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Photo Degradation of Cotnaminants of Emerging concern (CECs) under Simulated Solar Radiation: Implications for their Environmental Fate

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PHOTO DEGRADATION OF CONTAMINANTS OF EMERGING CONCERN (CECs) UNDER SIMULATED SOLAR RADIATION: IMPLICATIONS FOR THEIR ENVIRONMENTAL FATE

A dissertation submitted in partial fulfillment of

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in

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by

Sudha Rani Batchu

2013

To: Dean Kenneth G. Furton
College of Arts and Sciences

This dissertation, written by Sudha Rani Batchu, and entitled Photo degradation of cotnaminants of emerging concern (CECs) under simulated solar radiation: Implications for their environmental fate, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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DEDICATION

I dedicate this thesis to my husband Venkat and loving daughter Hasini, without their understanding, support and love, it would be impossible to stand where I am today. I dedicate this dissertation to my parents and my brother, who stood by me in all parts of my life and taught me to do the right thing.

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ABSTRACT OF THE DISSERTATION

PHOTO DEGRADATION OF CONTAMINANTS OF EMERGING CONCERN (CECs) IN SIMULATED SOLAR RADIATION: IMPLICATIONS FOR THEIR ENVIRONMENTAL FATE

by

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Miami, Florida

Professor Piero R. Gardinali, Major Professor

Contaminants of emerging concern (CECs) are continuously being released into the environment mainly because of their incomplete removal in the sewage treatment plants (STPs). The CECs selected for the study include antibiotics (macrolides, sulfonamides and ciprofloxacin), sucralose (an artificial sweetener) and dioctyl sulfosuccinate (DOSS, chemical dispersant used in the Deepwater Horizon oil spill). After being discharged into waterways from STPs, photo degradation is a key factor in dictating the environmental fate of antibiotics and sucralose. Photodegradation efficiency depends on many factors such as pH of the matrix, matrix composition, light source and structure of the molecule. These factors exert either synergistic or antagonistic effects in the environment and thus experiments with isolated factors may not yield the same results as the natural environmental processes. Hence in the current study photodegradation of 13 CECs (antibiotics, sucralose and dicotyl sulfosuccinate) were evaluated using natural water matrices with varying composition (deionized water, fresh water and salt water) as well as radiation of different wavelengths (254 nm, 350 nm and simulated solar radiation) in

order to mimic natural processes. As expected the contribution of each factor on the overall rate of photodegradation is contaminant specific, for example under similar conditions, the rate in natural waters compared to pure water was enhanced for antibiotics (2-11 fold), significantly reduced for sucralose (no degradation seen in natural waters) and similar in both media for DOSS. In general, it was observed that the studied compounds degraded faster at 254 nm, while when using a simulated sunlight radiation the rate of photolysis of DOSS increased and the rates for antibiotics decreased in comparison to the 350 nm radiation. The photo stability of the studied CECs followed the order sucralose > DOSS > macrolides > sulfonamides > ciprofloxacin and a positive relationship was observed between photo stability and their ubiquitous presence in natural aquatic matrices. An online LC-MS/MS method was developed and validated for sucralose and further applied to reclaimed waters (n =56) and drinking waters (n = 43) from South Florida. Sucralose was detected in reclaimed waters with concentrations reaching up to 18 µg/L. High frequency of detection (> 80%) in drinking waters indicate contamination of ground waters in South Florida by anthropogenic activity.

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LIST OF ABBREVIATIONS AND ACRONYMS

ABBREVIATION	FULL NAME
AP	American Press
APCI	atmospheric pressure chemical ionization
CAS	chemical abstract service
CDDEP	Center for Disease Dynamics, Economics & Policy
CE	collision energy
CECs	contaminants of emerging concern
CH_2Cl_2	dichloromethane
CH_3CN	acetonitrile
CH_3OH	methanol
CID	collision induced dissociation
$CO_3^{\cdot-}$	carbonate radical
CW	canal water
DDT	dichloro-diphenyl-trichloroethane
DOC	dissolved organic carbon
DOSS	dioctyl sulfosuccinate
DP	degradation product
DWTPs	drinking water treatment plants
EDCs	endocrine disrupting chemicals
EPA	Environmental Protection Agency
ESI	electrospray ionization

FDA	Food and Drug Administration
H_2O_2	hydrogen peroxide
H_2SO_4	sulfuric acid
HESI	Heated Electrospray Ionization
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
HRMS	high resolution mass spectra
IT	ion trap
k	pseudo first order rate constant
K_{oc}	sorption coefficient
K_{ow}	octanol water partition coefficient
LC	liquid chromatography
LC-QqQ-MS/MS	liquid chromatography tandem mass spectrometry
MDL	method detection limit
MS	mass spectrometry
MSDS	material safety data sheet
NaOH	sodium hydroxide
NIH	Natioaal Institute of Health
NOM	natural organic matter
$^{\cdot}OH$	hydroxyl radical
$^1O_2$	singlet oxygen
$O_2^{\cdot -}$	Superoxide

OWCs	organic wastewater contaminants
PAHs	polyaromatic hydrocarbons
PFCs	perfluorinated compounds
pK_a	acid dissociation constant
PPCPs	pharmaceuticals and personal care products
ppm	parts per million
Q-TOF-MS	quadrupole time of flight mass spectrometer
Qys	quantum yields
RODW	reverse osmosis deionized water
ROS	reactive oxygen species
RSD	relative standard deviation
Rt	retention time
SPE	solid phase extraction
SRM	selected reaction monitoring
STPs	sewage treatment plants
SW	sea water
TOC	total organic carbon
USGS	United States Geological Survey
UV	ultra violet
WWTP	wastewater treatment plant
amu	Atomic mass units

CHAPTER 1

INTRODUCTION

1.1. Contaminants of Emerging Concern (CECs)

Contaminants of emerging concern (CECs) can be defined as any synthetic or naturally occurring chemical that is not routinely monitored in the environment but has the potential to enter the environment and cause known or suspected adverse ecological and (or) human health effects. In some cases, release of emerging chemical or microbial contaminants to the environment has likely occurred for a long time, but may not have been recognized until new detection methods were developed. In other cases, synthesis of new chemicals or changes in use and disposal of existing chemicals can create new sources of emerging contaminants (USGS, 2008). Some examples of CECs include pharmaceuticals and personal care products (PPCPs), natural and synthetic hormones, perfluorinated compounds (PFCs) and other persistent and toxic organohalogen compounds produced by modern society (Halden, 2010).

1.2. Sources of CECs in the environment

Sources of CECs include but are not limited to household sewer systems, hospital effluents, agricultural runoffs, manufacturing process, livestock, disposal of unused and expired products and from accidental releases like spills, etc. In humans or animals, CECs such as PPCPs are subjected to metabolic reactions like hydroxylation, cleavage or

glucuronation to be converted to nontoxic and more polar compounds which can be easily excreted through urine or faeces. However, a significant portion of many CECs will still be excreted as a mixture of unchanged substance and its transformation product. The rate of excretion of pharmaceuticals as the parent compound ranges from low (≤ 5 %) to high (≥ 70%) (Hirsch et al., 1999; Jjemba, 2006). Thus CECs could reach sewage treatment plant in relatively high concentrations. As an example, the artificial sweetener sucralose is now considered an emerging contaminant as a consequence of its increasing usage over the last decade. The slow metabolic degradation in humans (<10%) (Roberts et al., 2000) has led to its higher detections in treated and untreated waters (parts per billion level) and extremely high persistence in the environment, with half-life up to several years (Lubick, 2008; Lubick, 2009; Lange et al., 2012b). Several studies showed that sewage treatment plants are the main point discharge source for the release of CECs in the environment (Scheurer et al., 2009b; Sui et al., 2010; Ryu et al., 2011; Lacey et al., 2012). Another CEC which received attention after the BP Horizon oil spill in Gulf of Mexico is dioctyl sulfosuccinate (DOSS), an anionic surfactant that accounts for 30% v/v of 1.8 million gallons of Corexit® dispersants used in the recovery efforts (USG; NALCO, 2013a). It is considered as CEC mostly because of the large quantity used and also as a result of the lack of understanding of its ecotoxicology and environmental fate (Place et al., 2010).

1.3. Environmental occurrence

There is a growing concern on the increasing prevalence of CECs in the environment as indicated by the ever increasing number of publications appearing in leading scientific

journals. Many studies found PPCPs in the environment but the one that introduced the topic was the study by USGS that detected of 82 out of 95 organic wastewater contaminants (OWCs) selected in 80% of the 139 streams sampled across 30 states in the United States. The selected OWCs include antibiotics (21 and one metabolite), prescription drugs (12 and 2 metabolites), nonprescription drugs (3 and 2 metabolites), steroids and hormones (14) and other wastewater-related compounds (35 and 5 metabolites) (Kolpin et al., 2002). An investigation by American Press found parts per billion concentrations of antibiotics, anticonvulsants, mood stabilizers and sex hormones in the drinking water supplies of 24 major metropolitan areas (AP, 2008). Antibiotics are one class of PPCPs that are ecologically important and routinely detected in various environmental matrices like wastewater (Karthikeyan and Meyer, 2006; Gao et al., 2012), surface water (Scheurer et al., 2009b), ground water (Barnes et al., 2008; Jesus Garcia-Galan et al., 2010) and even drinking water (Watkinson et al., 2009) at concentrations reaching up to few µg/L. Sucralose is a chlorinated derivative of sucrose and used as an artificial sweetener in many consumer products worldwide. Because of its magnitude of usage over the last few years, sucralose is one of the most frequently detected artificial sweeteners in river, coastal and marine waters in concentrations reaching up to 1 µg/L (Loos et al., 2009b; Mead et al., 2009b). The surfactant used for the oil spill cleanup, DOSS was detected 300 km away from application site and 64 days after its application has been ceased (Kujawinski et al., 2011).

1.4. Significance of CECs in the environment

Detection of many CECs such as hormones, antibiotics, sucralose and DOSS in various water bodies is an alarming issue because of their potential to induce known or suspected adverse ecological and (or) human health effects.

Antibiotics are an important group of CECs because they have the potential to induce bacterial resistance in aqueous environments. Resistance of bacterial strains to widely used medical and veterinary antibiotics poses high risk to humans and livestock exposed to and infected with the resistant strains. Antibiotic resistance is widespread in wastewaters, environmental waters, marine sediments and in fish (Andersen and Sandaa, 1994; Witte, 1998; Miranda and Zemelman, 2001; Costanzo et al., 2005). Constanzo et al. showed that antibiotics can potentially inhibit microbial organisms responsible for denitrification when exposed to high concentrations (Costanzo et al., 2005). Nitrogen cycling bacteria in wastewaters also showed inhibition when exposed to oxytetracyclines at concentrations in the range of 100-250 μg/mL (Campos et al., 2001). More alarming is the fact that resistant genes can be mobilized between various environmental compartments and thus reach the food chain (Chee-Sanford et al., 2001).

Although sucralose has been measured in many water matrices its fate and ecotoxicological data on long term exposure were mostly unknown (Mawhinney et al., 2011b). One study reported that it has the potential to change organisms feeding behaviors, to interfere with plant photosynthesis by shutting down CO_2 uptake in algae (Lubick, 2008). Another study showed that sucralose is capable of influencing both

physiology and locomotion in *Daphnia magna* (Wiklund et al., 2012) at concentrations 0-500 μgL^{-1}. It is found in drinking water in concentrations reaching up to 1 μg/L, higher than any other known anthropogenic trace pollutants (Lange et al., 2012b) but no transformation products have been identified, so its ecotoxicological relevance is still largely unknown.

Another contaminant of concern is DOSS. Despite of its low to moderate toxicity to most aquatic species (George-Ares and Clark, 2000; Hemmer et al., 2010; Judson et al., 2010) and skeptical biodegradation (Kujawinski et al., 2011; Campo et al., 2013), it increases the dissolution of polyaromatic hydrocarbons (PAHs, carcinogens) in aqueous phase (Barron et al., 2003). Moreover in presence of sunlight, a multifold (1.5-48) increase in the toxicity of DOSS dispersed oil was observed in eggs and larvae of Pacific herring (Barron et al., 2003). The data pertaining to the fate and ecotoxicology are still scarce and further research needs to be done.

1.5. Relevance of the study

In order to produce an accurate environmental risk assessment for a contaminant, it is important to fully understand both fate and transformation in the environment. The risk posed depends not only on the initial concentration of the contaminant in the sewage treatment plant (STP) influent but also in its effluent water after the discharge (except DOSS as its application is limited to ocean waters). In STPs, contaminants could undergo hydrolysis, biodegradation and/or sorption to sewage sludge during their treatment. Contaminants which are not eliminated during the normal processes in the

STPs, will be discharged to the environment. In a combined test designed to check both biodegradation and sorption of 17 antibiotics in STPs, Gartiser et al. found that most of the antibiotics showed minimal or insignificant biodegradation and low to moderate sorption (Gartiser et al., 2007), thus leading to their frequent detections in STP effluents (Gulkowska et al., 2008; Gao et al., 2012). Scheurer et al. showed that sucralose was even persistent to advanced treatments employed in wastewater treatment plants including soil aquifer treatment and powdered activated carbon and thus making it a valuable tracer of wastewater intrusions (Scheurer et al., 2011). The data on the biodegradation of DOSS were limited and contradictory (Kujawinski et al., 2011; Campo et al., 2013). Kujawinski et al. reported that DOSS was not biodegradable, while Campo et al. confirmed the opposite. In general, if a contaminant has high concentration in the STP effluent but it can be degraded quickly in the environment, one might assume that the risk posed to the environment would be minimal. On the contrary if a contaminant has either high or low concentration in the STP effluents but is stable in the environment its concentration will likely build up with time and thus pose a greater environmental risk compared to the former contaminant. Thus environmental fate of contaminant (including their stability) is a very important aspect of risk assessment but it has not been extensively studied or fully understood. Since biodegradation and sorption are part of the sewage treatment and were unable to remove selected CECs, it is likely that a large portion of their removal in the environment would be by photodegradation.

1.6. Hypothesis

On the basis of the above mentioned evidences, the hypothesis for the current study is that "Photodegradation plays a significant role in dictating the persistence of CECs in the aquatic environment but its effects are dependent on the quality of the light source and interaction with the matrix components".

1.7. Objectives

1. Study the photolysis of target CECs in both pure water and natural waters to understand the effect of matrix components on the overall photolysis rate.
2. To compare the photolysis rates of CECs in different light sources to understand their behavior in sewage and/or drinking water treatment plant (based on UV 254 nm) and to predict their environmental half-lives (based on UV 350 nm and SunTest experiments).
3. To identify photo transformation products of selected CECs and to predict if the products would present similar structures to the parent molecule.

1.8. Experimental

1. To develop sensitive LC-MS/MS methods for the determination of selected CECs in pure and natural waters.
2. To perform photo degradation experiments in UV 254 nm, 350 nm and SunTest, in both pure and natural waters, to estimate the kinetic parameters.

3. To identify the photo transformation products of selected CECs, propose their structures and possible degradation pathways.

1.9. Target CECs

The CECs selected for this study include antibiotics (11), sucralose and DOSS. Antibiotics are chemotherapeutic agents used in human and veterinary medicine to prevent and treat microbial infections (Sarmah et al., 2006). Antibiotics are used as chemotherapeutic agents in the treatment of infectious diseases in humans, animals and plants. They are also used in sub-therapeutic doses in concentrated animal feed operations as growth promoters (Sarmah et al., 2006). Antibiotics can be classified on the basis of their structure, mode of action or spectrum of activity. Different classes of antibiotics include sulfonamides, quinolones, tetracyclines, macrolides, beta lactams and other miscellaneous ones (Moore, 2013). According to Center for Disease Dynamics, Economics & Policy the United States (US) is among the most intensive users of antibiotics in the world (CDDEP, 2013). United States Food and Drug Administration recently summarized the antibiotic consumption in food producing animals as 28.9-million pounds in 2009; this is about four times the human medical antibiotic use of 7.3-million pounds in the same year (FDA, 2009). The antibiotics selected for the study include Erythromycin, roxithromycin, ciprofloxacin, sulfamethoxazole, sulfadiazine, sulfathiazole, sulfamerazine, sulfamethazine, sulfamethizole, sulfachlorpyridazine and sulfadimethoxine. Sucralose is a chlorinated derivative of disaccharide sucrose, used an artificial sweetener in over 4,000 consumer products all over the world (Torres et al.,

2011). The most familiar consumer product of sucralose is Splenda, which contains 1% sucralose and 99% maltodextrin and dextrose. It is 600 times sweeter by weight than sucrose (Wiet and Miller, 1997). The structures and physical properties of all targeted CECs i.e., antibiotics, DOSS and sucralose were presented in their respective chapters.

1.10. Fundamentals of Photo degradation

According to the Grotthus-Draper law, a chemical must absorb radiation for photo chemical change to happen within the structure (Liefer, 1989). The absorption coefficient, also known as molar absorptivity constant (ε), is a measure of a molecule's ability to absorb a photon of a specific wavelength, as described in equation 1.

$$Abs = (I/I_0) = b\varepsilon[C] \qquad \text{(Eq. 1)}$$ where

I = intensity of light transmitted

I_0 = incident intensity

b = pathlength (cm)

ε = absorption coefficient (L mol^{-1} cm^{-1})

[C] = molarity of the compound (mol/L)

When a compound absorbs energy it gets excited and can undergo multiple physical and chemical processes such as fragmentation, intramolecular rearrangement, hydrogen abstraction and electron transfer (Murov, 1993; Larson, 1994). The efficiency of a photo transformation process can be expressed in terms of quantum yield (ϕ), which is the ratio

between the total number of molecules of the compound transformed by a chemical reaction per total number of photons absorbed by a given system resulting from the presence of the compound.

The quantum yield of a compound for a specific light source at a specific wavelength (Eg. 350 nm) can be calculated as the ratio of first order rate constant (s^{-1}) to the specific rate of light absorption by the compound using the following equations

$$K_s(\lambda_{350nm}) = \frac{E^{\circ}_p(\lambda_{350nm})\varepsilon\lambda_{350nm})[1-10^{-a(\lambda_{350nm})z}]}{a(\lambda_{350nm})z} \qquad \text{(Eq. 2)}$$

$$\phi_{350nm} = \frac{k_d^{'}[CEC]}{k_s(\lambda_{350nm})} \qquad \text{(Eq. 3)}$$

Where $k_d^{'}$ represents the time-based pseudo-first-order rate constant for the individual antibiotic at specific wavelength in a specific matrix. $K_s(\lambda)$ represents the specific rate of light absorption by the compound, $E^0_p(\lambda)$ the incident photon irradiance, $\varepsilon(\lambda)$ the molar absorption coefficient which measures the probability that a compound will absorb light at a certain wavelength), $a(\lambda)$ the solution absorbance, z the optical path length of the solution (1 cm), and ϕ the quantum yield. The incident photon irradiance can be measured with an Ocean Optics spectrophotometer using a cosine corrector. Two processes are responsible for the photolysis of organic compounds under natural conditions: direct photolysis and indirect photolysis.

$$A + h\nu \rightarrow A^* \rightarrow A' \qquad \text{(Direct photolysis) (Eq. 4)}$$

For direct photolysis, the compound of interest [A] absorbs energy, the rate of its transformation follows first order reaction and the rate law is:

$$\ln [A]_t = -kt + \ln [A]_0 \qquad \text{(Eq. 5)}$$

Where $[A]_t$ is concentration of A at time t and $[A]_0$ is the initial concentration of A. k is the direct photolysis first-order constant and its half–life ($t_{1/2}$) expressed by:

$$t_{1/2} = \frac{\ln 2}{k} \qquad \text{(Eq. 6)}$$

In indirect photolysis, energy is absorbed by a photosensitizer [B*] and initiates a series of reactions that results in the transformation of the compound (Equation 7) and is governed by second order reaction. But if [B*] remains fairly constant over the period of time, the rate law could be condensed to pseudo first order reaction and half-life can be calculated using the equation 6.

$$A + B^* \rightarrow [A\text{-}B]^* \rightarrow A' \qquad \text{(Indirect photolysis)} \quad \text{(Eq. 7)}$$

Indirect photolysis can happen through reactive oxygen species such as singlet oxygen, superoxide, hydroxyl radical and hydrogen peroxide (Lu et al., 2006). Nitrate and dissolved organic matter are good sources of many short lived photo oxidants in environmental waters.

1.10.1. Photosensitizers and oxidants

In natural waters, reactive oxygen species are commonly found at very low concentrations ranging from picomolar to micromolar concentrations (Table 1) (Burns et al., 2012). They are generated by photolysis, electron transfer or through energy transfer reactions (Bartosz, 2006; Lu et al., 2006). For example, singlet oxygen and hydrogen peroxide are generated from excited photosensitizers such as humic substances (Draper and Crosby, 1983; Lu et al., 2006). Superoxide can be generated from the photodegradation of dissolved organic matter (Richard and Canonica, 2005). Hydroxyl radical can be generated from various sources including but not limited to photolysis of nitrate and nitrite ions, photo Fenton reactions, dissolved organic matter (Zafiriou, 1977; Stangroom et al., 1998; Calza et al., 2012; Zeng et al., 2012). Humic acids in natural waters play a significant role in the photodegradation of organic molecules. They can competitively absorb incident photons showing an inner filter effect and thus leading to attenuation of rates. Alternatively, they can get excited to form triplet states and either generate many transient reactive species or photosensitize the target molecule, leading to an increase in photolysis rates. The overall effect of natural organic matter on the photolysis of an organic molecule is a combination of these two opposite effects (Stangroom et al., 1998). Thus the photolysis rate of a contaminant in the environment cannot be easily interpreted, because different enhancement/attenuation mechanisms may operate simultaneously. Hence experiments must be performed with natural waters to better imitate the prevailing conditions in the environment.

Table 1. Steady state concentrations of various reactive oxygen species in natural waters

ROS	Steady state concentration in natural waters (M)*
Singlet oxygen ($^1O_2$)	10^{-12} to 10^{-13}
Superoxide ($O_2^{\cdot-}$)	10^{-9} to 10^{-12}
Hydrogen peroxide (H_2O_2)	10^{-7} to 10^{-11}
Hydroxyl radical ($^{\cdot}OH$)	10^{-15} to 10^{-18}
Carbonate radical ($CO_3^{\cdot-}$)	10^{-13} to 10^{-15}

*(Burns et al., 2012)

CHAPTER 2

Photodegradation of antibiotics in simulated solar radiation: Implications for their environmental fate

2.1. Introduction

Antibiotics have been found in surface water at concentrations ranging from 30-1900 ng/L (Batt and Aga, 2005; Brown et al., 2006; Hernandez et al., 2007; Watanabe et al., 2008; Zuccato et al., 2010). Development of antimicrobial resistance is the most important issue related to the occurrence and persistence of antibiotics in the environment (Costanzo et al., 2005; Martinez, 2008) because of the possibility of transfer of resistant genes to the native bacterial population. In aqueous environments, some antibiotics undergo photodegradation relatively easily (Trovo et al., 2009), in comparison to others such as macrolides (Tong et al., 2011) and it is because the rate of photolysis depends on many factors like light source, pH of the water, latitude and season.

In the recent years, photodegradation of antibiotics was studied using different light sources like UV light (Pereira et al., 2007), solar light simulator (Ge et al., 2010) and natural sunlight (Avisar et al., 2010) for a variety of water matrices such as laboratory grade water, fresh waters (from rivers and lakes) and salt water (Sirtori et al., 2010). Most of the researchers studied photo degradation under the influence of buffers, metals like Fe^{+3}, humic acids or titanium dioxide (Belden et al., 2007; Vione et al., 2009; Avisar et al., 2010; Paul et al., 2010; Anquandah et al., 2011) and thus the rate constants obtained may not be the best representative of typical natural environmental scenarios. The goal of

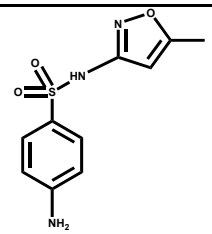
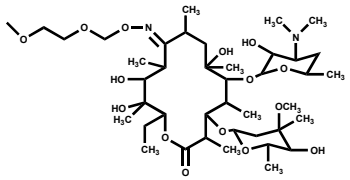
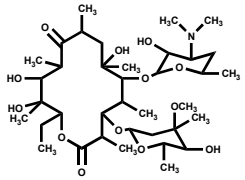
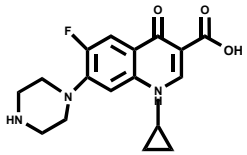
the present study is to conduct experiments under conditions that could better reflect real environmental settings. Therefore, irradiations were carried out using four selected antibiotics in pure and natural waters with no chemical additives and as a function of three light sources (UV 254 nm, UV 350 nm and Solar simulator). Detailed information on the selected antibiotics is shown in Table 2.

2.2. Experimental Section

2.2.1. The selected antibiotics

The four reference antibiotics selected for this study represent main antibiotic groups which were frequently detected in the environment. Sulfamethoxazole [1] is a sulfonamide antibiotic, commonly used in human beings in synergistic combination with trimethoprim (under the trade name Bactrim) to treat urinary tract infections and in veterinary medicine as growth promoters (Crosby, 1991). Roxithromycin [2] and erythromycin [3] are macrolide antibiotics widely used to treat respiratory tract infections (Cals et al., 2008). Ciprofloxacin [4], a man made fluoroquinolone antibiotic. It is one of the most popular fluoroquinolone used in human medicine (Giger et al., 2003).

Table 2. Target antibiotics, structures, CAS and their classification

Name	Structure	CAS	Classification
Sulfamethoxazole [1]		723-46-6	Sulfonamide antibiotic
Roxithromycin [2]		80214-83-1	Macrolide antibiotic
Erythromycin [3]		114-07-8	Macrolide antibiotic
Ciprofloxacin [4]		85721-33-1	Fluoroquinolone antibiotic

2.2.2. Chemicals

Standards of Sulfamethoxazole [1], roxithromycin [2], erythromycin [3] and ciprofloxacin [4] and sulfathiazole were purchased from Sigma-Aldrich (St. Luis, MO, USA). Sulfathiazole was used as an internal standard for quantification and was also purchased from Sigma-Aldrich (St. Luis, MO, USA). Standard stock solutions (100 µg/ml) of all the compounds were prepared in methanol and stored in the dark at <4 °C.

Ultrapure Water (H_2O), methanol (CH_3OH), dichloromethane (CH_2Cl_2), acetonitrile (CH_3CN) and hexane (C_6H_{14}) (all of optima grade or equivalent) and whatman GFF glass fiber filters were obtained from Fisher Scientific (Fair Lawn, NJ). Reverse osmosis deionized water (RODW) was produced in the laboratory using a Milli-Q RG water system from Millipore.

2.2.3. Sampling sites/Sample collection

Canal water (fresh water, CW) was collected from Tamiami Canal at its confluence with the Miami River. Seawater (SW) was taken from the shore at Bill Baggs State Park at Key Biscayne, Miami, FL. Canal and salt water characteristics are shown in Table 3. Natural waters were filtered using a 0.2-micron membrane fiber filter to remove any particles and microorganisms and stored in the dark at <4°C until the experimental solutions were prepared, typically within a month.

Table 3. Characteristics of canal water and salt water used in the experiment

Parameter	Canal water	Salt water
pH	8.1	7.9
Dissolved organic carbon (mg-C/L)	10.4	1.37
Electrical conductivity (µs)	544	88000
Fe^{3+} (µg/L)	187	109
Salinity (ppt)	0.2	36

2.2.4. Comparison of light sources employed in the study

Photodegradation experiments were conducted using Rayonet UV photochemical reactors (Southern New England Ultraviolet Co., Branford, CT) and a Sun Test XLS Tabletop

Xenon Exposure System (ATLAS Material Testing Technology LLC, Chicago, Illinois, USA) and natural Sunlight.

The photochemical reactor was used with 16-mercury vapor lamps (Ultra Violet (UV) 254 nm) or black light phosphor bulbs (UV 350 nm). UV 254 nm radiation is the most used light source to induce photolysis of a wide variety of organic compounds for wastewater treatment (Thomas, 2003) and hence included in the present study to assess the likelihood of the antibiotic to survive water treatment in the treatment process. UV 350 nm is comparable to the range of UVA region (315 nm-400 nm) of sunlight and hence commonly used to predict the photodegradation of pharmaceutical compounds in environment (Lam et al., 2003; Radjenovic et al., 2009; Sturini et al., 2009).

Sun light plays an important role in determining the persistence and environmental fate of antibiotics. The intensity of natural sunlight depends on latitude (Li et al., 2002), depth of the photic zone and overcast conditions. Because of this, experiments conducted with sunlight take longer and may pose a challenge in comparing the data between different studies. Hence experiments were conducted with a SunTest, which is a surrogate of natural sunlight. The SunTest XLS produces continuum of wavelengths from 300 nm to 800 nm using a properly fitted Xenon lamp on the top of the exposure chamber. The xenon lamp was used with its maximum intensity (750W/cm^2) for macrolide antibiotics i.e., erythromycin and roxithromycin and at its minimal intensity (250 W/cm^2) for sulfamethoxazole and ciprofloxacin.

A comparison of light sources employed in this study is shown in Figure 1. The spectra were recorded using with Ocean Optics spectrophotometer with cosine corrector. Mercury vapor lamps produce intense radiation at 254 nm where as black phosphor bulb emits polychromatic radiation with a maximum at 365 nm. The wavelength distribution and the intensity of the Xenon lamp are very similar to that of natural sunlight (Diepens and Gijsman, 2007). Compared with the spectrum of real sunlight measured on a sunny day at Miami, FL, the light distribution of SunTest xenon lamp matched sunlight very well, especially in the most relevant 300 nm-400 nm range.

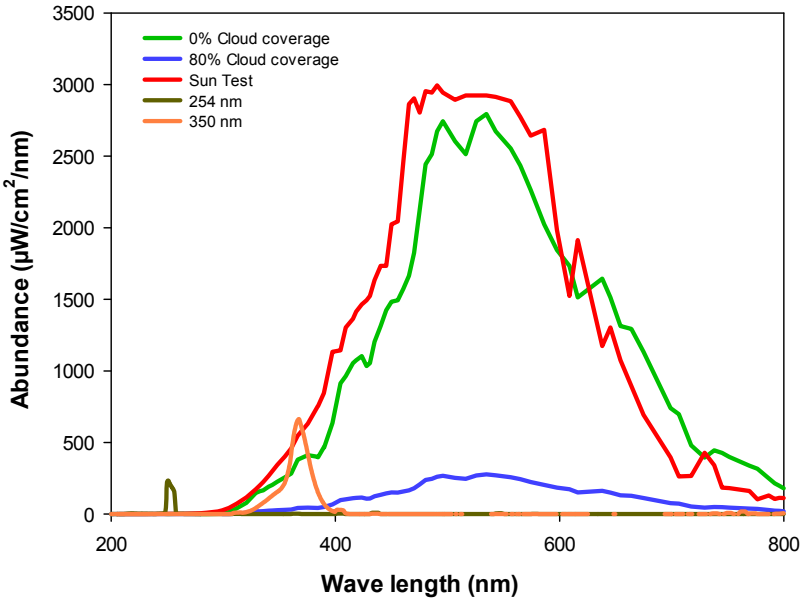


Figure 1. Comparison of light sources used in the study

2.2.5. UV absorbance spectra versus type of water matrix

The pH of water matrices used in this study are listed in Table 3 and range from 5.5 (RODW) to 8.1 (CW). The pH of the matrix can influence speciation of the antibiotic, absorbance and hence the potential for photolytic degradation when irradiated at a specific wavelength. Thus there could be a relationship between pH of the water sample, speciation (governed by pKa), and its absorbance. In addition to pH, other two major differences between matrices are dissolved organic content (DOC, CW being rich) and ionic strength (SW being the highest). Natural organic matter present in the water sample may act as photosensitizer or as a quencher, therefore changing the rate of photolysis of substrate under study. As the rate of photolysis of a compound in natural waters is likely be influenced by the combination of DOC, pH and ions in the medium, to study their integrative effects on absorption, the absorption spectra were recorded using standard solutions of 1-4 (at 5 μg/mL) in RODW, CW and SW. UV–visible measurements of the water samples were carried out with 1 cm quartz UV-visible cells at room temperature (20°C), using a Shimadzu UV-2101PC UV–visible double beam spectrophotometer. MilliQ water was used as a reference.

2.2.6. Quantum yields

It is expected that pH influences speciation and thus absorbance of the molecule. Therefore quantum yields (QYs) were experimentally calculated for the direct photolysis of the studied antibiotics in 254 and 350 nm light source in both RODW and natural waters using the procedure proposed by Pereira et al. (Pereira et al., 2007). Molar

absorption coefficients of 1-4 at 254 nm and 350 nm was calculated by dividing the absorbance at a specific wavelength by its molar concentration (Eq. 1).

Quantum yields were calculated as the ratio of first order rate constant (s^{-1}) to the specific rate of light absorption by the compound (equation 2 & 3) in Chapter 1. The abundance intensity of 16-mercury vapor lamps (UV 254 nm) and black light phosphor bulbs (UV 350 nm) is 233 and 844 $\mu W/cm^2/nm$, respectively.

2.2.7. Sample irradiation

The experimental working solutions of all antibiotics were prepared by diluting each stock solution to 1mg/L with the three types of water: a) RODW b) CW and c) SW. Seven 30 ml- quartz tubes (Southern New England Ultraviolet Co., Branford, CT) were used for running experiments in the photochemical reactor and one tube filled with 30 ml of RODW was used as a blank. Dark controls (3 tubes) were totally covered with aluminum foil to prevent light exposure. The remaining three tubes were left uncovered and exposed to light. All the tubes were placed on a merry-go-round to ensure uniform irradiation in the Rayonet UV photochemical reactor chamber. For experiments in SunTest, 25 mL of the experimental solutions were placed in six UV transparent polyethylene bags (*Nasco* WHIRL-PAK 2 OZ.). Similar to the UV photolysis experiments 3 control bags were kept in the dark by covering them with aluminum foil and the remaining three were left uncovered. One bag filled with RODW acts as blank. All bags were then floated in a water bath with circulating water during exposure in SunTest to keep the solution at a constant temperature (25°C).

At specified time intervals, 500 µl aliquots were withdrawn into 2 mL amber glass vials, sulfathiazole (500 ng/mL) added and samples were analyzed subsequently by liquid chromatography tandem mass spectrometry (LC-MS/MS).

2.2.8. Liquid Chromatography/Mass Spectrometry

A Thermo LCQ advantage max Ion trap Mass spectrometer (IT-MS) equipped with electrospray ionization source (ESI) and connected to surveyor LC system (Thermo Finnigan, San Jose, CA) was used for sample analysis. Separations were achieved on a Luna C18 column (150 cm x 4.6 mm x 5 µ particle size) equipped with a Luna C18 guard column, both purchased from Phenomenex (Torrance, CA). The pump was operated at a flow rate of 0.5 mL/min. The column oven temperature was 30 °C, and the full loop injection volume was 20 µL. The separation was performed using a simple binary gradient mobile phase consisting of methanol (A) and water with 0.3% formic acid (B) (Table 4). The ESI-IT-MS/MS was operated in positive ion mode. The capillary temperature was 315 °C, and the spray voltage was 4.5 kV. Nitrogen was used as a sheath gas and as an auxiliary gas at a flow rate of 59 and 20 arbitrary units, respectively. Collision energy is an important factor in the collision induced dissociation (CID) process by which fragment ions are produced. Optimized values of collision energy and fragment ions monitored for each antibiotic are shown in Table 5. Two SRM transitions were monitored for the identification and quantitation of target analytes. The SRM chromatograms obtained of canal water fortified at a concentration of 100 µg/L are shown in Figure 2.

Table 4. Summary of the gradient program for the chromatographic separation

Time (min)	% Solvent A	%Solvent B (0.3% formic acid in water)
0	20	80
13	90	10
16	20	80
25	20	80

Table 5. Optimized parameters for the detection of all analytes and internal standard in MS/MS SRM mode.

Analyte	ESI parameter					
	Precursor ion m/z	m/z	SRM 1	SRM 2	Optimal Collision energy	Rt (min)
Sulfamethoxazole	$[M+H]^+$	254	188	194	32	11.5
Roxithromycin	$[M+H]^+$	837	679	522	32	11.7
Erythromycin	$[M+H]^+$	734	558	576	30	10.4
Ciprofloxacin	$[M+H]^+$	332	314	288	55	5.87
Sulfathiazole	$[M+H]^+$	256	156	190	30	8.21

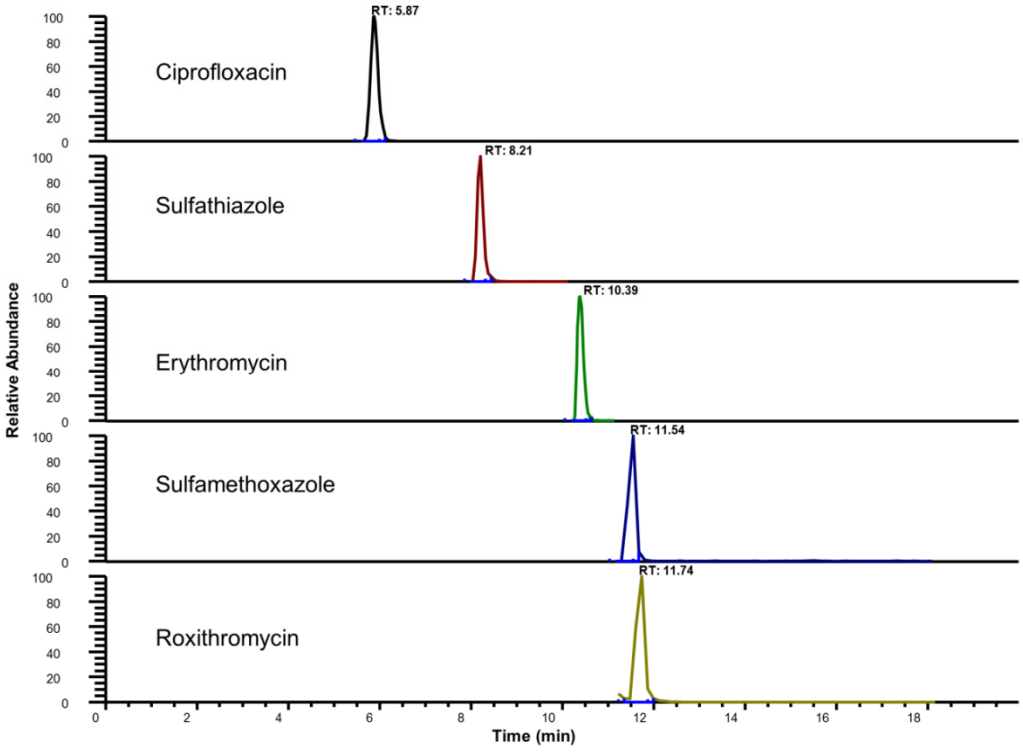


Figure 2. SRM chromatograms of canal water fortified with target antibiotics and internal standard at a concentration of 100 µg/L.

2.3. Results and discussion

2.3.1. UV absorbance spectra versus type of water matrix

For any organic compound to be photolabile, it must be able to absorb photons of the incident light. The spectra in Figure 3 were plotted after blank matrix subtraction and it shows that all compounds absorb UV light from 200-350 nm. However, at the same concentration (5 µg/mL) the intensity as well as the position of the absorption maximum is different among the antibiotics tested which can be attributed to the structural differences between them. The absorbance maximum (λ_{max}) for sulfamethoxazole [1] is

255nm, ciprofloxacin [4] has two λ_{max} values at 275 nm and 325 nm in RODW while both macrolides do not show any absorbance maxima. The pH of the water sample alters the absorbance spectrum as evidenced in ciprofloxacin. Ciprofloxacin λ_{max} at 276 nm in RODW (pH 5.5) underwent hypsochromic shift to 270 nm in CW (pH 8.1) and the same trend was observed by (Avisar et al., 2010). Though the absorbance is different between RODW and natural waters, the shape of the spectrum matches well for all antibiotics except in low UV range (200-230 nm).

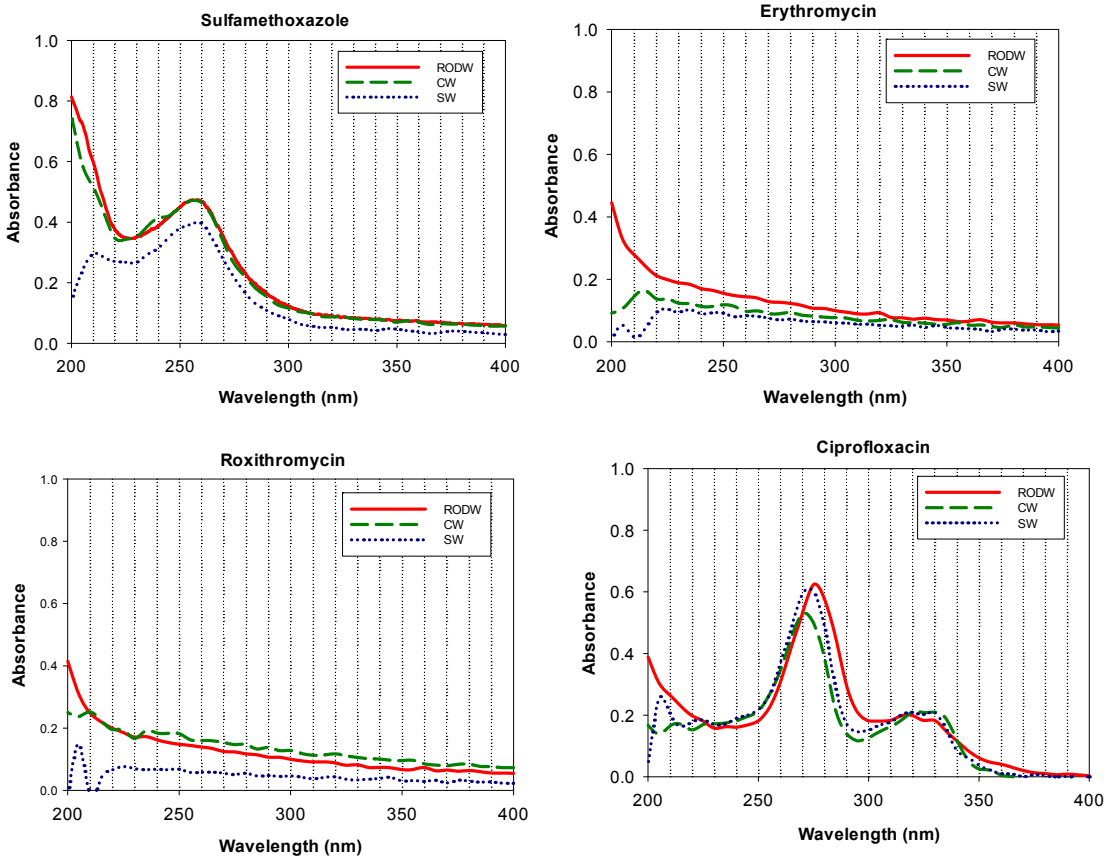


Figure 3. Absorbance spectra of 5 µg/mL solutions of sulfamethoxazole, roxithromycin, erythromycin and ciprofloxacin in various matrices.

2.3.2. Photolysis rate constants, nature of matrix and Quantum yield

Photolysis decay curves of antibiotics in different light sources was constructed by plotting Ln (Ct/C0) versus time of irradiation, where Ct is the concentration of substrate at any given point of time and C0 is the initial substrate concentration. Sigmaplot v11.0 software was used to plot the degradation curves and each data point shown in the graph is an average of the triplicate measurements using standard deviation as error bars. As the substrate decay can be fit to pseudo first order reaction the slope of the plot gives pseudo first order rate constant (k). Because the pH of the natural waters in the environment (6-9) falls within ±3 units of pKa of target analytes, it affects speciation of the compound. The pKa values (SRC, 2013), speciation and pseudo first order rate constants of 1-4 are listed in Table 6. In natural waters all antibiotics exist predominantly in cationic form except sulfamethoxazole [1]. For [1], the degradation under 254 nm is so fast that no substrate was left after 1 minute of irradiation. Hence a calculation was made to obtain a half-life of at least 2 minutes and accordingly four lamps were used instead of 16. Even with four lamps, the half-live in all matrices is less than 2 minutes and it was not possible to distinguish the difference in rates among the three matrices (Fig. 4a). The rate constants shown in Table 6 were corrected for 16 lamps. The kinetic data from 350nm and SunTest shows that [1] is more stable at natural water pH as it exists as anion (Boreen et al., 2004) which is in good agreement with other studies (Avisar et al., 2010) (Fig. 4b,c). It suggests that photolysis rate is dependent mainly on its speciation of [1] in the given water sample. The molar absorption coefficient obtained at 254 nm and pH 7.9 (SW) in this study was 19958 M^{-1} cm^{-1} and is in the same order of magnitude to the one

reported by Baeza and Knappe in a solution of controlled pH 7.85 at 254 nm (16580 M^{-1} cm^{-1}) (Baeza and Knappe, 2011). For sulfamethoxazole, the single wavelength quantum yields measured at 350 nm showed opposite pH dependence similar to photolysis rates i.e., the higher the pH of the water sample the lower is the quantum yield. This same trend observed in another study (Boreen et al., 2004).

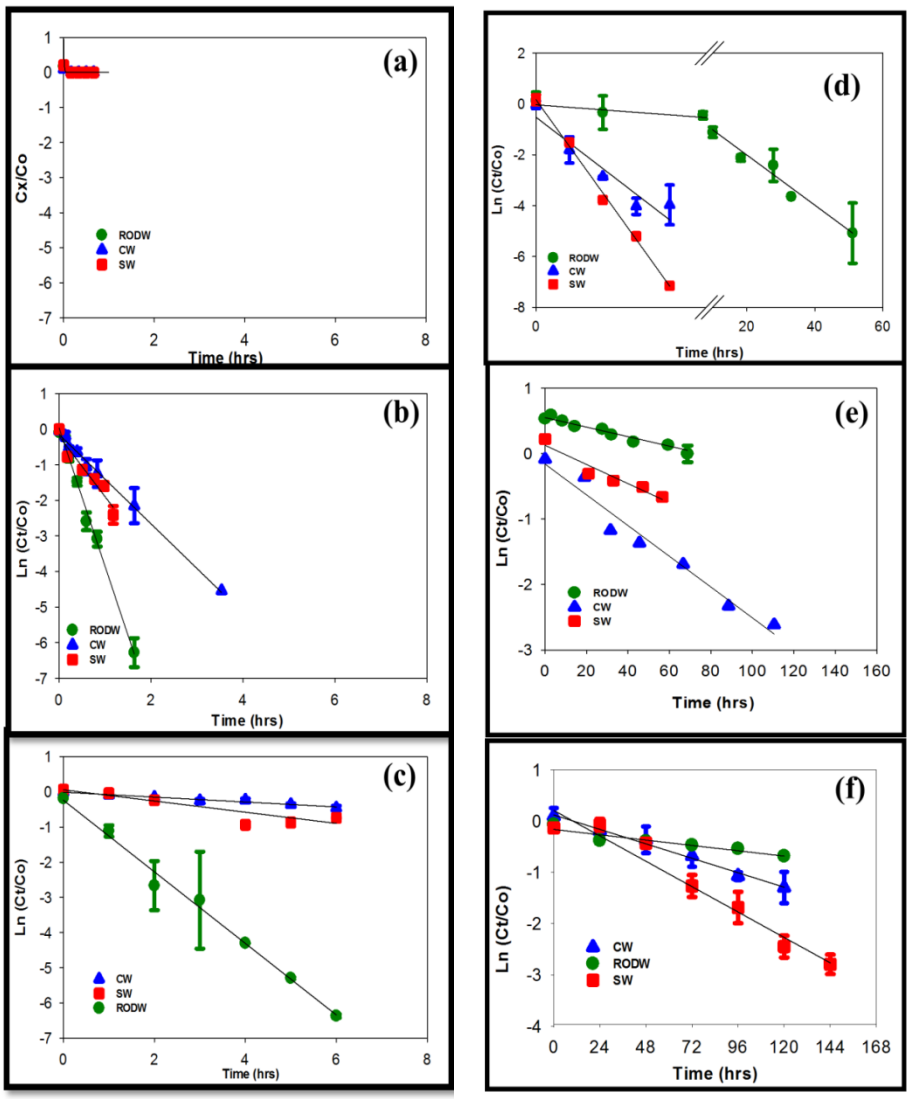


Figure 4. Photolysis decay curves of sulfamethoxazole at a) 254 nm b) 350 nm c) solar simulation; roxithromycin at d) 254 nm e) 350 nm f) solar simulation. RODW = reverse osmosis deionized water CW = canal water SW = salt water.

Table 6. Molar absorptivity coefficient and quantum yields of (1) Sulfamethoxazole (2) Roxithromycin (3) Erythromycin (4) Ciprofloxacin in RODW and natural water matrices measured under [a]254 nm [b]350 nm. pKa values were obtained from SRC database (accessed on 1/18/2013). Pseudo first order rate constants measured at [c]254 nm [d]350 nm [e]SunTest RODW = reverse osmosis deionized water SW = salt water CW = canal water. SH_2^+ = acidic form SH= neutral form S^- =basic form.

Compound	pK_a	Matrix	Protonation state	Molar absorption coefficient [a] (M^{-1} cm^{-1})	Quantum Yield[a] (mol $einstein^{-1}$)	Molar absorption coefficient[b] (M^{-1} cm^{-1})	Quantum Yield[b] (mol $einstein^{-1}$)	Rate constant (hr^{-1})[c]	Rate constant (hr^{-1})[d]	Rate constant (hr^{-1})[e]
1	1.6, 5.8	RODW	SH_2^+	24018	1.2309	3834	1.07530	73.8	15.5	3.05
		SW	S^-	19958	1.3686	2372	0.73740	73.8	5.02	0.209
		CW	S^-	23909	1.2367	3537	0.37500	73.6	6.79	0.477
2	9.0	RODW	SH_2^+	24139	0.0012	11175	0.00020	0.103	0.00730	0.00570
		SW	SH_2^+	9599	0.0492	4973	0.00070	1.01	0.0231	0.0117
		CW	SH_2^+	27153	0.0109	15999	0.00040	1.81	0.0146	0.0212
3	8.7	RODW	SH_2^+	21717	0.0003	10218	0.00020	0.0217	0.00640	0.0123
		SW	SH_2^+	11778	0.0030	6611	0.00040	0.272	0.0231	0.00990
		CW	SH_2^+	16582	0.0045	8181	0.00070	0.133	0.00904	0.00290
4	5.9, 8.9	RODW	SH_2^+	14911	0.1590	4074	0.15680	7.58	2.44	0.415
		SW	SH_2^+	17650	0.8321	2446	1.55120	16.1	27.0	1.60
		CW	SH_2^+	17729	0.2970	1684	4.02300	45.0	14.9	3.03

In spite of absence of absorbance maxima in UV range, macrolides [2-3] showed higher degradation in experiments with mercury vapor lamp (Fig.4d, 5a). The rates at 254 nm were up to 125 fold higher than those at 350 nm and SunTest (Table 6). Photolysis of the roxithromycin in natural waters alongside photolysis in RODW revealed significant enhancement in the rates in natural waters relative to that observed in RODW as illustrated in Figure 4 (d, e, f). This indicates other possible indirect photochemical process in the natural waters. The fresh water matrix (CW) has high dissolved organic carbon that can absorb radiation over a wide range of wavelengths, gets photo excited; photosensitize roxithromycin. Photosensitized NOM can generate many transient yet powerful photo oxidants like hydroxyl radical (Burns et al., 2012) which could augment the photolytic degradation of roxithromycin. Rapid oxidation of roxithromycin by hydroxyl radical during ozonation process was previously documented (Dodd et al., 2009). Humic acid enhanced photolysis rates in natural waters under simulated and natural solar light was also observed in another macrolide molecule azithromycin (Tong et al., 2011).

The increased rates observed in salt water (SW) may possibly be attributed to abundant chloride ions, which in presence of hydroxyl radical ($^{\cdot}OH$) form $Cl_2^{\cdot-}$ (Vione et al., 2005), a strong oxidant for the removal of antibiotics in surface waters (Chamberlain and Adams, 2006). However the exact mechanism ($^3DOM^*$ or $^{\cdot}OH$ or $Cl_2^{\cdot-}$) responsible for indirect photolysis process in natural waters can be identified by the use of sensitizers and/or quenchers and was not performed here as this is not the main objective of the study.

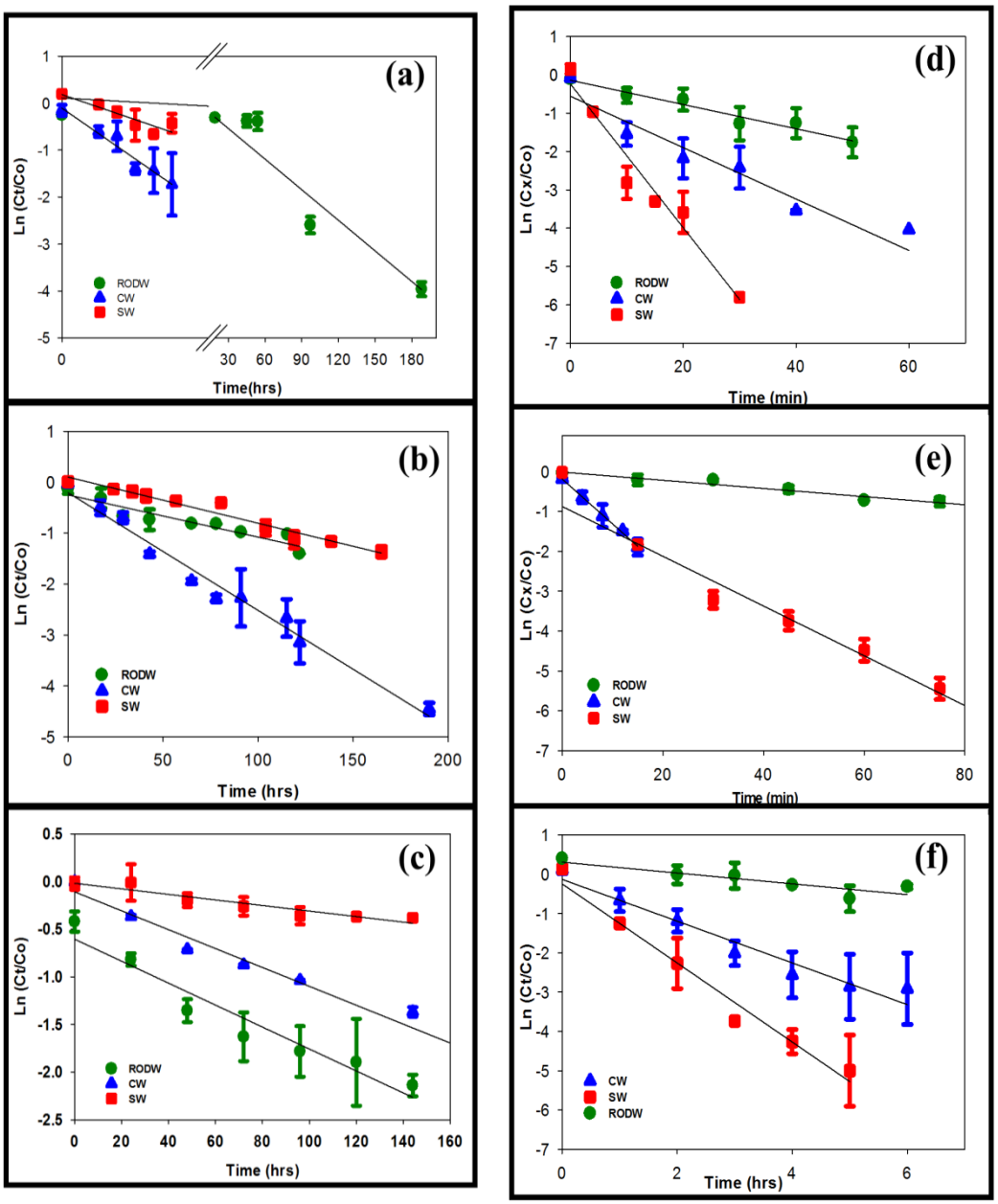


Figure 5. Photolysis decay curves of erythromycin at a) 254 nm b) 350 nm c) solar simulation; ciprofloxacin at d) 254 nm e) 350 nm f) solar simulation. RODW = reverse osmosis deionized water CW = canal water SW = salt water.

Because erythromycin and roxithromycin are macrolide antibiotics, one would expect similar photochemical behavior for both of them. However, kinetic data shows otherwise i.e. rates of photolysis of roxithromycin in natural waters relative to RODW were

enhanced in all light sources whereas rates were not always higher in natural waters for erythromycin. Variation in the behavior between roxithromycin and erythromycin can be attributed to the structural difference at C-9 substituent position (Table 2), which is also responsible for the observed differential effects on growth inhibition in *H. influenzae* (Mabe et al., 2004a). Macrolides degraded with a half-life of 3-10 days in natural waters in solar simulation which is in agreement with previous studies reporting half-lives in the range of 1-40 days (Vione et al., 2009; Tong et al., 2011). In natural waters (pH: 7.9-8.1), carboxylic functional moieties (with pK_a of 2.5-5) of humic acids would be deprotonated, and phenolic functional groups (with pK_a of 8-10) would be partially deprotonated. At pH 7.9 (CW), approximately 93% roxithromycin present as neutral form and 7% of roxithromycin is in cationic form whereas 86% erythromycin present as neutral form and 14% of erythromycin is in cationic form. The % of neutral form is reduced by 6% for both macrolides by increasing the pH to 8.1 (SW). Even though percentage of cationic form is higher in erythromycin, protonation of imine functional group in roxithromycin demonstrates a localized positive charge, and possibly developed strong electrostatic attractions with negative charges in the humic acids and lead to higher removal of roxithromycin than erythromycin. Differences in the behavior of antibiotics towards dissolved humic substances was previously reported (Ding et al., 2013). In the natural environment, sunlight intensity is greatly reduced with increasing depth and hence these macrolides would be expected to persist longer than what this study calculated.

Similar to roxithromycin, ciprofloxacin also showed enhanced degradation (2-11 fold higher) in natural waters compared to RODW (Fig. 5d, e, f). The increase in the photolysis in natural waters could be attributed to the [Cip-Fe^{3+}] complex formation

resulting in oxidative degradation of the antibiotic, commonly observed for all fluoroquinolone antibiotics (Zhang and Huang, 2007). Ciprofloxacin degrades very quickly with a half-life of about 14 min in CW. Under very similar conditions (Sun Test with an irradiance intensity of 250 W/cm^2, river waters) a structurally similar fluoroquinolone antibiotic, enrofloxacin was estimated to degrade with a half-life of 7 min (Sturini et al., 2009). Lam et al. reported a half-life of 11-15 min in deionized water when using xenon lamp with maximum intensity (765 W/cm^2) (however they also reported that the filter is not very efficient in removing wavelengths < 290nm and hence there is systematic error in the results) (Lam et al., 2003). These results are comparable to the data presented in this study (100 min at 765 W/cm^2). Molar absorption coefficients obtained in this study at 254 nm in RODW (14911 M^{-1} cm^{-1}) matches very well with that reported by Pereira et al. under the same conditions (254 nm, laboratory-grade water, 12400 M^{-1} cm^{-1}) (Pereira et al., 2007).

In a previous study by Panditi et al. for the analysis of antibiotics in reclaimed and surface waters in South Florida sulfamethoxazole and erythromycin were detected at rather higher concentrations (up to 400 ng/L) and more frequently (80-100%) (Panditi et al., 2013). Sulfamethoxazole's higher frequency of detection despite of its low photo stability may be explained based on its high consumption both in humans and animals. The exact consumption statistics for therapeutic drugs in United States are not available and the annual estimations for human health and agriculture are controversial (Mellon et al., 2001). Macrolides are of high ecological concern because of their high resistance to bio- (Gartiser et al., 2007) and photo degradation (this study), their high toxicity towards blue green algae, (EC50 = 0.002 mg/L for erythromycin in *P. subcapitata*) (Isidori et al.,

2005) and their ability to enzymatically inhibit the cytochrome P-450 leading to adverse drug reactions in humans (Sagir et al., 2003). A positive correlation between macrolides use and penicillin non-susceptible and macrolide resistant pneumococcal isolates was reported by Albrich et al. (Albrich et al., 2004). Even though both macrolides were equally photostable, roxithromycin is rarely detected in surface waters as it was clinically approved for use in United States recently (NIH, 2010).

2.3.3. Metabolite identification and degradation pathway of roxithromycin

Antibiotics were designed to cause a physiological effect. They have specific biological effects not only on humans and/or animals, but also on non- target aquatic organisms. In the environment they will be subjected to different attenuation processes including photolysis. In the process of degradation it may form stable metabolites that still retain biological activity (Bonvin et al., 2012) or that can increase the sensitivity of bacterial strains (Palmer et al., 2010). Some of the degradation products of antibiotics detected may not have any antibacterial activity, for example oxidation of trimethoprim by ferrate (VI) lead to the formation of oxidation products with no biological activity (Anquandah et al., 2011). The degradation products were identified (m/z 307, 305, 197, 111 and 171) and structures were proposed by our laboratory. The same product m/z 305 was also observed in the photolysis of trimethoprim in simulated solar radiation (Shi, 2006). Since the biological activity of the metabolites is largely unknown and could range from none to almost equivalent to parent, they must also be included in evaluating the overall effect of antibiotics on the ecosystem.

Of the four antibiotics studied, macrolides are highly photo stable, implying their persistence in the aquatic environments (Isidori et al., 2005). Previous studies reported the degradation products of erythromycin under many conditions (Kim et al., 2004a; Kim et al., 2004b) and hence degradation products of roxithromycin was the focus of this study. Samples irradiated at 254 nm in RODW were used for the preliminary identification of products. The column and mobile phase conditions were same as before. A mass spectrometer (MS) was operated in full scan mode and the mass range monitored was 150-900 amu. Two new masses detected only in irradiated samples were m/z 734 and m/z 576. The kinetic profiles of formation of the degradation products are shown in Figure. 6 and the proposed pathway is shown in Figure. 7.

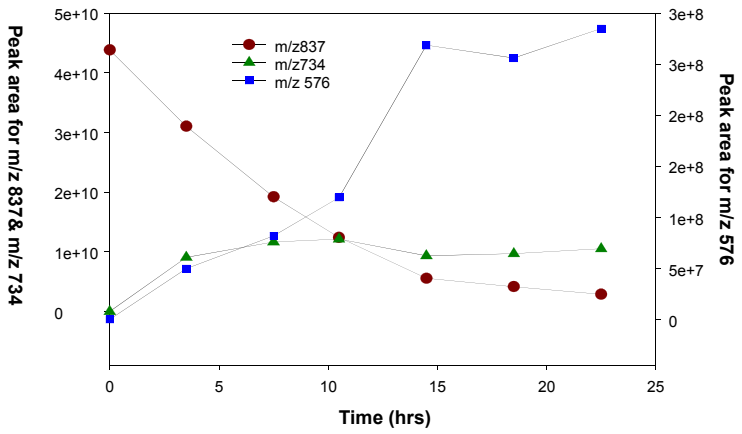
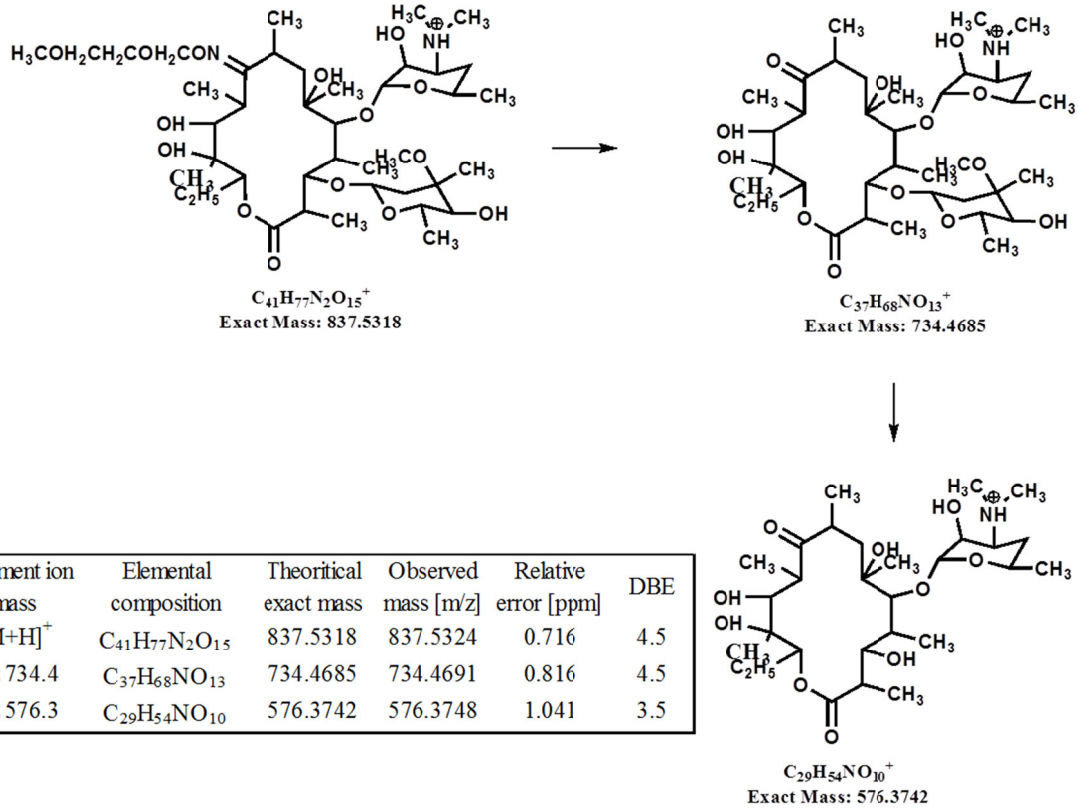


Figure 6. Kinetic profiles of roxithromycin and its degradation products



Fragment ion mass	Elemental composition	Theoritical exact mass	Observed mass [m/z]	Relative error [ppm]	DBE
$[M+H]^+$	$C_{41}H_{77}N_2O_{15}$	837.5318	837.5324	0.716	4.5
m/z 734.4	$C_{37}H_{68}NO_{13}$	734.4685	734.4691	0.816	4.5
m/z 576.3	$C_{29}H_{54}NO_{10}$	576.3742	576.3748	1.041	3.5

Figure 7. Possible photo transformation pathways of roxithromycin in water under 254 nm irradiation

Accurate mass measurements were performed on a Thermo Scientific LTQ orbitrap. The major degradation product (m/z 734.4685) was not in the controls and corresponds to erythromycin. Formation of erythromycin from roxithromycin in natural waters has never been reported. The second degradation product (m/z 576.3742) was formed by the loss of the cladinose sugar moiety from the major degradation product and had been reported in the literature (Kim et al., 2004a). It should be noted that in both degradation products the lactone ring and desosamine sugar moiety remain intact. These metabolites are mainly responsible for macrolides antimicrobial activity through the formation of hydrogen bonds with rRNA (Mabe et al., 2004b).

2.4. Conclusion

Antibiotics are highly water soluble (log Kow = -0.52 to 3) and are resistant to biodegradation (Gartiser et al., 2007). However, the results from photochemical degradation experiments showed that photodegradation had a potential impact on the environmental fate of antibiotics. The extent of photolysis was largely dependent on the pH of the sample, dissolved organic content, [Cl^-] and the irradiation source. Results indicate that the selected antibiotics will be subject to a wide range of photodegradation rates with sulfamethoxazole degrading relatively quickly, ciprofloxacin degrading moderately and macrolides degrading much more slowly in the environment. Under 254 nm irradiation, it was found that roxithromycin gets converted to erythromycin, which is also biologically active. Moreover macrolides have the ability to pass on antimicrobial properties to the degradates (Radjenovic et al., 2009). In lieu of these facts, it is of great importance to include the monitoring of degradates to fully assess the risks associated with antibiotic released from treated wastewater streams entering the natural environment. The photolysis rates reported in this work were measured under continual irradiation in isolated systems and thus represent higher limit, as the degradation in natural systems may be significantly reduced based on overcast conditions, the depth of the photic zone and on the composition of the water matrix.

CHAPTER 3

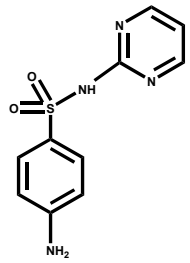
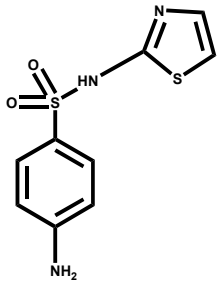
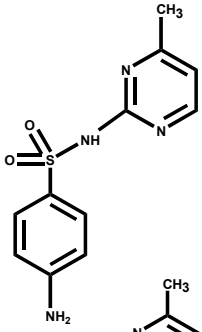
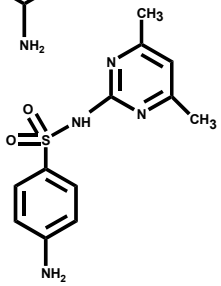
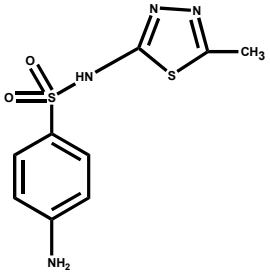
Photodegradation of sulfonamide antibiotics and in simulated and natural Sunlight: Implications for their environmental fate

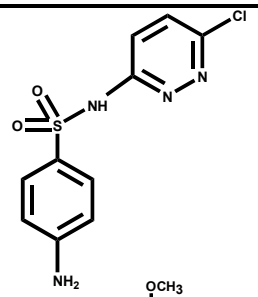
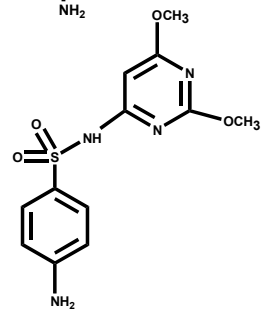
3.1. Introduction

Pharmaceutical and personal care products have been increasingly detected in drinking, surface and wastewater effluents globally (Halling-Sorensen et al., 1998; Calamari et al., 2003) because of their inefficient removal in sewage treatment plants (Gulkowska et al., 2008). Although several classes of compounds have been detected, antibiotics are of particular concern as a result of their ability to spread bacterial resistance among native bacterial populations (Costanzo et al., 2005). Among antibiotics, sulfonamides are one of the most frequently detected class, in concentrations that could reach up to 1.9 μg/L (Kolpin et al., 2002). Sulfonamides are used in aquaculture, as herbicides (Hirsch et al., 1999), in animal husbandry (Crosby, 1991; Hirsch et al., 1999) and to treat respiratory and urinary tract infections in humans (Huovinen, 2001). Although there were no water standards established for monitoring antibiotics in waters, the levels found in the environmental waters are not trivial and the effect of chronic exposure to low levels of mixtures of antibiotics is largely unknown. This is in part because of the lack of information on the environmental fate of antibiotics (Backhaus and Grimme, 1999). Photochemical degradation is likely an important degradation pathway in controlling the fate of antibiotics in the environment. Ultra violet 254 nm is mostly commonly used for disinfection purposes in drinking water treatment plants and solar simulators are widely

used to predict photolysis under Sunlight. Accordingly, degradation of sulfonamides has been studied in UV light (Pereira et al., 2007; Avisar et al., 2010), solar simulators (Garcia-Galan et al., 2012) and in natural sunlight (Boreen et al., 2004). Photodegradation of sulfonamide antibiotic is highly dependent on the composition of the water sample (Eg. pH, dissolved organic matter, $[Cl]^-$, Fe^{3+}) and thus researchers have studied photolysis in controlled pH (using buffers(Khaleel et al., 2013), humic substances (Boreen and McNeill, 2005) and in oxidants like hydrogen peroxide and free chlorine (Chamberlain and Adams, 2006; Pereira et al., 2007). The above mentioned parameters may likely have a synergetic or antagonistic effect on the rate of photolysis and thus changing the extent and type of photolysis, for example photodegradation of five membered sulfonamides is solely due to pH dependent direct photolysis (faster) whereas in nitrate-enriched waters it is because of the combination of direct and indirect photolysis (slower) (Boreen et al., 2004). Accordingly the study was performed using reverse osmosis deionized water (RODW) and 2 natural waters that have wide variation in their composition. Detailed information on the selected antibiotics is shown in Table 7.

Table 7. Target sulfonamide antibiotics, structures, CAS and their classification

Name	Structure	CAS
Sulfadiazine (5)		68-35-9
Sulfathiazole (6)		72-14-0
Sulfamerazine (7)		127-79-7
Sulfamethazine (8)		57-68-1
Sulfamethizole (9)		144-82-1

Name	Structure	CAS	Classification
Sulfachlorpyridazine (10)		80-32-0	Sulfonamide antibiotic
Sulfadimethoxine (11)		122-11-2	Sulfonamide antibiotic

3.2.Experimental Section

3.2.1. Chemicals

Standards of Sulfadiazine, sulfathiazole, sulfamerazine, sulfamethazine, sulfamethizole, sulfachlorpyridazine and sulfadimethoxine were purchased from Sigma-Aldrich (St. Luis, MO, USA). Sulfamethoxazole-d4 was used as an internal standard for quantification and was purchased from Toronto Research Chemicals (Toronoto, Canada). Standard stock solutions (100 µg/ml) of all the compounds were prepared in methanol and stored in the dark at <4 °C.

3.2.2. Sampling sites and light sources

The information on the water matrices (RODW, CW and SW) and light sources (UV 254 nm, 350 nm and SunTest) was given in the previous chapter (Section 2.2.3 and 2.2.4).

Additionally, experiments were conducted under natural sunlight from July 20th-July 30th 2012, in North Miami, FL (25°N latitude and 80°W).

3.2.3. UV absorbance spectra and quantum yields

The general structure of sulfonamide antibiotics is shown below (SH in Fig. 8) and they all consist of a benzene ring, an amine moiety ($–NH_2$), and a sulfonamide group ($–SO_2NHR$). They differ in the N-bound substituent of the sulfonamide linkage. The selected sulfonamides have pK_a values ranging from 2-2.9 and 5.3 to 8 for pKa_1 and pKa_2, respectively. Figure 8 shows the speciation of a generic sulfonamide antibiotic (Boreen et al., 2004).

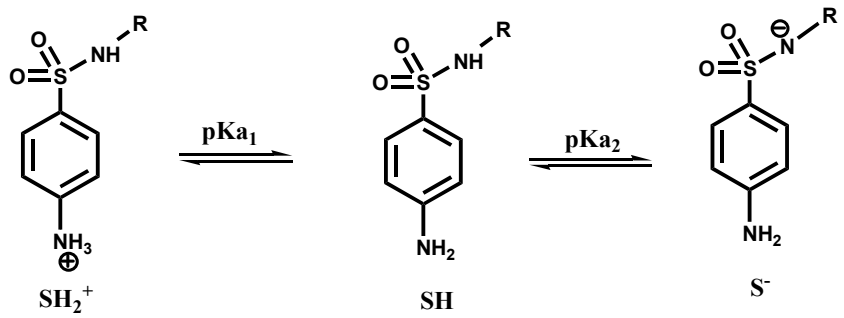


Figure 8. Protonation states of the sulfa drugs SH_2^+ = cationic form, SH = neutral form, S^- = anionic form

On the basis of the pH of the water matrices used in this study (5.5-8.1), it is expected to see different speciation in each matrix, which has a relation to the molecule absorbance and thus photochemical behavior. Hence the UV absorption spectra were measured in all three types of water samples (RODW, CW and SW).

3.2.4. Sample irradiation

Sample irradiation at 254 nm, 350 nm and in solar simulator were exactly similar to the ones described in the previous chapter (section 2.2.7). Natural sunlight experiments were conducted from July 20th-July 30th 2012, in North Miami, FL (25°N latitude and 80°W). 25 mL of experimental solutions (100 µg/L) were placed in five UV transparent polyethylene bags (2 dark controls, 2 treatments, 1 blank) (*Nasco* WHIRL-PAK 2 OZ.). Dark control was wrapped with aluminum foil. All bags were placed in a plastic basket (36 cm*24 cm*12cm) with side holes for water circulation and then floated in the pond which is right in front of the building. The Intensity of the sunlight was measured twice a day (20 measurements randomly taken at different times of the day) using an Ocean Optics spectrophotometer with cosine corrector and the average of all measurements was used to normalize the intensity of SunTest to sunlight and kinetic data were corrected accordingly. Aliquots were collected at specified time intervals, fortified with internal standard and analyzed by LC-MS/MS.

3.2.5. Liquid Chromatography/Mass Spectrometry

This method was based on an Online SPE method published by Panditi and Batchu et al. (Panditi et al., 2013). Separation of analytes was achieved on a Hypersil gold column (150 mm x 2.1 mm x 3 µm) equipped with guard column of the same material and from the same manufacturer (Thermo Fisher Scientific, San Jose, CA). The full loop injection volume was 20 µL. The HPLC was operated at a flow rate of 0.25 mL/min using a binary gradient mobile phase consisting of acetonitrile (A) and 0.1% formic acid in LC-MS water (B) shown in Table 8. Detection of analytes was performed on a TSQ Quantum

Access triple quadrupole Mass Spectrometer (Thermo Scientific, San Jose, CA, USA) equipped with an Ion Max API Heated Electrospray Ionization (H-ESI) source operated in positive ionization mode. For salt water samples, the effluent from column has been diverted to waste for the first 1.2 min to prevent salts from entering MS. For all analytes, optimum ionization conditions and selected reaction monitoring (SRM) transitions were selected by infusing a 5 mg/L individual standard solution through a syringe pump at a flow rate of 50 µL/min. For all analytes, $[M+H]^+$ was selected as the parent ion. Subsequent identification of the two most abundant fragment ions and selection of the optimum collision energies (CEs) was carried out in the product ion scan mode. Optimized parameters for quantitative analysis of antibiotics are shown in Table 9. The capillary temperature was 315 °C, and the spray voltage was 4.5 kV. Nitrogen was used as a sheath gas and as an auxiliary gas at a flow rate of 39 and 20 arbitrary units respectively. The reconstructed SRM chromatograms of a salt water sample fortified at 100 µg/L are shown in Figure 9. Xcalibur 2.1 software was used for data acquisition and quantification of analytes.

Table 8. Summary of the gradient program for the chromatographic separation

Time (min)	% Solvent A (Acetonitrile)	% Solvent B (0.1% formic acid in LC-MS water)
0.0	10	90
2.0	10	90
7.0	50	50
10.0	25	75
12.0	10	90
15.0	10	90

Table 9. Optimized parameters for the detection of all analytes and internal standard in MS/MS SRM mode.

Analyte	ESI parameter						
	Precursor ior	m/z	SRM 1	CE1	SRM 2	CE2	Tube lens
Sulfadiazine	$[M+H]^+$	251.065	92.396	35	156.054	16	82
Sulfathiazole	$[M+H]^+$	256.023	91.925	26	156.214	14	76
Sulfamerazine	$[M+H]^+$	265.067	92.131	28	108.434	25	86
Sulfamethazine	$[M+H]^+$	279.134	92.157	32	124.123	24	102
Sulfamethizole	$[M+H]^+$	271.027	92.235	34	156.165	12	92
Sulfachlorpyridazine	$[M+H]^+$	285.059	92.136	29	108.113	23	98
Sulfadimethoxine	$[M+H]^+$	311.122	92.138	33	108.098	29	100
Sulfamethoxazole-d4	$[M+H]^+$	258.004	96.191	32	112.101	32	86

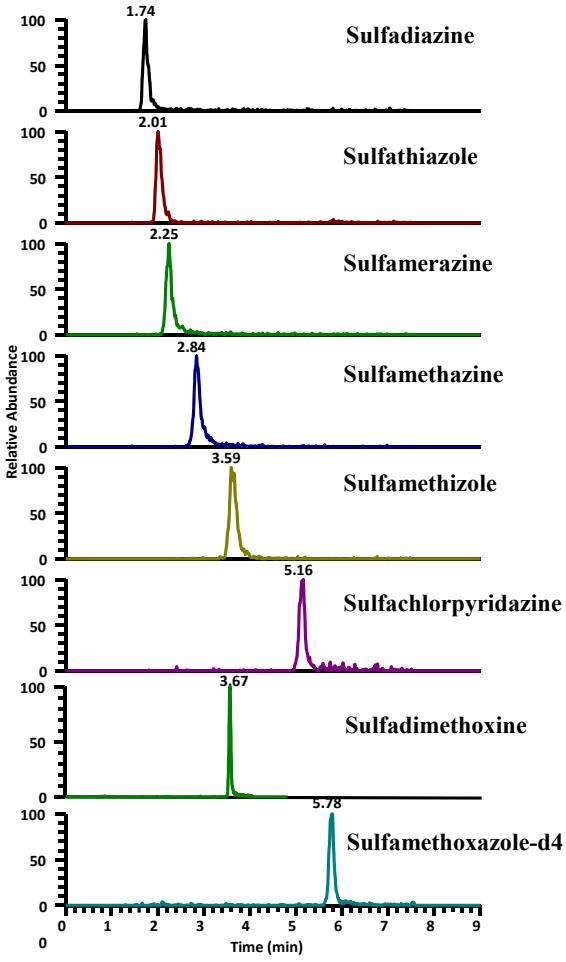


Figure 9. SRM chromatograms of salt water fortified with target antibiotics and internal standard at a concentration of 100 µg/L.

3.3. Results and discussion

3.3.1. UV absorbance spectra versus nature of the matrix

The pH of the natural waters range from 6-9 and one would expect to see the anionic form of sulfonamide as the prevalent species in the environment. The studied matrices differ not only in the pH but also in dissolved organic carbon concentration that could potentially change photolytic behavior of an antibiotic. Previous studies measured absorption spectra in buffers or at controlled pH but no one has studied the absorption spectra in natural water matrices to see the cumulative effect of pH, DOC and other key parameters like ionic strength. Hence my study measured the absorption spectra in natural waters alongside RODW and is shown in Figure 10-11. As expected, the extent of light absorption, absorbance maxima and the shape of the spectra was dependent on the type of matrix, which might lead to significant differences in the pseudo first order rate constants shown in the later parts. For example in sulfadiazine, the spectra was smoother in SW and absorption maximum shifted to 240 nm in DDW to 245 nm in CW, from 250 nm in SW.

3.3.2. UV 254 nm

All sulfonamides have absorption at 254 nm. A plot for natural log of photolysis decay of antibiotics (Ln Ct/C0) as a function of time yielded straight line (r^2>0.9) indicating that the experimental data can be fit to pseudo first order kinetics. Results indicate that sulfonamides degrade 80-100 fold faster at 254 nm compared to other light sources. All experiments at UV 254 nm were performed with 4 lamps instead of 16 to obtain half-

lives to be in the range of minutes. For ease of comparison, the rate constants obtained in different matrices (normalized to 16 lamps), are shown as grouped bar chart, in Figure 12.

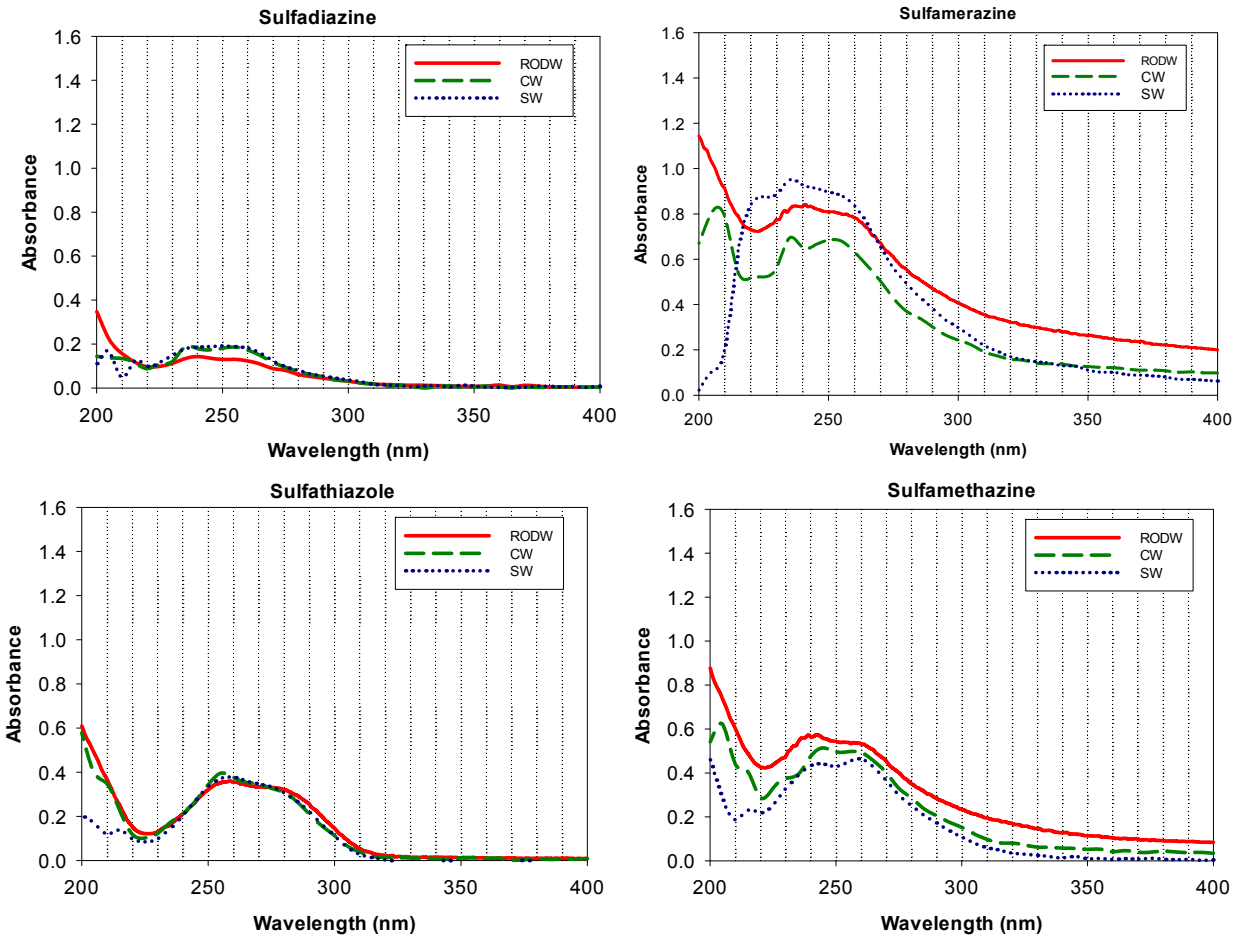


Figure 10. Absorbance spectra of 5 µg/mL solutions of sulfadiazine, sulfathiazole, sulfamerazine and sulfamethazine in various matrices

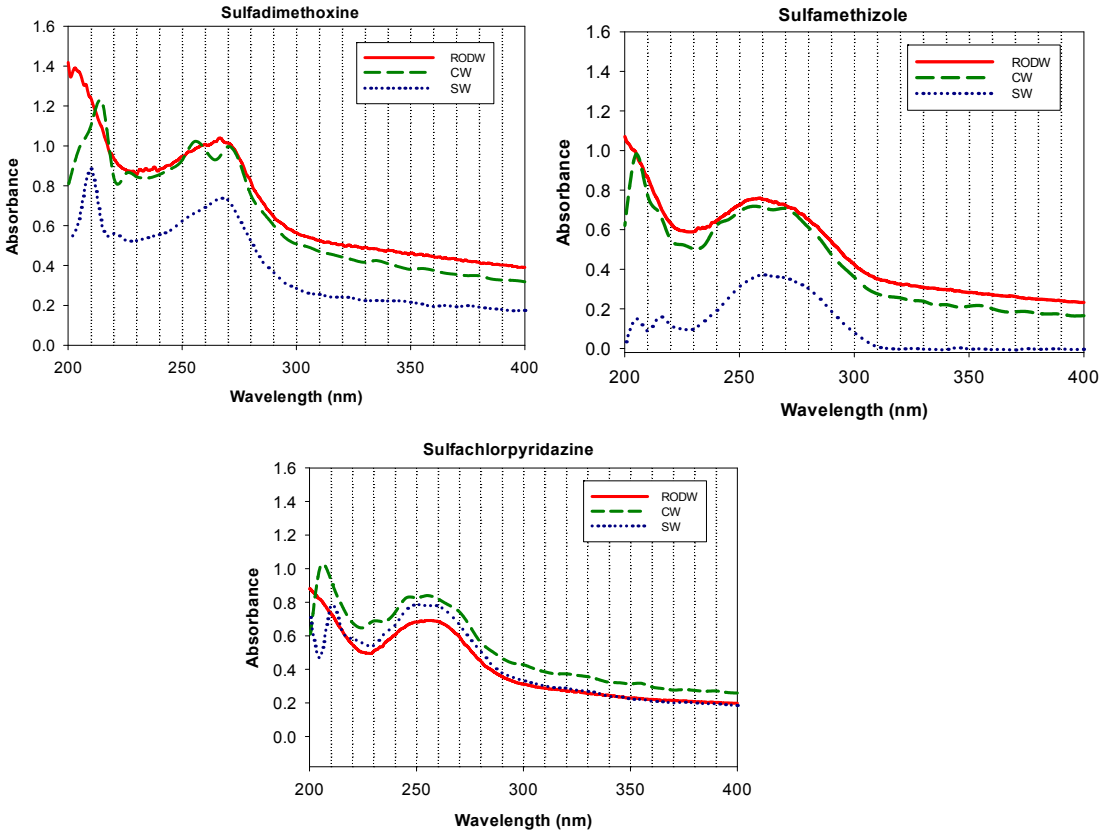


Figure 11. Absorbance spectra of 5 µg/mL solutions of sulfamethizole, sulfachlorpyridazine and sulfadimethoxine in various matrices

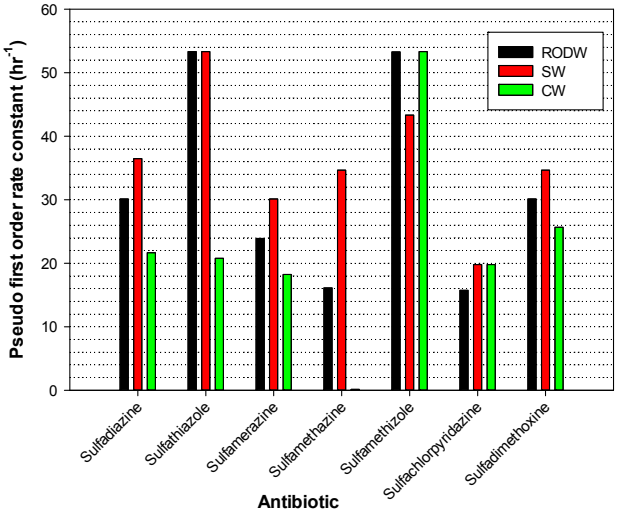


Figure 12. Comparison of pseudo first order rate constants obtained for sulfonamides at UV 254 nm

On the basis of the extent of photolysis in the studied matrices, all compounds can be classified into two categories: antibiotics showing a specific trend CW_k (rate constant in CW) < DDW_k < SW_k and antibiotics with no trend. Sulfadiazine, sulfamerazine, sulfamethazine and sulfadimethoxine fall in the first category showing similar trends in the rate of photolysis decay, which can be attributed to their similarity in N-bound substitution of the sulfonamide linkage i.e., pyrimidine ring. Of the seven sulfonamides tested sulfathiazole is photo chemically the least stable molecule and sulfachlorpyridazine is the most stable one. The molar absorption coefficients and the quantum yields calculated based on molecule's absorption at UV 254 nm and irradiance intensity of mercury vapor lamps are shown in Table 10 and the photolysis decay curves in each water matrix tested (for 4 UV 254 lamps) are shown in Figure 13-15. The rates obtained for sulfamethazine in CW were surprisingly lower (100-240 fold) than those measured in other matrices and thus was the most stable sulfonamide in fresh waters (Fig. 13- 15). For further confirmation the experiment was repeated which yielded similar results. Sulfathiazole degraded with a half-life less than 2 min in CW and hence was not shown in Figure 14. At UV 254 nm photo stability and quantum yields matches well i.e least stable compound has highest quantum yield (sulfathiazole) and most stable one has low quantum yields (sulfachlorpyridazine). The calculated half-lives of sulfonamides at UV 254 nm range from 1.0 – 2 minutes, with the exception of sulfamethazine in fresh waters (289 min) (Table 10), indicating that UV treatment applied in drinking water treatment plants would lead to complete removal of sulfonamides in drinking waters.

Table 10. Molar absorption coefficient, quantum yields and half-lives of sulfonamides in RODW and natural water matrices measured at UV 254 nm. pKa values were obtained from SRC database (accessed on 1/18/2013). RODW = reverse osmosis deionized water SW = salt water CW = canal water. SH_2^+ = acidic form SH= neutral form S^- =basic form.

Compound	pK_{a1}	pK_{a2}	Matrix	Protonation state	Quantum Yield (mol einstein^{-1})	Half-life (hr)
Sulfadiazine (5)	2	6.48	RODW	SH_2^+	1.3021	0.023
			SW	S^-	1.1626	0.019
			CW	S^-	0.6959	0.032
Sulfathiazole (6)	2.4	7.23	RODW	SH_2^+	1.0538	0.013
			SW	S^-	1.0172	0.013
			CW	S^-	0.7634	0.033
Sulfamerazine (7)	2.5	7	RODW	SH_2^+	0.3012	0.029
			SW	S^-	0.3680	0.023
			CW	S^-	0.2445	0.038
Sulfamethazine (8)	2.6	8	RODW	SH_2^+	0.2283	0.043
			SW	S^-	0.5405	0.020
			CW	S^-	0.0021	4.819
Sulfamethizole (9)	2.1	5.3	RODW	SH	0.7273	0.013
			SW	S^-	0.8106	0.016
			CW	S^-	0.6841	0.013
Sulfachlorpyridazine (10)	2.0	5.9	RODW	SH_2^+	0.1950	0.044
			SW	S^-	0.2341	0.035
			CW	S^-	0.2282	0.035
Sulfadimethoxine (11)	2.9	6.1	RODW	SH_2^+	0.3041	0.023
			SW	S^-	0.4007	0.020
			CW	S^-	0.2564	0.027

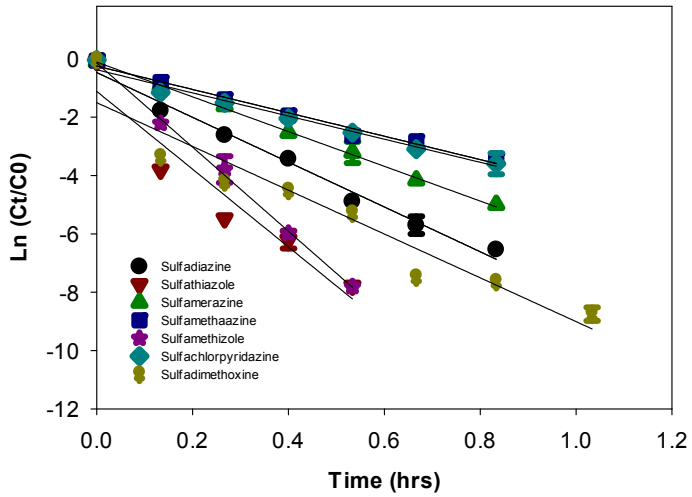


Figure 13. Photolysis decay curves of sulfonamides in RODW at UV 254 nm using 4 lamps

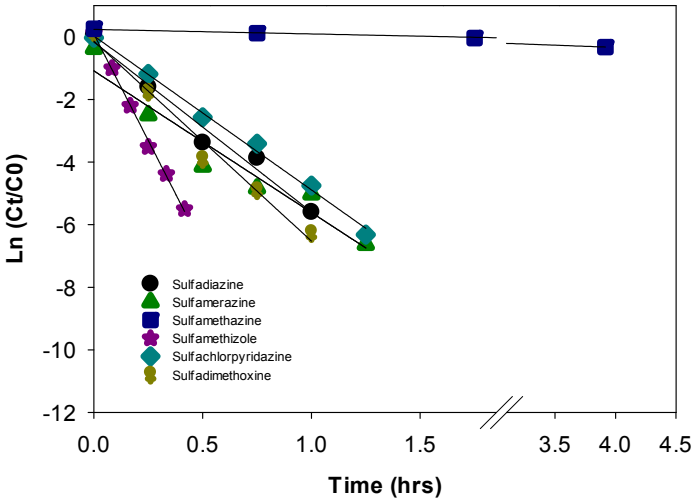


Figure 14. Photolysis decay curves of sulfonamides in canal water (CW) at UV 254 nm using 4 lamps

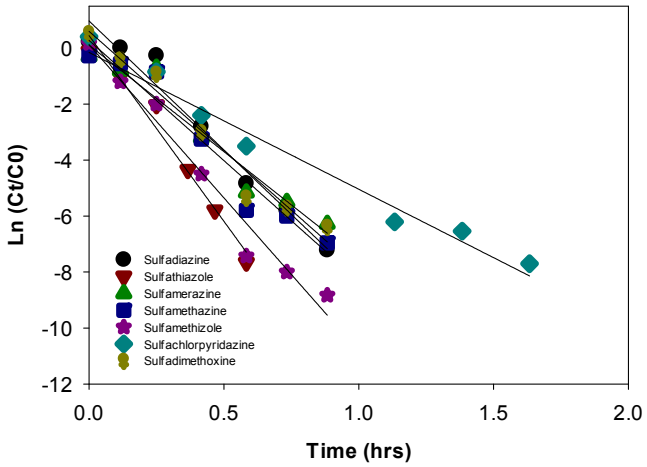


Figure 15. Photolysis decay curves of sulfonamides in salt water (SW) at UV 254 nm using 4 lamps

3.3.3. Photolysis decay at UV 350 nm, simulated solar and natural solar radiation

3.3.3.1. Comparison of rates in different light sources

The major objective of this part of the study is to understand the photo stability of sulfa drugs in natural environment as well as to compare degradation of sulfonamides in simulated and natural solar radiation to see if both light sources yield similar results or not. Sulfonamides behaved similarly in UV 350 nm, simulated and natural solar radiation. One would expect this result based on similarity of UV spectra (300-360 nm) among the light sources, as shown in Figure 1. The rate constants obtained in all light sources were plotted as grouped bar charts (Fig. 16) and half-lives were tabulated in Table 11. As predicted the rates were higher at 350 nm followed by solar simulation and natural sunlight. In all light sources, sulfathiazole was the least stable and sulfadimethoxine was the most stable compound.

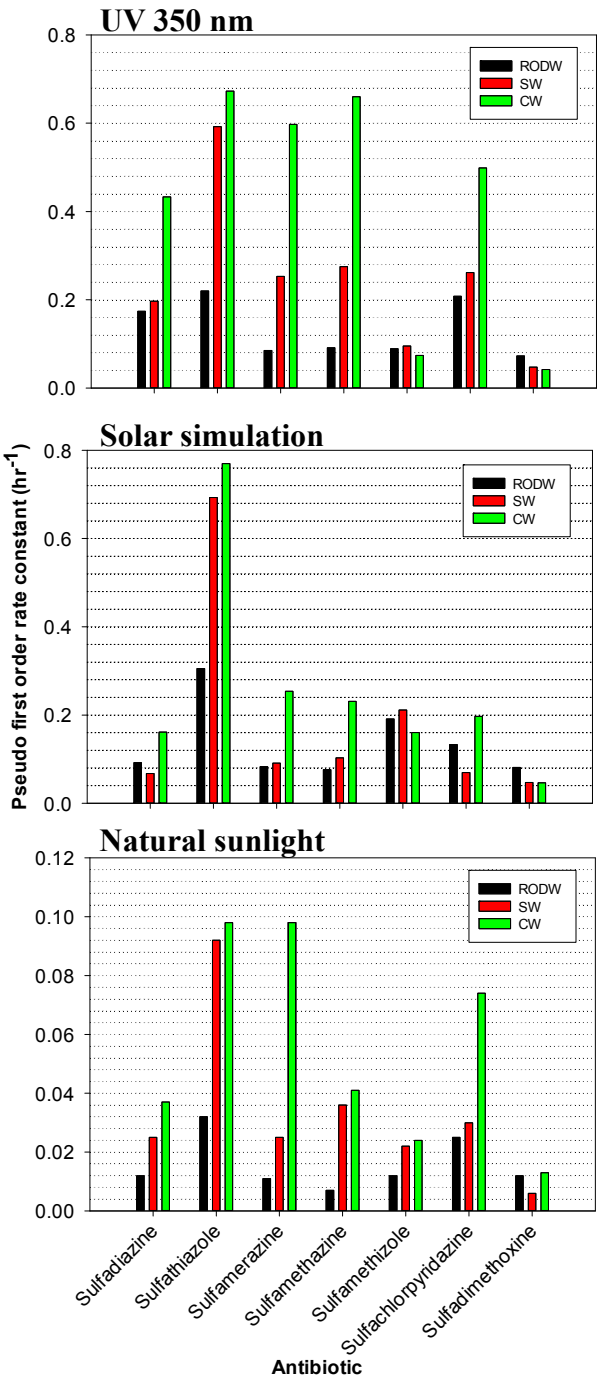


Figure 16. Comparison of pseudo first order rate constants obtained for sulfonamides at UV 350 nm, simulated and natural solar radiation (from top to bottom)

Table 11. Molar absorption coefficient and quantum yields of sulfonamides in RODW and natural water matrices measured at UV 350 nm. RODW = reverse osmosis deionized water SW = salt water CW = canal water. [a]350 nm [b]Simulated solar radiation [c]Natural sunlight.

Compound	Matrix	Molar absorption coefficient (M^{-1} cm^{-1})	Quantum Yield (mol $einstein^{-1}$)	Half-life (hr)[a]	Half-life (hr)[b]	Half-life (hr)[c]
Sulfadiazine	RODW	411	0.1044	3.98	10.1	107
	SW	416	0.1166	3.52	13.8	50.2
	CW	233	0.4565	1.60	5.78	34.7
Sulfathiazole	RODW	605	0.0900	3.15	3.05	40.1
	SW	-310	-0.4626	1.17	1.34	13.9
	CW	637	0.2616	1.03	1.21	13.0
Sulfamerazine	RODW	13885	0.0020	8.13	11.2	117
	SW	5852	0.0119	2.74	10.2	51.3
	CW	6700	0.0251	1.16	3.67	13.0
Sulfamethazine	RODW	6379	0.0040	7.59	11.5	178
	SW	505	0.1344	2.52	9.04	35.5
	CW	2919	0.0586	1.05	4.03	30.8
Sulfamethizole	RODW	15292	0.0019	7.78	4.88	103
	SW	-142	-0.1635	7.28	4.41	56.8
	CW	11556	0.0020	9.40	5.82	52.9
Sulfachlorpyridazine	RODW	13046	0.0050	3.33	7.00	51.0
	SW	12701	0.0064	2.65	13.4	43.0
	CW	17839	0.0096	1.39	4.73	17.3
Sulfadimethoxine	RODW	28813	0.0010	9.51	12.3	110
	SW	13388	0.0009	14.7	19.8	217
	CW	23669	0.0007	16.5	20.2	100

An average intensity of solar radiation was measured (n=20) during the daylight time (between 9:00 am - 5:30 pm) and used to normalize the rate constants from SunTest to solar radiation (Fig. 16 and Table 11). Pseudo first order rate constants calculated in simulated and natural solar radiation vary by 2-16 fold. The half-lives of sulfa drugs in sunlight ranged from ~1-9 days and matches well with previous studies which reported half-lives within a range of 1-17 days (Boreen et al., 2005).

3.3.3.2. Photolysis of sulfa drugs in freshwaters and the effect of DOC on the rate

Despite of many studies reported in the literature on the photolysis of sulfonamides, comparison to previous studies were not always possible due to the differences in matrix compositions. Photolysis of sulfa drugs in natural waters as well as those in RODW revealed a significant enhancement in the rate in fresh waters relative to that observed in RODW (Figure 16) in all light sources, which could be explained based on the differences in the pH and [DOC] between matrices. Compared to RODW, in fresh water (CW), pH increased by 2.6 units and [DOC] increased from none to 10.4 mg-C/L. pH effects the speciation of the molecule and thus absorption of photon (pH) and (DOC) acts as photosensitizer, explaining the observed increase of 1-9 fold in the rates for most of the sulfonamides (Fig. 17-18 for 350 nm, Figure 19-20 for SunTest and Figure 21-22 for sunlight).

The kinetic plots shown in Figures 19-20, 23 were based on the raw data obtained from SunTest; the results were then normalized to the natural sunlight and are shown in the Figure 16 and Table 11. In each figure, the differences in the photo stability of various sulfonamides can be seen in a given matrix as a function of the light source. The significant enhancement in the rates observed due to the integrative effects of pH and DOC can be supported by previous studies demonstrating an enhancement of 2-4 fold in the removal of sulfathiazole and sulfamethizole in natural sunlight in solutions when pH was increased from 5.2 to 8.6 (Boreen et al., 2004) and 2-fold higher rate observed with increasing concentrations of humic acid (from 5 to 50 mg/L) at a given pH in artificial sunlight for C-14-sulfadiazine (Sukul et al., 2008).

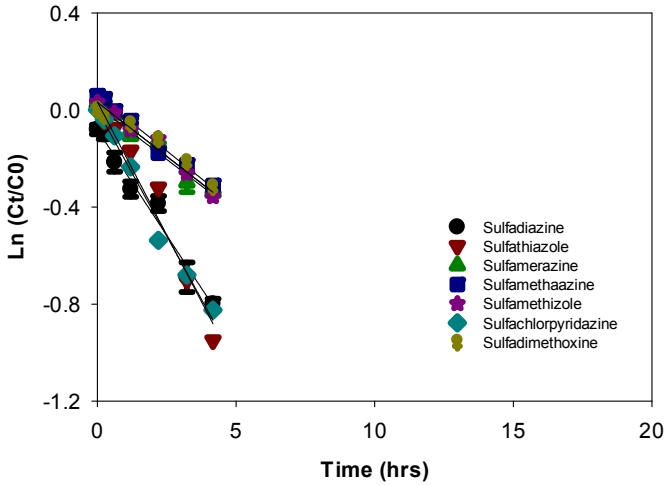


Figure 17. Photolysis decay curves of sulfonamides in RODW at UV 350 nm

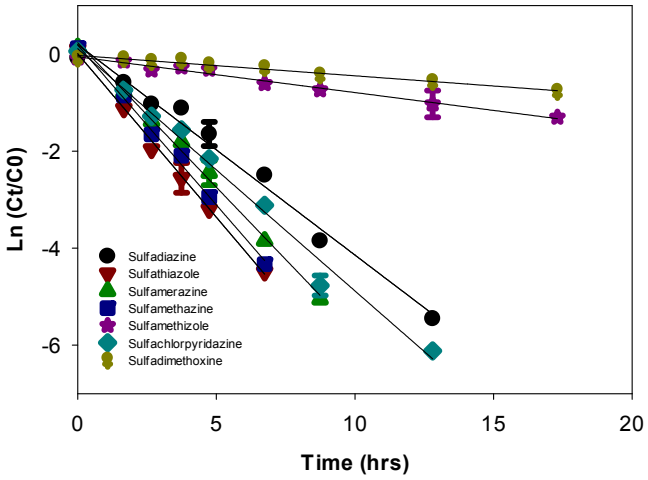


Figure 18. Photolysis decay curves of sulfonamides in CW at UV 350 nm

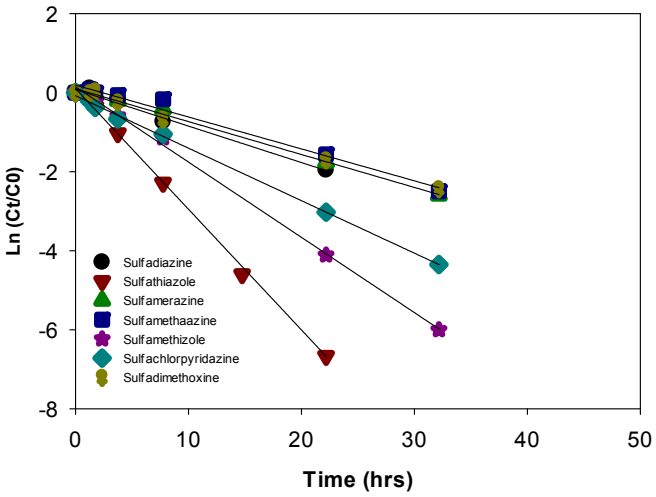


Figure 19. Photolysis decay curves of sulfonamides in RODW in solar simulation (with no normalization applied)

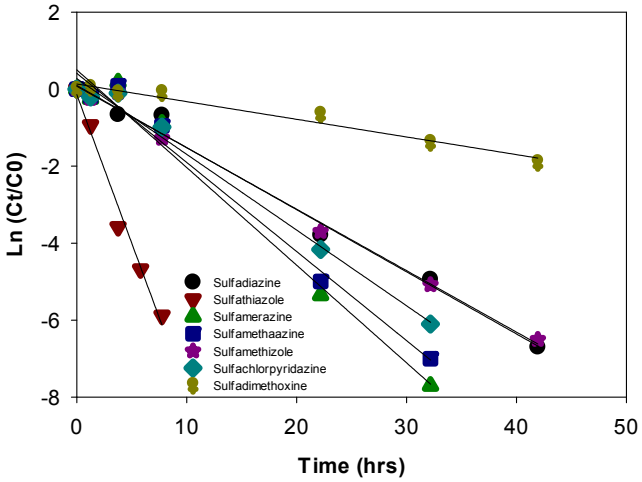


Figure 20. Photolysis decay curves of sulfonamides in CW in solar simulation (with no normalization applied)

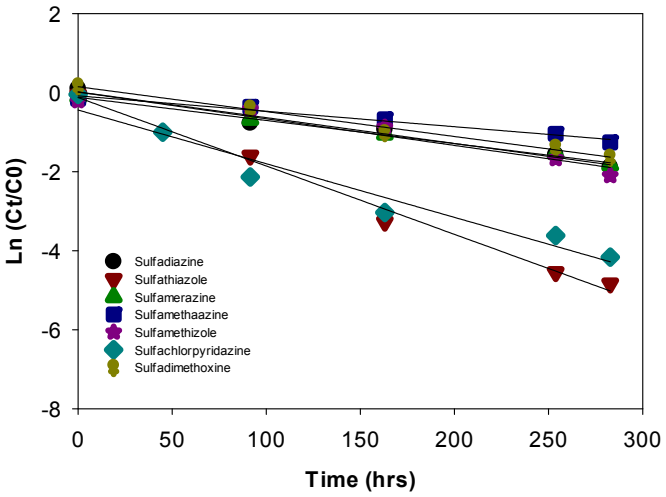


Figure 21. Photolysis decay curves of sulfonamides in RODW in natural sunlight

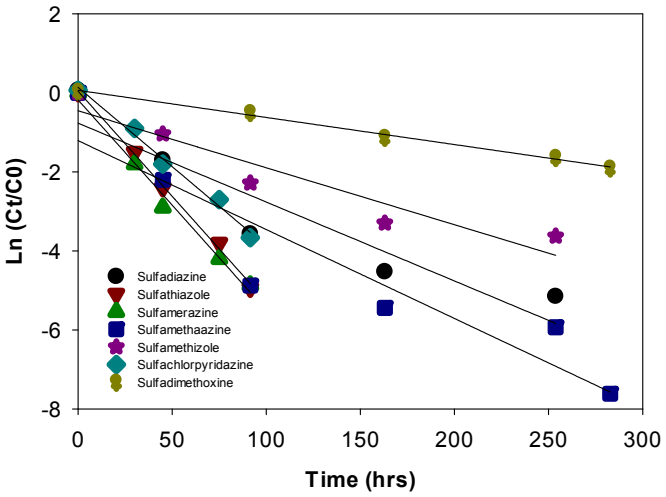


Figure 22. Photolysis decay curves of sulfonamides in CW in natural sunlight

In natural sunlight, Boreen et al. 2005 also observed higher photolysis rates (2-fold) in fresh waters compared to deionized water for sulfonamides (Boreen et al., 2005). But the rate of enhancement observed in the present study was higher and it might be due to the higher [DOC] in the fresh water matrix used in this study (10.4 versus 5.9 mg-C/L).

Therefore, it can be concluded that direct photolysis moderated by the pH is the predominant mechanism for sulfathiazole and sulfamethizole and indirect photolysis by natural organic matter is the predominant factor in the fate of sulfonamides with six membered ring substituents. Sulfadimethoxine decays rate was slow in CW relative to other sulfa drugs suggesting that its indirect photolysis was dominated by the inhibition effects of pH rather than photosensitizing effects of DOC.

3.3.3.3. Photolysis of sulfa drugs in salt waters and the effect of $[Cl^-]$ on the rate

The enhancement of photolysis rates in salt waters in comparison to RODW (Fig. 23-25) may be linked to pH as well as high $[Cl^-]$. The sensitizing effect of $[Cl^-]$ is the consequence of the formation of free chlorine in presence of $OH^{\cdot}$ (Vione et al., 2005) and due to singlet oxygen formation in a photochemical reaction were documented elsewhere (Ge et al., 2009). The increase in rate in SW relative to RODW is less than CW/RODW, further signifying the importance of DOC in the photolysis of sulfonamides.

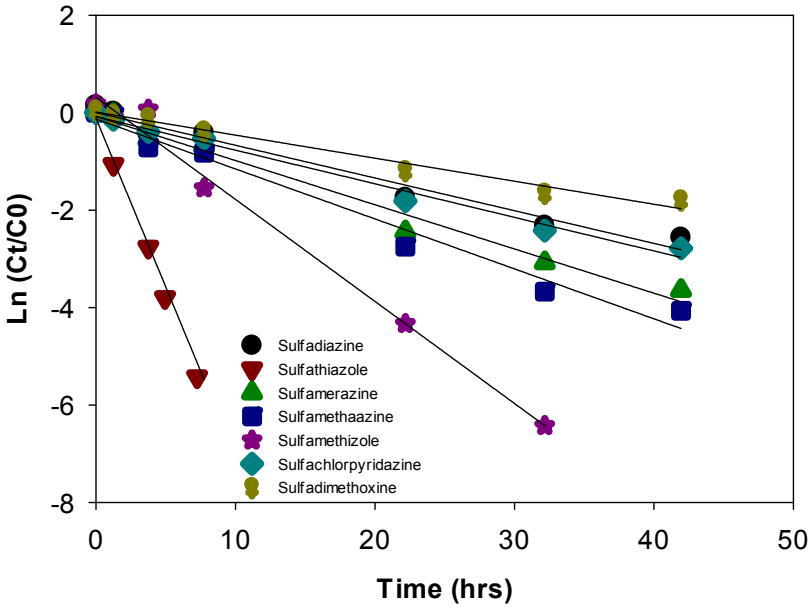


Figure 23. Photolysis decay curves of sulfonamides in SW in solar simulation (with no normalization applied)

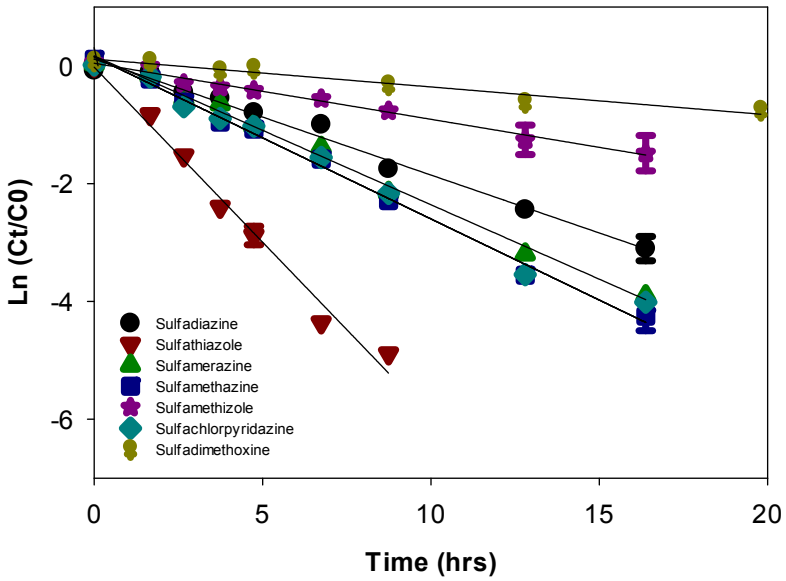


Figure 24. Photolysis decay curves of sulfonamides in SW at UV 350 nm

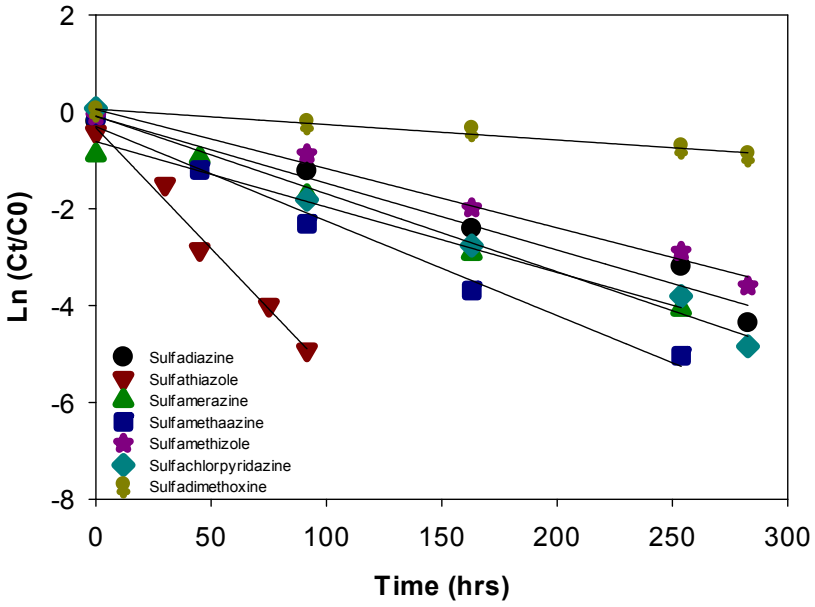


Figure 25. Photolysis decay curves of sulfonamides in SW in natural sunlight

3.3.3.4. Molar absorption coefficients and quantum yields

Whether all compounds exhibited pH dependent rate constants or not, they all showed differences in absorption spectra from one matrix to another. This prompted the calculation of molar absorption coefficients and quantum yields. Table 11 presents the molar absorption values calculated at UV 350 nm. They depend on the nature of matrix (or pH mainly) which was also confirmed by other studies (Avisar et al., 2010; Lester et al., 2012). Molar absorption coefficients were calculated on the basis of the absorbance values obtained after subtracting corresponding water matrix spectra from the target compound spectra. For both sulfathiazole and sulfamethizole, negative values were obtained for absorbance when spectra were measured in SW (Table 11). A positive correlation between the direct photolysis rate constant and quantum yield, both calculated

at 350 nm was observed for all sulfa drugs with six membered substitutions (sulfadiazine, sulfamerazine, sulfachlorpyridazine and sulfadimethoxine) except sulfamethazine. These finding are in good agreement with those of Boreen et al. 2005 who found that quantum yields were function of pH with higher quantum yields occurring at higher pH (Boreen et al., 2005).

3.4. Conclusion

The most important contribution of this study comes from the fact that half-lives were estimated in matrix compositions similar to the ones found in the natural environment. In natural waters sulfonamides are prevalent in anionic form which has different spectral properties from cationic or neutral form of the drug. Results show that the photolysis decay rate was dependent on the speciation of the molecule, pH of the water sample, dissolved organic matter and [Cl^-]. Half-lives in UV 254 nm were in the range of 2-5 min indicating that drinking water treatment plants equipped with germicidal lamps could potentially remove sulfonamides from drinking waters and is consistent with the fact there were no traces detected in drinking waters (Garcia-Ac et al., 2009; Watkinson et al., 2009). The calculated first order rate constants at UV 350 nm and SunTest were within the same magnitude (1-5 fold). Half-lives of sulfonamides in SunTest and in natural sunlight were of similar magnitude thus justifying the use of SunTest to predict the fate of contaminants in natural sunlight. In environmentally relevant conditions sulfathiazole was the least stable molecule and sulfadimethoxine was the most stable one and accordingly sulfadimethoxine is one of the most frequently detected sulfonamide in

surface waters (Garcia-Galan et al., 2011). Sulfonamides degraded with a half-life of ~1-9 days in natural sunlight, which could significantly change based on latitude, depth of the photic zone, overcast conditions and composition of the water matrix. Being polar, amphoteric and readily soluble in water, sulfonamides have high migration ability in the environment. Moreover they can be accumulated in various organisms of a food chain (Halling-Sorensen et al., 1998; Kummerer, 2001) and have the ability to cause drug-resistance pathogenic bacteria at lower concentrations in the environment, however information on the exposure period to cause bacterial resistance is largely unknown. It may be advisable to prescribe a sulfonamide that could be easily degraded compared to other highly stable sulfa drug provided they are equally efficient.

CHAPTER 4

Stability of Dioctyl Sulfosuccinate (DOSS) towards hydrolysis and its photodegradation under simulated laboratory conditions

4.1. Introduction

The Deepwater Horizon was a semisubmersible offshore oil drilling rig, which exploded and sank in the Gulf of Mexico on April 20th 2010. It initiated the largest marine oil spill in US history before it was completely sealed on September 19th 2010 (Campbell and Clifford, 2010). In the remediation efforts of oil spills, chemicals called dispersants were commonly used (Li et al., 2009). They break up the oil into small droplets that diffuse throughout the total water column, thereby reducing the local concentration of the oil and its imminent toxicity towards the aquatic organisms. Two such dispersants authorized by U.S. EPA in the remediation efforts were Corexit® EC9500A and EC9527A (formerly Corexit® 9500 and 9527) (USEPA, 2013). At least 1.8 million gallons of dispersants were applied on the surface as well as underwater during the response and recovery process (USG). Dioctyl sulfosuccinate sodium salt (DOSS, CAS number 577-11-7, Fig. 1) is contained (21 ± 2)% and (22 ± 5)% in Corexit® 9500 and Corexit® 9527, respectively (Ramirez et al., 2013). Other components of Corexit® EC9500A are hydrotreated light petroleum distillates (10-30% w/w) and propylene glycol (1-5% w/w) (NALCO, 2013a) whereas Corexit® EC9527A contains mainly 2-butoxyethanol (30-60 %w/w) (NALCO, 2013b). Based on the limited information available, the Corexit® formulations have only low to moderate toxicity to most aquatic species (George-Ares and Clark, 2000; Hemmer

et al., 2010; Judson et al., 2010), studies describing their environmental fate are scarce (Thibodeaux et al., 2011). Garcia et al. have shown that DOSS is susceptible to biodegradation in both aerobic and anaerobic conditions in fresh waters (Garcia et al., 2009). The studies on the bio degradation of DOSS in salt waters are contradictory. Kujawinski et al. reported that DOSS was detected 300 km from the well 64 days after the Corexit application has ceased, indicating that this compound is not biodegradable and any decrease in the concentration could be attributed only to dilution effects (Kujawinski et al., 2011). A more recent study by Campo et al. found that DOSS was biodegraded by 98% in 8 and ~38 days in cultures from surface and deep Gulf of Mexico waters (at 25°C and 5°C, respectively) (Campo et al., 2013). In the light of these findings it is important to monitor the DOSS and its degradation products in the environment as the released oil dispersant moves and transforms. The stability of DOSS towards photolysis and hydrolysis was never studied, and is thus the main objective of present study. Accordingly the photo stability of DOSS was studied in UV light (at 254 and 350 nm) and in simulated and natural solar radiation. The photolysis was studied in natural salt water (SW) through comparisons to those in deionized water in order to assess the importance of indirect versus direct photolysis processes.

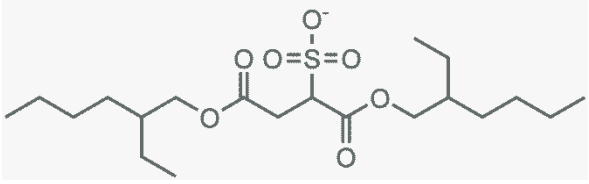


Figure 26. Structure of DOSS

4.2. Experimental Section

4.2.1. Chemicals

Certified DOSS and DOSS-$^{13}C_4$ standards were purchased from Cambridge Isotopes Laboratories (Andover, Massachusetts, USA.). Stock and working solutions of standards were prepared in acetonitrile. Optima LC/MS grade formic acid, acetonitrile and water were purchased from Fisher Chemical (Fairlawn, New Jersey, USA). Sodium hydroxide and sulfuric acid (certified A.C.S. grade) and ammonium hydroxide (trace metal grade) were also purchased from Fisher Scientific. Artificial seawater was prepared using the commercially available Instant Ocean® sea salt to 3.5% w/v.

4.2.2. Sampling sites and light sources

The information on the water matrices (RODW and SW) and light sources (UV 254 nm, 350 nm and SunTest) was given in the previous chapter (Section 2.2.3 and 2.2.4).

4.2.3. Sample irradiation

Standard solutions of DOSS (100 µg/L) were prepared in RODW and Salt water (SW). Sample irradiation processes at 254 nm, 350 nm and in solar simulator were exactly similar to the ones described in the previous chapter (section 2.2.7 and 3.2.4). At regular time points 1000 µL of test solution was collected into a 2-mL LC amber vial containing 484 µL of acetonitrile, fortified with DOSS-$^{13}C_4$ (15.8 µL, 1.9 mg/L) and subsequently analyzed by LC-MS/MS.

4.2.4. Hydrolysis experiments

Sodium hydroxide reagent solution (0.01M) was prepared by dissolving 40 mg of the solid into 100 mL of solution. 0.01M sulfuric acid was prepared by dissolving 55.6 µL of 95.8% pure H_2SO_4 (specific gravity: 1.84 g/mL) in 100 mL of water. Test solution of standard DOSS (80 µL, 1.5 mg/L) in RODW was transferred to two vials each containing 5 mL of 0.01M sulfuric acid and 0.01M sodium hydroxide, respectively. Control solutions (no acid or base added) were prepared by spiking standard DOSS (80 µL, 1.5 mg/L) into 5 mL of RODW. The same procedure was repeated with artificial seawater. After 24 hrs reaction time, the pH of the samples was changed to 7 with formic acid or ammonium hydroxide. Then, 1000 µL of test solution was placed in a 2 mL amber vial containing 484 µL of acetonitrile, fortified with DOSS-$^{13}C_4$ (15.8 µL, 1.9 mg/L) and subsequently analyzed by LC-MS/MS.

4.2.5. Liquid Chromatography/Mass Spectrometry

The HPLC analysis was performed using an Accela quaternary pump equipped with a HTC-PAL autosampler system (Thermo Scientific, USA). Liquid chromatography was carried out using a Hypersil Gold aQ column C18 (50mm × 2.1mm, 3 µm) equipped with Hypersil Gold aQ pre-column (10mm x 4.6mm x 3 µm) (Thermo Scientific, USA). Injection volume was 20 µL. The separation was performed in 10 minutes with a flow rate of 325 $\mu L min^{-1}$ using a binary gradient mobile phase consisting of 0.1% formic acid in acetonitrile (A) and 0.1% formic acid in water (B) according to the program in Table

12. Instrument control and data acquisition was performed using the Xcalibur 2.1 software (Thermo Scientific, USA).

4.2.6. Mass Spectrometry

Detection of analytes was performed on a TSQ Quantum Access QqQ Mass Spectrometer equipped with an Ion Max API Electrospray Ionization (ESI) Source (Thermo Scientific, USA). The ESI source was operated in negative mode for the detection of DOSS, DOSS-$^{13}C_4$. The capillary temperature was 300 °C and the capillary voltage was 4.0 kV. Nitrogen was used as a sheath gas and as an auxiliary gas at a flow rate of 10 and 25 arbitrary units, respectively. The tube lens value was 90 v. Data were acquired in selective reaction monitoring mode (SRM) and optimized values of collision energy and fragment ions information are provided in Table 13. The flow from the LC was diverted to waste for the first minute to prevent the accumulation of salts into the mass spectrometer source. A typical chromatogram for a spiked seawater sample is shown in Fig. 27. The analytical methodology developed here was published as one of three high sensitivity LC-MS/MS methods used for the analysis of DOSS in different stages of an oil spill response (Ramirez et al., 2013).

Table 12. Summary of the mobile phase compositions for the chromatographic separation.

Time (min)	% A (0.1% formic acid acetonitrile)	% B (0.1% formic acid in LC-MS water)
0	2	98
0.9	2	98
3.7	98	2
5.6	98	2
5.9	2	98
10	8	98

Table 13. Summary of the retention times, MS/MS parameters, and precursor and product ions observed for each of the target compounds.

Analyte	ESI parameter				
	Precursor ion m/z	SRM 1	CE 1	SRM 2	CE2
DOSS	421.034	81.18	27	227.107	24
DOSS-$^{13}C_4$	425.3	81.091	27	231.108	24

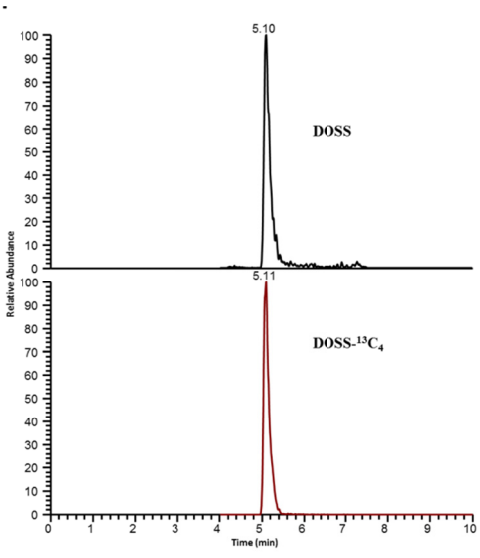


Figure 27. SRM chromatograms of DOSS and DOSS-$^{13}C_4$ in seawater at a spike level of 0.78 μgL^{-1} and 20 μgL^{-1}, respectively.

4.3. Results and discussion

4.3.1. Hydrolysis experiments

The hydrolysis samples were analyzed in a triple quadrupole mass spectrometer for the preliminary identification of products followed by high resolution mass spectrometer (HRMS) confirmation in a Quadrupole Time of Flight Mass Spectrometer (Q-TOF-MS). The flowchart diagram for the preparation and analysis of hydrolysis samples is shown in Fig. 28. The mobile phase conditions, column and MS operating conditions were adopted from the LC-MS/MS method used for the analysis of photodegradation samples.

Full scan MS and two MS/MS scan modes (neutral loss and parent ion scans) were used for structure identification. Using full scan mode (m/z: 50-450), only one product was identified by subtracting spectra of the control sample from the corresponding acid or base treated sample. In parent ion scan, MS was programmed to scan for precursor ions producing a product ion with m/z 81 (fragment a, Fig. 29) whereas in neutral loss experiments, MS scanned for the precursor ion that decomposes into a product ion with the loss of a neutral molecule i.e., 130 amu (fragment b, Fig. 29). Both DOSS and its hydrolysis product presented signals in both neutral loss (M-130) and product ion scans (m/z = 81) indicating that the hydrolysis product preserved the sulfonate moiety and at least one intact octyl group. The chromatograms of parent ion scan and the MS spectrum are shown in Figure 30. With this information, along with its observed m/z ratio (309.1), the hydrolysis product was identified as the product of a des-octylation reaction via substitution with a hydroxyl group (Fig. 29).

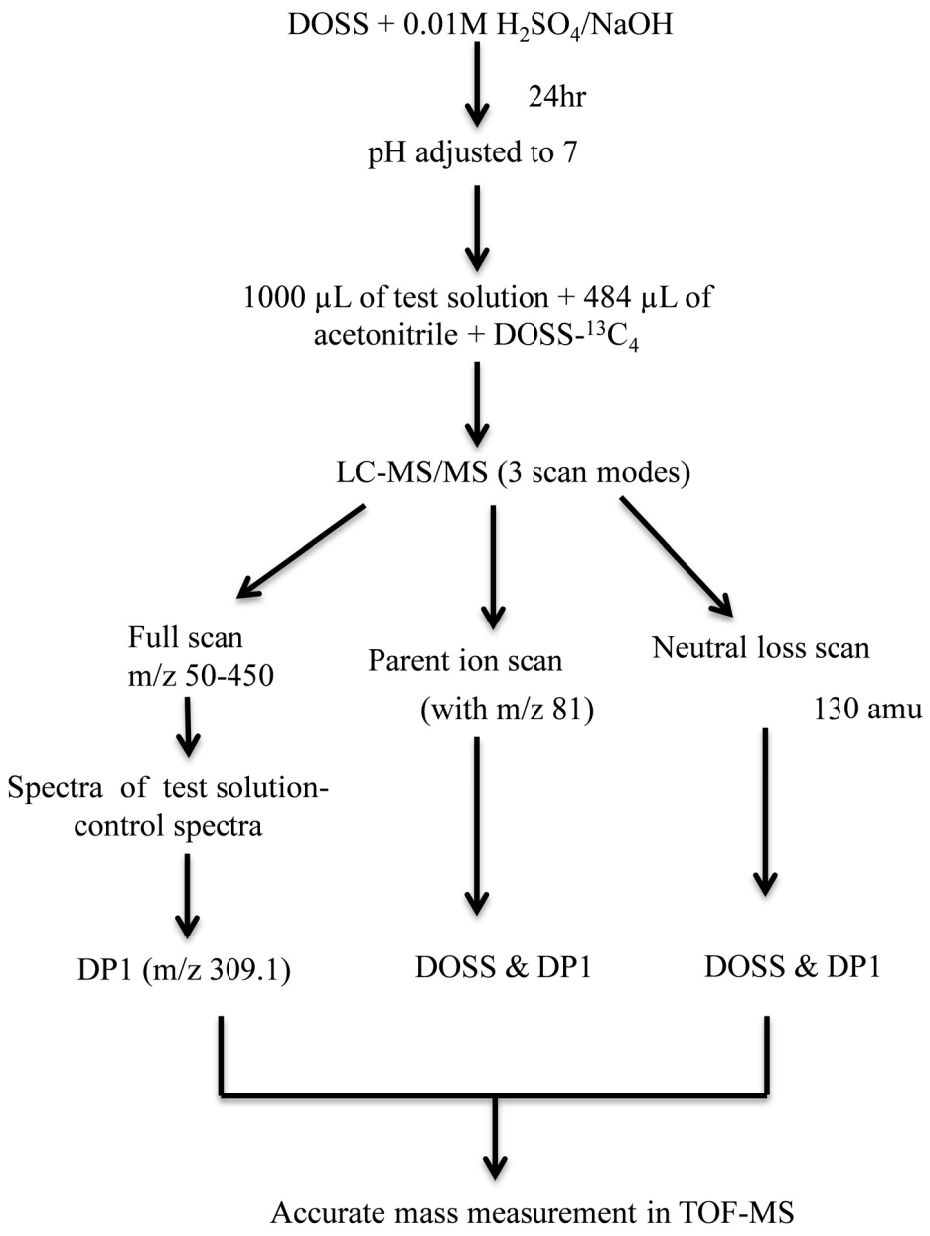
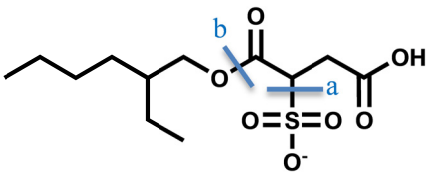


Figure 28. Flow chart diagram for the identification of DOSS hydrolysis products



a= m/z 81, b=m/z 130

$C_{12}H_{21}O_7S^-$

Exact Mass: 309.1013

Figure 29. Structure of DP1. Fragments a and b, masses monitored for the neutral loss experiments

The chromatograms of parent ion scan and the MS spectrum are shown in Figure 30. With this information, along with its observed m/z ratio (309.1), the hydrolysis product was identified as the product of a des-octylation reaction via substitution with a hydroxyl group (Fig. 29).

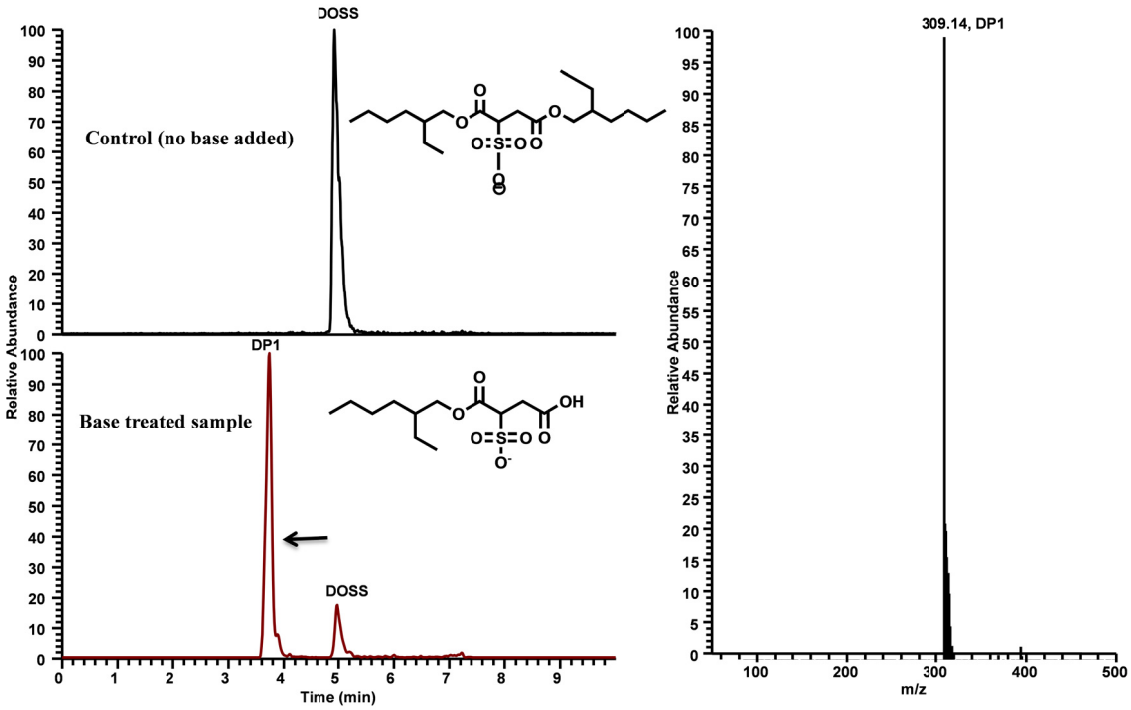


Figure 30. ESI- full scan chromatograms of control DOSS and base hydrolysis sample (left) after 24 hours of reaction. MS/MS spectra of DP1 obtained in triple quadrupole mass spectrometer (right)

To verify the proposed structure accurate mass measurements were performed in the Q-TOF in negative mode, which showed a molecular ion with m/z 309.1017. The proposed structure has a molecular formula of $C_{12}H_{21}O_7S$ with m/z 309.1013. The error between both the masses (in ppm) was calculated using the equation 8 and is +1.29 ppm.

$$ppm = \frac{1.0x10^{6}(measured\ mass - theoritical\ mass)}{theoritical\ mass} \quad \text{(Eq. 8)}$$

The proposed fragmentation pathway (Fig. 31) and the spectra for DP1 (m/z 309.1017) are shown in Fig. 32-33. A search in the Chemspider database using the experimental exact mass only yielded two possible structures (diisobutylsulfosuccinate and dibutylsulfosuccinate). None of these compounds would produce the $[M-130]^-$ signal in the neutral loss scan, therefore the accurate mass confirms the proposed structure.

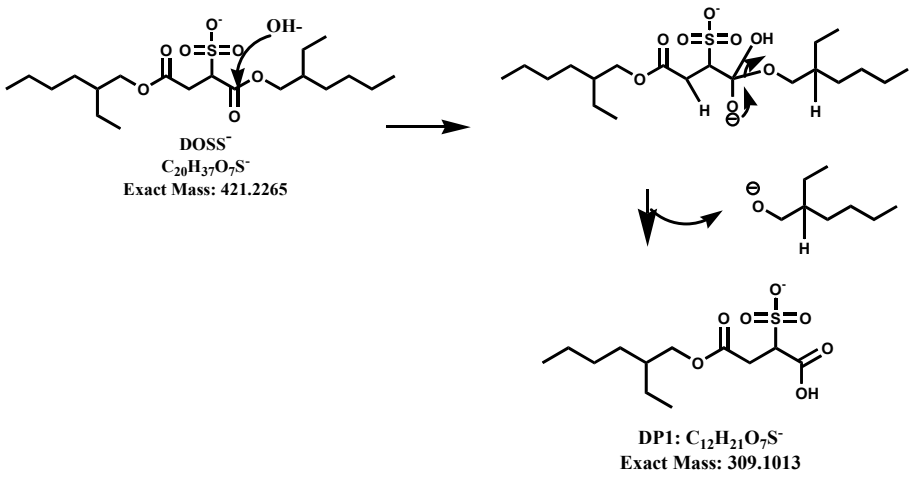


Figure 31. Proposed scheme for the formation of DP1 (m/z 309.1014) from the parent molecule

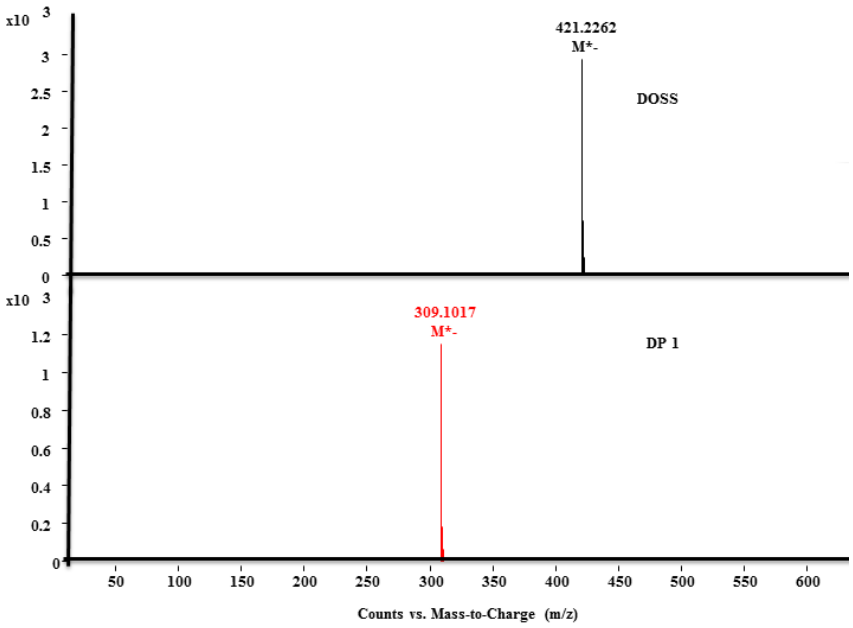


Figure 32. High resolution mass spectra of DOSS and its hydrolysis product DP1 obtained in Q-TOF mass spectrometer

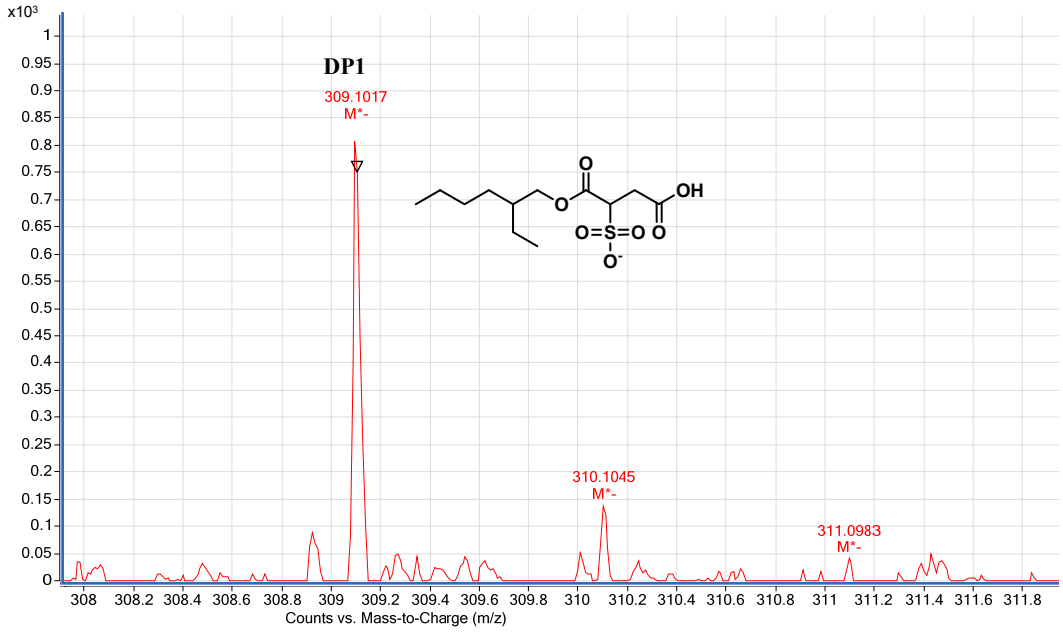


Figure 33. HRMS spectra of DP1 (m/z 309.1017), with x-axis zoomed over the mass range 308 to 312 amu

The same DP1 was also observed in acid hydrolysis but at much lower concentration, indicating that DOSS undergoes preferential hydrolysis under alkaline conditions. Although ester hydrolysis in presence of strong base leading to the formation of carboxylic acid is very well studied, very few studies documented alkaline hydrolysis of DOSS (USEPA, 1991; Mukherjee et al., 1994; Cross, 1998). The same hydrolysis product was reported in biodegradation experiments of DOSS in laboratory microcosms by Campo et al. although no further confirmation of this product was performed during the study (Campo et al., 2013).

4.3.2. Photodegradation studies

The photolysis of DOSS was studied by using RODW and SW solutions in different light sources (254nm, 350nm and SunTest Xenon lamp). The photodegradation of DOSS was followed by monitoring the loss of the molecular ion as a function of irradiation time. The plots of Ln (Ct/C0) versus time (Ct is the concentration of DOSS at a given time and C0 is initial concentration) is linear, suggesting photolysis of DOSS is a first order reaction. Degradation at the most energetic wavelength (UV 254 nm) was rather fast with half-life 0.6 hrs for RODW and 3.4 hrs for SW. The 6-fold reduction of DOSS photolysis in salt water (Fig. 34) suggests a predominant indirect photolysis process that could be attributed to a photo generated species such as the hydroxyl radical. Previous studies found a positive correlation between [$OH^{\cdot}$] and photolysis rate of anionic surfactants (Leu et al., 1998; Lin et al., 1999; Daneshvar et al., 2002; Horvath and Huszank, 2003). Under a different set of experimental conditions (pH 10.5, DOSS = 300 mgL^{-1}, H_2O_2 = 300 mM), DOSS had a half-life of 0.14 hrs at UV 254 nm (Olmez-Hanci et al., 2011) and the

enhanced rates observed in comparison to the present study could be due to the higher concentration of hydroxyl ions, generated by H_2O_2. As expected, degradation in this study was much slower with half-lives of 17, 14 days at 350 nm and 5, 7 days in SunTest for RODW and SW, respectively (Fig 34). The kinetic parameters of DOSS in various light sources are shown in Table 14. The half-life values in SunTest were based on continuous irradiation, although photolysis rates may be significantly lowered by sorption, limited photic zone depth and overcast conditions in the environment. Accordingly, Kujawinski et al. reported that DOSS was persistent in Gulf waters up to 64 days after its application had ceased (Kujawinski et al., 2011). There is no significant enhancement or attenuation of DOSS photolysis decay in natural water (SW) compared to RODW, implying that effect of matrix components (dissolved organic matter and chloride ion) is minimal in these light sources. The irradiation samples obtained at UV 254 nm (RODW) were run in HRMS which confirmed the formation of same DP 1 observed in hydrolysis experiments.

Table 14. Kinetic data of DOSS under different light sources

Light source	Matrix	k (h^{-1})	r^2	$t_{1/2}$ (h)
UV 254 nm	RODW	1.1	0.995	0.605
UV 254 nm	SW	0.22	0.995	3.43
UV 350 nm	RODW	0.0017	0.995	407
UV 350 nm	SW	0.0021	0.994	330
SunTest	RODW	0.0059	0.974	116
SunTest	SW	0.0044	0.981	157

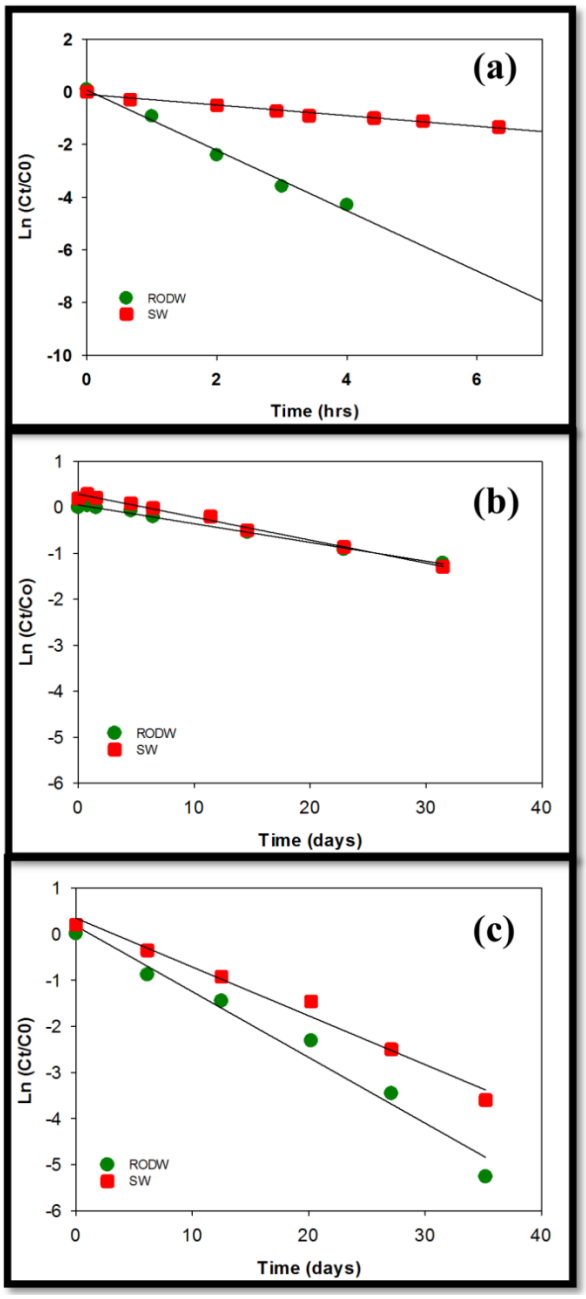


Figure 34. First order curves of DOSS at UV 254 nm, 350 nm and SunTest. Note: X axis is in hours (for 254 nm) and in days (350 nm and SunTest)

4.4. Conclusion

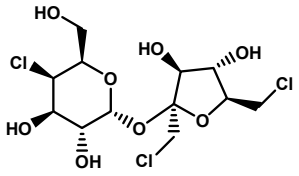
DOSS degraded rather quickly at 254 nm and moderately at both 350 nm and under solar simulation. The estimated half-life of DOSS in simulated solar radiation was ~ 6 days suggesting that it will remain longer in the natural environment. DOSS was introduced both at the surface and under the water at a depth of 3000 feet in response to Deepwater Horizon oil spill. In the surface waters, DOSS will be removed by photo-, bio degradation and sorption. In deep ocean waters, photodegradation could be insignificant due to limited depth of photic zone. Moreover studies by Campo et al. with microbial cultures isolated from the surface and deep sea of Gulf of Mexico showed that biodegradation of DOSS was slower in deep waters relative to the surface waters (Campo et al., 2013). Hence, DOSS would be persistent in deep ocean waters. DOSS was converted to its hydrolysis product (DP1, m/z 309.1017) in acidic, alkaline media and in UV light. The same hydrolysis product was also reported in biodegradation experiments with cultures isolated from the surface of Gulf of Mexico. However, it was not detected in experiments with deep sea cultures (Campo et al., 2013). Data pertaining to the fate and ecotoxicology for DOSS are still scarce and nonexistent for DP1 and thus needs further research.

CHAPTER 5

Online solid phase extraction liquid chromatography tandem mass spectrometric (SPE-LC-MS/MS) method for the determination of Sucralose in reclaimed waters and its photo degradation in natural waters from South Florida

5.1. Introduction

Artificial sweeteners are widely added to foods, drinks, personal care products and pharmaceutical formulations replacing sugar in low calories diet. One artificial sweetener that has gained popularity and has been used in over 80 countries worldwide is sucralose. (E. Brorström–Lundén, 2008; Mead et al., 2009b). It is used in 4000 consumer products globally (Torres et al., 2011). Sucralose (4-chloro-4-deoxy-α, D-galactopyranosyl-1, 6-dichloro-1, 6-didexoy-β, D-fructofuranoside, CAS number 56038-13-2, pKa 11.8) is a chlorinated disaccharide which originates from the non-chlorinated compound sucrose (Fig. 35).



Sucralose
Exact mass:396.0146

Figure 35. Structure of Sucralose

After decades of use of artificial sweeteners, recent studies have documented their widespread occurrence in various environmental waters, such as wastewater, groundwater, surface water, bank filtrate and drinking water (Brown et al., 2006; Loos et

al., 2009b; Mead et al., 2009b; Scheurer et al., 2009b; Ferrer and Thurman, 2010a; Mawhinney et al., 2011b; Minten et al., 2011b; Oppenheimer et al., 2011a). Although previous studies on sucralose have proved its safety for human consumption, they suggest that sucralose it is extremely persistent, with a half-life in water of up to several years, depending on pH and temperature (Grice and Goldsmith, 2000). The compound is thermally stable and not extensively adsorbed or metabolized in humans resulting in the majority (98%) being excreted unchanged. Sucralose enters the environment mainly because of incomplete removal during wastewater (conventional primary and secondary) treatment (E. Brorström–Lundén, 2008) and recent publications have shown that sucralose could be a valuable tracer to monitor impact of wastewaters in the environment (Oppenheimer et al., 2011a)

As a consequence of the unintended but widespread presence in the aquatic environment and the fact that long-term health effects resulting from chronic exposure to low levels of sucralose are largely unknown (Mawhinney et al., 2011b), the presence of μgL^{-1} concentrations of the sweetener in the environment has raised concern, especially since it could affect organisms feeding behaviors (Lubick, 2008). More alarming is the suggestion that sucralose could interfere with plant photosynthesis by shutting down CO_2 uptake (Lubick, 2008). The ecotoxicological effects of sucralose still need to be examined systematically but initial studies with *Daphnia magna* and gammarids exposed to increasing concentrations of sucralose (0-500 μgL^{-1}) showed that both physiology and locomotion were influenced by exposure to sucralose suggesting that sublethal effects rather than acute toxicity may be the mechanism to consider (Wiklund et al., 2012).

The literature contains a minimal amount of research on the degradation of sucralose. Abiotic hydrolysis of sucralose does not appear to be a dominate mechanism of degradation where less than 1% of initial sucralose was shown to degrade into two chlorinated monosaccharides (1,6-dichloro-1,6-dideoxy-D-fructose and 4-chloro-4-deoxy-D-galactose) after a 1 year incubation in a pH 3 solution at 25 °C. Experiments at higher, more relevant pH (4 and 6) showed no hydrolysis (Grice and Goldsmith, 2000). The first study to examine biotic degradation of sucralose found that the compound could be degraded in soil although the specific microorganism(s) responsible for the degradation were not clearly identified (Lappin-Scott et al., 1987), Labare and Alexander found that sucralose can be mineralized in natural environments, such as lake sediments (4.4–18.8%, 96–126 days), sewage (23.2%, 123 days), and surface waters (1.1–4%, 42–132 days) to lesser extents than in soils (32.6–60.4%, 20–101 days) (Labare and Alexander, 1993, 1994). The intermediates of soil microbial degradation of sucralose proposed by Labare and Alexander (Labare and Alexander, 1993, 1994) either the aldehyde or the uronic acid of sucralose, could not be detected in soil incubation experiments by Soh et al. (Soh et al., 2011a). The occurrence of such intermediates is still to be reported in environmental samples (Lange et al., 2012b). In incubation experiments with sweeteners in soils, Buerge et al. reported one of the shortest half-lives (DT50) at 9 days (Buerge et al., 2009b). Previous studies showed that sucralose is not oxidized by UV light or visible light (Soh et al., 2011a; Torres et al., 2011). Calza et al. reported that 90% sucralose underwent photocatalyzed degradation in 30 min, in presence of 200 mg/L under a solar simulator (Calza et al., 2013). But no studies were reported on the

degradation of sucralose under environmentally relevant conditions and thus it is one of the objectives of current study.

Robust analytical methods for assessing sucralose's environmental fate are crucial. To date, artificial sweeteners have been determined by HPLC with reverse phase chromatography using different buffer systems, ion pairing reagents and specific derivatization procedures (Ferrer and Thurman, 2010a) and by GC-MS (Mead et al., 2009b). More recently, studies employing liquid chromatography–electrospray ionization-tandem mass spectrometry (LC–ESI-MS/MS) have been published for the analysis of sucralose in water samples by direct injection (higher detection limits) or offline SPE (Loos et al., 2009b; Scheurer et al., 2009b; Ferrer and Thurman, 2010a; Mawhinney et al., 2011b; Minten et al., 2011b; Ordonez et al., 2012a). Nowadays the use of online SPE has shown important improvements such as higher sensitivity, analysis of smaller sample volumes, limited sample loss, no carry-over and robust and reproducible detection, while increasing sample throughput (Neset et al., 2010; Heeb et al., 2012; Panditi et al., 2013; Quinete et al., 2013).

5.2.Experimental

5.2.1. Chemicals

Sucralose was purchased from Sigma–Aldrich (Oakville, ON, Canada). Sucralose- d6 (98% purity) was used as internal standard and was obtained from Santa Cruz Biotechnology Inc, (Santa Cruz, CA, USA). Optima LC/MS grade formic acid,

acetonitrile and water were purchased from Fisher Scientific (Fairlawn, New Jersey, USA). Membrane filters (0.45 µm and 0.2 µm pore size) were purchased from Millipore (Billerica, MA). Ultrapure water (16 MΩ cm^{-1}) was generated from a Nanopure Infinity Ultrapure Water system. Stock solutions of 1 mg/mL were prepared in acetonitrile for both sucralose and sucralose- d6. All stock solutions were kept in the dark at −18 ºC.

5.2.2. Sample collection sites

Reclaimed water samples (n=56) were collected from the sprinklers at least twice monthly at Florida International University (FIU) Biscayne Bay Campus (North Miami, Florida, USA) from January 2011 to December 2011 except February and September 2011, where only one sample was collected per month. All samples were taken directly from the sprinkler systems after they were flushed for at least 5 minutes. Drinking/Tap water samples (n=43) were collected from residents' homes and shopping centers located in the Miami-Dade County area between August and October 2011 . Information of sampling locations is shown in Table 15. After collection, all samples were immediately transported on ice to the laboratory, filtered through a 0.45 µm glass fiber filter and then through a 0.2 µm membrane filter to minimize any potential biodegradation. Filtered samples were stored in the dark at -18ºC until analysis. Photodegradation experiments were performed using the two most common end members for treated wastewater releases, natural canal water and seawater. The information on the water matrices (RODW, CW and SW) was given in the previous chapter (Section 2.2.3).

Table 15. Drinking water sample name, sampling date and location

Sample name	Sampling date	Latitude	Longitude
DW001	8/22/2011	25°46'20.91"N	80°22'6.15"W
DW002	8/22/2011	26° 1'15.59"N	80° 8'30.79"W
DW003	8/22/2011	25°54'20.54"N	80° 9'31.89"W
DW004	8/23/2011	25°57'3.51"N	80° 9'58.40"W
DW005	8/23/2011	26° 0'45.46"N	80° 9'0.06"W
DW006	8/23/2011	25°45'34.04"N	80°21'54.13"W
DW007	8/23/2011	25°52'33.72"N	80° 7'32.80"W
DW008	8/24/2011	25°33'36.96"N	80°21'4.39"W
DW009	8/28/2011	25°55'53.30"N	80° 7'30.34"W
DW010	9/6/2011	25°53'59.72"N	80° 9'0.36"W
DW011	9/13/2011	25°56'35.70"N	80° 8'21.82"W
DW012	9/13/2011	25°54'16.94"N	80°11'31.66"W
DW013	9/13/2011	25°51'49.99"N	80° 7'28.05"W
DW014	9/13/2011	25°51'4.95"N	80°17'54.93"W
DW015	9/13/2011	25°46'17.43"N	80°22'19.79"W
DW018	9/13/2011	25°45'46.76"N	80°22'53.87"W
DW019	9/13/2011	25°45'31.28"N	80°21'56.43"W
DW020	9/13/2011	25°40'11.52"N	80°26'32.74"W
DW021	9/13/2011	25°45'39.59"N	80°22'34.44"W
DW022	9/13/2011	25°46'1.62"N	80°22'26.60"W
DW023	9/13/2011	25°59'33.45"N	80°15'7.17"W
DW024	9/13/2011	25°45'56.37"N	80°23'46.87"W
DW026	9/13/2011	25°46'58.22"N	80° 8'23.03"W
DW027	9/13/2011	25°53'50.73"N	80°18'47.40"W
DW028	9/13/2011	25°46'36.45"N	80°22'27.53"W
DW030	9/13/2011	25°45'44.70"N	80°15'49.34"W
DW031	9/13/2011	25°46'14.82"N	80°11'51.36"W
DW032	9/20/2011	25°45'16.06"N	80°13'13.16"W
DW033	9/29/2011	25°54'14.88"N	80°17'57.88"W
DW035	10/1/2011	25°47'0.12"N	80°20'11.02"W
DW037	10/1/2011	25°50'21.18"N	80°22'14.67"W
DW038	10/1/2011	25°50'24.33"N	80°19'7.10"W
DW039	10/1/2011	25°53'57.52"N	80°11'39.34"W
DW040	10/2/2011	26° 7'16.37"N	80°10'47.11"W
DW042	10/2/2011	25°28'39.66"N	80°27'56.37"W
DW044	10/2/2011	25°37'36.85"N	80°24'53.63"W
DW045	10/2/2011	25°28'44.81"N	80°25'50.04"W
DW046	10/2/2011	25°33'56.77"N	80°22'56.38"W
DW047	10/2/2011	25°36'50.11"N	80°18'58.73"W
DW050	10/2/2011	25°40'20.88"N	80°19'21.63"W
DW052	10/16/2011	25°50'49.93"N	80°11'4.68"W
DW053	10/16/2011	25°47'47.93"N	80°11'23.91"W

5.2.3. Online preconcentration

A Thermo Equan online SPE system was used for the determination of sucralose in reclaimed and drinking waters. An Accela 1000 was used as analytical HPLC pump and an Accela 600 was used as loading pump (Thermo Scientific, San Jose, CA, USA). The analytical separation was carried out using a Hypersil Gold PFP column (100 mm × 2.1 mm, 1.9 μm) while the SPE pre-concentration column was a HyperSep Retain PEP (20mm × 3.0mm I.D) from the same manufacturer. Instrument control and data acquisition was performed using the software Xcalibur 2.1 (Thermo Scientific, USA). An HTC-PAL autosampler (Thermo Scientific, San Jose, CA, USA) was set to perform up to 10 mL injections. The automated online SPE clean-up and pre-concentration step was performed using only 10.0 mL of untreated water samples with little sample preparation. Two six-port switching valves were used for all analysis. Drinking and reclaimed water samples were prepared by adding 10 μL of 500 ng/mL sucralose-d6 and the final volume was made up to 10.5 mL with the sample.

The online procedure consists of a divert valve on the mass spectrometer which is programmed by the data system to control the loading and elution of the two LC columns. In the Load Position, 10.0 mL of sample was injected into a 10.0 mL loop and then loaded onto a SPE column by the loading LC pump (Accela 600), followed by a wash step to remove interferences (flow rate 2 mL min^{-1}). The target compounds were retained in the SPE column and the matrix that is not retained during the extraction process was directed to waste while simultaneously the analytical pump equilibrates the analytical column in the starting gradient conditions. After 5.3 min, when the valve was

switched to Inject Position, the solvent flow through the HyperSep Retain PEP column is reversed, and the analytes were then back flushed onto a Hypersil Gold PFP column for separation and quantitation by APCI-MS/MS. After 10 min, the switching valve was returned to the loading position to allow the extraction column to be re-equilibrated with water. Valve switching events as well as the gradient program are summarized in Table 16-17 for both drinking and reclaimed waters, respectively. The samples were kept at 10°C in the autosampler. The total run time per sample was 12 min.

Table 16. Gradient program for sucralose determination in drinking water. Top: loading pump gradient Bottom: analytical pump gradient

Time (min)	%A	%B	Flow (µL min-1)
0	100	0	2000
5	100	0	2000
5.3	100	0	1000
6.3	0	100	1000
8.3	0	100	1000
10	0	100	1000
11	100	0	1000
12	100	0	1000

Time (min)	% A	% B	Flow ($\mu L\ min^{-1}$)
0	10	90	250
2	90	10	250
5.3	90	10	400
8	10	90	400
12	10	90	250

A= LC-MS water; B=acetonitrile

Table 17. Gradient program for sucralose determination in reclaimed water. Top: loading pump gradient Bottom: analytical pump gradient

Time (min)	%A	% B	%C	Flow (µL min-1)
0	100	0	0	1000
1.2	100	0	0	1000
2	0	50	50	100
6	0	50	50	100
7.5	0	50	50	1000
8.5	0	50	50	1000
8.6	100	0	0	1000
11	100	0	0	1000

Time (min)	% B	% C	Flow ($\mu L\ min^{-1}$)
0	10	90	250
6	90	10	250
8	90	10	250
9	10	90	250
11	10	90	250

A= LC-MS water; B=acetonitrile, C=0.1% formic acid in LC-MS water

5.2.4. Sample irradiation

Photodegradation experiments were conducted using Rayonet UV photochemical reactors (Southern New England Ultraviolet Co., Branford, CT) and a SunTest which is a solar simulator. The experimental solution of sucralose was prepared by diluting the stock solution to 0.2 µg/mL with three types of water: reverse osmosis-deionized water (RODW, natural freshwater (CW) and seawater (SW). Sample irradiation procedures were similar to the ones described in previous chapters. At specified time intervals, 380 µL of the samples were transferred to a 2 mL LC amber vial, and fortified with sucralose-d6 internal standard (10 µg/mL) to a final volume of 400 µL. Samples were thoroughly mixed and analyzed directly by LC-MS/MS. No degradation was observed in the dark

controls. Degradation curves were plotted as [ln (C_t/C_0)] versus time (hrs) using the Sigma Plot software v 11.0. A SunTest XLS Tabletop Xenon Exposure System (ATLAS Material Testing Technology LLC, Chicago, Illinois, USA) was used to predict the degradation rates of sucralose in natural sunlight under environmental conditions. The xenon lamp was used with its maximum abundance intensity (750W/cm^2). Sample irradiation procedure was similar to the one explained in previous chapters.

5.2.5. Direct injection LC-MS/MS method

A direct injection method was used for the analysis of photodegradation samples. Separation was performed on a Hypersil Gold PFP column (100 mm × 2.1 mm, 1.9 μm) in 11 minutes with a flow rate of 250 μL/min using a binary gradient mobile phase consisting of acetonitrile (C) and 0.1% formic acid (D) in water according to the following program: gradient from 10% to 90% C in 6.0 min and held it for 2 min, and gradient back to 10% C in one min and held the gradient for 2 min. Column effluent was diverted to waste for the first 2.2 minutes in order to flush out the salt from the samples. A sample chromatogram is shown in Fig. 36.

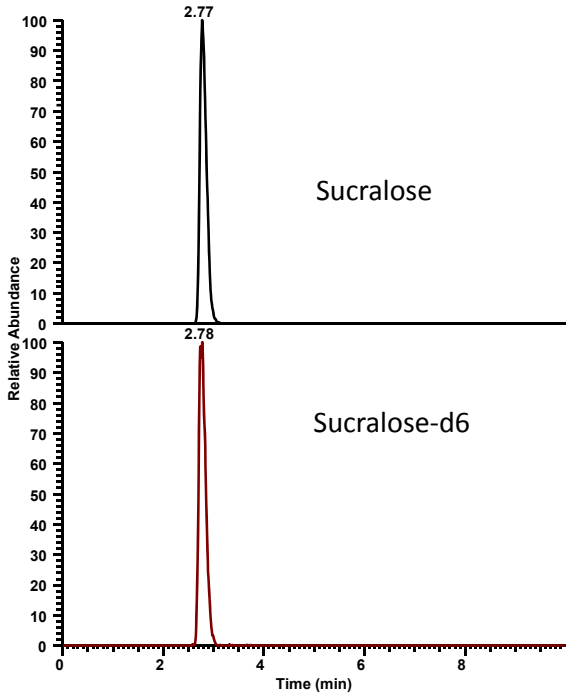


Figure 36. LC-MS/MS chromatograms of sucralose in salt water at 200 ng/L in direct injection method

5.2.6. MS/MS detection

In both methods analytes were detected on a TSQ Quantum Access QqQ Mass spectrometer equipped with an Atmospheric Pressure Chemical Ionization (APCI) source (Thermo Scientific, San Jose, CA, USA) operated in the negative mode. The optimized MS parameters were obtained by direct infusion of 10 µg/mL of standard solutions through a syringe pump at a flow rate of 50 µL min^{-1}. The standard solution was mixed with the mobile phase using a T-connector before being introduced into the APCI source. The APCI vaporizer temperature and capillary temperature were 350 and 300 °C respectively, with a discharge current of 5 kV. Sheath gas and auxiliary gas (N_2) were used at a flow rate of 35 and 10 arbitrary units, respectively, and collision gas (Ar)

pressure of 1.5 mTorr. The two transitions monitored for sucralose were 396.995 → 359.115 (collision energy CE, 12) and 396.995 → 361.056 (CE, 13) and for sucralose-d6 were 403.006 → 365.450 (CE, 15) and 403.006 → 367.140 (CE, 12). Instrument control and data acquisition was performed using Xcalibur 2.1 software (Thermo Scientific, San Jose, CA, USA).

5.3. Results and Discussion

5.3.1. Method development

The initial online SPE LC-APCI-MS/MS developed for determination of sucralose following available literature conditions produced MDLs of 2.7 µg/L for reclaimed waters and 0.7 µg/L for drinking waters using 1 mL of sample volume. These MDLs are adequate for wastewaters but not for surface and drinking waters. Therefore, the method was further optimized for detection of sucralose in the ppt levels (ng/L) normally observed in U.S. drinking waters (Mawhinney et al., 2011b).

During the optimization process it was observed that the combination of water and formic acid was causing adverse ion suppression in the negative mode. Suppressive effects of formic acid were also observed for the analysis of other compounds such as endosulfan (Quinete et al., 2013). Therefore formic acid was eliminated and the mobile phase was switched to acetonitrile and water resulting in a 20-fold increase in sensitivity. Moreover, the sample volume was increased to 10 mL. The method was then able to detect sucralose at ng/L levels for all matrices. This is the first method to achieve these levels without the

use of any modifier. When applying the methodology for tap water and reclaimed water samples, it was observed a high co-eluting interference with the analyte, which was not seen when formic acid was, used (Fig 37 a, b). In LC-MS water, no interferences were observed (Fig 37 c). Separation of the interference peak from the analyte was performed by the use of a differential flow rate (Table 16; Fig 37 d, e). Typical chromatograms of sucralose in LC-MS water, tap water and reclaimed water can be seen in Figure 37.

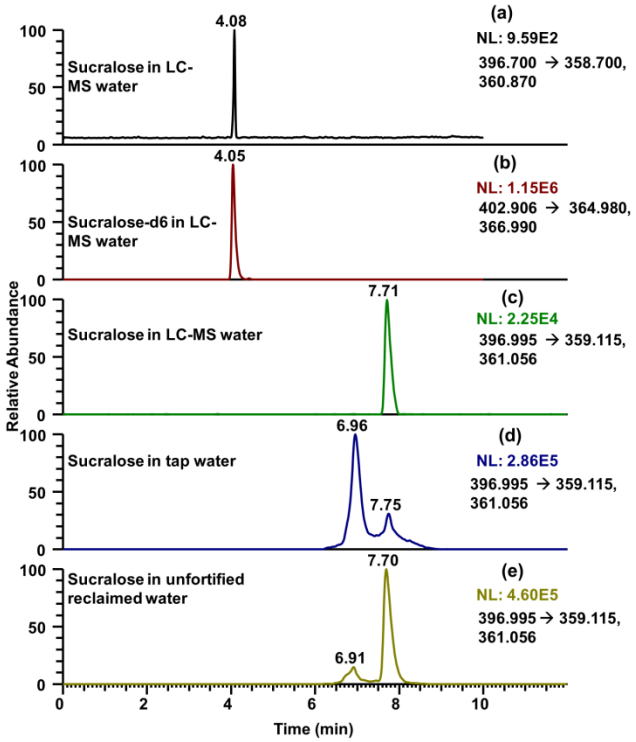


Figure 37. LC-MS/MS chromatograms of a) LC-MS water fortified with sucralose at 200 ng/L and formic acid was used as modifier b) LC-MS water fortified with sucralose-d6 (internal standard) at 50 µg/L and formic acid was used as modifier c) LC-MS water fortified with sucralose at 100 ng/L d) tap water fortified with sucralose at 100 ng/L e) unfortified reclaimed water.

5.3.2. Method Performance- Online SPE-LC-MS/MS

The online SPE-LC/MS/MS method for waters samples was validated in terms of specificity, linearity, limit of detection, matrix recoveries and inter-day precision of the technique.

The chlorine-isotopic pattern for a molecule containing three chlorine atoms such as sucralose, results in four spectral peaks with abundances 100, 95, 31 and 3% for M, M+2, M+4 and M+6, respectively (Linstrom and Mallard, 2005). The specific chlorine-isotopic pattern of the sucralose molecule was observed and shown in Fig. 38. The most abundant ion that was capable of giving a product ion spectrum was chosen (m/z 397) for quantitation. Two SRM transitions were monitored for accurate identification and quantification of sucralose.

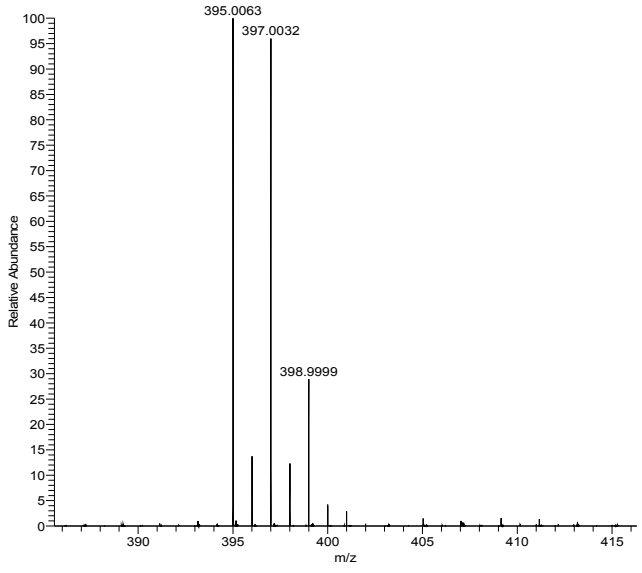


Figure 38. Mass spectrum of sucralose molecular ion cluster from an authentic standard

An 8 point calibration was prepared by spiking varying levels of sucralose working standard solution in LC/MS grade water in the concentration range of 10 ng/L to 2000 ng/L. Calibration curves were built with the relative response ratio (area of sucralose divided by area of sucralose-d6) as a function of the analyte concentration. Linear response was observed in all cases (R^2> 0.99).

Method detection limit (MDL) was calculated from the standard deviation of seven spiked reclaimed and drinking water samples. Standard deviation of seven replicates was multiplied by the student t value at the 99% confidence interval (six degrees of freedom, t value, 3.143), according to procedures outlined by the USEPA (USEPA, 2010). The matrix was spiked (n=7) at 50 ng/L, 100 ng/L and 200 ng/L with the resulting MDLs of 4.5 ng/L, 8.5 ng/L and 0.5 µg/L in deionized water, drinking and in 1:10 diluted reclaimed waters, respectively. Using this method, the MDL for the determination of sucralose in reclaimed waters improved by 6-fold. Matrix matched recovery (n=5) were assessed by spiking reclaimed and drinking water at 50 µg/L and 200 ng/L, respectively. Because real sample matrices may contain target analytes, non-spiked samples were also analyzed and the concentration found was subtracted from the spiked sample concentration. Recoveries ranged from 91 to 108 % (96.7±5.27), 85 to 107 % (96.2±10.9) and 85 to 113 (97±9.7) in deionized water, drinking and reclaimed waters, respectively.

For a repeatability study of the LC-MS method, duplicates and replicate determinations of spiked standard mixture were carried out on the same day (intra-day analysis) and on different days (inter-day analysis). The calculated relative standard deviation (RSD)

ranged from 3 to 13% and 9 to 12% for intra-days and the inter-days in drinking water and reclaimed water, respectively.

Previous studies using offline sample preparation in surface water, drinking water, groundwater and sewage effluents reported MDLs in the range of 10 ng/L to 25 µg/L (Loos et al., 2009b; Scheurer et al., 2009b; Ferrer and Thurman, 2010a; Mawhinney et al., 2011b; Minten et al., 2011b; Oppenheimer et al., 2011a; Ordonez et al., 2012a), which are consistent with the present study. Heeb et al. and Neset et al. have previously reported an online SPE LC-MS/MS determination of sucralose in wastewater, surface and drinking water; however presenting higher detection limits (Heeb et al., 2012) or similar detection with larger volume of sample (20mL) (Neset et al., 2010).

5.3.3. Applicability of the method to environmental samples

The developed online-SPE-LC-MS/MS methods were applied for the analysis of reclaimed water (n=56) and drinking water samples (n=43). Concentrations below MDL were considered as not detected for the purpose of average calculation. Average concentrations and frequency of detection of sucralose in reclaimed water are presented in Table 18. Sucralose was detected in 82% of the reclaimed water samples with concentrations ranging from 4.1 µg/L to 18 µg/L. The monthly average concentration was 9.1 ± 2.9 µg/L. The concentrations found in reclaimed water are comparable with previously published levels of sucralose in wastewater (Minten et al., 2011b; Ordonez et al., 2012a) and at least 10 times higher than concentrations found in surface and

groundwaters (Loos et al., 2009b; Scheurer et al., 2009b; Ferrer and Thurman, 2010a; Mawhinney et al., 2011b; Minten et al., 2011b; Oppenheimer et al., 2011a).

Table 18. Average concentration and frequency of detection of sucralose in reclaimed water samples.

Month	Samples/month	Frequency of detection (%)	Monthly averages (μg/L)
Jan-11	5	80	8.00±3.44
Feb-11	1	100	10.2
Mar-11	8	88	9.63±4.05
Apr-11	4	100	6.96±1.18
May-11	8	100	9.32±2.85
Jun-11	9	89	8.53±2.02
Jul-11	6	33	5.89±2.47
Aug-11	4	75	10.6±6.35
Sep-11	2	50	12.1
Oct-11	2	100	9.42±1.16
Nov-11	4	100	10.1±1.34
Dec-11	3	67	8.92±2.75

Based on the results obtained, month wise distribution of sucralose in reclaimed water was uniform; with no observed temporal trend and statistically difference between the wet season (April to October) and the dry season (November to March) as seen in Figure 39. This result indicates that conventional Wastewater Treatment Plants (WWTP) were not efficient in removing sucralose. In fact, previous study by Brorstorm-Lunden et al. reported removal efficiency <10% for sucralose in corresponding waste water samples (Brorström–Lundén et al., 2008). The chlorinated structure of the sucralose seems to be resistant to microbial degradation even in the mixed media of a sewage treatment facility, which explains its persistence through wastewater treatment processes in municipal plants (Soh et al., 2011a; Torres et al., 2011) and likely resulting in the high concentrations observed in reclaimed water.

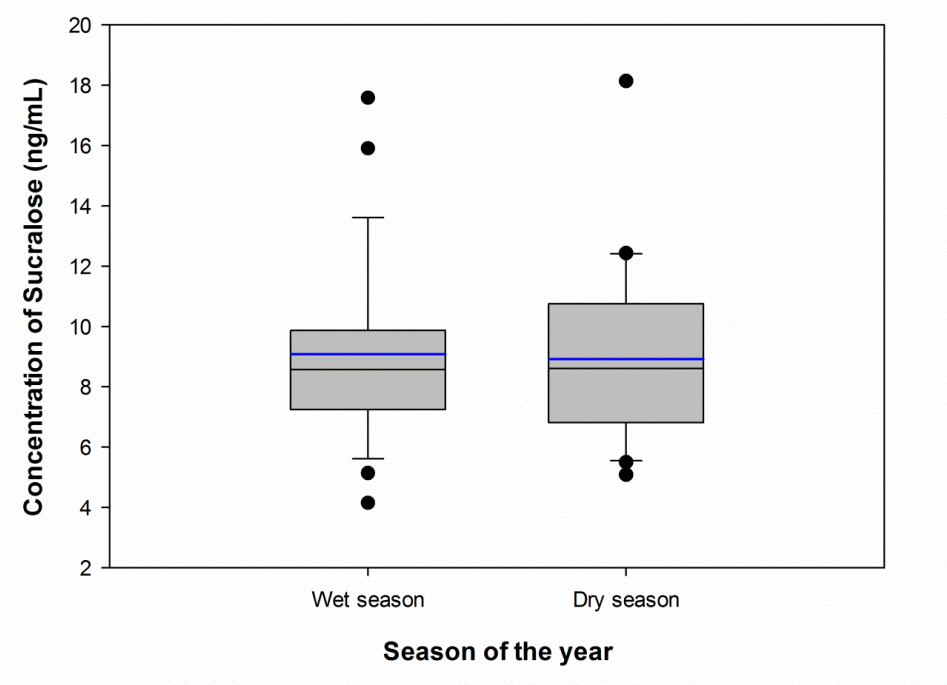


Figure 39. Distribution of sucralose in reclaimed waters in various seasons. The boundaries of box plot cover 25th-75th percentile, the center line indicates median of the sample population, error bars (whiskers) above and below the box refer to 90th and 10th percentiles. The blue line in each box plot indicates mean of the sample population.

Mass loadings (mg/day) of sucralose was calculated by multiplying its average concentration and daily flow rate of the STP effluent during the sampling period (380 000 m3/day). Since the serving population of Miami Dade North District wastewater treatment plant was not directly available, it was estimated by using the population of Miami Dade County (USCB, 2011) and average daily flow rate of STP effluents of Miami Dade North District wastewater treatment plant (112.5 mgd). The estimated mass load per capita of sucralose was 4.37 mg/person/day, which is in good agreement with the U. S. average.

Sucralose has become the most detected unregulated chemical and artificial sweetener in wastewater, surface water and groundwater samples (Lange et al., 2012a). A worldwide comparison of the occurrence of sucralose in STPs and wastewaters is presented in Table 3 and the mass load per capita in the different countries were estimated (Brorstrom-Lunden et al., 2008; Brorström–Lundén et al., 2008; Buerge et al., 2009a; Scheurer et al., 2009a; Neset et al., 2010; Minten et al., 2011a; Morlock et al., 2011; Oppenheimer et al., 2011b; Scheurer et al., 2011; Torres et al., 2011; Berset and Ochsenbein, 2012; Ordonez et al., 2012b; Gan et al., 2013). The average effluent daily flow for China and European Union (EU) was calculated based on the general guideline suggested by Imhoff (1985), which estimates that 200 L of waste is produced per capita (Imhoff and Imhoff, 1985; UNEP, 2000; Heeb et al., 2012). The estimated mass load per capita (mg/person/day) was high for U.S.A (5.0), moderate for the EU (2.1) and very low for China (0.37). The higher value observed for U.S. can be explained based on higher consumption of sucralose and its early introduction into market (1998) compared to other countries (Switzerland: 2005; Sweden: 2004; Germany: 2005; China: 2009) (Lange et al., 2012a). The mass load per capita on a global scale (2.1) was calculated similarly and is comparable to EU and lower than US.

Table 19. Summary of previous studies on detection of sucralose in sewage and waste water effluents

Number	Study	Concentration detected (ng/L)	Country
1	Bronstrom-Lunden et al. 2008	3500	Sweden
2	Bronstrom-Lunden et al. 2008	4900	Sweden
3	Neset et al. 2010	2400	Sweden
4	Buerge et al. 2009	4470	Switzerland
5	Scheurer et al. 2009	800	Germany
6	Torres et al. 2011	2800	USA
7	Ordonez et al. 2012	49600	Spain
8	Oppenheimer et al. 2011	27000	USA
9	Scheurer et al. 2011	18000	Germany
10	Morlock et al. 2011	6449	Germany
11	Minten et al. 2010	11000	Sweden
12	Berset and Ochsenbein 2012	3641	Switzerland
13	Gan et al. 2013	1850	China
14	Current study	9100	USA

Spatial distribution of sucralose concentration in various drinking water sample locations is shown in Figure 40, with different colors ranging from white (< MDL) to red (>250 ng/L). No specific trend was observed between the concentration of sucralose in drinking waters and sampling location. Sucralose was frequently detected (88%) in drinking water samples with an average concentration of 111± 95 ng/L. The highest concentration observed in drinking water was 465 ng/L. The drinking water samples were collected from areas served by three major drinking water treatment plants in Miami-Dade, Hialeah and John E. Preston plant, Alexander Orr Jr. plant and South Dade water supply system. The Hialeah and John E. Preston plant serves most Miami-Dade residents living between the Miami-Dade-Broward County line and SW 8th Street.

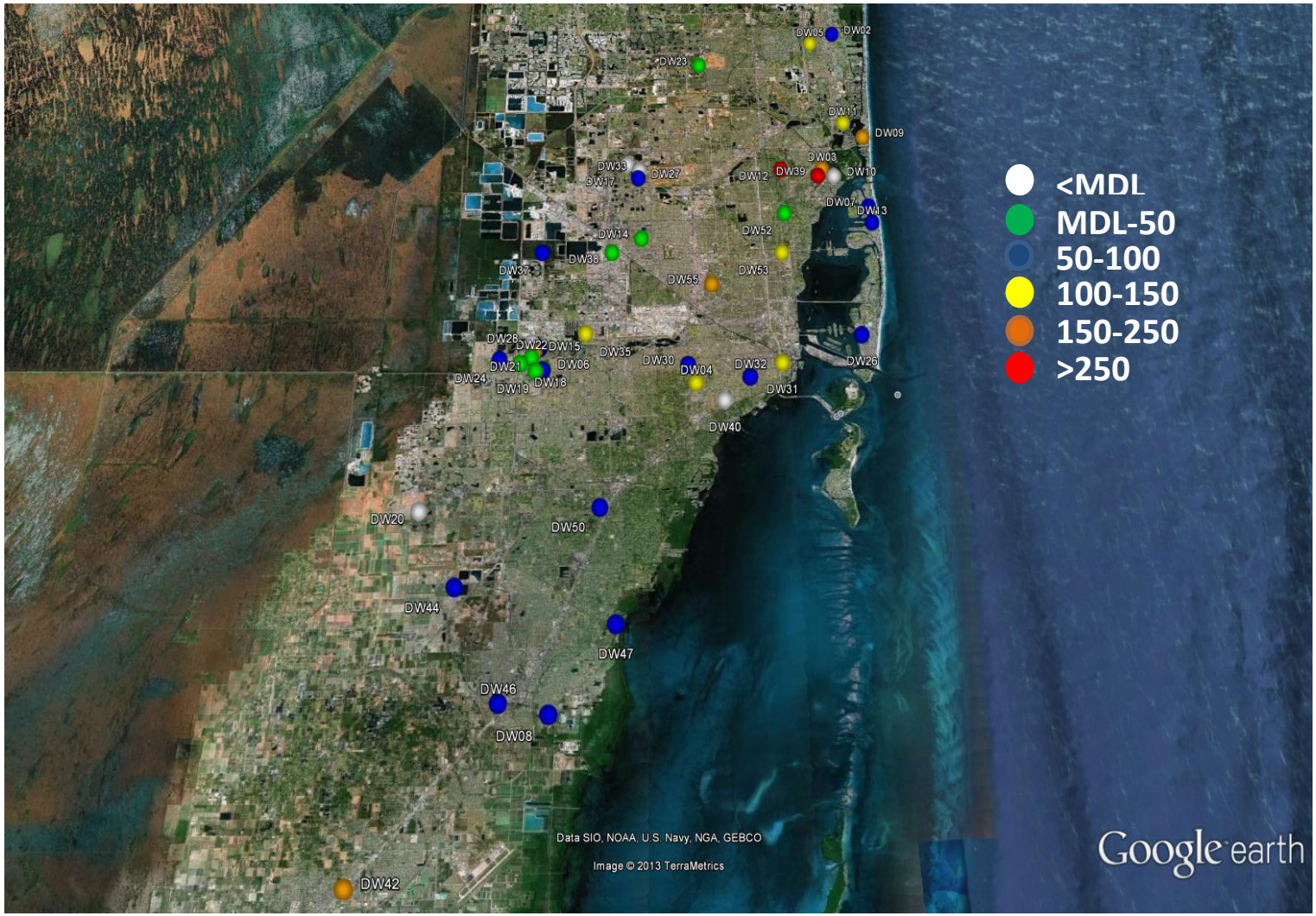


Figure 40. Distribution of sucralose in Miami-Dade County drinking waters.

The Alexander Orr, Jr. water treatment plant, serves most County residents living between SW 8th Street and SW 264th Street. The other drinking water treatment plant is South Dade Water Supply System, which is comprised of five smaller water treatment plants that serve residents south of SW 264th Street in the unincorporated areas of the County (MiamiDade). A plot of drinking water treatment plant versus sucralose concentration in samples collected from the areas served by the selected treatment plant is shown in Figure 41 and the sample size in each plant was shown in parenthesis above the error bar. Further, means of Hialeah and John E. Preston plant samples and The Alexander Orr, Jr. plant samples were compared using a t-test and results indicated that the means of the two drinking water treatment plants were not statistically different (Table 20). Thus the source of variation in the concentration of sucralose among samples might be caused by the time of sample collection, residence time of the waters before distribution in drinking water treatment plant and most likely be by point sources of contamination.

Table 20. Results of t-test using the samples of 2 major drinking water treatment plants in Miami-Dade County

Group Name	*N*	*Missing*	*Mean*	*Std Dev*	*SEM*
Hialeah and John E. Preston plant	20	0	127.598	120.054	26.845
Alexander Orr Jr. plant	12	0	74.222	27.453	7.925

t = 1.507 with 30 degrees of freedom. (P = 0.142)

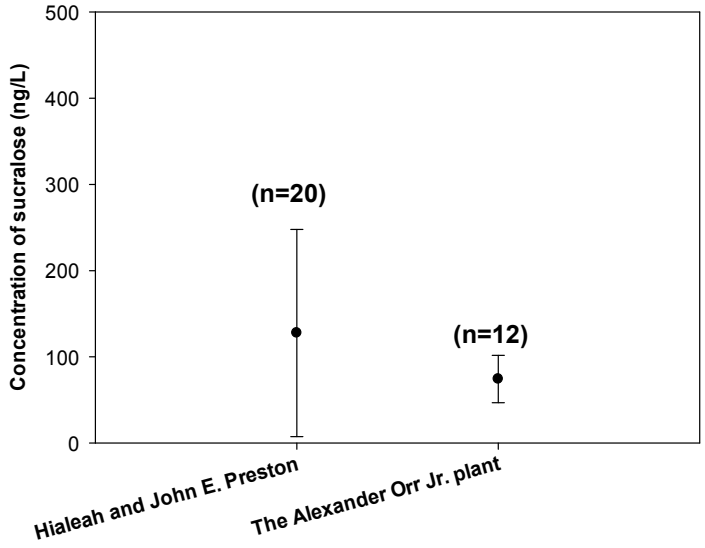


Figure 41. Comparison of samples from two major drinking water treatment plants of Miami-Dade County

Levels of sucralose in drinking water samples were similar to surface and groundwater in Europe (Loos et al., 2009a; Scheurer et al., 2009a; Mawhinney et al., 2011a; Minten et al., 2011a). Recent reports on U.S. ground and drinking waters (Ferrer and Thurman, 2010b; Mawhinney et al., 2011a) showed concentration of sucralose in the μg/L range, relatively higher than those reported in Europe and in the present study.

For results obtained in U.S. waters, the concentrations reported here in drinking water samples (up to 465 ng/L) were lower than in surface water (up to 10,000 ng/L), drinking water (up to 2400 ng/L) and groundwater (up to 2400 ng/L) from previous studies (Mead et al., 2009a; Ferrer and Thurman, 2010b; Mawhinney et al., 2011a; Oppenheimer et al., 2011b). These results suggest that levels of sucralose found in drinking and ground

waters are comparable to surface water, demonstrating that this compound can be of great concern even for Drinking water Treatment Plants (DWTPs) with groundwater sources.

The occurrence of sucralose in groundwater and therefore drinking water would likely be an effect of surface water, contaminated with a nearby WWTP, being drawn into alluvial wells (Ferrer and Thurman, 2010b). Therefore, it is reasonable to expect that human exposure to sucralose through tap water consumption may be widespread in the U.S.

5.3.4. Photodegradation study of sucralose

The photolysis decay curves of sucralose at UV 254 nm, UV 320-380 nm and SunTest are shown in Figure 42 and the kinetic parameters obtained are shown in Table 21. Results clearly indicate that in all light sources, the extent of sucralose degradation is mainly dependent on the type of matrix used i.e., highest rate in RODW and lowest in salt water. In the most energetic light source used in the study (UV 254 nm) no degradation was seen in SW (Fig 41 a).

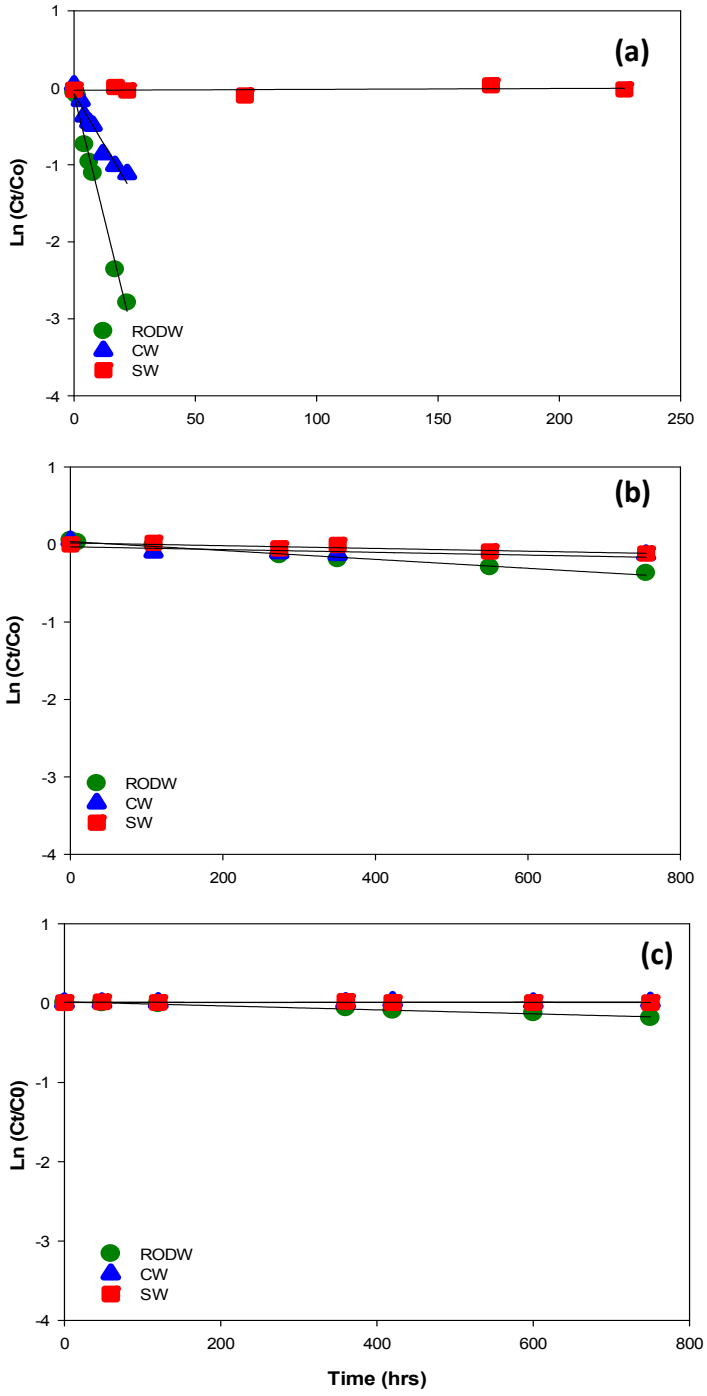


Figure 42. Photolysis decay curves of sucralose in a) UV 254 nm light source b) UV 320-380 nm light source c) Sun Test.

Similar results were observed by Torres et al i.e., minimal degradation (<8%) of sucralose in phosphate buffer was seen within 24 hours of exposure under UV 254 nm (Torres et al., 2011). Pronounced stability of sucralose was also evident in the UV treatment of wastewaters studied by Torres et al. where no sucralose degraded even after 24 hrs at 254 nm (Torres et al., 2011). At UV 320-380 nm and in Sun Test (Intensity: 750 W/cm2), sucralose was persistent to photolysis in both natural water matrices (SW and CW). Soh et al reported that no degradation was seen for 1µM sucralose exposed to UV 254 and 320-380 nm for 5 hours (Soh et al., 2011b). After one month of continuous irradiation only <16% degraded indicating that sucralose will be extremely persistent under natural conditions (Fig. 41 b, c).

Table 21. Kinetic parameters of photodegradation experiments

Light source	Matrix	k (h^{-1})	r^2	$t_{1/2}$ (h)	% degradation after a month
UV 254 nm	RODW	0.129	0.991	5.37	100
UV 254 nm	CW	0.053	0.948	13.1	100
UV 254 nm	SW	0.0001	0.053	>200	0
UV 320-380 nm	RODW	0.0006	0.984	1155	33
UV 320-380 nm	CW	0.0002	0.464	>750	16
UV 320-380 nm	SW	0.0002	0.818	>750	12
SunTest	RODW	0.0003	0.979	2310	18
SunTest	CW	0	0.218	NA	0
SunTest	SW	0	0.021	NA	0

5.3.5. Identification of photodegradation intermediates

Sucralose degraded significantly in the most energetic light source (254 nm) in RODW and CW and hence used to study the photolysis products. The samples (RODW)

irradiated under mercury vapor lamp were analyzed in full scan mode. A new peak showed up at its maximum intensity in the sample irradiated for 2.75 hrs and its intensity slowly decreased to 50% of the maximum intensity until the last time point measured (70.4 hrs, Fig. 43). This peak was absent in control samples. However, the MS spectra did not show the exact m/z information (Fig. 43). The same samples were reprocessed in a Q Exactive Benchtop Quadrupole-Orbitrap (Thermo Scientific, San Jose, CA, USA) using same the analytical column and mobile phase conditions used above. The Q Exactive was operated in full scan mode with negative atmospheric pressure chemical ionization (APCI). The APCI vaporizer temperature and capillary temperature were 350 and 300 °C respectively, with a discharge current of 5 kV. Sheath gas and auxiliary gas (N_2) were used at a flow rate of 30 and 10 arbitrary units, respectively. The S-lens RF level was 90, the resolution for the full scan experiment was 70 000 and the mass range monitored was 35-500 amu. The peak observed in triple quadrupole mass spectrometer was not observed in orbitrap.

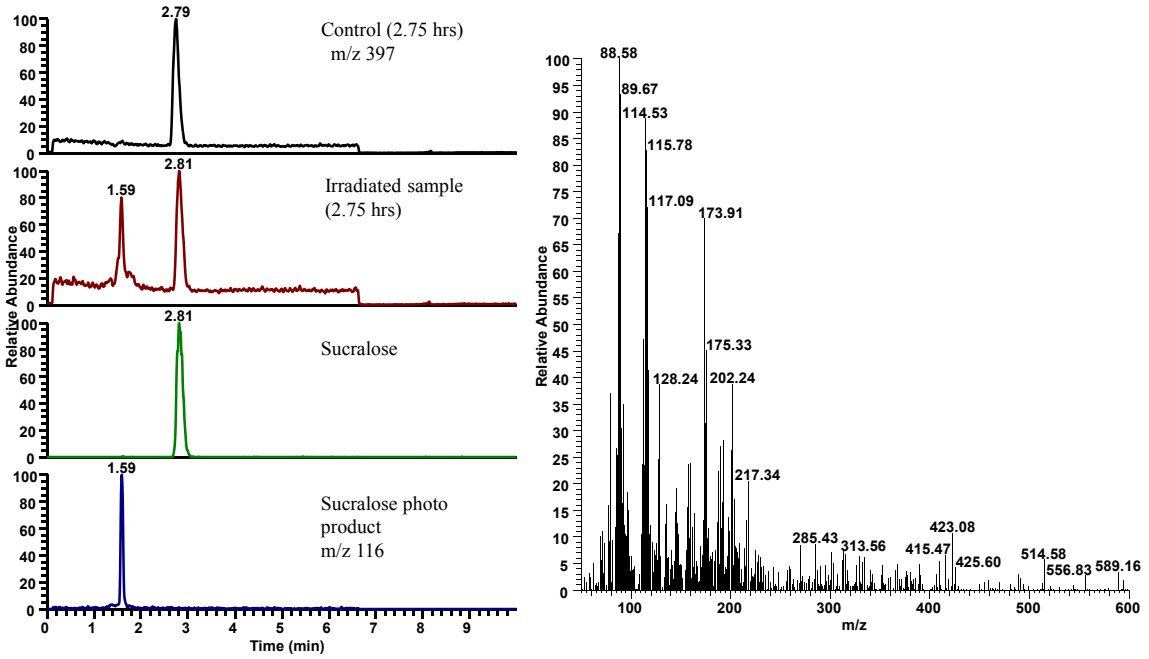


Figure 43. Chromatograms of sucralose and its photolysis product in control and irradiated samples (after 2.75 hrs, left); mass spectra of sucralose photolysis product (R_t = 1.59 min, right)

5.4.Conclusion

An automated online SPE LC-APCI/MS/MS was developed and validated for the determination of sucralose at low ng/L levels in water samples. The method was successfully applied to drinking and reclaimed water from South Florida, U.S.A. The method detection limits are 8.5 ng/L and 2.7 µg/L in drinking and reclaimed waters, respectively. In all matrices tested, the recovery of sucralose ranged from 85-113%. The method was successfully applied to drinking and reclaimed water from South Florida, U.S.A. Sucralose was frequently detected (> 80%) in all studied samples with concentrations up to 18 µg/L. The mass load per capita of sucralose released in STPs effluent was estimated in the U.S. and is two times higher than the global and European

Union. The maximum concentration of sucralose detected in drinking waters is 465 ng/L. Based on a study conducted by Fujimaru et al., it has been estimated that 79.5 mg of sucralose is required to make 1L of water weakly sweet (Fujimaru et al., 2012). Thus, considering the calculated global mass load of sucralose discharged by the STPs it would still take 8.43.E+13 years to have all ocean waters become sweet. The ubiquitousness of sucralose in the aquatic environment is however of great concern, especially since little information is known to date about its potential long term ecological effects.

At 254nm, sucralose degraded significantly in pure and freshwaters but was very stable in salt waters. Significant photolysis in freshwaters might be due to high [DOC] and Fe^{3+} in the matrix. Sucralose was extremely photo stable at environmentally relevant conditions. Its high resistance to photodegradation, minimal sorption and high solubility could explain the higher frequency of detection and levels found in this study. These results corroborate with previous findings indicating that sucralose could be a great tracer of anthropogenic wastes in to the environment.

CHAPTER 6

Conclusions

A most important contribution of the present study is that experiments were performed in natural matrices without any enrichment such as buffers and/or oxidants, thus the results could be very useful in risk assessment of CECs in the environment. It was proved that photo degradation plays a major role in dictating the fate of CECs and the efficiency of photo transformation depends mainly on pH of the water body (and thus speciation of the contaminant) and matrix composition.

The half-lives of all studied CECs are summarized in Table 22. Ciprofloxacin was the least stable CEC followed by sulfa drugs. Among sulfonamides, sulfadimethoxine was the most stable one with a measured half-life of 4-9 days in the environment and hence one of the most frequently detected sulfonamides in surface waters (Garcia-Galan et al., 2011). For sulfonamides, the half-lives obtained in simulated and real sunlight vary by 2-16 fold which was used to predict the half-lives of other CECs in natural sunlight. The predicted half-lives are shown in grey color in the table. The macrolide antibiotics (erythromycin and roxithromycin) are moderate to highly persistent in the aqueous matrices. Moreover, they have high toxicity towards blue green algae (Isidori et al., 2005), can inhibit the cytochrome P-450 leading to adverse drug reactions in humans (Sagir et al., 2003) and form degradation products that are microbiologically active (Radjenovic et al., 2009).

Table 22. Summary of half-lives of studied CECs

	Half-lives of studied CECs (hrs)											
	UV 254 nm			UV 350 nm			Solar simulation			Natural sunlight		
	RODW	SW	CW	RODW	SW	CW	RODW	SW	CW	RODW	SW	CW
Sulfamethoxazole	0.00939	0.00939	0.00942	0.0447	0.138	0.102	0.227	3.32	1.45	2.04	29.8	13.1
Roxithromycin	6.73	0.686	0.383	94.9	30.0	47.5	122	59.2	32.7	1094	**533**	**294**
Erythromycin	31.9	2.55	5.20	108	30.0	76.7	56.3	70.0	239	507	**630**	**2151**
Ciprofloxacin	0.0914	0.0430	0.0154	0.284	0.0257	0.0465	1.67	0.434	0.229	15.0	3.91	2.06
Sulfadiazine	0.0230	0.0190	0.0320	3.98	3.52	1.60	10.1	13.8	5.78	107	50.2	34.7
Sulfathiazole	0.0130	0.0130	0.0333	3.15	1.17	1.03	3.05	1.34	1.21	40.1	13.9	13.0
Sulfamerazine	0.0290	0.0230	0.0380	8.13	2.74	1.16	11.2	10.2	3.67	117	51.3	13.0
Sulfamethazine	0.0430	0.0200	4.82	7.59	2.52	1.05	11.5	9.04	4.03	178	35.5	30.8
Sulfamethizole	0.0130	0.0160	0.0130	7.78	7.28	9.40	4.88	4.41	5.82	103	56.8	52.9
Sulfachlorpyridazine	0.0440	0.0350	0.0350	3.33	2.65	1.39	7.00	13.4	4.73	51.0	43.0	17.3
Sulfadimethoxine	0.0230	0.0200	0.0270	9.51	14.7	16.5	12.3	19.8	20.2	110	217	100
DOSS	0.605	3.43	N/A	407	330	N/A	116	157	N/A	1044	**1413**	**N/A**
Sucralose	5.37	13.1	>200	1155	>750	>750	2310	ND	ND	20790	**ND**	**ND**

RODW = reverse osmosis deionized water CW= canal water SW=salt water N/A =not studied ND= no degradation seen

CECs which are very persistent are shown in bold; predicted half-lives are highlighted in grey

DOSS had a predicted half-life of 58 days in surface ocean waters and formed a structurally similar degradation product whose fate and ecotoxicology were unknown to date. Since bio-and photo degradation were minimal in the deep sea, it would persist much longer in deep ocean waters, suggesting a high risk for benthic organisms. Sucralose was the most persistent CEC studied showing absolutely no degradation in relevant environmental sources. It was detected in all types of water matrices including drinking waters. The maximum concentration of sucralose detected in Florida drinking waters was 465 ng/L. It has been recently identified as an ideal tracer to monitor anthropogenic activity in aqueous environments (Lubick, 2009). The estimated mass load per capita of sucralose released by the STPs was 5.0 mg/day/person in the U.S. which was 2-fold higher than the global and European Union mass load. This results can be explained based on higher consumption and early introduction into U.S. markets. Finally the order of photo stability of studied CECs was sucralose >>> DOSS >> macrolides > sulfonamides = ciprofloxacin. Sucralose, DOSS and macrolides were very persistent (shown in bold numbers in the table) and should be further studied.

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PUBLICATIONS AND PRESENTATIONS

Venkata Reddy Panditi**, Sudha Rani Batchu**, Piero R. Gardinali. Online solid phase extraction-liquid chromatography-electrospray-tandem mass spectrometry determination of multiple classes of antibiotics in environmental and treated waters. Analytical and Bioanalytical Chemistry (In Press, DOI: http://dx.doi.org/10.1007/s00216-013-6863-8)

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