiple studies investigating the sorption behavior of CBZ in both activated sludge [30] and soil/sediment systems [31]. The sorption behavior of CBZ in water/sediment system was investigated by Loffler et al. and CBZ was found to display a moderate affinity for the sediment, which is consistent with its lipophilicity [31]. In general, sorption of CBZ to sludge was investigated and determined to be minimal. In recent studies, PAC has been used to enhance CBZ sorption during activated sludge treatment resulting in a higher degree of biodegradation [29]. Such observations may be explained by the fact that PAC addition effectively increased the retention time for CBZ and this may have allowed the bacterial community to act on the compound.

William et al. observed adsorption and desorption of CBZ from irrigated soils and suggested initial removal of CBZ via adsorption from irrigation water may be significant. Desorption characteristics would limit the mobility of CBZ through soil profile and it was shown that CBZ has a low leaching potential in sewage effluent for irrigated use [32]. During these experiments, CBZ degradation was not observed [33, 34].

1.5.3: Chemical and Electrochemical Processes

Chemical processes are commonly used in wastewater treatment plants for many applications including advanced oxidation and disinfection. While processes such as ozonation and photolysis have been investigated for CBZ removal efficiency, there have been only two studies focusing on chlorination of CBZ [24, 35].

Ozonation

Ozone treatment of CBZ has been studied for both drinking water and wastewater applications. In drinking water facilities, ozonation has been shown to readily transform CBZ with some mineralization observed [36]. At the lab scale, the removal efficiency of CBZ by ozonation has been shown to be greater than 90% with ozone doses as low as 0.5 mg/L ozone [37]. Studies along the Detroit River in Ontario, Canada have found that ozone treatment was highly effective for removing CBZ and other pharmaceutical compounds [38]. These results indicate that ozonation is may be a better option for treatment of CBZ than conventional water treatment processes [38, 39].

With respect to wastewater treatment, Ternes et al. conducted pilot-scale studies examining the effect of ozonation on CBZ in wastewater effluent. At does of up to 10-15 mg/L ozone and a contact time of 18 minutes, CBZ was no longer detectable [40]. Mcdowell et al. showed that ozone reacts with the double bond in the carbamoyl ring and forms several by-products. These by-products, consisting mainly of quinazoline functional groups [41], 1-(2-benzaldehyde)-4-hydro-(1H,3H)-quinazoline-2-one (BQM), 1-(2-benzaldehyde)-(1H,3H)-quinazoline-2,4-dione (BQD), and 1-(2-benzoic acid)-(1H,3H)-quinazoline-2,4-dione (BaQD) [41] were not completely mineralized in the study.

In addition to ozonation, recent studies have attempted to combine ozonation with anodic oxidation by boron-doped diamond. The studies achieved close to 100 % CBZ removal solely from anodic oxidation with little to no contribution by ozonation [42]. Such result conflicting reports may occur from inconsistent experiment protocols that alter conditions during ozonation/anodic oxidation processes.

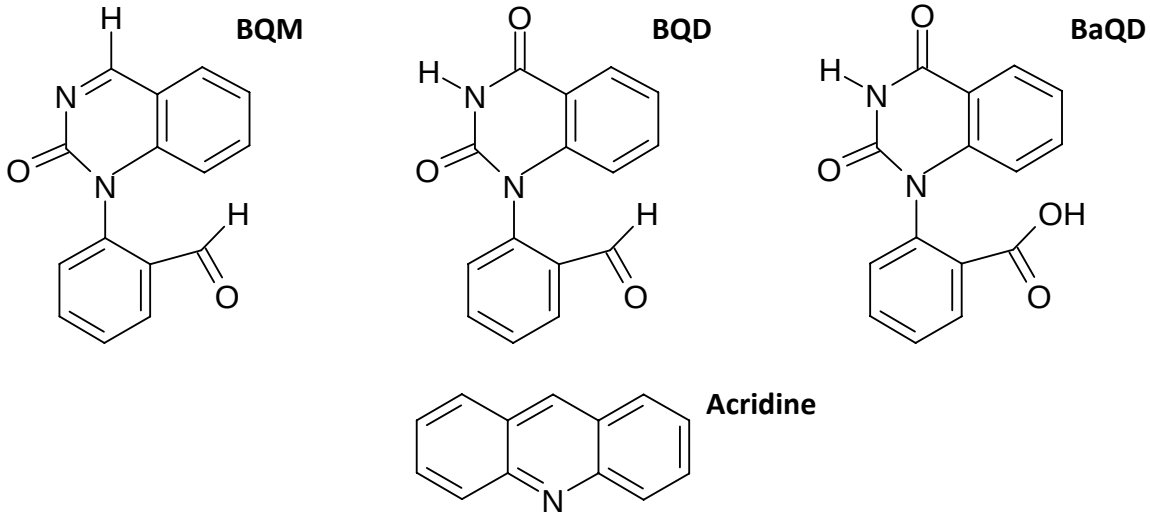


Figure 1. Structures of three carbamazepine ozonation products and acridine

1.5.4: Photolysis and Photocatalysis Studies

In recent years, photocatalysis has been studied and has become one of the more promising technologies that may be applied towards treatment of wastewater [43-46]. Frequently, titanium dioxide (TiO_2) is used in combination with photolysis sources. As TiO_2 is cheap, non-toxic, active and stable over a wide range of pH values, it has been noted as a good option for photocatalyst treatment. Treatment using this technology involves oxidation of the compound via either indirect action by surface-bound hydroxyl radicals or directly via valence-band hole reactions [43-46]. In general, photocatalysis with TiO_2 has been shown to be a suitable method for elimination of CBZ [47] with varying efficiency achieved based upon the source of TiO_2 material [48].

UV photolysis of CBZ has also been studied because UV disinfection systems are common in both drinking water and wastewater treatment. By itself, UV is not effective for CBZ removal, but when combined with H_2O_2 the process efficiency increases substantially. This combination of photolysis and advanced oxidation processes can result in the formation of acridine as a main by-product. As acridine is a mutagenic and carcinogenic compound, further treatment of the byproducts are needed for this treatment option to be feasible [49, 50].

1.5.5: Chlorination of CBZ

Okuda et al. studied the degradation of pharmaceuticals in wastewater treatment. From this study, both UV and chlorination when following biological treatment were inefficient processes for the removal of most pharmaceuticals including CBZ [24]. Gibs et al. performed another study that examined the fate of CBZ in the presence of free chlorine. CBZ was persistent over time after chlorination of water at the studied drinking water treatment plant. This research was performed using drinking water treatment plant effluent that contained total and free chlorine at 1.3 and 1.2 mg/L, respectively. Duplicate samples (preserved and unpreserved) were analyzed over a 10 day reaction period. Results from this experiment suggest a negligible rate of reaction between free chlorine and CBZ [35]. At present, other than these two incomplete studies, there is an absence of literature detailing the fate of CBZ in the presence of chlorine. As chlorination continues to be a primary method for wastewater effluent disinfection, it is imperative that further studies be performed to examine the potential reactions between CBZ and free chlorine.

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Chapter 2: Chlorination of Carbamazepine

2.1: Introduction

Large quantities of pharmaceutical compounds are manufactured and used worldwide [1]. Numerous recent reports have documented the presence of pharmaceutically active compounds in municipal sewage treatment plants as well as in surface and ground waters [1-4]. These pharmaceuticals reach the environment via many different paths, including hospital waste, household wastewater effluents, and landfill leachate [3]. As the ecotoxicological effects of many of these compounds are currently unknown, it is important that we try to minimize the input of these pharmaceuticals to the environment.

Compounds that are recalcitrant to wastewater treatment and water treatment are of great interest to the field of environmental engineering. One such recalcitrant compound is carbamazepine (CBZ). CBZ is a highly polar pharmaceutical that is commonly used as an anticonvulsant and mood stabilizing drug [5-8]. CBZ is sold under many commercial names such as Tegretol [9] and Karazepin [10]. CBZ has been noted to be resistant to removal by conventional wastewater treatment processes and this recalcitrance may stem from its complex structure [2, 11-13]. The structure and chemical properties of CBZ are shown in Table 1. The fate of CBZ in the environment is expected to be pH independent because its pK_a (=13.9) is quite high [14]. As the median pH in the natural environment ranges from 6 to 7, CBZ will primarily be present in its protonated form ($CBZH^+$).

CBZ is one type of Pharmaceutical and Personal Care Product (PPCP) that has been documented to be released into the environment primarily due to human usage. The presence of CBZ in wastewater can be attributed to excretion via urine/feces or as a result of the indiscriminate disposal of pharmaceuticals into sanitary sewers. CBZ has been detected in various water and wastewater systems around the world. In Germany, CBZ was found in sewage at concentrations up to 625 ng/L in both influent and effluent [2]. In South Korea, CBZ was detected in >80% of the surface waters tested [15]. Within the United States, CBZ has been noted to have a 20% chance of being detected in groundwater [15]. Such studies in a range of different waters suggest that CBZ is ubiquitous in aquatic environments impacted by

humans.

Most pharmaceuticals are removed from wastewater by biological processes [16, 17]. CBZ is exceptional in that it is recalcitrant to biological removal. Investigations of CBZ degradation in different wastewater treatment plants have shown the inefficient removal of CBZ during wastewater treatment [18, 19]. Degradation of CBZ was studied in sequencing batch reactors (SBR) at both lab and field scale. The results indicated no removal of CBZ in both experiments [20]. Lab-scale activated sludge systems have also shown insignificant removal of CBZ [19]. Investigations using membrane bioreactors, which have been shown to be more effective than conventional activated sludge for the treatment of some PPCPs, also showed no biodegradation of CBZ [19, 21-23].

Chemical treatment processes are widely used in wastewater and drinking water treatment. Because of its recalcitrance to biotransformation, the chemical reactivity of CBZ is of great interest. Reactions between ozone and CBZ are the most frequently studied. At the lab scale, the removal efficiency of CBZ in flocculated drinking water by ozonation has been shown to be greater than 90% with ozone doses as low as 0.5 mg/L ozone [24]. Studies along the Detroit River in Ontario, Canada found that ozone treatment was highly effective at depleting CBZ and other pharmaceutical compounds [25]. In contrast to ozonation, post-chlorination of biologically treated wastewater has shown insignificant degradation of CBZ [16]. In distribution system water (1.2 mg/L free chlorine), no removal of CBZ was observed over a 10 day holding time [26].

Chlorination of settled secondary effluent is widely used in US wastewater treatment plants [27]. If CBZ could be effectively removed during chlorination it would be highly beneficial because chlorination is well understood in terms of operation and maintenance. Were CBZ removal by chlorination effective, we would not have to build new basins or processes to remove CBZ. However; the two chlorine-CBZ studies mentioned previously showed no degradation of CBZ. Okuda et al. studied the degradation of pharmaceuticals in wastewater treatment processes. From this study, UV and chlorination following biological treatment were inefficient processes as related to removal of most pharmaceuticals including CBZ [16]. Gibs et

al. performed another study that examined the fate of CBZ in the presence of free chlorine. CBZ was persistent over time after chlorination of water at the studied drinking water treatment plant. This research was performed using drinking water treatment plant (DWT) effluent which contained total and free chlorine at 1.3 and 1.2 mg/L respectively. Duplicate samples (preserved and unpreserved) were analyzed over a 10 day reaction period. Results from this experiment indicate a negligible rate of reaction between free chlorine and CBZ [26]. In these two studies, the presence of other organic compounds may result in free chlorine consumption. Free chlorine thus changes to an inactive form and no reaction can occur between free chlorine and CBZ.

The objects of this study were to better understand the free chlorine-CBZ reactions and to investigate the kinetics and products of these reactions under controlled conditions. The ultimate goal of this work is to study these reactions under realistic conditions at lab scale and use them to understand CBZ fate in wastewater treatment plants. Our results indicate that in the absence of organic material that CBZ reacts with free chlorine. Furthermore, several intermediates and products were detected in this study. The results show that the reactivity of free chlorine with CBZ depends on the solution pH and on the temperature. When pH decreases, the reactivity increases, and similarly when temperature increases the CBZ loss rate increases. These results imply that CBZ should be degraded during chlorination if sufficient free chlorine is present.

2.2: Materials and Methods

2.2.1: Reagents, Equipment and Glassware Preparation

Reagent grade water was purified via deionization and distillation. CBZ was obtained from MP Biomedicals (Solon, OH) and was used without further purification. CBZ stock solutions (10 g/L) were prepared by dissolving solid CBZ in reagent grade methanol or acetone (Thermo Fisher Scientific, Pittsburgh, PA). Free chlorine stock solutions were prepared by diluting purified grade sodium hypochlorite (4-6%; Thermo Fisher Scientific, Pittsburgh, PA) with reagent grade water. pH was monitored with a Fisher Scientific model 60 pH meter

coupled with a Thermo-Orion Ross PerpHect combination electrode. Sodium sulfite and sodium bicarbonate were purchased from Fisher Scientific (Fair Lawn, NJ) and 10, 11-dihydrocarbamazepine was used as internal standard for CBZ and purchased from Alfa Aesar (Ward Hill, MA). Glassware for all experiments was prepared by soaking overnight in a 10% nitric acid bath followed by soaking in a concentrated chlorine bath for at least three hours. The cleaned glassware was then rinsed with copious amounts of distilled water and air-dried prior to use.

2.2.2: Free Chlorine Analysis

Free chlorine concentrations were determined using the DPD/FAS colorimetric method [28]. A modified version of this method was utilized for chlorine decay experiments. For these analyses, 4 mL of sample was removed from 40 mL headspace-free sample vials to provide enough room for the addition of the colorimetric reagents. Subsequently, 2 mL of N,N-dimethyl-p-phenylenediamine (DPD) indicator (4.19 mM) and 2 mL of phosphate buffer (0.507 M PO_4^{3-}) were added to each reaction vessel to quench the reaction. The vessel contents were well mixed and the indicator color was allowed to develop for one minute. Each solution was then transferred to a 1 cm glass cuvette and absorbance readings were taken on a UV-Vis spectrophotometer at a wavelength of 515 nm and compared to a calibration curve (1-20 μM). Each concentration was measured in triplicate. The calibration curve was developed by serial dilution of concentrated free chlorine stock. The titer of the stock was frequently measured by the manual titration method outlined in Standard Methods for Analysis of Water and Wastewater [28].

2.2.3: Chlorination of CBZ at Various CBZ/HOCl Ratios and at Different pH.

Unless otherwise specified, all experiments and analyses were performed in triplicate. Chlorination experiments were performed using a reaction matrix consisting of buffered reagent grade water (2 mM $NaHCO_3$ in reagent grade water). Known concentrations of CBZ and free chlorine were added to headspace-free 40 mL amber vessels. These suspensions were

mixed by shaking the vessels. The glass beads were added so that mixing by hand could proceed effectively. In these experiments, initial CBZ concentrations ranging from 20.3 to 45.7 μM were treated with free chlorine over a concentration range of 2.0- 5.0 μM at pH values between 5.5 and 10. Upon mixing, the free chlorine concentration was determined at various time points by analyzing aliquots from sacrificial vials using the modified DPD photometric method described previously.

2.2.4: Experiments to Determine CBZ Transformation Kinetics

Kinetic experiments monitoring CBZ loss were performed at initial CBZ and free chlorine concentrations of 40.4 μM and 1220 μM, respectively (1:30 molar ratio of CBZ to free chlorine). The solution pH was varied between 6 and 9 at 0.25 pH unit increments using diluted sodium hydroxide (1N NaOH) and sulfuric acid (1N H_2SO_4). Sample aliquots, obtained at various timepoints, were supplemented with sodium sulfite (1.5× molar excess of sodium sulfite) to quench the free chlorine. These samples were then analyzed using the HPLC-UV and LC-MS techniques detailed below.

2.2.5: Analysis of CBZ and CBZ byproducts.

Samples obtained from the CBZ kinetic experiments were stored at 4°C for preservation prior to analysis. For HPLC-UV quantification of CBZ, an internal standard, (10, 11-dihydrocarbamazepine; 10 mg/L) was added to 2 mL sample aliquots and the combined solution was then subjected to HPLC separation using a Hewlet Packard Liquid Chromatography (HPLC) model 1090. Peak separation was achieved using a Betabasic-18 column (100 mm in length, 2.1 mm ID, 3 μm particle size) obtained from Thermo Fisher Scientific (Pittsburgh, PA) and a mobile phase consisting of 75% methanol and 25% distilled water applied at 0.2 μL/min over a 10 minute period. The column temperature was held constant at 40 °C and the total injection volume was 200 μL. UV detection was performed at 254 nm and 281 nm and the data was analyzed using Chemstation software.

2.2.6: Identification of Daughter-Products

Solid Phase Extraction (SPE)

Samples were solid phase extracted to remove the salts that interfere with LC-ITMS analysis. OASIS® HLB cartridges (500 mg) were preconditioned with 12 mL of Nanopure water followed by 6 mL of acetonitrile (ACN). These samples were collected and then shipped to the University of Buffalo for extraction and mass spectrometry analysis. Samples were loaded onto the cartridges at a rate of 2 mL/min, and the cartridges were eluted twice with 4 mL ACN. The elutate was reduced to 0.2 mL via evaporation under a gentle stream of nitrogen. Nanopure water was added to each sample to a final volume of 1.0 mL. The extraction recovery using this procedure was over 95% for CBZ (data not shown).

Mass-spectrometry Analysis

Extracted samples were analyzed using an LCQ Advantage^{TM} liquid chromatograph / ion trap mass spectrometer system (LC-ITMS) equipped with an electrospray ionization (ESI) source (Thermo Finnigan, San Jose, CA). All analyses were performed under positive ionization mode. A reversed phase column, BetaBasic-18 C-18 column, 100×2.1 mm internal diameter with 3 µm particle size, with a UNIPHASE guard cartridge, 10×2.1 mm internal diameter with 3 µm particle size, was used for separation. Both columns were purchased from Thermo Hypersil-Keystone (Bellefonte, PA). The column oven temperature was set at 30 °C, and the full roof injection volume and the flow rate of the mobile phase were 10 µL and 200 µL/min, respectively. The gradient mobile phase used to separate analytes consisted of ACN (mobile phase A), methanol (mobile phase B) and water with 0.3% formic acid (mobile phase C). The initial condition of this gradient, 5% A , 20% B and 75% C, was held for 1 min, after which component B was increased to 75% and C was decreased to 20% over 9 min . Then B was increased to 90% and C was decreased to 5%. Then B was decreased to 5% and C was increased to 90% over 1 min. This last condition was held for 5 min, resulting in a total analysis time of 21 min for each sample.

The mass spectrometer was tuned at 250 °C of capillary temperature and 5.0 kV of spray voltage. Nitrogen was used as a sheath gas at a flow rate of 24 arbitrary units. The acquisition time was divided into two different segments. The first segment consisted of a full scan for IOP and TMP, and the second segment was for a full MS/MS scan event for IOP and TMP with the collision energy of 44% and isolation width of 1.0.

2.3: Results and Discussion

2.3.1: Introduction

Degradation of CBZ is significant in the presence of free chlorine. Few studies have previously investigated the kinetics of CBZ loss in the presence of free chlorine under drinking and wastewater treatment conditions. Although Gibs et al. [26] suggested that reduction of CBZ during chlorination of drinking water was negligible, the results from our present study suggest otherwise. Our results indicate significant degradation of CBZ in the presence of free chlorine (**Figure 2**). As the ratio of CBZ to free chlorine was increased, reduction of CBZ was observed to decrease further suggesting that free chlorine reacts with CBZ.

The typical chlorine concentrations that are used in drinking water and wastewater treatment are 4 and 6 mg/L as Cl_2, respectively. The typical detected CBZ concentration in drinking water and wastewater treatment are 110 ng/L[26] and 850 ng/L[29], respectively. Therefore; estimated molar ratio of CBZ to free chlorine in drinking water and wastewater based on these CBZ and free chlorine doses are 1:36000 and 1:7000, respectively. These ratios are much lower than the ratios used in our experiments. However, the reactivity of free chlorine with other organic compounds are not accounted in these ratios. The actual ratio of CBZ to free chlorine would be higher than the estimated ratios and the actual ratios need to be investigated. We believe that our experimental ratios were suitable to determine the CBZ loss kinetics because >50% loss was observed for the 1:10 and 1:30 ratios.

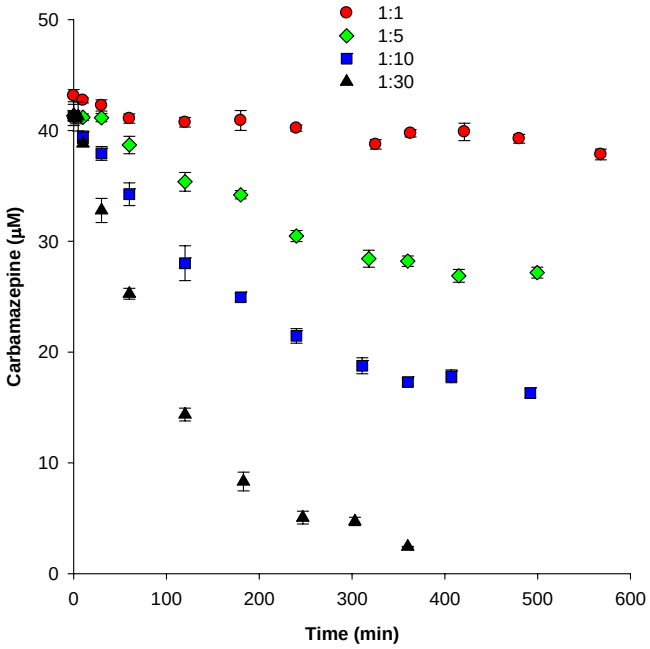

Figure 2: Carbamazepine loss in the presence of free chlorine. Reaction conditions: pH 6, $[CBZ]_0$ = 40.4 μM (=9.6 mg/L); $[\text{Free Chlorine}]_0$ = 40.4- 1210 μM (=2.87-85.91 mg/L as Cl_2); $[NaHCO_3]$ = 2 mM (=168 mg/L).

2.3.2: Development of a Kinetic Model to Describe the Reaction between CBZ and Free Chlorine.

As the stoichiometry for the reaction between free chlorine and CBZ has not been reported in the literature to date, we developed the following simple kinetic model (**Equation 1**).

$$\mathbf{a\,Carbamazepine} + \mathbf{b\,Free\,Chlorine} \xrightarrow{k} \mathbf{products} \qquad (1)$$

If equation 1 holds, the loss of CBZ over time is proportional to the loss of free chlorine (FC; **Equations 2** and **3**),

$$\frac{d[CBZ]_T}{dt} \alpha \frac{d[FC]}{dt} \qquad (2)$$

$$\frac{1}{a}\frac{d[CBZ]_T}{dt} = \frac{1}{b}\frac{d[FC]}{dt} \quad (3)$$

where a and b are the stoichiometric coefficients for CBZ and FC, respectively.

2.3.3: Free Chlorine Loss Kinetics

To determine rate coefficients for the reaction between CBZ and free chlorine, experiments were conducted in the presence of 10-fold excess CBZ. Under these conditions, a pseudo-first-order approximation describing the loss of chlorine was assumed to be appropriate. Interestingly, however, two distinct reaction regimes were visible – a rapid initial reaction and a slower longer-term consumption of free chlorine (**Figure 3**). Regression of the data yielded two pseudo-first-order curves describing the initial and slow kinetic reactions. Two distinct regimes may reflect a complex series of reactions. The rapid initial rate is probably a direct reaction between CBZ and free chlorine, and the subsequent slow reaction may be due to the involvement of intermediates in the reactions. Similar behavior has been observed in the reactions between complex natural organic matter (NOM) with free chlorine or monochloramine. Duirk et al. investigated monochloramine loss in the presence of NOM and observed an initially rapid monochloramine loss followed by a slow monochloramine loss [30]. Therefore, it may also be true that the CBZ-free chlorine reaction is complex and results in two different rates.

For our current purposes, only the initial rates were used to predict the relationship between CBZ and free chlorine. Such an assumption presumably allows us to reduce the complexity of the reactions between free chlorine and CBZ. We note that initial rates should be minimally affected by the potential build-up of products that we suspect affect the free chlorine-CBZ reaction at later times [31].

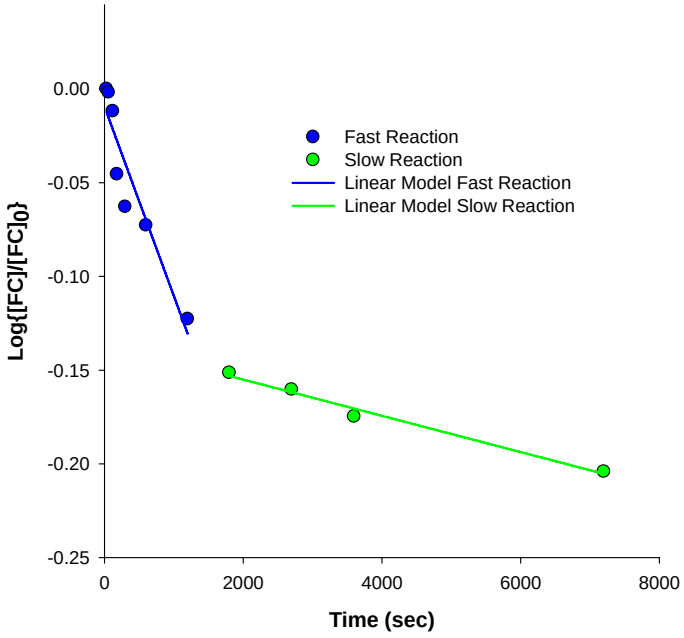

Figure 3: Two distinct reaction regimes. Log {[FC]/[FC]$_0$} vs. time. Reaction conditions: pH 7, [CBZ]$_0$ = 40.4 μM (=9.6 mg/L); [Free Chlorine]$_0$ = 4μM (=0.29 mg/L as Cl_2); [$NaHCO_3$] = 2 mM (=168 mg/L).

Pseudo-first-order rate coefficients (k_{obs1}; s^{-1}) were determined at several pH values using the method of initial rate (**Figure 4**). These pseudo-first-order rate coefficients are related to the overall second-order rate coefficient (k_{FC1}; $M^{-1}s^{-1}$) as follows,

$$\frac{d[FC]}{dt} = -k_{FC1}[CBZ]_T^{\alpha}[FC]_T^{\beta} \quad (4)$$

$$k_{obs1} = k_{FC1}[CBZ]_{T,t=0}^{\alpha} \quad (5)$$

where $[CBZ]_T$ (=[CBZ]+[CBZ-H^+]) represents the sum of CBZ and protonated CBZ (CBZ-H^+), $[FC]_T$ is the total concentration of free chlorine (= [HOCl]+[OCl^-]), and $[CBZ]_{T,t=0}$ is the initial excess CBZ concentration.

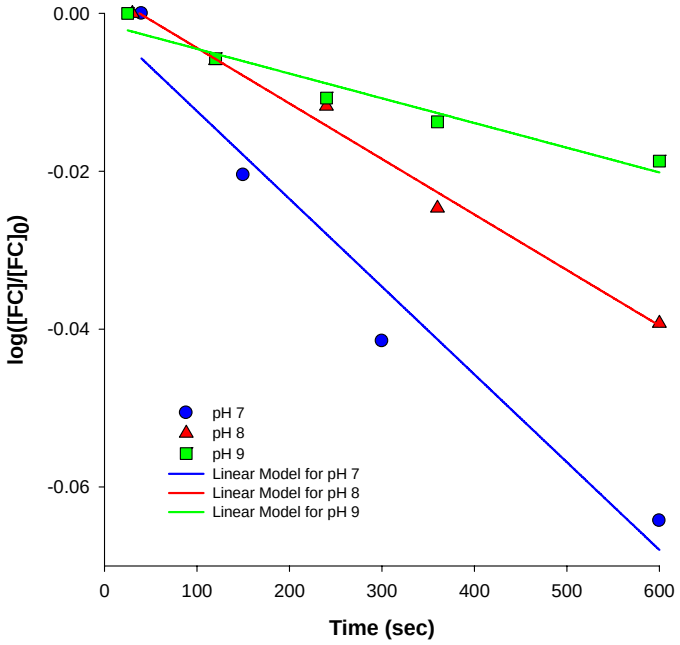

Figure 4: Method of initial rates. Log {[FC]/[FC]$_0$} vs. time. Reaction conditions: [CBZ]$_0$ = 40.4 μM (=9.6 mg/L); [Free Chlorine]$_0$ = 2μM (=0.14mg/L as Cl_2); [$NaHCO_3$] = 2 mM (=168 mg/L).

Order with Respect to Free Chlorine

Assuming that free chlorine is reduced by a first-order reaction and that the reaction can be described using a pseudo-first-order expression, we assume β=1 resulting in a simplification of (**Equation 4**) as presented in logarithmic form:

$$\log[FC] = -k_{obs1}t + \log[FC]_0 \qquad (6)$$

To verify this assumption, pseudo-first-order rate coefficients (k_{obs1} values) were determined for a wide range of $[FC]_0$ at fixed pH and constant $[CBZ]_{T,t=0,}$. Results from these experiments indicate that the k_{obs1} values were approximately equal within a 95% confidence level. The average value of k_{obs1} is (8.40±0.85)x10^-5 1/sec (**Figure 5**). This result indicates that the assumed first-order dependency on free chlorine during the first phase of reaction is valid (β =1).

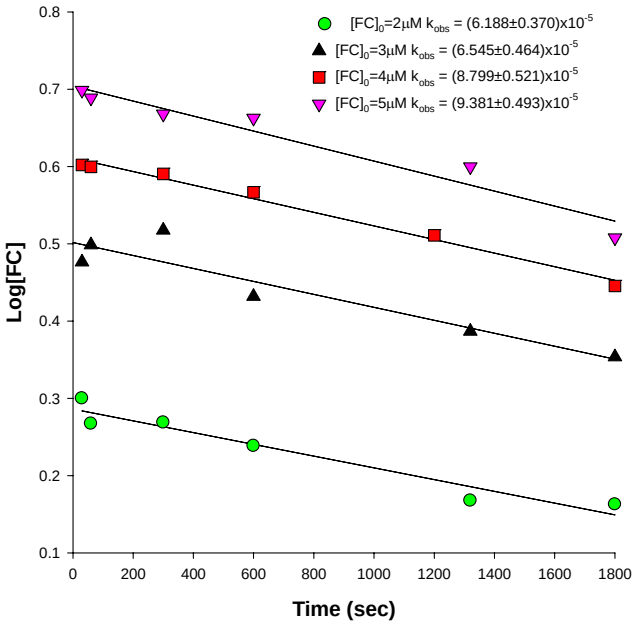

Figure 5: Determination of reaction order with respect to free chlorine. Errors in k_{obs1} values correspond to 95% confidence intervals. Reaction conditions: [Free Chlorine]$_0$ = 2-5 µM(=0.14-0.72mg/L as Cl_2); [CBZ]$_0$= 40.6 µM (=9.6 mg/L); [$NaHCO_3$] = 2 mM (=168 mg/L); pH 7.

Order with Respect to Carbamazepine

Evaluation of the reaction order (α) with respect to CBZ was performed though linearization of **Equation 5.**

$$\log\{k_{obs1}\} = \log\{k_{FC1}\} + \alpha \log[CBZ]_{T,t=0} \qquad (7)$$

Experiments were conducted at a fixed $[FC]_0$ concentration while varying the $[CBZ]_{T,t=0}$ concentration at pH 7. Regression of the data yielded an α value equal to 1.09 ± 0.11 suggesting that the reaction with respect to CBZ is first order (**Figure 6**). As such, we propose the free chlorine kinetic model expressed below to describe the reaction between CBZ and free chlorine,

$$\frac{1}{a}\frac{d[CBZ]_T}{dt} = \frac{1}{b}\frac{d[FC]}{dt} = -k_{FC}[CBZ]_T[FC] \qquad (8)$$

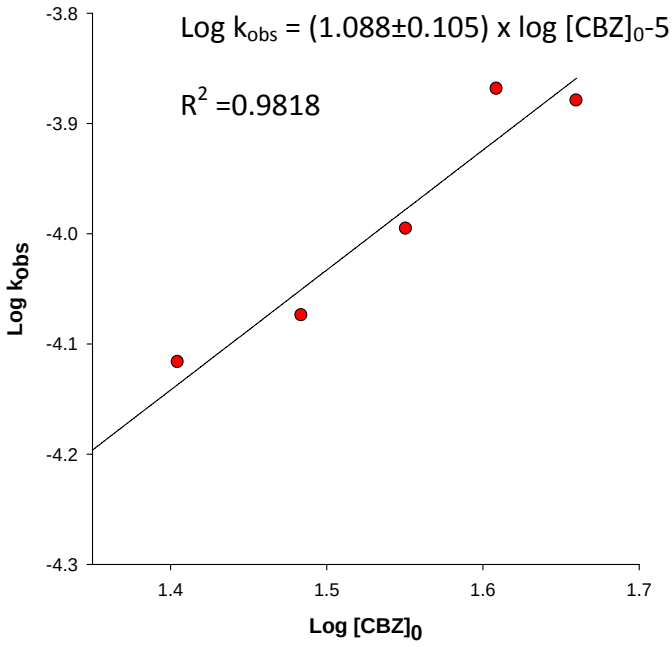

Figure 6: Determination of reaction order with respect to carbamazepine. Reaction conditions: $[Free\ Chlorine]_0$ = 2 µM (=0.14 mg/L as Cl_2); $[CBZ]_0$= 20.1-45.7 µM (=4.75 – 10.8 mg/L); $[NaHCO_3]$ = 2 mM (=168 mg/L); pH 7.

Order with Respect to $[H^+]$

To evaluate effect of $[H^+]$, k_{obs1} values were determined under varying pH conditions (pH 5.5 to 10) and log-log plots of the k_{obs1} values versus the $[H^+]$ concentration (**Figure 7**) were constructed. Since the reaction between CBZ and free chlorine is pH dependent, it was expected that the logarithm of the measured reaction rates would increase with a decrease in pH. Such a pH dependence can be rationalized by the acid-base speciation of CBZ and free chlorine.

Given the speciation of these compounds, potential reactions include:

$$CBZH^+ \xleftrightarrow{K_{a,CBZ}} CBZ + H^+ \qquad (9)$$

$$HOCl \xleftrightarrow{K_{HOCl}} OCl^- + H^+ \qquad (10)$$

$$CBZ + HOCl \xrightarrow{k_1} products \quad (11)$$

$$CBZH^+ + HOCl \xrightarrow{k_2} products \quad (12)$$

$$CBZ + OCl^- \xrightarrow{k_3} products \quad (13)$$

$$CBZH^+ + OCl^- \xrightarrow{k_4} products \quad (14)$$

At pH < $K_{a,CBZ}$, CBZ will primarily be in the protonated form ($CBZH^+$) and we thus neglect reactions with the neutral form. Accordingly,

$$\frac{d[FC]}{dt} = -k_{HOCl}[CBZH^+][HOCl] - k_{OCl^-}[CBZH^+][OCl^-]$$

$$[HOCl] = \alpha_{0,HOCl}[FC]_T \text{ and } [OCl^-] = \alpha_{1,OCl^-}[FC]_T$$

$$\frac{d[FC]}{dt} = -k_{HOCl}\alpha_{0,HOCl}[FC]_T[CBZH^+] - k_{OCl^-}\alpha_{1,OCl^-}[FC]_T[CBZH^+]$$

$$\frac{d[FC]}{dt} = -(k_{HOCl}\alpha_{0,HOCl} + k_{OCl^-}\alpha_{1,OCl^-})[CBZH^+][FC]_T \quad (15)$$

where $\alpha_{0,HOCl}$ is the ionization fraction of hypochlorous acid (HOCl) and α_{1,OCl^-} is the ionization fraction for OCl^-

Based on this reaction mechanism, k_{obs1} takes the following form:

$$k_{obs1} = (k_{HOCl}\alpha_{0,HOCl} + k_{OCl^-}\alpha_{1,OCl^-})[CBZH^+] \quad (16)$$

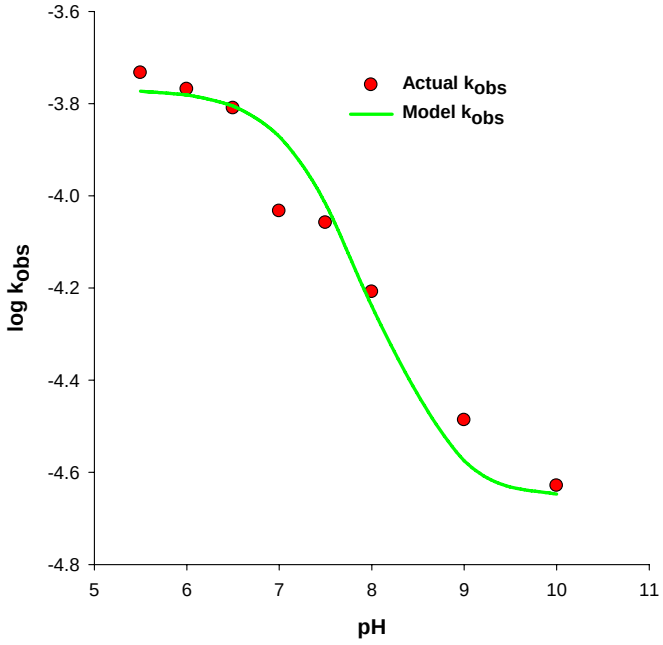

Figure 7: Experimental and predicted k_{obs} values. Logk_{obs} vs. pH. Reaction conditions: [Free Chlorine]$_0$ = 4 µM (=0.28 mg/L as Cl_2); [CBZ]$_0$ = 40.6 µM (=9.6 mg/L); [$NaHCO_3$] = 2 mM (=168 mg/L).

To determine the pH independent kinetic rate coefficients k_{HOCl} and k_{OCl^-}, the Excel Solver program was applied to Equation 16 by using the estimated k_{obs} in order to determine k_{HOCl} and k_{OCl^-} values at each pH (pH5.5-10). The average k_{HOCl} and k_{OCl^-} values obtained are 4.19 and 0.54 ($M^{-1}s^{-1}$), respectively. These estimates lead to a model that can predict k_{obs1}, as given in equation 17.

$$k_{obs1} = (4.19 * \alpha_{0,HOCl} + 0.54 * \alpha_{1,OCl^-})[CBZH^+] \quad (17)$$

The k_{obs},model was plotted relative to the estimated k_{obs} values to determine how well the model predicted experimental data (**Figure 7, Model**). The model fit the experimental data very well. This result indicates that our k_{HOCl} and k_{OCl^-} values are reasonable for use in the simple model.

During these experiments, the highest pH used was pH 10, which is lower than the reported pK_a of CBZ. As a result, $CBZH^+$ is the key species in the reaction with free chlorine. Since pH ranges from 6 to 7 in natural and engineered environments, $CBZH^+$ can also be assumed to be the dominant species. As such, we expect our model to predict the reactions that will occur under these conditions. If we consider cases where the pH is below 6, $[H^+]$ might also have an effect on the reaction. Under these conditions Cl_2 gas may form and affect the predicted reaction. It is anticipated though that the reaction would proceed more rapidly in the presence of gaseous Cl_2 due to its higher reactivity than free chlorine [31].

2.3.4: Transition from Chlorine Kinetics to Prediction of Carbamazepine Decay

To estimate parameters a and b in Eq. 3, experiments were performed at different CBZ to free chlorine molar ratios (1:1, 1:5, and 1:10). The experiments were conducted at pH 6 and both [CBZ] and [FC] were measured as a function of time (**Figures 8A, B and C**). As indicated in these figures, CBZ and free chlorine loss followed similar patterns. For 1:1 ratio, the reaction intermediates may react with free chlorine and affect the free chlorine loss profile. Therefore; free chlorine loss is faster than CBZ loss after 10 minutes reaction time. As excess chlorine was present during the 1:5 and 1:10 experiments, the reaction intermediates are assumed to not significantly impact the free chlorine loss profile in those cases. This assumption appears reasonable for 1:5 and 1:10 molar ratios since the kinetics of CBZ loss and free chlorine loss are similar after 3 minutes for each of these cases. We can therefore conclude that parameters *a and b* should have equal values since both CBZ and free chlorine followed similar trends in 1:5 and 1:10 molar ratio. Thus,

$$\frac{d[CBZ]_T}{dt} = \frac{d[FC]}{dt} = -k_{FC}[CBZ]_T[FC] \qquad (18)$$

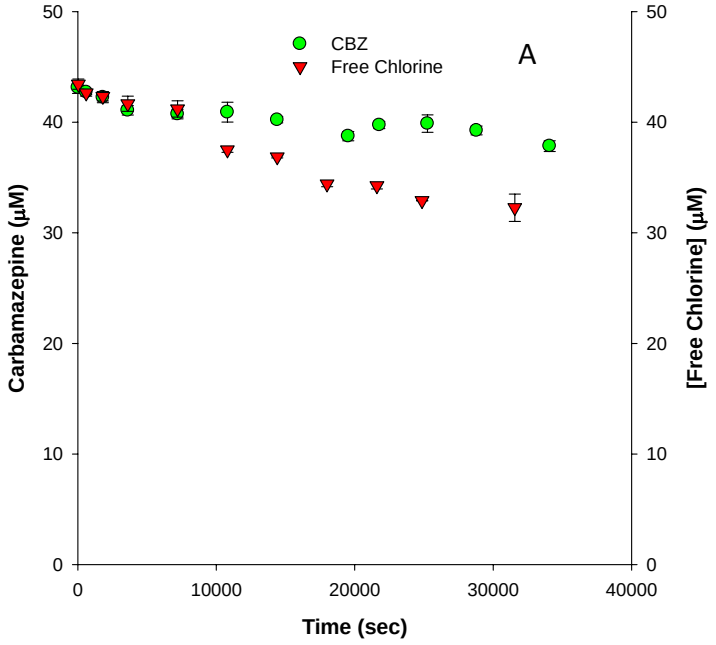

Figure 8A: Carbamazepine and Free Chlorine losses in 1:1 molar ratio $[CBZ]_0$ to $[FC]_0$. Reaction conditions: $[FC]_0$ = 40.4 µM (=2.87 mg/L as Cl_2);

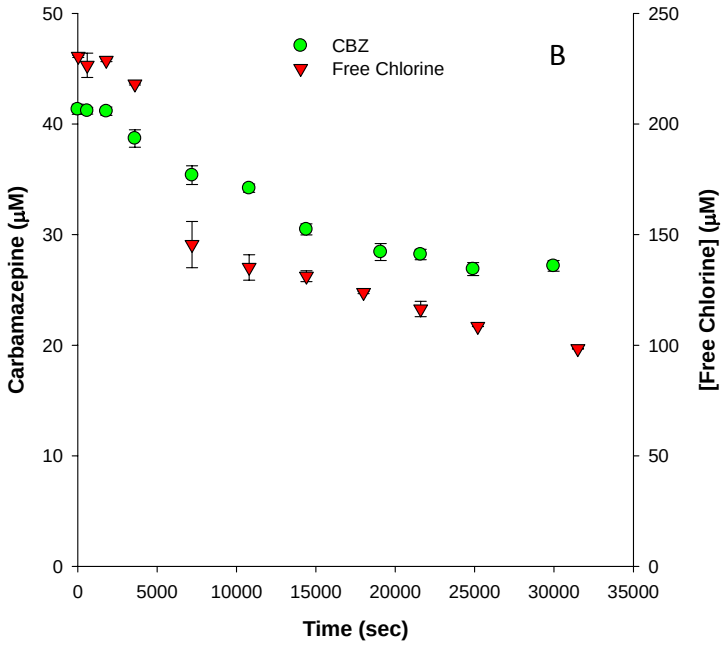

Figure 8B: 1:5 molar ratio $[CBZ]_0$ to $[FC]_0$. Reaction conditions: $[FC]_0$ = 202 µM (=14.3 mg/L as Cl_2);

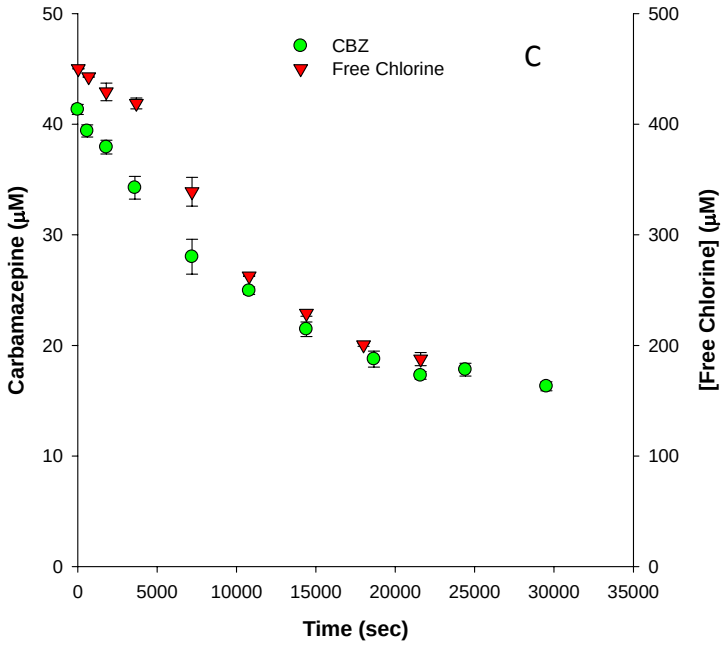

Figure 8C: 1:10 molar ratio $[CBZ]_0$ to $[FC]_0$. Reaction conditions: $[FC]_0$ = 404 µM (=28.7 mg/L as Cl_2); $[CBZ]_0$= 40.6 µM; $[NaHCO_3]$ = 2 mM (=168 mg/L); pH 6.

2.3.5: Kinetic Model Evaluation

To evaluate the validity of the model given by equations 9-14, data generated from the model was compared to experimental data (**Figures 9**). Free chlorine loss was over predicted by the model as compared to the experimental data and CBZ loss was also over predicted (**Figure 9**). Such results suggest that initial rates may not be appropriate to predict both CBZ and free chlorine losses. To properly predict CBZ and free chlorine losses, more complex models need to be developed. One approach may be to consider that losses need to reflect the slow reaction kinetics.

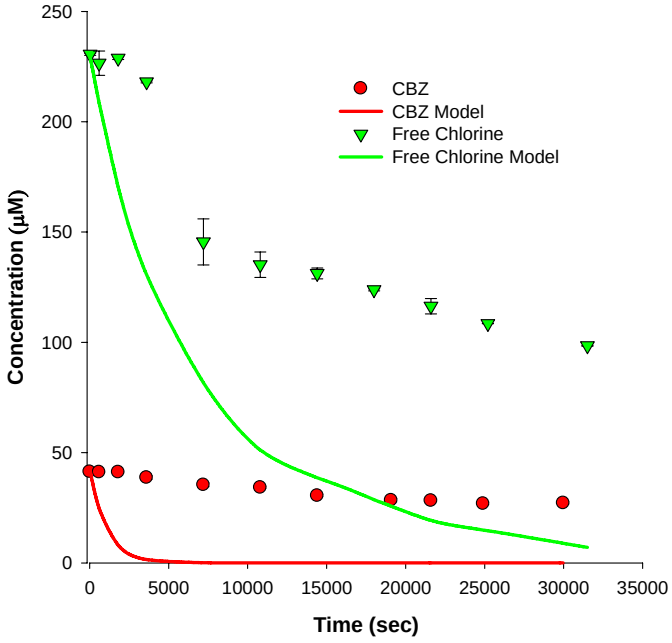

Figure 9: Free chlorine experimental data, carbamazepine experimental data and prediction models. Reaction conditions: [Free Chlorine]$_0$ = 202 µM (=14.3 mg/L as Cl_2); [CBZ]$_0$= 40.6 µM (=9.6 mg/L); [$NaHCO_3$] = 2 mM (=168 mg/L); pH 6.

2.3.6: Carbamazepine Kinetics

To determine the CBZ kinetic coefficients, experiments were conducted in the presence of excess free chlorine (30×) at various pH values (5.5 to 9). In contrast to the free chlorine kinetics in the presence of excess CBZ, the overall rates appear to follow first-order kinetics over the course of the reaction. The pseudo-first-order rate coefficients ($k_{obs,CBZ}$; s^{-1}) are related to the overall carbamazepine second-order rate coefficient (k_{CBZ}; $M^{-1}s^{-1}$) as follows,

$$\frac{d[CBZ]_T}{dt} = -k_{CBZ}[CBZ]_T[FC]_0 \quad (19)$$

$$k_{obs,CBZ} = k_{CBZ}[FC]_0 \quad (20)$$

The first-order rate coefficients were determined from **Figure 10**. From these results, the $k_{obs,CBZ}$ decreases when pH increases until pH 7 after which the values are constant. The

cause of this strange profile is not currently understood, but presumably reflects the complexity of the CBZ-free chlorine reactions.

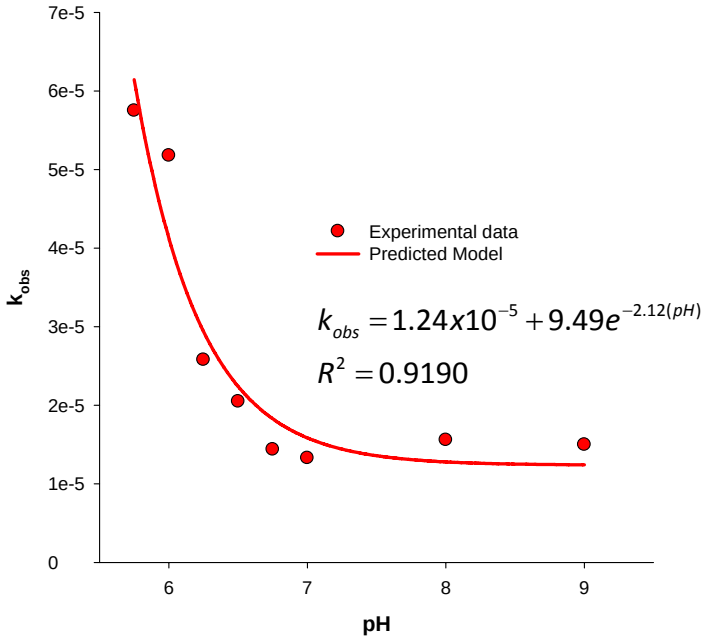

Figure 10: Carbamazepine kinetic Log k_{obs} vs. pH. Reaction conditions: [Free Chlorine]$_0$ = 1200 µM (=85.2 mg/L as Cl_2); [CBZ]$_0$= 40.6 µM (=9.6 mg/L); [$NaHCO_3$] = 2 mM (=168 mg/L).

To compare the kinetic model to real data at pH 6, $k_{obs,CBZ}$ was calculated using the equation in Figure 9. The calculated $k_{obs,CBZ}$ is $4.078x10^{-5}$ s^{-1} and the equation 20 can be used to predict the k_{CBZ} which equals to 0.034 $M^{-1}s^{-1}$. CBZ loss as modeled using equation 19, fit the experimental data fairly well. Under 1:10 and 1:30 molar ratios, the model started to under predict CBZ loss past 1800 seconds whereas for the 1:5 ratio experiment, the model fit the experimental data (**Figure 11**). From these results, it appears that the proposed model (**equation 19**) is suitable to predict CBZ loss.

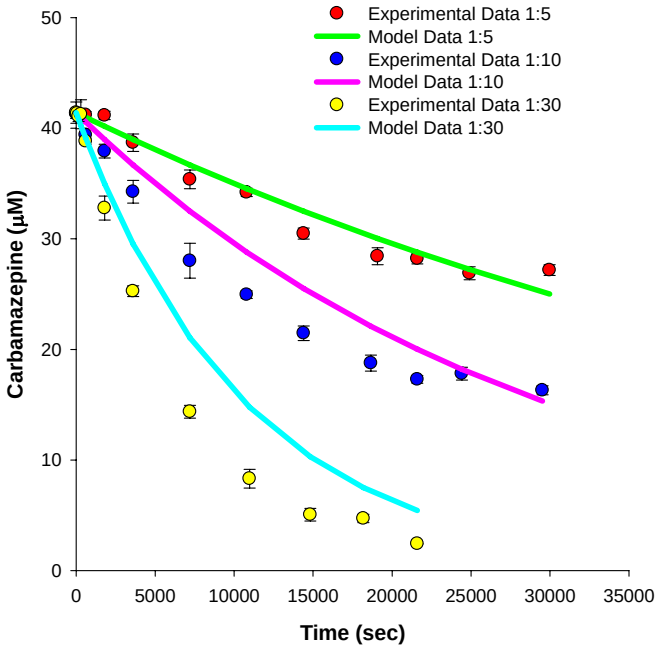

Figure 11: Carbamazepine experimental data and predicted model for 1:5, 1:10, and 1:30 molar ratio of CBZ to free chlorine. Reaction conditions: [Free Chlorine]$_0$ = 200-1200 µM (=; [CBZ]$_0$= 40.6 µM; [$NaHCO_3$] = 2 mM; pH 6.

2.3.7: Temperature Effect on Carbamazepine Reaction Kinetics

The effect of temperature on CBZ loss was investigated using a CBZ to free chlorine molar ratio of 1:30 and four different temperatures (22, 30, 40, 50°C). These experiments were performed at pH 7. Results from this experiment indicate that as the temperature was increased, the reactivity between CBZ and free chlorine also increased (**Figure 12**). To evaluate the relationship between temperature and kinetic rates, the natural log of k_{obs} was plotted versus the inverse of temperature (**Figure 13**).

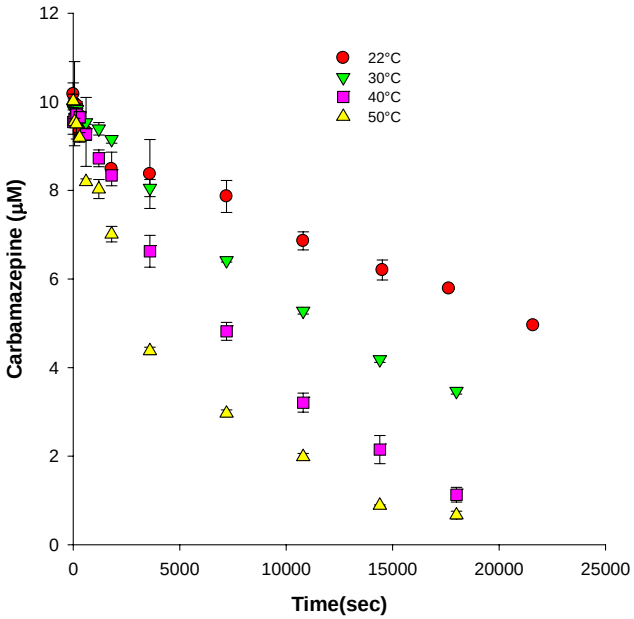

Figure 12: Carbamazepine loss in the presence of free chlorine at different temperatures. Reaction conditions: pH 6, [CBZ]$_0$ = 40.4 μM(= 9.6 mg/L); [Free Chlorine]$_0$ = 1210 μM (=85.9 mg/L as Cl_2); [$NaHCO_3$] = 2 mM (=168 mg/L).

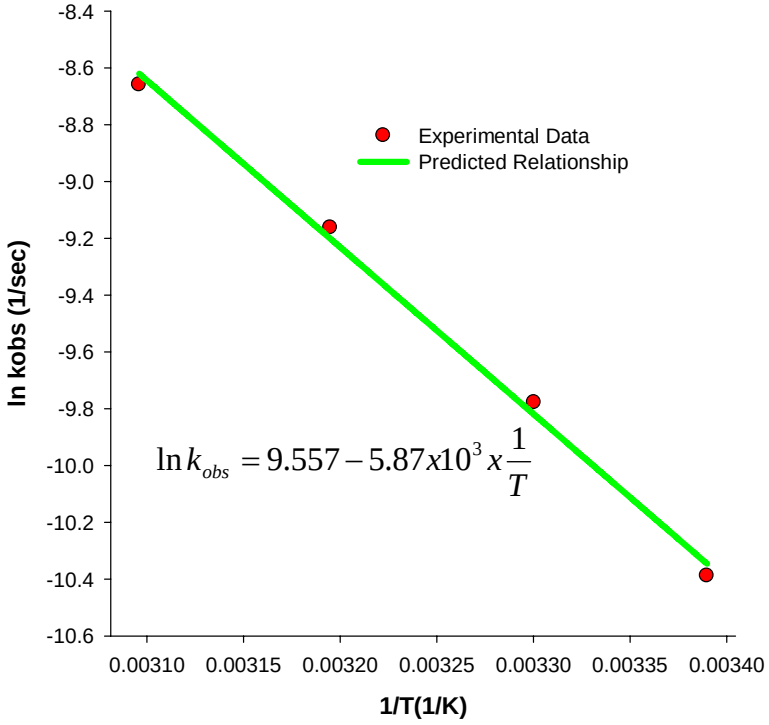

Figure 13: Temperature effect experimental data. Ln k_{obs} versus 1/T. Reaction conditions: pH 6, [CBZ] $_0$ = 40.4 μM (= 9.6 mg/L); [Free Chlorine] $_0$ = 1210 μM (=85.9 mg/L as Cl_2); [$NaHCO_3$] = 2 mM (=168 mg/L).

The Arrhenius equation is given by equation 23,

$$k = Ae^{-E_a/RT} \quad (23)$$

and the natural logarithm of the Arrhenius equation yields

$$\ln(k) = -\frac{E_a}{R} \times \frac{1}{T} + \ln(A) \quad (24)$$

where k = rate constant, R = gas constant, E_a = activation energy, and A = pre-exponential factor

The equation in **Figure 13** can be rearranged to the natural logarithm form (**Equation 24**). E_a and A values are 48.8 KJ/mol and 1.41 x 10^4 s^{-1}, respectively. Based on these results, Arrhenius expressions for adjusting k_{obs} and k_{FC} as a function of temperature were developed:

$$k_{obs,CBZ} = 14143e^{-48.81/RT} \quad (25)$$

$$k_{CBZ} = 0.571e^{-48.81/RT} \quad (26)$$

Equation 25 could be used to predict the CBZ loss under real world temperature for drinking water and wastewater treatments by determining the k_{obs} value. The typical temperature in drinking water is about room temperature (22°C). Therefore; predicted $k_{obs,CBZ}$ value is 3.23x10^{-5} s^{-1}. The typical temperature for wastewater effluent is higher than drinking water (~35°C). So, the predicted $k_{obs,CBZ}$ in wastewater applications is 7.47 x 10^{-5} s^{-1}.

2.3.8: Product Identification

HPLC-UV results

Four possible intermediates were detected using HPLC-UV at 254 nm (**Figure 14**). Peaks #1, #2, #3, and #4 showed up at 3.5, 4.2, 4.7 and 5 min retention time. Peaks also were monitored as a function of time under 9 different pH conditions (pH5.5-9). The areas of the unknown peaks were normalized to the area of the internal standard peak ("unknown/IS") and were plotted along with CBZ concentration on the same graph to determine patterns associated with formation and loss of intermediates.

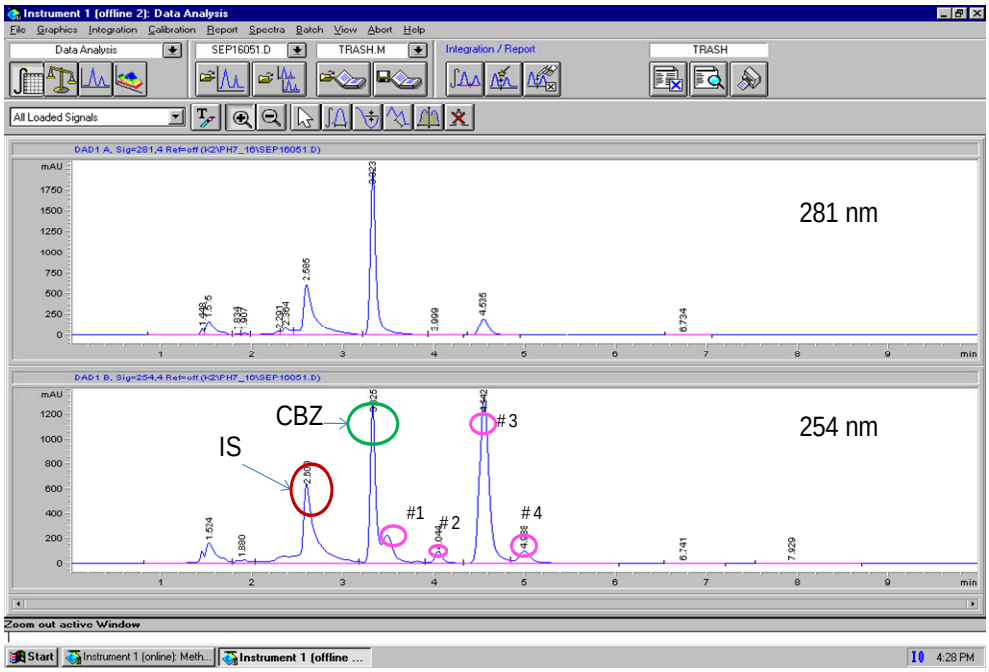

Figure 14: Sample HPLC-UV chromatogram showing CBZ and metabolites generated during chlorination experiments. Reaction conditions: pH 7, [CBZ]$_0$ = 40.4 μM (= 9.6 mg/L); [Free Chlorine]$_0$ = 1210 μM (=85.9 mg/L as Cl_2); [$NaHCO_3$] = 2 mM (=168 mg/L).

The patterns of intermediate formation and loss at different pH values are different and indicate that pH is an important variable in these reactions (**Figures 15-18**). For the 3.5 min peak at low pH (**Figure 15**), the intermediate was formed and initially increased with time until reaching a maximum value after which it decreased. This same intermediate formed and

remained constant at higher pH values except pH 9 when a consistent increase in the amount of the intermediate was observed with time. This suggests that the intermediate reacts with remaining free chlorine and forms other intermediates. Reactivity is faster at lower pH because of the presence of more HOCl than OCl^-.

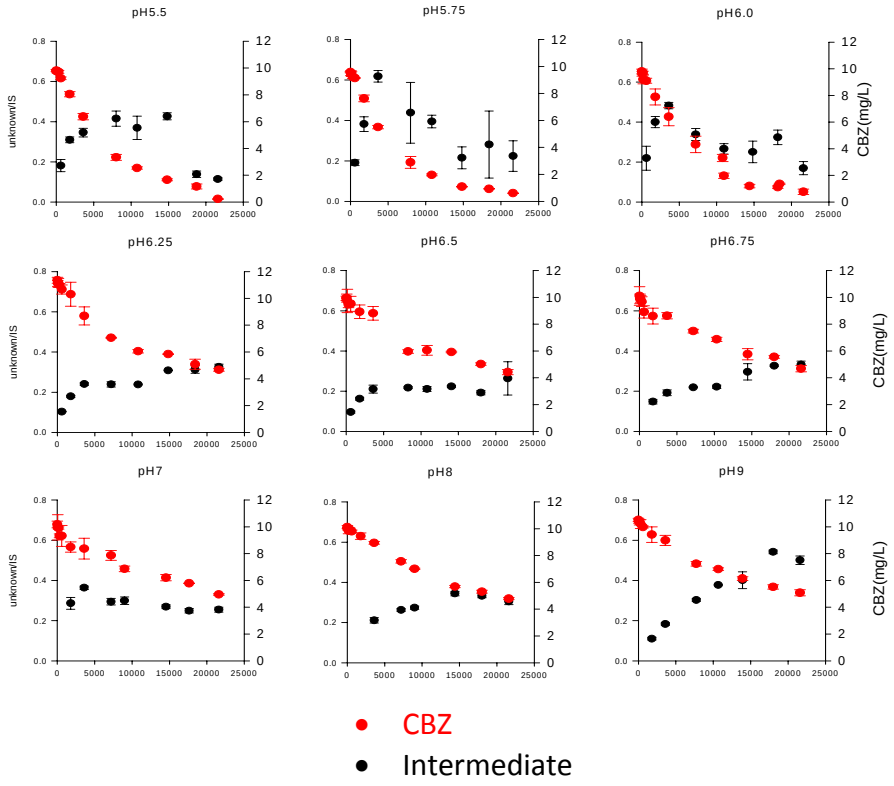

Figure 15: Intermediates at 3.5 retention time from HPLC-UV. Reaction conditions: $[CBZ]_0$ = 40.4 μM (= 9.6 mg/L); $[Free\ Chlorine]_0$ = 1210 μM (=85.9 mg/L as Cl_2); $[NaHCO_3]$ = 2 mM (=168 mg/L).

For the 4.2 min peak, no intermediate was formed at pH 5.5 or 5.75 (**Figure 16**). An intermediate was detected at pH 6.0 and higher and generally increased in concentration over time. The degree of concentration increase also grew as pH increased. This result suggests that this intermediate may be a final product because the reduction of the intermediate does not occur.

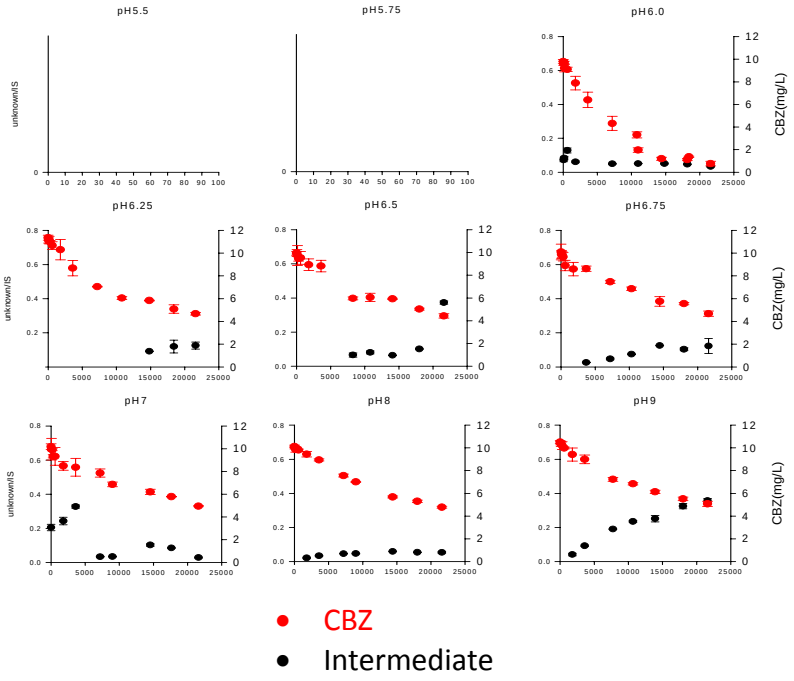

Figure 16: Intermediates at 4.2 retention time from HPLC-UV. Reaction conditions: $[CBZ]_0$ = 40.4 μM (= 9.6 mg/L); $[Free\ Chlorine]_0$ = 1210 μM (=85.9 mg/L as Cl_2); $[NaHCO_3]$ = 2 mM (=168 mg/L).

The 4.7 min intermediate peak formed and remained stable over time at pH 5.5 through 6.75, and increased thereafter up to a pH of 9. This response is shown in Figure 17 and indicates that this intermediate forms more readily at higher pH values.

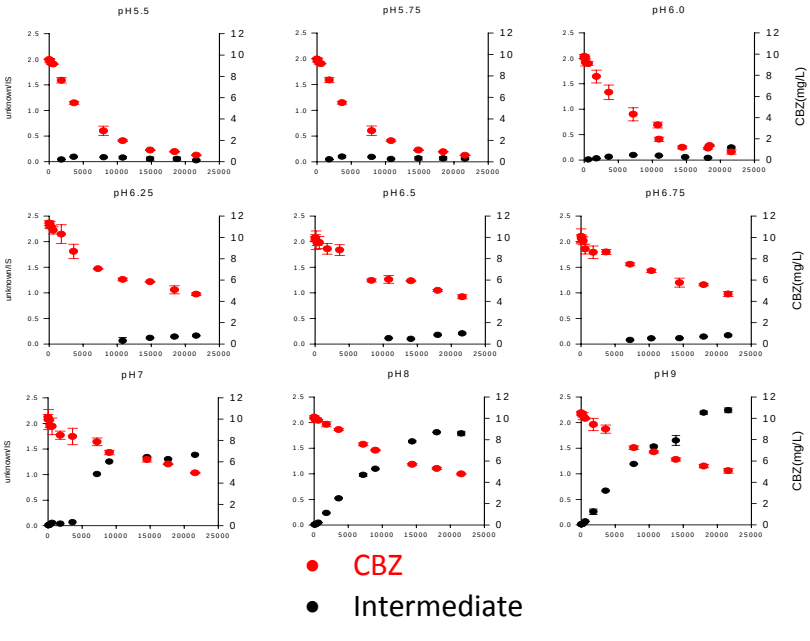

Figure 17: Intermediates at 4.7 min retention time. Reaction conditions: [CBZ]$_0$ = 40.4 μM (= 9.6 mg/L); [Free Chlorine]$_0$ = 1210 μM (=85.9 mg/L as Cl_2); [$NaHCO_3$] = 2 mM (=168 mg/L).

The 5.0 min intermediate peak was observed at only three pH conditions (pH 6, 7 and 8) (**Figure 18**). Production of this intermediate increased over time for these pH values. The pattern is odd, and suggests that something may be affecting the production of this intermediate.

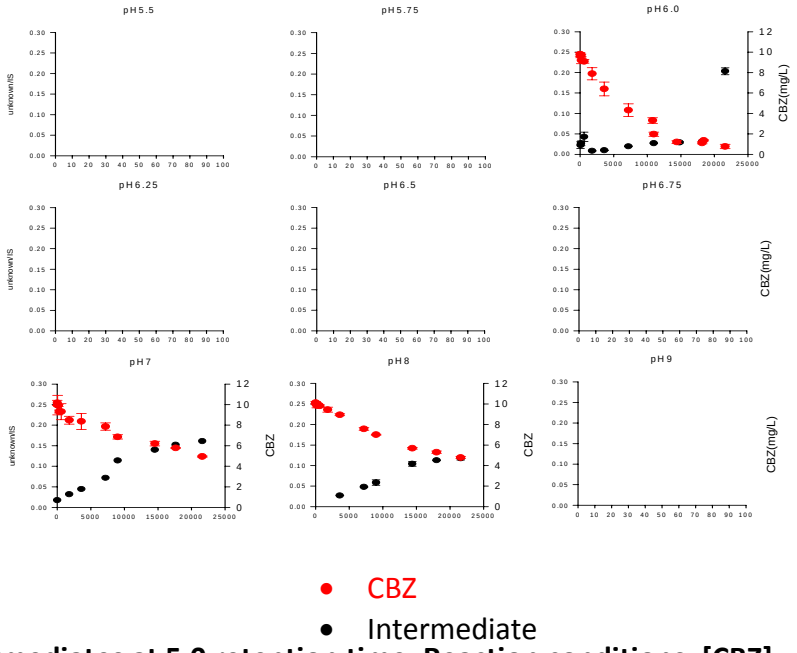

Figure 18: Intermediates at 5.0 retention time. Reaction conditions: [CBZ]$_0$ = 40.4 μM (= 9.6 mg/L); [Free Chlorine]$_0$ = 1210 μM (=85.9 mg/L as Cl_2); [$NaHCO_3$] = 2 mM (=168 mg/L).

<u>LC-ITMS results</u>

LC-ITMS analysis of the metabolites yielded mass to charge (m/z) ratios of each peak corresponding to 194.2, 208.1, 222.1, and 252 m/z. The chromatogram is shown below (**Figure 19**). Proposed structures of these intermediates are shown in **figure 20**. Of the four metabolites detected, two have been identified to a reasonable degree. Iminostilbene ($C_{14}H_{11}N$) which forms during the breakdown of CBZ corresponds to 194.2 m/z. This compound has a similar structure to CBZ except the amine group is no longer present. 10,11-dihydro-10,11-epoxycarbamazepine (CBZ-EP) is another common metabolite that is commonly detected in the

environment and corresponds to 252 m/z. The identities of the other two metabolites are yet to be determined.

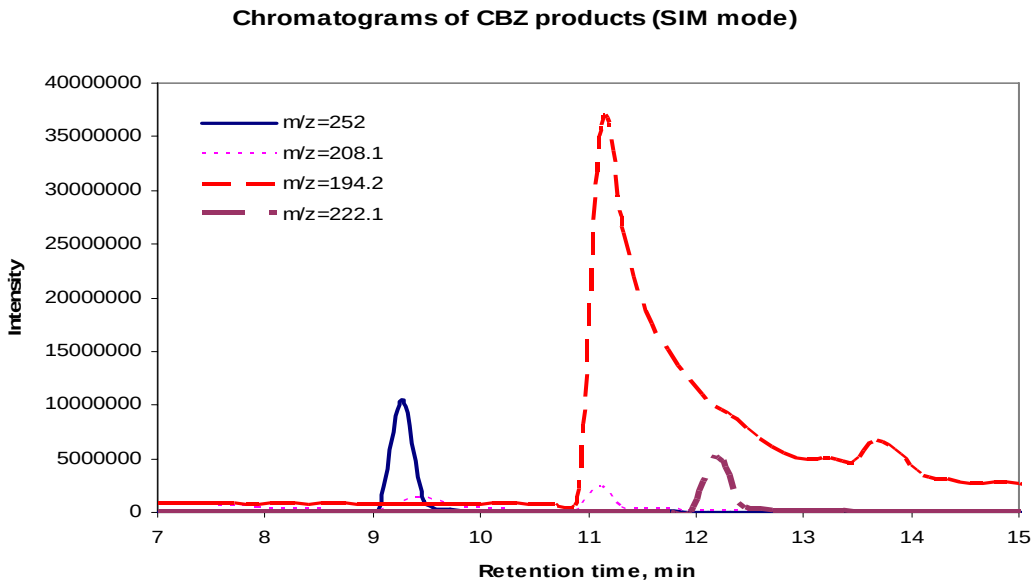

Figure 19: Sample LC-ITMS chromatogram metabolites generated from chlorination experiments. Reaction conditions: pH 7, $[CBZ]_0$ = 40.4 μM (= 9.6 mg/L); $[Free\ Chlorine]_0$ = 1210 μM (=85.9 mg/L as Cl_2); $[NaHCO_3]$ = 2 mM (=168 mg/L).

Proposed reaction pathways are based on the intermediates that have been detected and monitored under excess free chlorine conditions (**Figure 20**). One possible reaction pathway has been observed where an activated oxygen atom binds to the CBZ structure to form 10,11-epoxide metabolite of carbamazepine CBZ-EP (**Figure 20, A**). In the other pathway, CBZ is reduced to 222 m/z (M222) which is then reduced to 193.4 m/z (Iminostilbene). M222 may also then be reduced to compounds corresponding to 208 m/z. Two peaks have the same m/z suggesting the presence of two different compounds. As the identity of these compounds has not been established, the relative quantities of these intermediates could not be established.

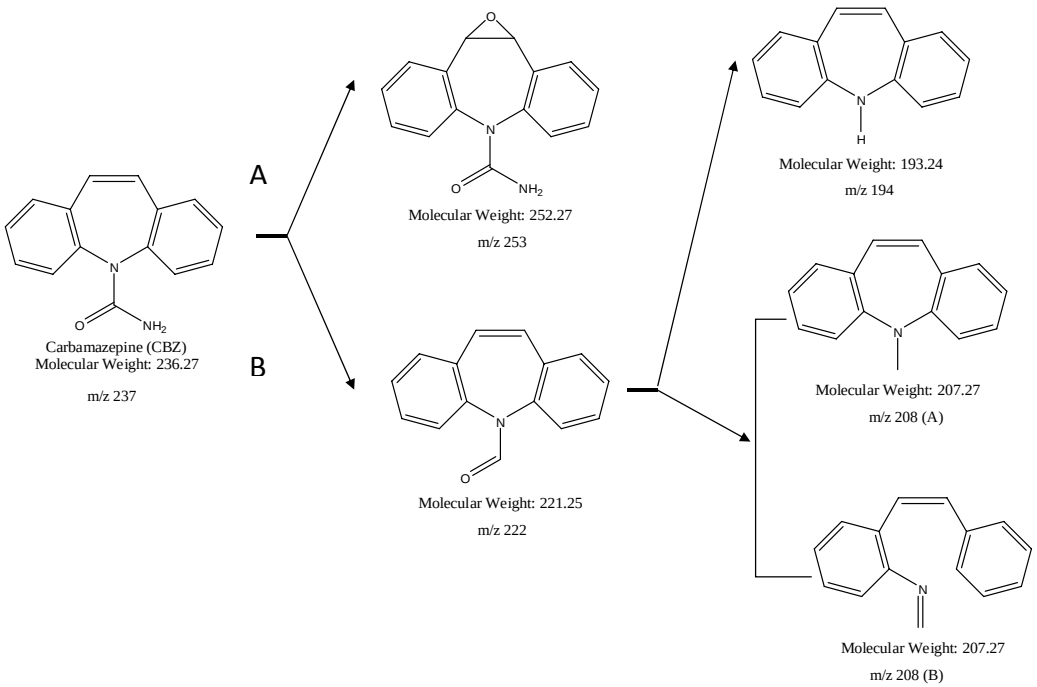

Figure 20: Possible intermediate pathways of carbamzepine with free chlorine reaction.

2.4: Engineering Significance of Results

The results are relevant and useful to the scientific community for the following reasons:

1. Previous studies in the literature showed that CBZ is not removed by free chlorine during disinfection. However, our results indicate that CBZ reacts with free chlorine and forms intermediates under the conditions used in this study. The reaction between CBZ and free chlorine is also affected by pH and temperature. The rate of CBZ loss increases when pH decreases. The CBZ loss rate is proportional to an increase in temperature. When the temperature increases, the rate of CBZ loss also increases. Therefore, this information will be useful for designing a chlorination process that can be effective at removing CBZ. Understanding the reaction between CBZ and free chlorine is a first step to understanding how bio-recalcitrant pharmaceuticals react with free chlorine. Then we can develop the optimum chlorination process to remove most bio-recalcitrant compounds.

2. Intermediates are formed during chlorination, but the intermediates and products have not been identified. Characterizing the intermediates and byproducts is needed to help determine if these compounds are hazardous.

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