UVC induced photo-removal of sulfamethoxazole (SMX), levofloxacin (LEVO), 17α-ethinylestradiol (EE2) and levonorgestrel (LNG) in wastewater

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ABSTRACT

The potential development of antibacterial resistance and endocrine disruption has lead to increased research investigating the removal of antibiotics and synthetic (or natural) hormones from water by various methods. Biodegradability of these compounds was generally reported to be low and these pharmaceuticals were frequently detected in sewage and wastewater treatment plant (WWTP) effluents. Therefore, alternative or complementary removal techniques to conventional wastewater treatment are necessary in order to mitigate potential health hazards associated to these contaminants. Investigation of advanced oxidation processes (AOPs) for removal of variety of pharmaceutically active compounds has received great interest in the recent years.

In the Ph.D. thesis presented here, results associated to photocatalytic removals under UVC radiation of two antibiotics: sulfamethoxazole (SMX) and levofloxacin (LEVO) and two synthetic hormones: 17-α-ethinylestradiol (EE2) and levonorgestrel (LNG) are presented. UVC radiation is chosen since it is commonly used in water sterilization facilities.

It was shown that photolysis is the dominant mode of removal of SMX during photocatalysis under UVC radiation. Even though, photolytic removal of this compound was faster, higher mineralization efficiency was reported for photocatalysis. Both UVC mediated treatments lead to the generation of products which are more toxic than the parent compound as determined by *Daphnia magna* toxicity tests.

There are currently no data on the photocatalytic removal of LEVO under UVC radiation, and this Ph.D. thesis provides the first set of data in literature about its photocatalytic removal. The results showed that the direct photolytic removal of this compound is not significant, and during photocatalysis more than 97% of LEVO is removed after 120 minutes of irradiation. The effectiveness of photocatalysis is shown by comparing these results to its removal by ozonation (another AOP). Ozonation leads to the generation of persistent products which are removed subsequently by photocatalysis. The generated products of photocatalytic treatment of LEVO were shown to contain no residual antibacterial activity. The applicability of this type of treatment to waters containing LEVO was verified. Concerns for increased antibacterial

resistance of pathogens in receiving waters can be mitigated by employing this treatment.

EE2 and LNG are commonly used in combination in oral contraceptive pills. EE2 is a synthetic estrogen commonly detected and was shown to have adverse endocrine disrupting effects at environmentally relevant concentrations. LNG is a synthetic progestin, research on its occurrence and potential health effects just started to be explored in the recent years. This Ph.D. thesis provides for the first time in literature, the photolytic and photocatalytic removal of LNG as an individual contaminant as well as in mixtures of EE2 and in a real pharmaceutical wastewater. Photo-removal experiments showed that LNG is significantly more sensitive to UVC photolysis than EE2. In complex reaction matrices (where more than one contaminants is present), higher photolytic removal efficiencies of LNG were observed, whereas for EE2 photocatalytic removal was always more significant than its photolytic removal. Similar photocatalytic reaction rates and efficiencies were observed for both compounds in the wastewater, suggesting that their simultaneous removal is possible and photocatalysis can be used for similar wastewaters to reduce hormone content.

This Ph.D. thesis provided detailed investigation of photocatalysis and photolysis as alternative removal methods for a wide range of pharmaceutical compounds. The non-selective oxidation capacity of oxidizing species generated during photocatalysis is verified. The versatility of photocatalysis is underlined by its strong performance in removing a variety of pharmaceutically active compounds.

RESUME

Le développement potentiel de la résistance aux antibiotiques et de la perturbation endocrinienne a menée à une intensification de la recherche sur l'enlèvement des antibiotiques et des hormones naturelles et synthétiques par divers méthodes. Ces composés ne se biodégradent pas rapidement et ils ont été fréquemment détectés dans l'affluent et l'effluent des stations d'épuration des eaux usées. Alors, des méthodes de rechange ou complémentaires aux techniques conventionnelles de traitement des eaux usées sont nécessaires pour réduire les risques potentiels à la santé associés à ces contaminants. La recherche sur les procédés d'oxydation avancés (POA) pour l'enlèvement d'une gamme diverse de composés pharmaceutiques actifs a attiré beaucoup d'attention dans les dernières années.

Dans la thèse de doctorat présentée ici, les résultats générés suite à l'enlèvement photocatalytique par radiation UVC de deux antibiotiques, sulfamethoxazole (SMX) et levofloxacin (LEVO), et de deux hormones synthétiques, 17α-éthynilestradiol (EE2) et levonorgestrel (LNG), sont présentés. La radiation UVC a été choisi parce qu'elle est couramment utilisée dans l'industrie pour la désinfection de l'eau.

Il a été démontré que la photolyse est le mode principal d'enlèvement de SMX par radiation UVC. L'enlèvement photolytique a été plus rapide, cependant un taux de minéralisation plus élevée a été reporté pour la photocatalyse. Le test de toxicité à base de *Daphnia magna* a démontré que ces deux traitements génèrent des sous-produits qui sont plus toxiques que le composé parent.

Présentement, il n'y a pas d'information sur l'enlèvement photocatalytique de LEVO, et cette thèse fourni les premières données de la littérature. Les résultats ont démontré que l'enlèvement photolytique direct de ce composé n'est pas significatif. Durant la photocatalyse plus de 97% de LEVO est éliminé après 120 min d'irradiation. L'efficacité de la photocatalyse est démontrée en comparant ces résultats à l'enlèvement obtenu par ozonation (POA). L'ozonation génère des sous-produits persistants qui sont par la suite éliminées par la photocatalyse. Les sous-produits de la photocatalyse de

LEVO ne possèdent pas d'activité antibactérienne résiduelle. L'applicabilité de ce type de traitement aux eaux contenant LEVO a été vérifiée.

Le 17α-éthynilestradiol (EE2) et levonorgestrel (LNG) sont habituellement utilisés en combinaison dans les pilules anticonceptionnelles. EE2 est un œstrogène synthétique couramment détecté et il est capable de produire des effets nocifs de perturbation endocrinienne. LNG est un progestagène synthétique et la recherche sur sa présence dans l'environnement et ces effets sur la santé ont commencé à être étudiés récemment. Cette thèse fournit pour la première fois dans la littérature, les premiers résultats de l'enlèvement photolytique et photocatalytique de LNG comme contaminant individuel et aussi dans des mélanges avec EE2. Les expériences d'enlèvement par photolyse ont démontré que LNG est significativement plus sensible que EE2 à la photolyse. Dans des matrices réactionnelles complexes, des taux élevées d'enlèvement photolytique de LNG ont été observées lorsque pour EE2 l'enlèvement photocatalytique a été toujours plus significatif. Des vitesses de réaction et efficacités similaires ont été observées pour les deux composés dans l'eau usée, ce qui suggère que leur enlèvement simultanée est possible et la photocatalyse peut être utilisée pour des eaux semblables pour réduire leur teneur en hormones.

Cette thèse de doctorat fourni une investigation détaillée de la photolyse et de la photocatalyse comme des méthodes de rechange pour l'enlèvement d'une gamme variée de composés pharmaceutiques. La versatilité de la photocatalyse est soulignée par sa forte performance lors de l'enlèvement d'une variété des composés pharmaceutiques actifs.

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Dedicated to my mother, Gul Aysen Nasuhoglu

TABLE OF CONTENTS

List of Tables

Table 4.1- MRM ion transitions, de-clustering potential (DP) and collision energies for analysis 43
Table 4.2- Calculated minimum apparent quantum efficiencies of photolytic removal of SMX ... 48

List of Figures

CONTRIBUTIONS OF AUTHORS

The role of each author during the preparation of the three manuscripts presented in this Ph.D. thesis is described below.

Manuscript 1 – Photo-removal of sulfamethoxazole (SMX) by photolytic and photocatalytic processes in a batch reactor under UV-C radiation (λ_{max} = 254 nm)

Deniz Nasuhoglu: designed and carried out the experiments, performed sample and data analysis, wrote the manuscript and responded to reviewers.

Dimitrios Berk: contributed to the experimental design and revised the manuscript.

Viviane Yargeau: contributed to the experimental design and revised the manuscript.

Manuscript 2 – Removal of the antibiotic levofloxacin (LEVO) in water by ozonation and TiO_2 photocatalysis

Deniz Nasuhoglu: designed and carried out the experiments, performed sample and data analysis, wrote the manuscript and responded to reviewers.

Angela Rodayan: designed and carried out ozonation experiments.

Dimitrios Berk: contributed to the experimental design and revised the manuscript.

Viviane Yargeau: contributed to the experimental design and revised the manuscript.

Manuscript 3 – Removal of 17α-ethinylestradiol (EE2) and levonorgestrel (LNG) in pharmaceutical wastewater under UVC radiation

Deniz Nasuhoglu: designed and carried out the experiments, performed sample and data analysis, wrote the manuscript and responded to reviewers.

Dimitrios Berk: contributed to the experimental design and revised the manuscript.

Viviane Yargeau: contributed to the experimental design and revised the manuscript.

1. INTRODUCTION

Frequent occurrence of pharmaceutical compounds in aquatic environments is an issue of global concern. Extensive use of these compounds resulted in their occurrence in sewage and subsequently their detection in aquatic environments due to their limited removal in wastewater treatment plants (WWTP) [1-5]. Numerous reports confirm the presence of pharmaceuticals in surface and groundwater at concentrations in the ng/L to µg/L range [6-11].

Two important classes of pharmaceutical compounds that are frequently encountered in wastewater effluents are antibiotics and hormonally active compounds (especially estrogens). Several antibiotic compounds were demonstrated to lead to increased antibiotic-resistant pathogens in wastewater [12, 13] while at environmentally relevant concentrations, natural and synthetic estrogens were shown to cause adverse effects on fertility of aquatic organisms [14, 15].

It is frequently reported that the removal efficiencies of these compounds in WTTPs are low; thus current research focuses on evaluating the possibility of applying advanced oxidation processes (AOPs) as complementary or alternative methods to conventional wastewater treatment for removal of these pollutants of emerging concern. Examples of AOPs for wastewater treatment include reactions with hydrogen peroxide (H_2O_2) with or without UV irradiation, ozonation (O_3), O_3/UV, photo-fenton, sonolysis and heterogeneous photocatalytic oxidation (PCO). Hydroxyl radicals created by UV radiation of a semi-conducting material have very high oxidizing potential and are capable of oxidizing a variety of compounds. In contrast to AOPs such as ozonation and hydrogen peroxide treatment where oxidants are consumed, heterogenous PCO use near UV and visible light as the energy source to create alternative reaction mechanisms for oxidation. Water and its dissolved oxygen content provide the necessary media for creation of oxidizing species.

Photolytic and photocatalytic removal of several antibiotics and hormones have been receiving great interest in the recent years. Complete removal of parent compound, removal of antibiotic activity, reduction in toxicity and removal of estrogenic activity

was reported [16-52]. Based on these promising reports, PCO was suggested as a potential wastewater treatment method to mitigate the impact of these contaminants or emerging interest on the environmental and public health.

Research in PCO of emerging pollutants is still in its growing phase. More data on removal efficiencies in complex water matrices, identification of degradation products, determination of toxicity of treated samples to variety of organisms and removal of pharmaceutical activity (antibiotic or estrogenic) are necessary to assess applicability of PCO for reduction of environmental impact of these contaminants of emerging concern.

The Ph.D. thesis presented here is focused on investigating the applicability of photocatalysis to the removal of two antibiotics: sulfamethoxazole (SMX) and levofloxacin (LEVO), and two synthetic hormones: 17a-ethinylestradiol (EE2) and levonorgestrel (LNG) in pure water and complex matrices such as industrial wastewater.

2. LITERATURE REVIEW

2.1 Sources of pharmaceutical contamination of aquatic environments

The most important ways that drugs enter the environment are through human and animal excreta, the improper disposal of unused or expired drug products, hospital effluents, and in some cases through waste effluents of manufacturing plants. Of these, animal excreta are the major source of environmental contamination by drugs, as most of the drugs used in veterinary medicine end up in manure. Hospital wastewater and wastewater from manufacturers and landfill leachates may also contain significant concentrations of pharmaceuticals [53]. Figure 2.1 outlines varieties of routes the pharmaceuticals find their way in the environment.

Pharmaceuticals are compounds synthesized in such a way that they do not accumulate in the organism. After medication they are mostly excreted unchanged, slightly transformed, or conjugated to polar molecules. For instance, sulfonamides are excreted via urine one or two days after administration as both the parent compound and the derivative with an acetylated amino group [54]. These conjugates can easily cleave during sewage treatment in such a way that the parent compound can be released into the effluent of the municipal sewage treatment plants. It has generally been reported that removal of these compounds is low in wastewater treatment plants by biodegradation or by adsorption [4, 5]. Therefore, aquatic environments are contaminated by these compounds via treated wastewater effluents [10, 55-59].

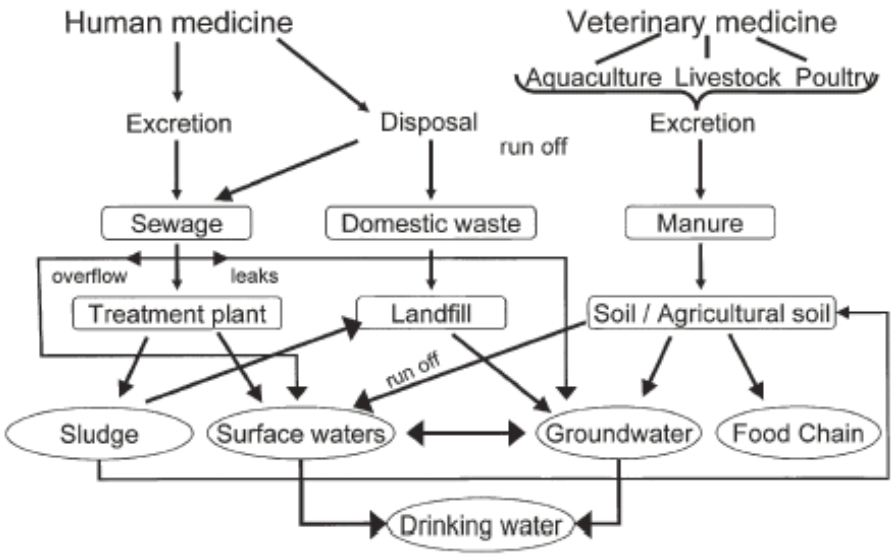

Figure 2.1- Principal routes of human and veterinary drugs to aquatic environments [60]

2.2 Occurrence of pharmaceuticals

It is estimated that the annual global consumption of pharmaceutical compounds is well over thousands of metric tons. Due to the advances in analytical techniques, in recent years increasing number of these compounds have been detected in various surface and ground water samples all over the world from ng/L to µg/L range [1, 7, 8, 11]. The contamination of surface and ground waters subsequently lead to the presence of these compounds even in treated municipal drinking water [6].

Even though there is lack of information in peer reviewed literature on the concentrations of pharmaceuticals discharged in wastewater of manufacturing plants, a study of the effluent of a wastewater treatment plant serving about 90 bulk drug manufacturers in Patancheru, India [61] reveals highest levels of pharmaceuticals reported in any effluent as seen in Table 2.1. The lack of regulations regarding the concentration of drugs in industrial wastewater suggests that numerous plants producing or processing pharmaceuticals release every year significant amounts of pharmaceuticals to the environment.

Table 2.1- Concentration of drugs in the effluent of a wastewater treatment plant serving 90 bulk drug manufacturers in Patancheru [61]

Compound	Type of drug	Range (µg/ l)
Ciprofloxacin	Antibiotic- Fluoroquinole	28000 - 31000
Losartan	Antigiotensin II receptor antagonist	2400 - 2500
Cetirizine	H_1- receptor antagonist	1300 - 1400
Metoprolol	β_1- andrenoreceptor antagonist	800 - 950
Enrofloxacin	Antibiotic- Fluoroquinole	780 - 900
Citalopram	Serotonin reuptake inhibitor	770 - 840
Norfloxacin	Antibiotic- Fluoroquinole	390 - 420
Lomefloxacin	Antibiotic- Fluoroquinole	150 - 300
Enoxacin	Antibiotic- Fluoroquinole	150 -300
Ofloxacin	Antibiotic- Fluoroquinole	150 -160

2.3 Ecotoxicological effects and concerns

The current literature about the ecotoxicological effects of pharmaceutical compounds for humans deal mainly with the acute toxicity in standardized tests focused generally on aquatic life. Effective concentration (EC) and lethal concentration (LC) of drugs for aquatic species are determined to measure toxicity (EC_{50} is the median concentration that causes 50 % of the maximal response; LC_{50} is the required concentration to kill half the members of the investigated population). The measured environmental concentrations of each individual pharmaceutical are 100 – 1000 times less than the acute effect concentrations. This suggests that acute toxicity would be a concern mostly in case of spills [62-65]. Data, however, are limited when it comes to studying the toxicological effect of mixtures of pharmaceuticals. Cleuvers [66, 67] investigated the ecotoxicological impact of mixtures of numerous pharmaceutical compounds in two studies. The acute toxicities of mixtures of diclofenac, ibuprofen, naproxen, acetylsalicylic acid, carbamazepine and clofibric acid were evaluated using *Daphnia magna* and algal tests. The mixtures were prepared at concentrations where single compounds showed no or little effect. Synergistic effects were observed for mixtures containing clofibric acid and carbamazepine as well as for the mixture of

ibuprofen and diclofenac. These findings indicated that even if these compounds are found in the environment at levels that they individually show no effect, it is highly likely for aquatic organisms to experience toxicity via concentration addition effect.

As mentioned in the previous section two major concerns are related to antibiotics and hormones. It is widely accepted that presence of antibiotics leads to antibiotic resistance in bacteria present in the environment and in conventional wastewater treatment. Decades of antibiotic use and unregulated discharge resulted in increased resistance for a variety of bacteria causing certain infections to be untreatable by regularly prescribed antibiotics [68]. This resistance developed by microorganisms is reported to be favoured by long term exposure to low concentrations of antibiotics [69]. The possible transfer of resistant genes from benign to pathogenic bacteria and consequently to humans is a major risk [70]. Even though no direct effect of contamination of aquatic environments by antibiotic compounds on human health was established increase in bacterial resistance to antibiotic agents was commonly reported [12, 13, 71-73].

Occurrences of natural and synthetic hormones in aquatic environments also cause a major concern because of their demonstrated effect on fertility and development of aquatic organisms by interfering with the endocrine system at ng/L concentrations [74, 75]. Up to 80 % of the estrogenic activity was shown to be removed by conventional activated sludge treatment [76-78]; however there is still concern due to the high biological potency of many of these compounds even at trace amounts. There are numerous reports investigating the impact of conventional WWTP effluents containing estrogenic compounds. Even at low concentrations (below 50 ng/L) populations of fish were shown to be severely affected by presence of estrogens. Collection of fish upstream and downstream of a WWTP effluent discharge showed that frequency of collecting male fish upstream was twice that of downstream and from the fish collected downstream 18 – 22 % of male fish showed intersex characteristic whereas no intersex characteristics were observed upstream [79]. Increased vitellogenin (protein naturally expressed only in egg-laying female fish) production in male fish was also demonstrated when fish were exposed to WWTP effluent [80]. Currently, there is no definite evidence that reproductive problems associated to aquatic organisms due to

presence of estrogenic compounds in natural waters will also be observed in humans. However, decreases in the birth of healthy baby boys and increases in male genital defects are speculated to be consequences of the presence of endocrine disruptors in the environment. Higher risk of developing adult reproductive problems was also linked to exposure to endocrine disruptors during fetal life [81].

2.4 Alternative waste treatment methods

The extent of elimination of pharmaceuticals in conventional wastewater treatment plants is generally studied by measuring the influent and effluent concentrations. For example, the average elimination of carbamazepine varied between 7 and 8 % [82], X-ray contrast media were not significantly eliminated [83], very high elimination of ibuprofen, ketoprofren , naproxen and diclofenac (94 -100%) were found in three STPs in the U.S.A by Thomas and Foster [84] whereas removal of diclofenac was limited to only 26 % in another study by Lindqvest et al. [85]. There is large variability in the reported performance of WWTPs for removing pharmaceuticals but generally incomplete or low removals are observed. Thus, alternative treatment methods are necessary for better, and ideally complete, removal of these compounds and their biological activity in order to mitigate their impact on the environment and public health.

2.4.1 Advanced oxidation processes (AOPs)

AOPs have received great interest in recent years as complementary or alternative treatment strategies to conventional wastewater treatment. AOPs are generally characterized by the generation of hydroxyl radicals. The hydroxyl radical is the second strongest oxidant, after fluorine. Rate constants for reactions involving hydroxyl radicals in aqueous solution are generally in the order of $10^6 – 10^9$ mol L^{-1} s^{-1} [86, 87]. Hydroxyl radicals can be generated by the use of UV radiation in the presence of oxidants such as ozone or hydrogen peroxide or by the use of a photocatalyst (e.g. TiO_2). It is also possible to generate hydroxyl radicals without radiation as in the case of Fenton processes, which only employ iron salt and hydrogen peroxide. Even though

generation of hydroxyl radicals occur during ozonation, the ozone molecule itself is also a strong oxidizing agent shown to oxidize many polar compounds containing amino groups, hydroxyl groups, carbon-carbon double bonds and aromatic carbons [88]. Processes that employ UV radiation to generate hydroxyl radicals are referred to as light oxidation processes and others are referred to as dark oxidation processes.

Majority of the dark oxidation treatments of emerging pollutants found in literature employ ozonation. Removals higher than 90 % were reached for most compounds such as antibiotics, endocrine disruptors, pesticides [89-94] but clofibric acid and x-ray contrast media showed considerable resistance [95]. Removals of emerging pollutants by Fenton reactions were not as extensively studied [96]. Among light oxidation processes UV/H_2O_2 and TiO_2 photocatalysis were the ones most employed to degrade emerging pollutants.

2.4.2 Photolysis

Direct photolysis in water involves the transformation of a chemical due to the direct absorption of a photon [97-101]. Direct photolysis has to be differentiated from indirect photolysis. Indirect photolysis in natural water, may involve the transformation of a chemical due to energy transfer from naturally occurring photosensitizers [97, 98, 102]. More commonly, indirect photolysis involves the transformation of a chemical due to reactions with transient oxidants such as hydroxyl radicals, molecular oxygen in a singlet electronic state (singlet oxygen), and peroxy radicals [97, 103-113]. Both photosensitizers and transient oxidants result from the absorption of photons by dissolved organic matter (DOM) and nitrate ion [97, 113-116]. In some cases both direct and indirect photolysis can contribute significantly to the dissipation of a chemical in natural waters.

The direct and indirect photo-transformation of chemicals in natural water bodies is a complex process which depends on factors such as: the chemical structure and electronic absorption spectrum of the chemical; the concentration, composition, and absorption spectra of DOM; and the intensity of incident radiation to which the chemical, DOM and nitrate are exposed.

To undergo transformation via direct photolysis, a chemical molecule must first absorb a photon. The absorption of a photon by a molecule leads to a transition from an electronic ground state to an electronically excited state of the molecule. The energy of the photon must correspond to the difference between the ground and a possible excited electronic state of the molecule for it to be absorbed by the molecule [97].

The absorption of a photon is a necessary, but generally not a sufficient condition, for a molecule to experience transformation via direct photolysis [97, 99]. The absorbed energy must first be sufficient to cause the transformation via bond cleavage, rearrangement, oxidation, or reduction of the compound. .

2.4.3 Photocatalysis

Photocatalytic oxidation refers to the degradation of organic pollutants through the creation of hydroxyl radicals (·OH) which are formed when a semi-conducting material (e.g. TiO_2) is illuminated with UV in an aqueous environment. Photocatalytic oxidation has the potential of complementing traditional wastewater treatment methods such as activated sludge when contaminants of concern are resistant to biodegradation. Recently there has been a growing interest in applying photocatalysis to treat waters containing micropollutants such as pharmaceuticals and personal care products. This section includes the properties of most commonly used photocatalyst (TiO_2) and the reaction mechanisms.

In crystals, multiple atomic or molecular orbitals are combined to form broad energy bands. In the absence of light, the valence band is fully occupied by electrons whereas the conduction band is unoccupied or partly occupied by electrons. The energy difference between the conduction band and the valence band is called the band gap. Metals have very small band gaps whereas semi-conductors have larger band gaps and insulators have very large band gaps. The first step in photocatalytic oxidation is the absorbance of UV-light ($\lambda < 390$ nm) by a semi-conductor. If absorption of a photon occurs with energy equal to or greater than the band gap energy (E_{bg}), transfer of an electron from the valence band to the empty conduction band is achieved thus creating an electron deficiency in the valence band, defined as a valance band hole (h_{vb}^{+}) which is a strong oxidant. The promoted electron in the conduction band is defined as the

conduction band electron (e_{cb}^-). These generated electron / hole pairs are responsible for the reduction / oxidation reactions that occur on the surface of the catalyst.

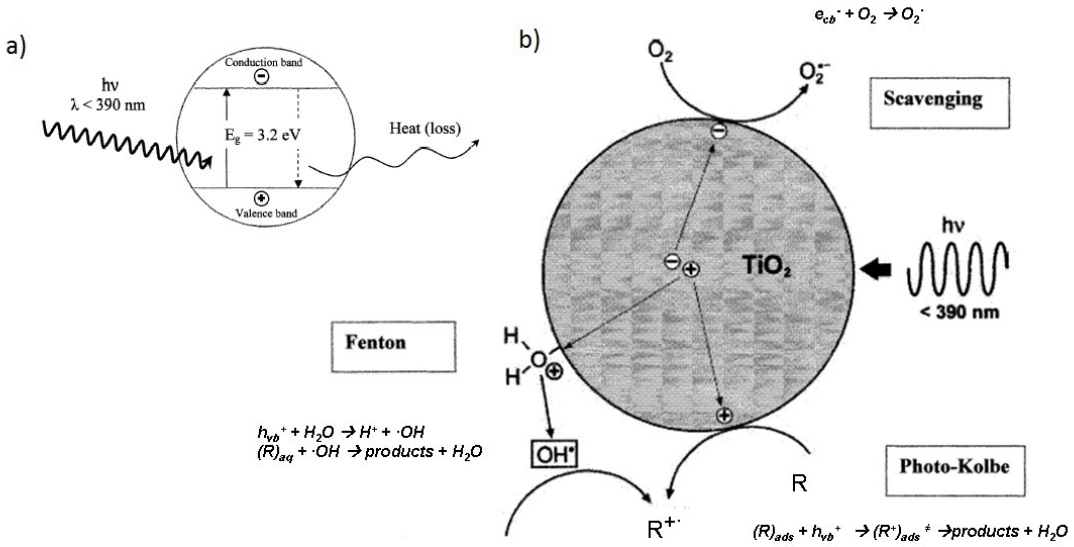

Figure 2.2- Schematic showing a) the excitation of an electron from valence to conduction band as well as possible relaxation due to recombination of electron and hole pairs b) Mechanism of TiO_2 photocatalysis [117]

The general proposed mechanism for the photocatalytic process where titania (TiO_2) is used as a photocatalyst can be outlined by the following steps (Figure 2.2):

1. Absorption of photons with $h\nu \geq E_{bg}$ (band gap) of titania

   $$TiO_2 + h\nu\ (< 390nm) \rightarrow e_{cb}^- + h_{vb}^+ \quad \textbf{(1)}$$

   Recombination of electron / hole pairs is possible within a few nanoseconds [118]

   $$e_{cb}^- + h_{vb}^+ \rightarrow heat \quad \textbf{(2)}$$

2. Electron scavenging by the adsorbed O_2, creating super oxide radicals (O_2^-) :

   $$O_2 + e_{cb}^- \rightarrow O_2^{\cdot -} \quad \textbf{(3)}$$

3. Formation of hydroxyl radicals by oxidation of adsorbed water or hydroxide ions:

   $$h_{vb}^+ + H_2O \rightarrow H^+ + \cdot OH \quad \textbf{(4)}$$

$$h_{vb}^{+} + OH^{-} \rightarrow \cdot OH \quad \textbf{(5)}$$

4. Neutralization of super oxide radicals by protons

$$O_2^{\cdot -} + H^{+} \rightarrow HO_2^{\cdot} \quad \textbf{(6)}$$

5. Transient hydrogen peroxide formation:

$$2\, HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \quad \textbf{(7)}$$

6. Decomposition of hydrogen peroxide through either UV or reaction with conducting band electrons if hydrogen peroxide is adsorbed onto the catalyst to create hydroxyl radicals [119]. It is important to note that generally hydrogen peroxide is found only at trace amounts [120] and if introduced into the system to yield excess amounts, it results in formation of peroxo compounds on the surface of the TiO_2 particles which inhibit the photocatalytic activity of the catalyst [121].

$$H_2O_2 + h\nu \rightarrow 2\, \cdot OH \quad \textbf{(8)}$$

$$H_2O_2 + e_{cb}^{-} \rightarrow \cdot OH + OH^{-} \quad \textbf{(9)}$$

7. Oxidation of organic molecules by hydroxyl radical attack

$$(R)_{aq} + \cdot OH \rightarrow products + H_2O \quad \textbf{(10)}$$

8. Direct reaction of adsorbed organic molecules with valence band holes to create intermediate radical cation of the organic molecule followed by hydrolysis to degradation products.

$$(R)_{ads} + h_{vb}^{+} \rightarrow (R^{+})_{ads}^{\neq} \rightarrow products \quad \textbf{(11)}$$

Titania (TiO_2) plays an important role in heterogenous photocatalysis and is one of the most widely used semi-conductors because of its activity, photostability, non-toxicity and commercial availability. Silicon (Si), zinc oxide (ZnO), cadmium sulfide (CdS), zinc sulfide (ZnS), strontium titanate ($SrTiO_3$) and iron oxide (Fe_2O_3) are other semi-conductors that can also be used as photocatalysts.

TiO_2 is generally prepared by flame hydrolysis of $TiCl_4$ and exists in three crystal forms: anatase, rutile and brookite. Anatase TiO_2 can be transformed into rutile

form at temperature above 900 K. The brookite form does not show photo activity where as anatase and rutile forms can be activated by light. There is growing evidence that anatase is more active than rutile for photocatalytic oxidation [122, 123]. The position of oxygen ions on the exposed crystal surface of anatase shows a triangular arrangement allowing effective absorption of organics. This favourable structural arrangement is not available for rutile [124]. Even though anatase is reported to be the more active form of titania, reports suggest that a pure anatase sample would not necessarily lead to the best photocatalytic performance [125, 126]. The presence of rutile phase introduces mesoporosity and a wider pore size distribution. Rutile / anatase combination also promotes charge pair separation and inhibits recombination. TiO_2 has high surface area and good corrosion stability. A vast majority of investigations reported in literature employ Degussa P-25 TiO_2, some of the properties of which are given in Table 2.2 below.

Table 2.2- Properties of TiO_2 Degussa P25 [122]

CAS Number	13463-67-7
Crystalline structure	80 % anatase 20% rutile
BET surface area	50 ± 15 m^2/g
Total pore volume	0.063 cm^3/g
Average particle size	25 - 35 nm
Band gap (E_{bg})	3.2 eV

2.5 Choice of pharmaceuticals and data reported on their removal by AOPs

As previously mentioned, the major environmental concerns with the presence of pharmaceuticals in the environment are associated with antibiotics and hormones. In the present study, substances belonging to these two groups were selected to evaluate the performance of photocatalysis as a method for the removal of these compounds and their transformation products. The two antibiotics are sulfamethoxazole (SMX) and levofloxacin (LEVO), and two hormones are 17-α-ethinylestradiol (EE2) and levonorgestrel (LNG) was chosen since it is commonly prescribed, commonly detected in WWTP effluents and in surface waters while LEVO was chosen since it is a newer drug and information on its occurrence and removal by treatment methods are scarce.

EE2 and LNG were selected due to their presence in the pharmaceutical wastewater provided by our industrial collaborator, WYETH, St-Laurent, Canada. Apart from its presence in the wastewater, EE2 is very commonly prescribed, is frequently detected in wastewater treatment plant effluents and has been shown to induce major reproductive problems in aquatic organisms. Photocatalysis of EE2 has been studied but information on its removal in complex matrices has yet to be obtained. Occurrence of progestins such as LNG only recently received attention therefore there is no information available about its removal by advanced oxidation methods. More information about the occurrence, environmental effects and removal during conventional and advanced treatment of all chosen compounds are summarized in the following sections.

2.5.1 Sulfamethoxazole (SMX)

SMX (Figure 2.3) is a synthetic antibiotic commonly used in humans for treatment of bronchitis and urinary tract infections and as well as veterinary medicine. It belongs to a group of antibiotics called sulfonamides. In 2007, it was the 6th most commonly prescribed drug, generally in combination with trimethoprim in Canada [127].

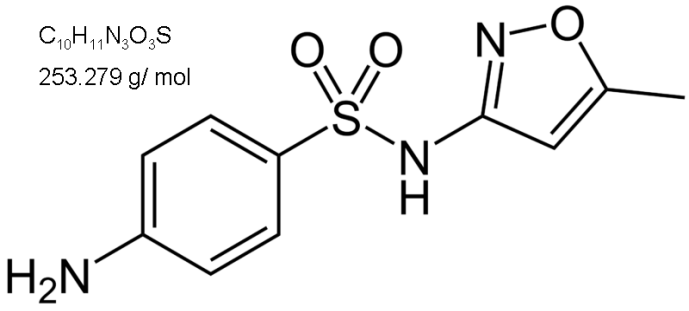

Figure 2.3- Molecular structure, weight and formula of SMX

SMX has been commonly detected in surface waters in the United States [6, 10] and Canada [11, 55, 56] and in WWTP effluents [55, 58] at concentrations ranging from ng/L to µg/L levels. Because of its occurrence in surface waters, it can be concluded that WWTP is not effective for the removal of SMX. In fact average removal of SMX compiled over data gathered from a vast number of conventional WWTPs (worldwide) was reported to be 60 ± 20% [128].

The use of AOPs to remove SMX in water and wastewater was extensively studied. There are many reports on ozonation [23, 24, 129-134], Fenton and photo-fenton [31, 135-138] and photolysis and photocatalysis [16, 21, 22, 24, 28, 52] for the removal of SMX. Major findings of reports investigating the photolytic and photocatalytic removals of SMX are mentioned in the following paragraphs.

A major portion of the existing literature reports the removal of SMX under UVA radiation. UVA radiation is commonly employed since it is the photocatalytically effective part of the spectrum of solar light, while the majority of the UVC radiation in filtered by the ozone layer. Therefore, there is great interest to harness UVA radiation for photocatalytic treatment of wastewater in areas with high solar light availability. The photocatalytic degradation of four sulfonamides (including SMX) during illumination under UVA radiation (λ_{max} = 366 nm) with TiO_2 catalyst was examined by Baran et al. [21]. Complete removal of all compounds within 300 minutes of irradiation was achieved (180 minutes for SMX); however 30 – 70% of the initial organic carbon remained even after 300 minutes, suggesting the persistence of the organic intermediate degradation products. SMX was found to be initially toxic to *Chlorella vulgaris* with EC_{50} value of 1.6 mg/L, while the degradation products were shown to have inhibitory and stimulatory effects; however the overall growth inhibition associated to the products was lower than the parent compounds.

The oxidation of SMX and related sulfonamide compounds by UVA ($324 < \lambda < 400$ nm) photocatalysis using TiO_2 was also investigated by Hu et al. [28]. SMX adsorption onto TiO_2 was shown to be insignificant. Photodegradation of SMX due to only UVA or visible light was reported to be minimal. From these literature results it can be concluded that UVA or visible light photolysis is not suitable for wastewaters containing SMX. 25 mg/L of SMX was found to be completely removed under photocatalysis (0.1 g/L TiO_2) after 60 min of irradiation. Reactions were found to follow pseudo-first order kinetics. It was demonstrated that UVA photocatalysis can be an effective approach for degrading SMX, especially in natural waters with alkaline pH and low natural organic matter concentrations.

The effect of irradiation wavelength on removal of SMX during photolysis and photocatalysis was investigated by Bayarri et al. [22]. The processes were carried out

under two irradiation setups, one with wavelengths greater than 235 nm (UV-ABC) and one above 300 nm (mainly UVA and UVB). SMX was found to degrade faster under UV-ABC radiation when no TiO_2 was present (complete removal at 200 min) compared to when TiO_2 was present. Both UV-ABC mediated photoprocesses were shown to remove SMX faster than UV-AB mediated processes. Highest mineralization efficiency was found for UV-ABC photocatalysis of 25% after 400 minutes of irradiation. Based on the comparison of HPLC chromatograms of UV-ABC photocatalysis, UV-ABC photolysis and UV-AB photocatalysis, it was proposed that UV-ABC photocatalysis goes through a different reaction pathway than UV-AB photocatalysis. It was suggested that during UV-ABC photocatalysis, photolysis shows a significant synergistic effect in removing SMX.

For areas with high solar light availability it is important to carry out photocatalytic removal experiments under solar light conditions. Under simulated solar light photocatalysis ($\lambda > 280$ nm) for an initial SMX concentration of 100 mg/L, 83% of SMX was shown to be removed after 360 min of irradiation at 0.5 g/L TiO_2 by Abellan et al. [17]. Removal of SMX by photolytic process was only 38%. No TOC removal was observed for photolysis; however this value was 22% for photocatalysis. Aromatic content of SMX was shown to be removed as irradiation time increased under photocatalysis.

Combinations of processes using ozonation, UVA photolysis and UVA photocatalysis were applied to water containing SMX (Beltran et al. [24]). 10 mg/L of SMX was shown to be removed by UVA photocatalysis (1.5 g/L TiO_2) at 15 minutes and by UVA photolysis at 30 minutes. Fastest SMX removal (10 minutes) and highest mineralization (more than 90% removal of total organic carbon in 60 min) was achieved for combined ozonation and UVA photocatalysis processes. Ecotoxicity tests of degradation products towards *Daphnia magna* showed that UVA photocatalysis leads to the generation of products more toxic than then the parent compound; however combined ozonation and UVA/TiO_2 treatment was capable of removing almost all of the associated toxicity. The report lacked information on how the treated samples were modified prior to toxicity determination by *Daphnia magna*. It was generally found that literature did not contain specific information on the methodology of this toxicity kit.

The general procedure for determination of EC_{50} values provided with the kit was mentioned but the fact that photo-degraded SMX solutions contain mixtures of unknown compounds was overlooked.

Even though most light mediated degradation experiments were performed at bench-scale, a recent report by Trovo et al. [136] investigated the removal of SMX by solar photo-fenton at pilot plant scale. Degradation and mineralization of SMX were strongly hindered in seawater compared to distilled water matrix. Rapid decay of 40% (10 mg/L SMX) during the first 45 minutes of irradiation was reported. However, after this point, photodegradation rate was significantly slower due to the accumulation of coloured reaction intermediates. No mineralization associated to photolytic removal of SMX was reported. Based on the photo-fenton experiments, addition of H_2O_2 up to 120 mg/L significantly reduced the toxicity of treated samples towards *Daphnia magna* and *Vibrio fischeri*.

Generally, photo-removal experiments of SMX are performed in pure water systems, but there are a few reports about removal of SMX in complex matrices. The removal of SMX in different water matrices (distilled water, distilled water + nitrite and seawater) by photolysis under simulated solar light conditions ($\lambda > 280$ nm) by Trovo et al. [139]. Findings about the photolytic SMX removal in distilled water matrix was the same as their previously mentioned work [136] and no enhancement by indirect photolysis was observed due to presence of nitrates. Complete removal in distilled water was achieved after 30 hours of irradiation; however in seawater after 10 hours of irradiation only 20% removal was reported. The proposed photo-transformation pathway was in agreement with the previously studied photolysis of SMX by Zhou et al. [140]. The reaction pathway was observed to follow the cleavage of the sulfonamide bond and the rearrangement of the isoxazole ring. Degradation products were shown to be more toxic than the parent compound towards *Daphnia magna* and *Vibrio fischeri*.

Recently, the effect of three different water matrices (ultrapure water, ground water and wastewater effluent) on removal of SMX by UVA photocatalysis was reported by Xekoukoulotakis et al. [52]. Complete removal of SMX (10 mg/L) was achieved after 45 min of irradiation (0.5 g/L TiO_2) and 90% TOC removal was reported after 120 min of irradiation. There was no significant effect of the water matrix on

removal rates of SMX; however at low pH, the photocatalytic degradation of SMX was favoured in all matrices. This was explained by the reduction of agglomeration of TiO_2 particles at acidic conditions [141], thus minimizing loss of active surface area.

2.5.2 Levofloxacin (LEVO)

LEVO (Figure 2.4) is a more recently developed antibiotic belonging to the fluoroquinolones (FQs), which are synthetic broad-spectrum antibiotics. The first and second generation quinolones are active against Gram-negative bacteria whereas the third and fourth generation quinolones have extended activity against Gram-positive bacteria as well. Ciprofloxacin belonging to the 2nd generation was the mostly prescribed quinolone in Europe in 2003. Currently the prescription trend is shifting towards levofloxacin and moxifloxacin both of which are 3rd generation quinolones [142]. There are limited reports on the presence of LEVO in aquatic environments; however other FQs such as ofloxacin and ciprofloxacin were commonly detected in effluents of hospital wastewaters, sewage and wastewater treatments plants at μg/L levels [10, 143-147]. The biodegradability of quinolones were shown to be very low [148] thus making conventional biological treatment methods ineffective for their removal.

It was found by Kim et al. [149] that LEVO did not show any acute toxicity to crustacean *Thamnocephalus platyurus* and fish species *Oryzias latipes*. According to the study conducted by Yamashita et al. [150], LEVO does not show acute toxicity to and *Vibrio fisheri* and *Daphnia magna,* either. However, in the same study it was shown LEVO to have chronic effects on the reproduction of *Daphnia magna* at 340 μg/L and to be toxic for microalgae in algal growth inhibition tests. Robinson et al. [151] performed toxicity tests with seven FQs on five aquatic organisms. Toxicity values ranged from 7.9 to 23,000 μg/L. The cyanobacterium *Microcystis aeruginosa* was the most sensitive organism. The authors also hypothesized that selective toxicity of FQs may cause disruptions in the aquatic community structure.

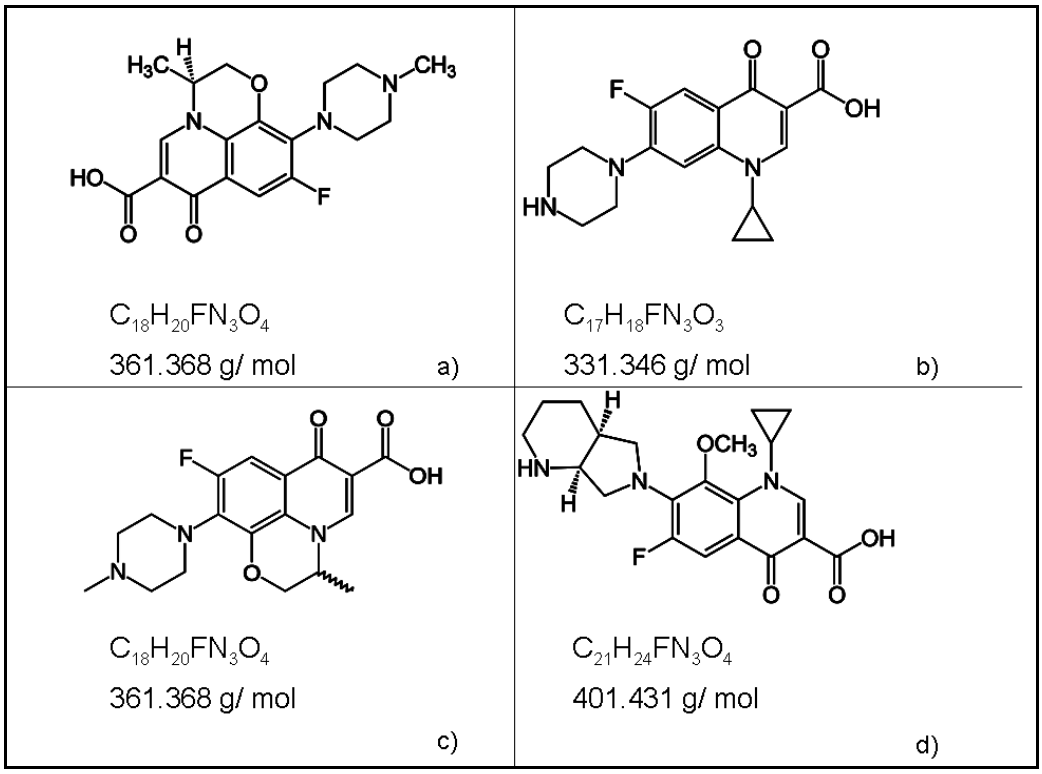

Figure 2.4- Molecular structure, weight and formula of a) LEVO b) Ciprofloxacin c) Ofloxacin d) moxifloxacin

Reports on the advanced oxidation of relatively older FQs such as ofloxacin and ciprofloxacin are abundant [18, 27, 37, 42, 43, 49, 50, 152-154]; however advanced oxidation of LEVO has seldom been investigated. The main results on ozonation of LEVO were very recently published by De Witte et al. [155]. Their report focused on the degradation rate and the formation of products. LEVO ozonation at different pH and different H_2O_2 amounts revealed a strong influence of pH on the degradation rate where as the H_2O_2 addition had only limited effect. Also a few other papers report the fate of this compound during UV radiation under close to sunlight conditions (> 290 nm) [156, 157]. Photochemical behavior of LEVO in pure and synthetic field waters was investigated by Lam and Mabury [156] along with three other pharmaceuticals. They reported that LEVO was sensitive to direct photolysis at irradiation wavelengths higher than 290 nm. They observed that in order to remove 50% of LEVO by photolysis only 20 minutes of irradiation was required. Significantly slower removals of LEVO were found for synthetic field water matrices. This was explained by the attenuation of light

by natural water constituents (e.g. nitrates, bicarbonates and dissolved organic matter). The only report on photocatalytic removal of LEVO was performed under UVA radiation by An et al. [19]. Reaction rate constants for reactions of LEVO with hydroxyl radicals and hydrated electrons were determined during UVA photocatalysis. They proposed reaction pathways and reported complete mineralization in 180 minutes. Because there are no other published data on photolytic or photocatalytic removal of LEVO, light induced treatment methods of other FQs (shown in Figure 2.4) are also included below.

As observed from literature data of SMX in the previous section, for FQs as well, majority of the photo-removal experiments are performed under UVA radiation or under solar light conditions. UVA (350 nm – 400 nm) induced photocatalytic removal of ofloxacin was investigated by Hapeshi et al. [27]. 40% conversion of the compound under photolytic conditions was achieved after 240 min (10 mg/L). However, under anoxic conditions photolytic removal was shown to be only 5%, suggesting that singlet-oxygen generated from dissolved oxygen contributes to the removal more than absorption of UVA light rather than direct photolysis. Complete removal of ofloxacin (10 mg/L) was observed after 240 min of photocatalytic treatment (250 mg/l TiO_2). After 90% removal of ofloxacin only 65% dissolved organic carbon (DOC) was reported, which confirmed the presence of persistent oxidation by products. Toxicity towards *Daphnia magna* revealed that early reaction intermediates induce considerable toxicity however the toxicity was shown to be eliminated upon prolonged treatment.

UVA induced TiO_2 photocatalysis of ciprofloxacin (CIPRO) was investigated by An et al. [18] to compare reactions of CIPRO with several free radicals : hydroxyl radicals, azide radicals, sulphate radicals and hydrated electron. It was shown that both oxidative and reductive processes could result in effective removal of CIPRO from environmental waters because both hydroxyl and azide radicals as well as hydrated electrons can react with CIPRO with high rate constants ($2.5 \pm 0.10 \times 10^{10}$, $2.90 \pm 0.12 \times 10^{10}$ and $2.68 \pm 0.15 \times 10^{10}$ M^{-1} s^{-1}, respectively).

A comparative study for the removal of CIPRO during UVA photolysis, UVA photocatalysis and visible light photocatalysis was performed by Paul et al. [42]. For a 30 mg/L initial CIPRO concentration, degradation rates followed the trend UVA-TiO_2 >

Vis-TiO_2 > UVA. UVA-TiO_2 was also proved to be the most energy efficient method when compared to other light mediated processes. LC-MS analysis led to the identification of organic products generally retaining core quinolone structure. Microbiological antibacterial activity assay (using *E. coli*) showed that for all treatment methods antibacterial activity was removed by increased irradiation time. They concluded that FQ deactivation is possible by these oxidation methods even though sufficient mineralization is not observed.

One of the few photo-removal studies of FQs under UVC radiation was investigated in a comparative study by Van Doorslaer et al. [48]. UVA and UVC induced photolytic and photocatalytic degradation of CIPRO and a newer generation FQ, moxifloxacin (MOX) was investigated. For photolytic removals of both compounds, it was found that UVC radiation led to faster removal of both compounds compared to UVA. Overall, MOX was found to be more photostable than CIPRO under UVA conditions and had similar removal rates during UVC photolysis. Photolytic reaction mechanism was also proposed. Significantly higher removal rates were observed for both compounds during both photocatalytic treatments when compared to photolytic processes, with UVC photocatalysis showing highest degradation rate.

Visible light mediated TiO_2 photocatalysis of CIPRO and three structural analogues were studied by Paul et al. [43]. UV photocatalysis (λ > 324 nm) was compared to visible light photocatalysis (λ > 400 nm). For all compounds UV photocatalysis was considerably faster and 55 % of total organic carbon (TOC) removal was observed for UV photocatalysis compared to none in visible photocatalysis. Both methods ensured complete removal of CIPRO (33 mg/L); however investigation of the participation of hydroxyl radicals to both degradation methods revealed that degradation of CIPRO is independent of hydroxyl radicals under UV photocatalysis and that holes played a more significant role in direct oxidation of CIP.

Even though, photo-removal of FQs in pure water systems is extensively investigated, there are a few reports on removal of a FQ in a complex matrix. Degradation of CIPRO in hospital wastewater was studied under ozonation and photodegradation by Vasconcelos et al. [49]. Medium pressure lamp was used as the radiation source (365 nm). Photodegradation in complex matrix took 300 min to

completely remove CIP (0.2 mg/L) and 30 minutes by ozonation and 60 minutes for TiO_2 photocatalysis using Degussa P25.

In another complex matrix photo-removal study of FQs, efficiency of photo-fenton and solar photocatalysis systems in removing ofloxacin in wastewater effluent was compared by Michael et al. [37]. Simulated solar light system was used as the irradiation source. Even though solar photo-fenton process was found to be more efficient in removing the parent compound as well as dissolved organic carbon, *Daphnia magna* toxicity tests revealed that solar photocatalysis products are considerably less toxic than solar photo-fenton products.

2.5.3 17α-ethinylestradiol (EE2)

EE2 (Figure 2.5) is a synthetic estrogen widely used in oral contraceptives and hormone replacement therapy. EE2 was the most commonly prescribed estrogen and 22nd most prescribed pharmaceutical in Canada in 2007 [127]. Occurrences of EE2 in STP effluents and in aquatic environments have been regularly reported [158-160]. It has been estimated to contribute to 35% to 50% of total estrogenicity of surface waters [161]. Average removal of EE2 compiled over data gathered from a vast number of WWTP was reported to be 65 ± 15% [128]. EE2 is an endocrine disrupting chemical, which binds to the estrogen receptors of aquatic organisms. It was shown to lead to a variety of estrogenic responses in fish. EE2 was reported to induce the expression of vitellogenin in male fish at 0.1 ng/L, at 0.1–15 ng/L to cause sex differentiation and life-long exposure to 5 ng/L lead to significant reduction in fish fertility [162, 163].

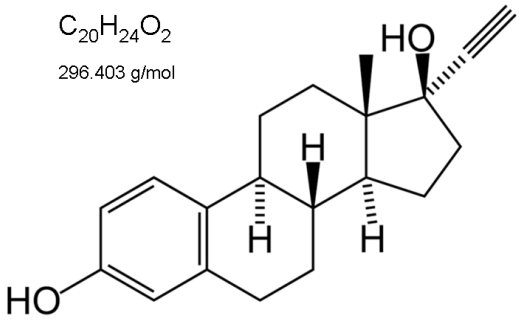

Figure 2.5- Molecular structure, weight and formula of EE2

AOPs such as ozonation [164-168], Fenton and photo-fenton oxidation[138, 169-171] on removal of natural and synthetic estrogens including E1, E2 and EE2 were previously studied while detailed findings associated to photolytic and photocatalytic removals of EE2 by various research groups are summarized below.

Some of the photo-removal reports of EE2 were investigated under UVA radiation. The photodegradation of EE2 induced by high-pressure mercury lamp ($\lambda >$ 313 nm) in aqueous solutions of EE2 was investigated in the presence of Fe^{+3} or algae (*Anabaena cylindrica*) by Liu et al.[35]. For an initial concentration of 5 mg/L of EE2, photolytic removal was limited to only 10% after 4 hours of irradiation. Photodegradation efficiency after 4 hours was shown to increase (20 %) with increasing Fe^{3+} concentration up to 10 μM. It was shown that EE2 was not biodegraded by the algae without irradiation (dark conditions). Significantly improved removal efficiency of EE2 after 4 hours (50%) was observed when the algae concentration was 9×10^{10} cell / L.

Removal of estrogenic activity of EE2, E2 and E1 under UVA photolysis and photocatalysis was demonstrated by Coleman et al. [26]. For photocatalytic experiments an immobilized TiO_2 reactor system was employed. Recombinant yeast estrogen assay was used to monitor the estrogenic activity during the treatment methods. Estrogenic activity for both all compounds was removed at the same rate under UVA photocatalysis (40 minutes). Overall, removal of estrogenic activity by UVA photolysis was slower than photocatalysis, and the time required for complete removal of estrogenic activity followed E2 (510 min) > E1 (360 min) > EE2 (120 min). Degradation intermediates were also identified and reaction pathways were proposed for photocatalytic degradation of EE2 with irradiating wavelength of 365 – 370 nm by Sun et al. [47]. They evaluated the effect of using methanol for preparing stock solutions of EE2 and showed that it acts as a hydroxyl radical scavenger, retards the photocatalytic degradation and alters the reaction pathway significantly. This phenomenon was also investigated by Karpova et al. [29, 30] and it was advised that using organic solvents to prepare estrogen stock solutions should be avoided. Strong inhibiting effect of presence of ethanol on photocatalytic removal rates of estrogens was observed.

Effect of the irradiation wavelength on photodegradation of EE2 was investigated by comparing monochromatic UVC radiation to irradiation at higher wavelengths (generally polychromatic) by various research groups. Removal efficiencies of EE2 under irradiation with UV disinfection lamp (λ= 254 nm, UVC) was compared to irradiation under high pressure mercury lamp (λ > 365 nm, UVA-Vis) by Liu et al. [34]. 80% removal of EE2 was observed under UVC radiation where no evident removal was achieved due to irradiation by high pressure lamp. Removal data was shown to fit to pseudo-first order kinetics for both processes. Increasing initial concentrations was shown to decrease photodegradation rates.

Effect of low pressure (LP) and medium pressure (MP) lamps on photolytic removal of endocrine disrupting chemicals bisphenol A, EE2 and E2 were investigated by Rosenfeldt et al. [46]. LP emitted monochromatic light at 254 nm and MP lamp emitted wide spectrum light at wavelength range of 200 – 300 nm. MP irradiation was shown to be more effective that LP irradiation but removals in both were significantly enhanced when 15 mg/L of H_2O_2 was added. A more recent study by the same research group [45] also confirmed the higher estrogenic activity removal during MP irradiation when compared to LP irradiation by using in vitro yeast estrogen screen assay. At the same UV fluence, 95% of estrogenic activity of EE2 was shown to be removed with MP irradiation compared to no removal under LP irradiation. It was also reported that transformation products present had significantly less estrogenic activities than the parent compound.

Another comparative study of photochemical transformation of E2 and EE2 dilute aqueous solution (0.15 mg/L) upon monochromatic (254 nm, UVC) and polychromatic ($\lambda \geq$ 290 nm) irradiation was reported by Mazellier et al. [36]. Quantum yield calculations revealed that polychromatic irradiation performs slightly better than UVC degradation of EE2. Detailed photoproduct analysis by coupling LC-MS and GC-MS analysis showed that for both compounds the irradiation wavelength did not influence the nature of products.

Photocatalytic oxidation of multi-component mixture containing E1, E2, E3 and EE2 under UVA (300 – 420 nm) and UVC radiations was recently reported by Li Puma et al. [32]. Removal rates of all estrogenic compounds under UVC irradiation were

found to be significantly faster than removals observed under UVA radiation. Removal rates of estrogens during UVA photocatalysis were shown to be faster than UVC photolysis but slower than UVC photocatalysis.

Based on these results of these wavelength effect studies, when operating at wavelengths above 300 nm photolytic removals are significantly reduced whereas under polychromatic irradiation at 200 – 300 nm higher photolytic removals are observed compared to monochromatic irradiation at 254 nm. This can be explained by the fact that EE2 has higher absorption in the 270 – 300 nm range.

Effect of having a complex reaction medium towards photo-removal of EE2 was investigated in a few reports. Results associated to the photocatalytic oxidation of estrogens EE2 and E2 in presence of urea, saccharose and urine as sanitary fraction of domestic sewage was reported in two research articles by Karpova et al. [29, 30]. Photocatalysis of the estrogens were studied under near UV irradiation ($\lambda > 365$ nm). According to their findings, complete removal of EE2 and E2 (0.5 mg/L) is achieved after 30 minutes photocatalytic treatment at very small TiO_2 concentrations (10 mg/L). Presence of urea was shown not to influence the removal rates of estrogens however saccharose and urine significantly retarded the removal rates.

Bioanalytical assessments of residual estrogenic activity were used to evaluate the performance of UVC/H_2O_2 process ($\lambda = 254$ nm) for mixtures of estrogenic contaminants in laboratory and natural river water by Chen et al. [172]. Four endocrine disrupting compounds (EDC) E2, EE2, bisphenol A and nonylphenol were spiked individually or as a mixture at μg/L – ng/L in laboratory or natural river water. The estrogenic activity removal rates were evaluated by in vitro yeast estrogen screen (YES) and in vivo by vitellogenin assays using *Oryzias latipes*. Additive synergistic effects of in vivo and in vitro estrogenic activity were found in EDC mixtures compared to single compound in solution. Estrogenic activity of EDC mixtures in river water was reported to be significantly higher than mixtures in DI water. Removal rates of estrogenic activity of mixtures were lower than rates observed for single compounds. Presence of natural water further slowed down estrogenic activity removal due to possible presence of hydroxyl radical scavengers.

2.5.4 Levonorgestrel (LNG)

LNG (Figure 2.6 a) is a synthetic progestogen (i.e. progrestin), used either alone or in combination with EE2 in a variety of hormonal formulations [173-176]. Occurrence of progestogenic compounds only recently received attention and currently there is very few data on the presence of progestogenic compounds in aquatic environments [177]. A recent study performed by Viglino et al. [178] confirmed the presence of LNG at a concentration of 150 ng/L and 30 ng/L in the influent and effluent of Montreal STP, respectively. LNG was found to affect men's fertility and exposure to high levels of LNG was shown to lead to azoospermia (no measurable sperm in semen) [179]. Recently, bioaccumulation of LNG in mussels was verified due to its high lipophilicity [180]. Reports on ecotoxicological effects of LNG are also found to be scarce; however another progestin norethindrone was shown by Paulos et al. [181] to produce a significant decrease in fecundity of Japanese medaka at aqueous concentrations ≥25 ng/L. A 21 day flow-through fathead minnow reproduction study also demonstrated that this compound causes a significant decrease in fecundity in the low ng/L range. Morphological changes in fathead minnow were also reported (i.e. female fin spots) suggesting that norethindrone exposure may have a potent androgenic effect on fish.

Since occurrence and fate of synthetic progestogens like LNG in the environment is only recently receiving attention, research on advanced oxidation methods for removal of LNG is very limited. Currently, there is only one article on the removal of LNG by AOPs. Second-order reaction rates of variety of pharmaceuticals, pesticides and endocrine disruptors with molecular ozone were compared in this study conducted by Broseus et al. [182]. They reported that, overall progestogens (norethindrone, levonorgestrel, progesterone, medroxyprogesterone) showed far slower reaction with ozone compared to estrogens (E1, E2 and EE2). Reports on advanced oxidation of natural progestogens are more available but still not extensive as other pharmaceuticals mentioned previously. Light induced advanced oxidation studies of structurally closely related compounds to LNG such as testosterone (androgen) and progesterone (natural progestogen) are summarized below.

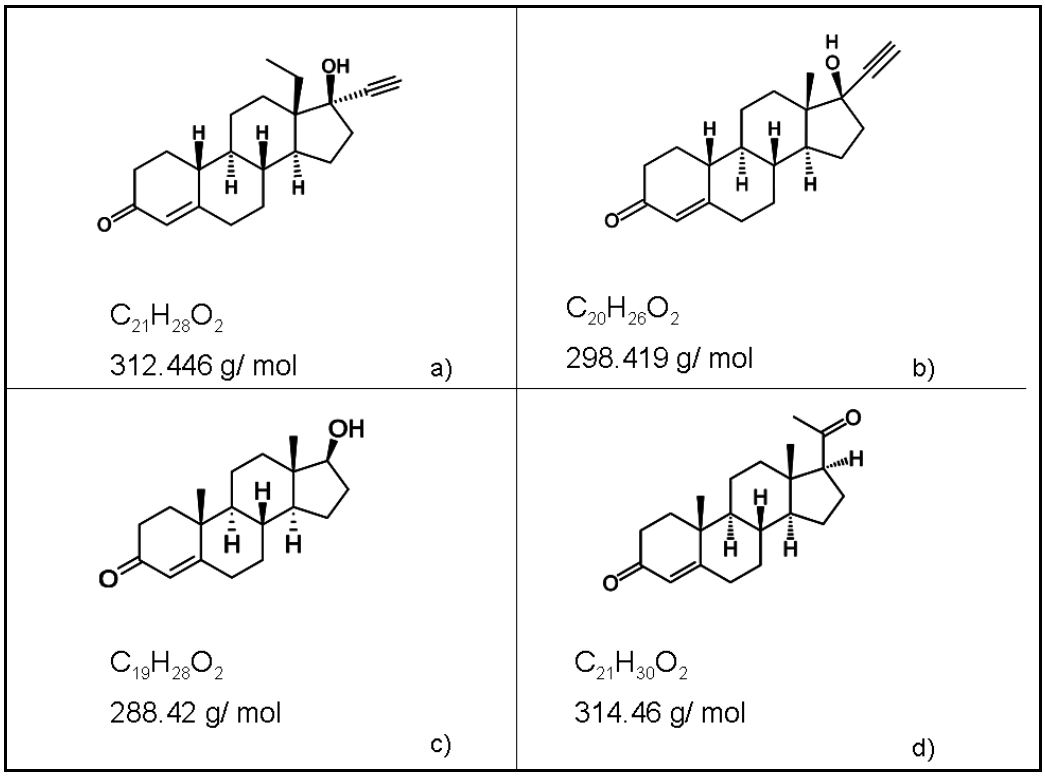

Figure 2.6- Molecular structure, weight and formula of a) LNG b) Norethindrone c) Testoterone d) Progesterone

Degradation of testosterone under irradiation wavelengths (254 and 313 nm) was investigated by Vulliet et al. [51]. Testosterone shows an intense absorption band centered around 244 nm (ε = 16,670 L mol^{-1} cm^{-1}) and a weaker absorption shoulder in the 280 – 320 nm region (ε = 110 L mol^{-1} cm^{-1}). The removal data of testosterone followed pseudo-first order reaction kinetics. Only 9 minutes of irradiation under UVC irradiation was required to remove 90% of the parent compound, while about 11 hours were required to achieve similar removal efficiency during irradiation at 313 nm. Same photoproducts were shown to be generated under both treatment methods. No differences in removal rates or transformation products were observed when the system was analyzed in natural water samples. The major product was found to be more photostable than the parent compound towards further photolytic removal.

Four reports were recently published by the same research group (Klamerth et al. and Miranda-Garcia et al.) using the same pilot compound parabolic collector solar plant to investigate removal of mixtures of contaminants of emerging concern including progesterone in synthetic fresh water, simulated wastewater effluent water and real

wastewater effluent by photo-fenton and solar TiO_2 photocatalysis [31, 38, 39, 138]. Initial study by Klamerth et al. [31] focused on the removal of mixture of nine emerging contaminants. Complete removal of progesterone over 145 minutes of irradiation under photocatalytic treatment (suspended TiO_2) was reported by Klamerth et al. and only 20 minutes were required for progesterone removal under photo-fenton reactions. Overall photo-fenton was found to be more efficient in removing majority of the compounds under solar irradiation. Presence of bicarbonates in the synthetic freshwater retarded removal rates significantly due to scavenging of hydroxyl radicals (300 min for complete removal of progesterone). Removal of the compounds in real wastewater effluent by photo-fenton was shown to be faster than in synthetic fresh water, due to presence of humic acid yielding solvated electrons and hydroxyl radicals upon irradiation [138]. They also reported that degradation products of the mixtures of compounds in the wastewater were shown to be more toxic than the initial mixture according to the tests with *Vibrio fisheri*.

Miranda-Garcia et al. evaluated [38, 39] the possibility of using immobilized TiO_2 on spherical beads in solar photocatalytic removal of a mixture of 15 compounds. Their initial bench scale experiments in a simulated solar radiation system resulted that immobilized TiO_2 system removed progesterone in less than 5 minutes. Once the effectiveness of the immobilized catalyst was proven, pilot plant scale experiments were conducted to compare performances of slurry (suspended TiO_2) to immobilized TiO_2. It was found that for all compounds immobilized system performed significantly better, reaching significantly higher removals. For example after 90 minutes of irradiation 90% of progesterone was removed and no further reduction was observed for the slurry system but on the other hand immobilized system was able to remove 90 % of progesterone within 23 minutes (complete removal in 32 minutes). The slurry system contained 5 mg/L of TiO_2 where as the 3.35 g of TiO_2 was immobilized for a total reaction volume of 10 L (0.335 g/L immobilized TiO_2). Even though it is expected for immobilized systems to experience reduction in surface area, there was more catalyst available in the immobilized system compared to slurry system. Their choice of very low TiO_2 concentration in the slurry was to avoid removal of compounds by adsorption.

Following their initial experiments in demineralised water mentioned above, removal of all compounds were satisfactorily shown in a real wastewater effluent as well [39].

A pilot scale photocatalytic membrane reactor employing UV/TiO_2 photocatalysis was evaluated for its ability to remove 32 pharmaceuticals including progesterone and testosterone as well as estrogens in river water by Benotti et al. [25]. Spectral output of the lamps employed in the system included bands at 254 and 185 nm. Photocatalytic removals of testosterone and progesterone were shown to be slower than estrogenic compounds (E1, E2 and EE2). Estrogenic activity tests performed by YES assay showed that none of the transformation products contained residual estrogenic activity. Photocatalytic removal efficiency was compared to direct photolytic removal and H_2O_2 enhanced photolytic removal. Based on the amount of energy required to reduce the concentration of a compound by one order of magnitude, H_2O_2 enhanced photolytic system was far more efficient than photocatalysis. However, they speculate that efficiency of photocatalytic systems could have been increased if higher TiO_2 concentrations were used (up to 1000 mg/L) compared to 50 mg/L of TiO_2 used in their study.

2.6 Summary of literature review

Due to the widespread use and poor removal of pharmaceuticals during wastewater treatment, they are commonly detected in wastewater effluents and therefore in natural waters. Especially the presence of antibiotics and hormones in the environment pose a great risk on the well being of aquatic wildlife, and there is increasing concern about potential impact on public health. These concerns have led to an increase in research in developing alternative treatment methods such as advanced oxidation processes (AOPs) for removal of the contaminants of emerging concern and/or removal of their biological activity. Among variety of AOPs, light mediated processes such as UV-TiO_2 photocatalysis continues to receive much interest. Possibility of using solar light and enhanced oxidation due to generation of hydroxyl radicals by presence of TiO_2, allow photocatalysis to be recognized as a potential treatment method in areas with high solar light availability. The majority of the reports on photocatalytic (or photolytic) removal of contaminants of emerging concern focuses on the advantages of

using solar radiation (or UVA) in order to establish inexpensive treatment methods. However, in areas such as Quebec, Canada, the availability of solar light is less but electricity is considerably less expensive. For such areas, investigating the applicability of UVC radiation by artificial lamps (e.g. germicidal) for this purpose seems to be a more feasible option. This type of treatment has the potential to be applicable as a pre-treatment step applied to industrial or hospital wastewater before their discharge to domestic sewage.

Photodegradation of SMX (with or without TiO_2) was studied by research groups mostly under UVA or simulated solar light irradiation. There was a lack of information on the possible synergistic effects of direct photolysis on photocatalysis during UVC radiation of this compound. The methodology for the determination of toxicity towards *Daphnia magna* was not clearly outlined for UV treated samples containing mixtures of unknown photodegradation products of SMX. For EE2, even though photolytic and photocatalytic removals at various wavelengths were investigated, its removal in mixtures containing progestins and in pharmaceutical manufacturing plant wastewaters was not reported. As for LEVO and LNG, there was significant scarcity of reports on advanced oxidation of these compounds. There was no previous report on quantification of hormone content of wastewater generated from oral contraceptive pill manufacturing plants. A table summarizing the key findings from related previous research can be found in Appendix I.

This study provides for the first time in literature findings associated with the removal of LEVO and LNG during UVC photolysis and photocatalysis. Characterization and matrix effect of pharmaceutical manufacturing plant wastewater on removal of EE2 and LNG are introduced only in this Ph.D. thesis. For SMX, synergistic effects of UVC on UVC photocatalysis are investigated in detail and *Daphnia magna* toxicity test procedure is modified. Removal of EE2 in mixtures and in complex wastewater matrix is also investigated.

Applying photocatalysis to point sources (such as hospital or manufacturing plant effluents) is an interesting area since the pollutants will be at higher concentrations and the volume to treat is smaller. The Ph.D. thesis presented here aims to fill the gaps in knowledge for some compounds (LEVO and LNG) and in applications

(photocatalysis of pharmaceutical wastewater containing high concentrations of synthetic hormone mixtures along with other constituents).

3. OBJECTIVES

The main hypotheses on which the objectives of this Ph.D. thesis were based are:

- Because the hydroxyl radicals generated by photocatalytic methods processes are very strong oxidizing agents even for recalcitrant compounds, major classes of pharmaceutical compounds are removed by photocatalysis in pure water as individual contaminants also in mixtures or in complex matrices such as industrial pharmaceutical wastewater.
- Photolysis contributes significantly to the removal of compounds having strong absorption bands at the wavelength of irradiation (UVC, 254 nm).
- Higher mineralization efficiencies can be obtained by photocatalysis compared to photolysis.
- Removal rates are slowed down as the matrix gets more complex because of a decreased light availability and increased competition for generated hydroxyl radicals

The specific objectives were:

- To determine the effect of the presence of TiO_2, mixtures of pharmaceuticals (as opposed to pure compounds) and of complex matrices such as wastewater on availability of light in a photolytic or photocatalytic treatment and on the level of removal of the target compounds
- To evaluate the effect of operating parameters such as dissolved oxygen and TiO_2 concentration on the removal of target compounds
- To determine the importance of the role of hydroxyl radicals in the removal of the target compounds
- To evaluate the residual biological activity (toxicity towards *Daphnia magna* or antibiotic activity) of the treated solution in order to estimate the toxicity of the transformation products formed during treatment.

The rest of the thesis consists of six more chapters. The objectives will be addressed in the chapters 4, 5 and 6 where the manuscripts regarding the photocatalytic removals of SMX, LEVO and EE2 - LNG, are provided respectively. Information not presented in the manuscripts regarding the schematic setup and the methodologies for Daphnia magna toxicity tests are included in Appendices II and III, respectively.

4. PHOTO-REMOVAL OF SULFAMETHOXAZOLE (SMX) BY PHOTOLYTIC AND PHOTOCATALYTIC PROCESSES IN A BATCH REACTOR UNDER UV-C RADIATION (λ_{max} = 254 nm)

4.1 Preface

One of the objectives of this thesis was to show that photocatalysis can be effectively used for the removal of antibiotics. Sulfamethoxazole (SMX) was chosen as the target molecule belonging in this class of compounds because of its frequent prescription and its common detection in wastewater plant effluents and in surface waters. This manuscript provides the detailed investigation of photolytic and photocatalytic removal of SMX under UVC radiation. Previously published research focused predominantly on the removal of this compound under UVA radiation.

SMX significantly absorbed in the UVC range so it was expected that photolysis would play a major role in the removal of this compound. The hypothesis mentioned in Chapter 3, that photolysis would contribute significantly to the removal of compounds which have maximum absorption in the wavelength of operation (UVC) is verified here by carrying out degradation experiments in the absence of TiO_2. Due to the high sensitivity of SMX to UVC radiation, increasing TiO_2 concentration only led to slower removal of this compound. Significantly higher chemical oxygen demand (COD) removals measured during photocatalysis compared to photolysis, validated higher mineralization efficiency of photocatalysis due to the generation of hydroxyl radicals and other oxidizing species. By monitoring the evolution of photo-products generated during photolysis and photocatalysis, it was shown that that the majority of the products were removed during photocatalysis whereas they remained in solution during photolysis. This result helped the evaluation of the beneficial effects of photocatalysis even though slower removal of SMX was observed for this type of treatment. The photocatalytic rate of removal of SMX was not influenced by varying dissolved oxygen concentration and by scavenging of hydroxyl radicals during photocatalysis. HPLC-MS analysis of treated samples revealed for both treatment methods major degradation

products were identical. These results helped us conclude that for SMX, photolysis was the dominant mode of removal and photocatalysis was efficient in removing the photolysis products. The synergistic effects of photolysis and photocatalysis under UVC radiation were thus verified. In order to fully assess the applicability of photo-removal treatment strategies for the removal of SMX, the toxicity of treated samples was determined by *Daphnia magna* toxicity kits revealing that for both treatment methods, the generated products were more toxic than the parent compound. Due to the lack of clarity in previous research about the methodology for the determination of *Daphnia magna* toxicity, a revised method was developed. These tests underlined the necessity of carrying out toxicity determinations to assess fully the performance of treatment technologies.

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Photo-removal of sulfamethoxazole (SMX) by photolytic and photocatalytic processes in a batch reactor under UV-C radiation (λ_{max} = 254 nm)

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4.2 Abstract

In this study, photolytic and photocatalytic removal of the antibiotic sulfamethoxazole (SMX) under UVC radiation (λ = 254 nm) was investigated. The light intensity distribution inside the batch photoreactor was characterized by azoxybenzene actinometry. The intensity of incident radiation was found to be a strong function of position inside the reactor. 12 mg/L of SMX was completely removed within 10 minutes of irradiation under UVC photolysis, compared to 30 minutes under TiO_2 photocatalysis. COD measurement was used as an indication of the mineralization efficiency of both processes and higher COD removal with photocatalysis was shown. After 6 hours of reaction with photolysis and photocatalysis, 24 % and 87 % removal of COD was observed, respectively. Two of the intermediate photo-products were identified as sulfanilic acid and 3-amino-5-methylisoxazole by direct comparison of the HPLC chromatograms of standards to those of treated solutions. Ecotoxicity of treated and untreated solutions of SMX towards *Daphnia magna* was also investigated. It was found that a 3:1 ratio of sample to standard freshwater and a high initial concentration of 60 mg/L of SMX were necessary to obtain reliable and reproducible results. The photo-products formed during photocatalytic and photolytic processes were shown to be generally more toxic than the parent compound.

Key words: Sulfamethoxazole, photolysis, photocatalysis, titanium dioxide, *Daphnia magna*

4.3 Introduction

Contamination of natural waters by pharmaceuticals and personal care products (PPCPs) is a rising issue of global concern. After their use, usually these substances are excreted only partially metabolized and end up in the sewage system. A great portion of these compounds are not removed by classical sewage and wastewater treatment plants and are eventually discharged into receiving waters [183, 184]. It has been found that there are significant amounts of pharmaceuticals present in the aquatic environments at up to the μg/L level [8, 11, 56, 185-187]. Even though these compounds are detected at very low concentrations in nature, it is highly likely for aquatic organisms to experience toxicity via concentration addition effect due to decades of unregulated discharge [66, 67].

Sulfamethoxazole (SMX, Figure 4.1) is a synthetic antibiotic commonly used in humans for treatment of bronchitis and urinary tract infections and as well as veterinary medicine. It is generally prescribed in combination with trimethoprim. In the year 2007, it was the 6th most commonly prescribed antibiotic combination in Canada [127]. Even though no direct effects on human health have been associated with this compound, concerns for increase in bacterial resistance to the antibiotic agents were also reported [71]. Due to the antibacterial nature of this compound, it shows resistance to conventional biological water treatment methods and is often found in sewage treatment plant effluents and wastewater plant effluents [58, 188], as well as in natural wasters [11].

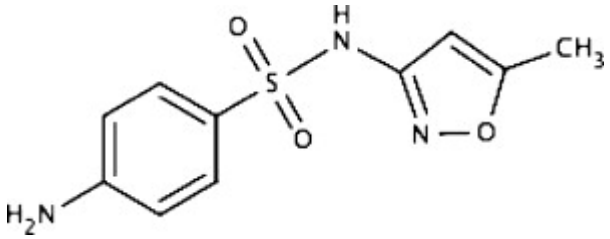

Figure 4.1- Chemical Structure of sulfamethoxazole (SMX)

Advanced oxidation processes (AOPs) have received great interest in recent years as complementary methods to conventional water treatment or as alternative treatment strategies for industrial wastewater prior to discharge into sewage or into aquatic environments. Among these AOPs; ozonation [95, 133, 134, 189, 190], fenton and photo-fenton oxidation [96, 135, 191]; photolysis and H_2O_2 enhanced photolysis [192-194]; heterogenous photocatalysis [41, 44] were frequently studied. During advanced oxidation of pharmaceutical compounds, intermediate compounds are formed that might show more toxic effects than the parent compound; therefore overall goal of treatment processes should be complete mineralization rather than just removal of the parent compounds or at least the transformation into non-toxic products.

Photolysis relies on the absorption of artificial or natural sunlight by a target molecule to undergo direct degradation to intermediates which can potentially further decompose to lead to mineralization. Ultraviolet (UV) radiation (especially UVC, $\lambda <$ 280 nm) is usually used for disinfection of drinking water and is increasingly used for sterilization of wastewater. Recent research employing only UV radiation (generally UVA, $320 < \lambda < 400$ nm and UVB, $280 < \lambda < 320$ nm) focuses generally on the understanding of photochemistry involved in the persistence and fate of pharmaceuticals in the natural aquatic environments [193].

Heterogenous photocatalysis involves the absorbance of UV-light ($\lambda < 390$ nm) by a semi-conducting material to produce electron / hole pairs which are responsible for the reduction / oxidation reactions that occur on the surface of the catalyst [195-197]. Titanium dioxide (TiO_2) is the most frequently used semiconductor in photocatalysis since it is biologically and chemically inert, cheap and non-toxic [122]. The oxidizing species generated during photocatalysis and responsible for degradation of compounds of interest are hydroxyl radicals (·OH), holes (h_{vb}^+) and superoxide radicals ($O_2^{\cdot -}$). Generally, a source of oxygen is required to scavenge for electrons, in order to reduce recombination of electron and holes.

There have been numerous reports on the heterogenous photocatalysis of SMX using UV lamps emitting radiation either in the UVA and UVB range [16, 17, 20-24, 28][. Very little work has been done on the efficiency or synergistic effects of photolysis to photocatalytic processes for SMX during UVC radiation. Higher

effectiveness of radiation at 254 nm compared to that at 350 nm towards the removal of salicylic acid and phenol was demonstrated by Matthews and McEvoy [198]. Similar results for phenol [199] and for 2-cholorophenol [200] were also reported.

The majority of the related work focuses on the advantages of coupling solar radiation with photocatalysis in order to establish inexpensive treatment methods. However, in areas such as the case of Quebec, Canada, there is less availability of solar power but electricity is cheaper when compared to other parts of the world, thus making it necessary to study the applicability of artificial radiation sources such as the case of UV-C radiation by germicidal lamps. This type of treatment could be applicable as a pre-treatment step to industrial or hospital wastewater before their release to municipal treatment facilities. Since UVC radiation is commonly used as a sterilization method in various treatment facilities, this research also allows to study the fate of pharmaceuticals during the sterilization and possible enhanced benefits if coupled with TiO_2 photocatalysis. The focus of this research is to show the parallel effects of photolysis and photocatalysis on photo-removal efficiency of SMX during UV-C radiation. The effect of initial concentration of SMX during photolytic removal is investigated. The effect of the presence of oxidizing species and dissolved oxygen is also studied to understand the major mode of removal of SMX. Residual toxicity and the extent of COD removal are measured to compare the two treatment methods. Finally several of the photoproducts are identified.

4.4 Materials and methods

4.4.1 Reagents

Sulfamethoxazole ($C_{10}H_{11}N_3O_3S$, > 99%), benzoquinone (> 99%), azoxybenzene (>98%) and sodium dihydrogen phosphate were obtained from Sigma–Aldrich, Canada. Aqueous stock solution (60 mg/L SMX) was prepared in reverse osmosis (RO) water and kept at 4 °C in the dark until the time of treatment. Commercial TiO_2 Degussa P25 (70% anatase and 30% rutile) was used as catalyst with an average particle size of 30 nm and BET surface area of 50 $m^2 g^{-1}$, according to the manufacturer (Evonik Degussa Canada Inc.). HPLC grade methanol, acetonitrile and isopropanol were purchased from Fisher Scientific, Canada. 95% ethanol was purchased from Commercial Alcohols

(Boucherville, Quebec, Canada). All the chemicals were used as received without purification.

4.4.2 UV-C irradiation experimental setup

Irradiation experiments were carried out in 2 L capacity cylindrical acrylic photoreactor (215 mm height, 108 mm diameter). The reactor walls were covered by aluminum foil to avoid exposure to UV radiation. 1.6 L of an aqueous solution of SMX was charged in the reactor for each experiment. The solution was irradiated by an Hg-Ar (Germicidal UV-C) lamp (Atlantic Ultraviolet Corp. GPH212T5L) located in the center of the reactor and protected in a quartz sleeve (maximum output at 254 nm) and mixing was achieved by magnetic stirring. Before each experiment required amount of titanium dioxide particles (0.01, 0.03, 0.05 and 0.5 g/L) were suspended in RO water and sonicated for 30 minutes to reduce agglomeration and create a more stable suspension. In photolytic degradation experiments no titanium dioxide was added to the reaction mixture.

Various concentrations of SMX ranging from 3 to 12 mg/L were tested for adsorption onto TiO_2 at a fixed concentration of 0.5 g/L. For adsorption experiments, Erlenmeyer flasks were filled with TiO_2 and SMX at the desired concentrations and were placed inside an incubator shaker set at 20 °C. After 24 hours, withdrawn samples were analyzed with HPLC and compared to the controls containing only SMX. The adsorption flasks and control flasks showed no difference in concentration concluding that the SMX is not adsorbed onto the TiO_2 under dark conditions.

In order to determine if the major removal mode of SMX is due to photolysis or to the presence of oxidizing species generated during photocatalysis the effect of dissolved oxygen concentration and scavenging of oxidizing species experiments were performed. Different concentrations of dissolved oxygen were obtained by bubbling air (8.8 mg/L) or pure oxygen (42 mg/L) to the reaction mixture. For close to anoxic conditions, the dissolved oxygen in the reaction mixture was purged off by continuously bubbling nitrogen for 2 hours before turning on the lamp and the flow of nitrogen was maintained through out the reaction time to maintain low dissolved oxygen levels (0.5

mg/L). In all cases, gases were introduced into the system by continuous bubbling through a sparger located at the bottom of the reactor.

The method of scavenging and concentrations of scavenging compounds were based on the results reported by Palominos et al. [41]. Isopropanol has been described as one of the best hydroxyl radical quencher due to its high reaction rate constant with the radical (1.9×10^9 mol $L^{-1}s^{-1}$) [201]. In this work, scavenging of hydroxyl radicals was achieved by adding isopropanol to the reaction mixture (12 mg/L SMX and 0.05 g/L TiO_2) at a molar concentration which was three orders of magnitude larger than the initial concentration of SMX. Benzoquinone is also commonly used to trap superoxide radicals by a simple electron transfer mechanism [41]. In the present work, it was introduced into the reaction mixture at a molar concentration ten times that of SMX.

4.4.3 Determination of incident light intensity distribution in the reactor

In order to quantify the intensity of incident radiation, azoxybenzene was used as a chemical actinometer. The method was modified from the technique developed by Bunce et al. [202]. A quartz cuvette with dimensions (1 cm x 1cm x 3.8 cm) was filled with 3 ml of 5 mM azoxybenzene in 95% ethanol. The cuvette was sealed by a stopper attached at the end of a 30 cm long rod and was then immersed inside the reactor at a desired height from the bottom of the reactor (5 to 15 cm) and at a desired distance (0.7 to 2.5 cm) away from the lamp. The solution was irradiated for a desired time of 0 to 8 minutes. At this point azoxybenzene is photorearranged to 2-hydroxyazobenzene. Three drops of 0.1 M KOH solution in 95% ethanol was added to convert the photoproduct to its anion form. The absorbance of the sample was taken at a wavelength of 458 nm. The molar extinction coefficient, ε for the product was 7800 L mol^{-1} cm^{-1}. Using Beer-Lambert's law

$$A = \varepsilon P l \qquad (1)$$

where, A is the absorbance measured, P is the concentration of the product (M) and l is the optical path length (equivalent to length of the cuvette, 1 cm) the concentration of the product was determined. The following equation provided by Bunce et al. [202] describes the relationship between the intensity of incident radiation per unit volume, I_o (

einsteins L^{-1} min^{-1}) to the concentration of product P, where A_o is the initial concentration of azoxybenzene (5 mM), t is time and φ_r is the quantum yield of azoxybenzene determined experimentally at 254 nm ($\varphi_r = 0.017$).

$$A_o \ln\left[1-\left(\frac{P}{A_o}\right)\right] = -\varphi_r I_o t \qquad (2)$$

The slope of the line – ln(1 – (P/A_o)) against t is used to calculate the intensity of incident radiation per unit volume. This value is converted to intensity of incident radiation (Einsteins/min) by multiplying with the volume of the solution irradiated in the cuvette (3 ml).

4.4.4 Ecotoxicity test with *Daphnia magna*

For toxicity testing of SMX solutions in RO water before and after UV treatment, *Daphnia magna* immobilization essays were carried out. Acute toxicity *Daphnia* tests were conducted using the commercial test kit DAPHTOXKIT F™ (MicroBioTests Inc, Gent, Belgium) following the procedures described in the kit. The control to test the viability of the supplied Daphnia population consisted of only standard freshwater (SFW). The organisms were considered viable as long as less than 10% of daphnia immobilized in the control. The test plate then was covered and incubated at 20 °C under dark. Potassium dichromate ($K_2Cr_2O_7$) was the reference chemical used. An EC_{50} 24 h of 1.23 mg L^{-1} was obtained for the reference compound which is within the range of the 0.6–2.1 mg L^{-1} stipulated in the ISO 6341 to ensure test validity (International Organization for Standardisation, 1996). For the toxicity determination of the samples taken during photolysis and photocatalysis, a dilution ratio of 3 to 1 (treated sample to SFW) was used. After 24 and 48 hours of exposure, dead or immobilized daphnids were counted and results were tabulated as % effect (percentage of immobilized organisms). Because the SMX solutions that were treated in the photolysis and photocatalytic experiments were made using RO water, the same ratio of 3 to 1 (RO water to SFW) was used for comparison and no inhibition was observed for the daphnids at this ratio.

4.4.5 Analytical Methods

Prior to any chemical analysis including toxicity tests, samples taken from the reactor were filtered by 0.22 μm syringe filters. SMX concentration was determined by an Agilent 1100 series high performance liquid chromatograph (HPLC) equipped with a Zorbax Eclipse C-8 column (3.5 μm, 4.6 x 150 mm). Eluents consisted of 20 mM sodium dihydrogen phosphate (NaH_2PO_4) buffer and acetonitrile using an eluent gradient from 30% acetonitrile to 50% over 10 minutes at a constant flow rate of 0.8 ml min^{-1}. The buffer was adjusted to a pH of 2.8 with phosphoric acid. Detection was made with a diode array detector (DAD) at wavelengths of 262 nm for SMX and 225 nm for the intermediates. COD testing of the samples was performed using a COD reactor (HACH DRB 200) and a spectrophotometer (HACH DR 2500). The method adapted by HACH from the ASTM D 1252-95. Dissolved oxygen and pH was measured by Thermo Scientific Orion 3-Star Benchtop pH and DO Meter. Absorbance of chemical actinometry solutions were determined by a UV- Vis spectrophotometer (Thermo Electron Corporation Evolution 3000).

Fractions collected on the HPLC Agilent 1200 were analyzed by mass spectroscopy by comparison with standards corresponding to products previously identified by Zhou and Moore [140]. Analyses were performed with an MDS/Sciex QTrap 500 mass spectrometer (Concord, ON, Canada) equipped with a TurboIon spray (i.e. ESI) ionization source operated in positive and negative ion mode. Acquisition was performed in multiple reaction monitoring (MRM) mode. Nitrogen was used as the curtain, nebulizer, auxiliary, and collision gases. The MRM ion transitions for both standards and selected optimized operating conditions for MS/MS are listed in Table 4.1. All data were acquired and processes using Analyst 1.4 software.

Table 4.1- MRM ion transitions, de-clustering potential (DP) and collision energies for analysis

Standards	Chemical Formula	Polarity	MRM Transition	DP (V)	Collision Energy (eV)
Sulfanilic acid	$C_6H_7NO_3S$	negative	171.5 → 79.8	-103	-25
3-amino-5-methylisoxazole	$C_4H_6N_2O$	positive	98.9 → 43.9	110	28

4.5 Results and discussion

4.5.1 Characterization of reactor conditions

Figure 4.2 a shows the distribution of light inside the empty reactor as a function of distance from the bottom of the reactor at a horizontal distance of 0.7 cm away from the lamp (shortest available distance). The effect of radial distance from the lamp on light intensity was studied in water which is the reaction medium. From the results presented in Figure 4.2 b there is about 70% reduction in light intensity if the distance from the lamp is increased from 0.7 cm to 2.5 cm at a vertical distance of 10 cm from the bottom of the reactor.

In order to assess the participation of photolysis during heterogeneous photocatalysis of SMX, the amount of light absorbed by the photocatalyst, TiO_2 was measured. Figure 4.3 shows the effect of having the photocatalyst in suspension on light intensity distribution inside the reactor. The maximum available light intensity in water occurs when no TiO_2 is present in the reaction mixture. As it is clearly observed from these results, almost all the available light is absorbed by the photocatalyst at a concentration of 0.5 g/L even at the location closest to the lamp. This result indicates that due to the presence of the titanium dioxide no significant amount of light would be available for SMX for it to undergo direct photolysis and also that the majority of the reactor operates in UV-dark.

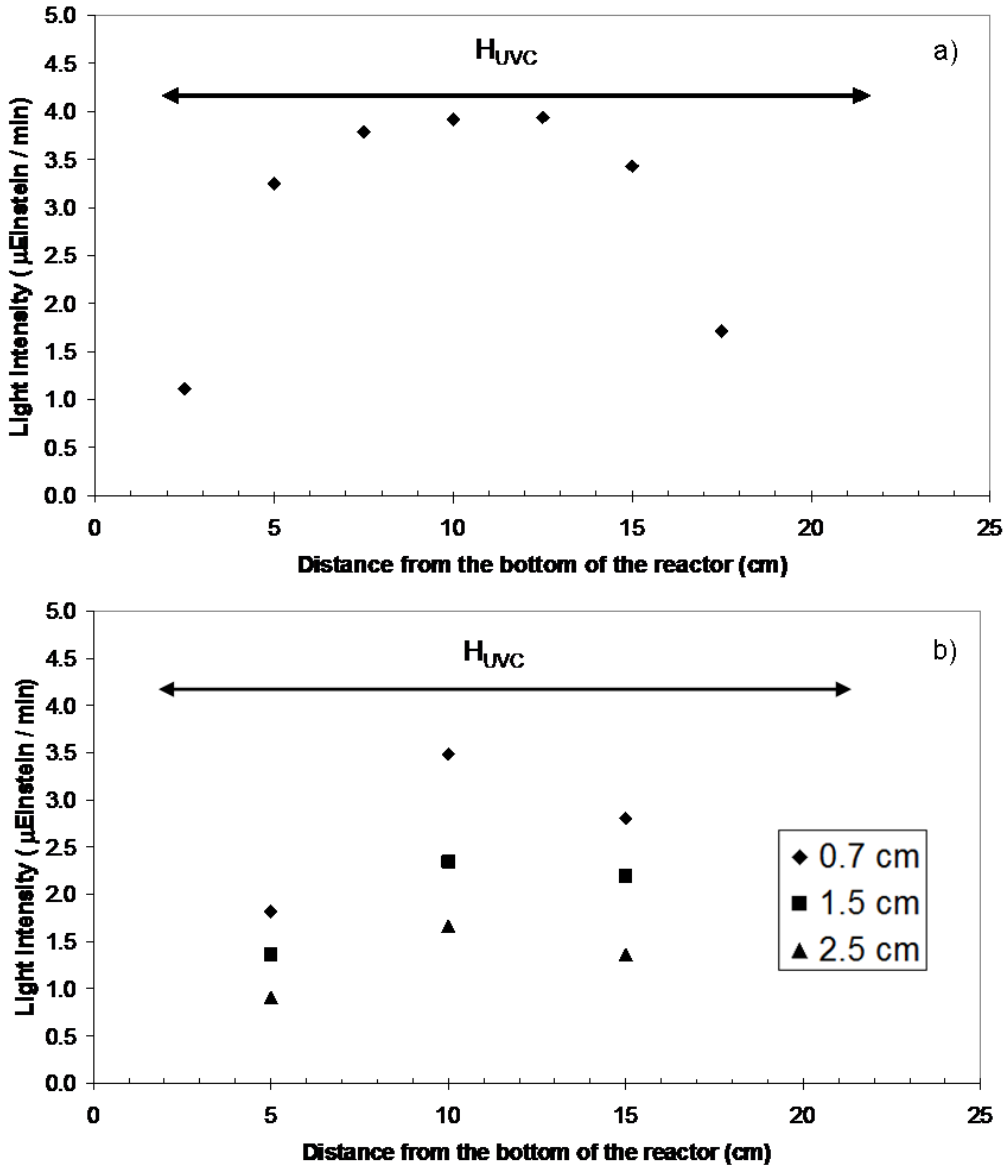

Figure 4.2- Light intensity measured: a) in air vertically as a function of distance from the bottom of the reactor at a radial distance of 0.7 cm away from the lamp, b) in RO water as a function of the vertical distance from the bottom of the reactor and of radial distance from the lamp. Height of the reactor = 21.5 cm, Position of the UVC lamp = 1.9 cm to 21.5 cm

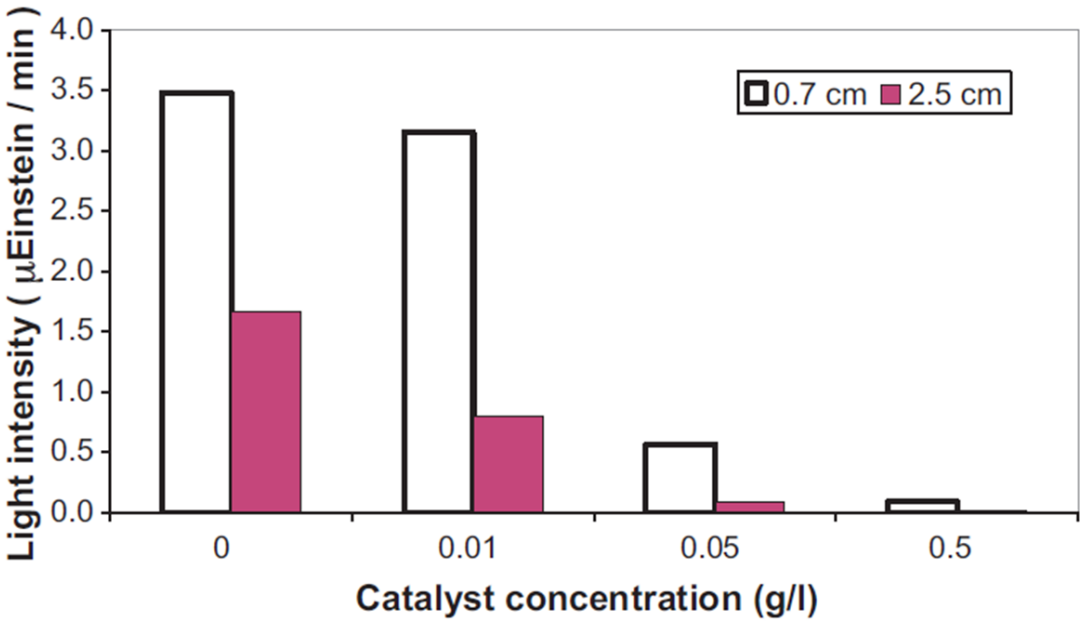

Figure 4.3- Light intensity measured as a function of catalyst concentration at 10 cm from the bottom of the reactor and at two radial distances

4.5.2 Evolution of SMX concentration during photolysis and photocatalysis

Figure 4.4 shows that the SMX removal rate diminishes as TiO_2 concentration increases under UVC radiation (λ = 254nm). By far the fastest removal is observed in the absence of the catalyst suggesting that the main mode of removal is photolysis. In order to distinguish the effect of photolysis and photocatalysis on the removal of SMX, the emission spectrum of the UVC lamp and the UV absorbance of SMX were compared in Figure 4.5. SMX absorbs up to 315 nm with a maximum absorbance at 262 nm, which is the region overlapping with the maximum radiation supplied by the lamp at a wavelength of 254 nm. Although the addition of TiO_2 seems to act as an inner filter, the beneficial effects of the presence of TiO_2 are discussed later.

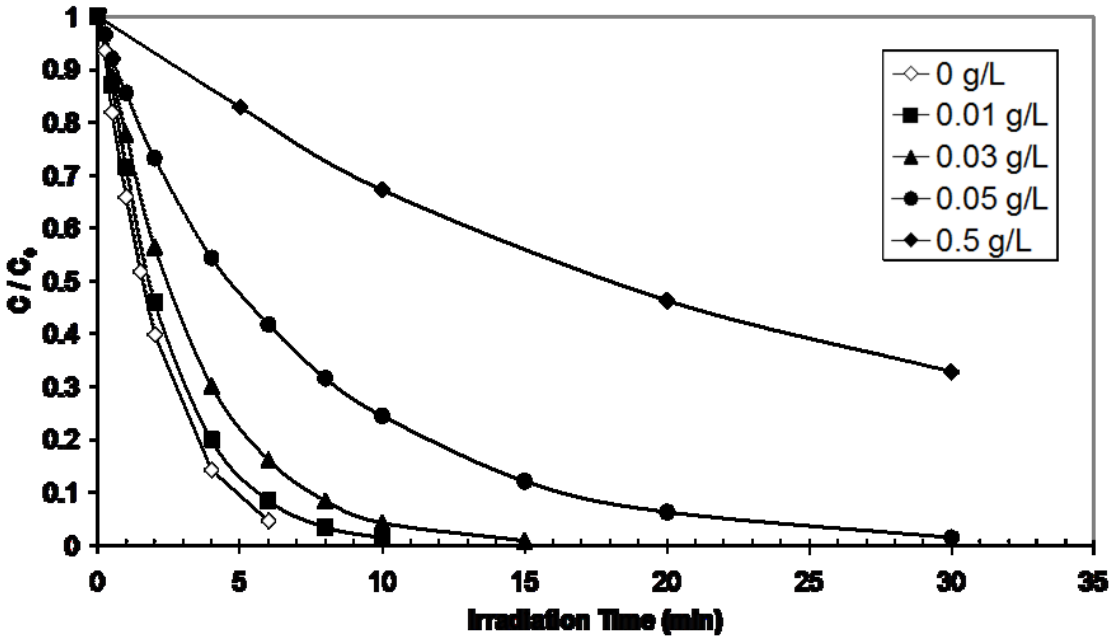

Figure 4.4- Effect of TiO_2 concentration on removal of SMX (C_o = 12 mg/L)

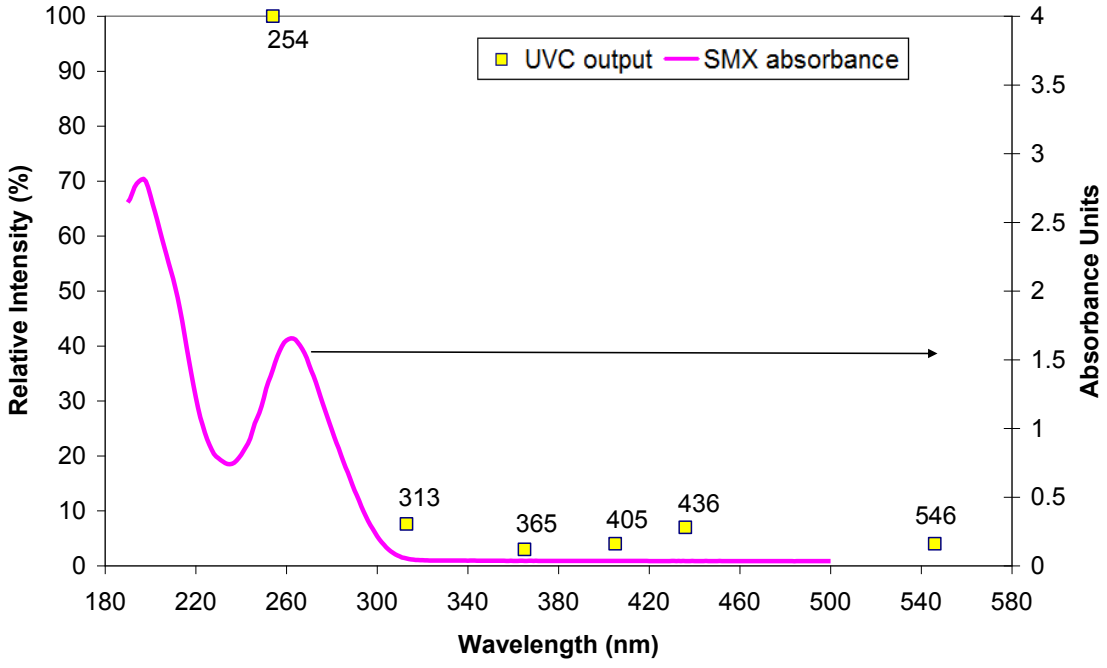

Figure 4.5- Comparison of the emission spectrum of UV-C lamp used with UV absorbance of SMX

4.5.3 Effect of initial concentration on SMX removal during photolysis

The effect of the initial concentration of SMX on the removal rate in photolysis is shown in Figure 4.6a. The exponential decay observed in Figure 4.4 and Figure 4.6a suggests the decomposition of SMX follows first order kinetics; however, the initial reaction rate data (Figure 4.6b) obtained from the derivatives estimated at the start do not support this observation as the reaction rates eventually reach a plateau at higher concentrations. This is further confirmed by the kinetic rate constants, k (min^{-1}) which were calculated by assuming first order reaction kinetics with respect to the SMX concentration and tabulated in Table 4.2. The decrease in the values of k with increasing SMX concentration is also an indication that the reaction of SMX with light is not first order. This plateau can be explained by the fact that at the higher concentrations of SMX, the available light becomes limiting. As shown by the azoxybenzene actinometry experiments, light is absorbed by the target compound as well as by the produced intermediates.

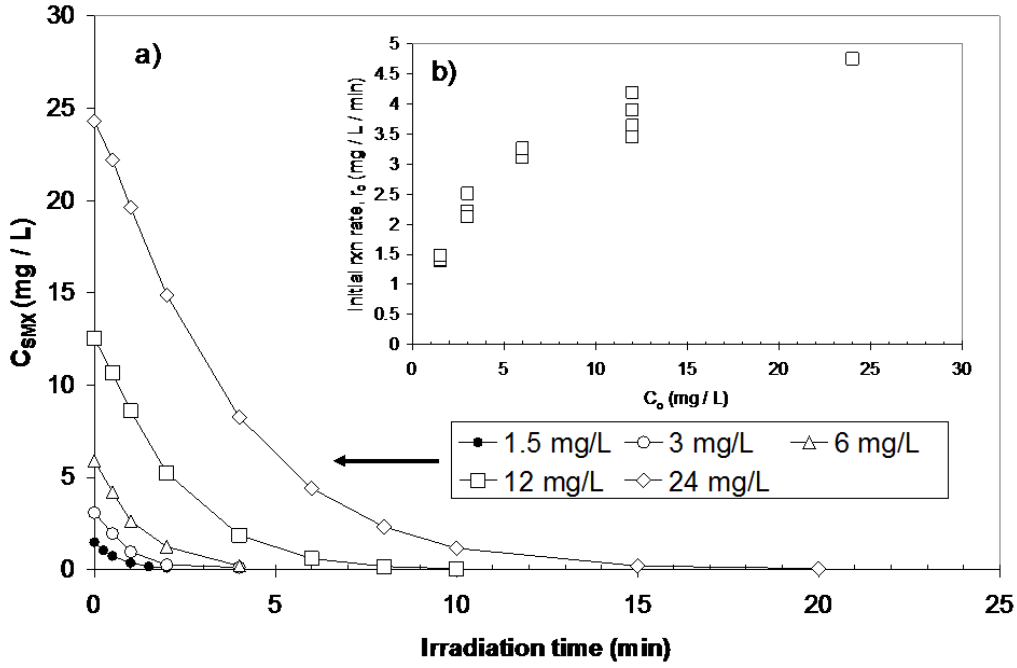

Figure 4.6- Effect of initial concentration of SMX on removal kinetics during photolysis: a) Evolution of SMX concentration at different initial SMX concentrations, b) Calculated initial reaction rates

The initial minimum apparent quantum efficiency for SMX, Φ_{app}^{min} (mol Einstein^{-1}) can be determined by normalizing the initial reaction rate, r_o (moles L^{-1} min^{-1}) with the maximum incident photon flux per unit volume I_o (Einsteins min^{-1} L^{-1}). Maximum incident photon flux per unit volume was measured at the mid section of the reactor at the closest available distance from the lamp and the value was approximately 1.3×10^{-3} Einsteins min^{-1} L^{-1}.

As shown in Table 4.2, Φ_{app}^{min} ranges from 0.41 to 1.41 mol Einstein^{-1} for some of the studied conditions. Quantum yields for photolysis has been reported as high as 7.9 mol Einstein^{-1} [22]. The values obtained in the present work fall in the same order of magnitude. It should be noted values presented here are a conservative estimate for the quantum efficiency. The intensity value in our calculations is the maximum value measured at a very close position to the lamp. The volume averaged light intensity the solution experiences would be much lower, thus increasing the calculated quantum yield values significantly.

Table 4.2- Calculated minimum apparent quantum efficiencies of photolytic removal of SMX

Type of Treatment	Catalyst Concentration (g L^{-1})	Initial Concentration of SMX (mg L^{-1})	Initial rxn rate, r_o (mg L^{-1} min^{-1})	Initial rxn rate, r_o (μM min^{-1})	1st order rxn rate constant, k (min^{-1})	Apparent quantum efficiency Φ_{app} (mol Einstein^{-1})
UVC	0	1.5	1.43	5.6	1.41	0.42
UVC	0	3	2.28	9.0	1.23	0.67
UVC	0	6	3.17	12.5	0.87	0.93
UVC	0	12	3.80	15.2	0.52	1.14
UVC	0	24	4.76	18.8	0.29	1.41

4.5.4 Effect of dissolved oxygen and oxidizing species on photocatalytic removal of SMX

The effect of the presence of oxidizing species such as hydroxyl radicals, superoxide radicals and holes on removal of SMX was studied by carrying out experiments to scavenge these species. Removal profiles when hydroxyl radicals and superoxides were scavenged are shown in Figure 4.7a. When the superoxide radicals were scavenged after 10 minutes of reaction time 32.2 (± 1.7) % of the initial SMX concentration was found to remain in solution. Scavenging of hydroxyl radicals lead to 26.2 (± 1.5) % of SMX remaining in solution after 10 minutes. When both oxidizing species were present (i.e. control) 24.2 (± 1.8) % of initial SMX was still present. Overlapping of the standard errors between hydroxyl radical scavenging and the control experiment suggests that there is no inhibitory effect on the removal of SMX due to scavenging of hydroxyl radicals, only slight inhibitory effects were observed when superoxides were scavenged.

Results of the effect of dissolved oxygen on the removal of SMX during photocatalysis are presented in Figure 4.7b. Experiments were carried out in the presence of air, pure oxygen and nitrogen corresponding to dissolved oxygen concentrations of 8.8, 42 and 0.5 mg/L. After 10 minutes of reaction 24.2 (± 1.8), 26.3 (± 1.2) and 32.3 (± 2.0) % of initial SMX remained in solution when air, nitrogen and oxygen were introduced, respectively. Overlapping of the standard errors between air and nitrogen purging reveals that the presence of dissolved oxygen does not contribute to the removal of SMX significantly. Contrary to what would be expected the introduction of oxygen did not improve the removal of SMX rather lead to a lower final removal than cases with lower dissolved oxygen concentrations.

Lack of considerable deviation from the general removal profile of SMX during photocatalysis when the above mentioned scavenging and dissolved oxygen experiments are performed, strongly suggests that the main mechanism of removal of SMX is by direct UVC irradiation (i.e. photolysis). Lower removal after 10 minutes of reaction observed when oxygen is used might be due to higher concentration of products (initial

rate of removal during oxygen is higher than nitrogen purging) accumulating as reaction proceeded, thus decreasing light availability for direct reaction with SMX.

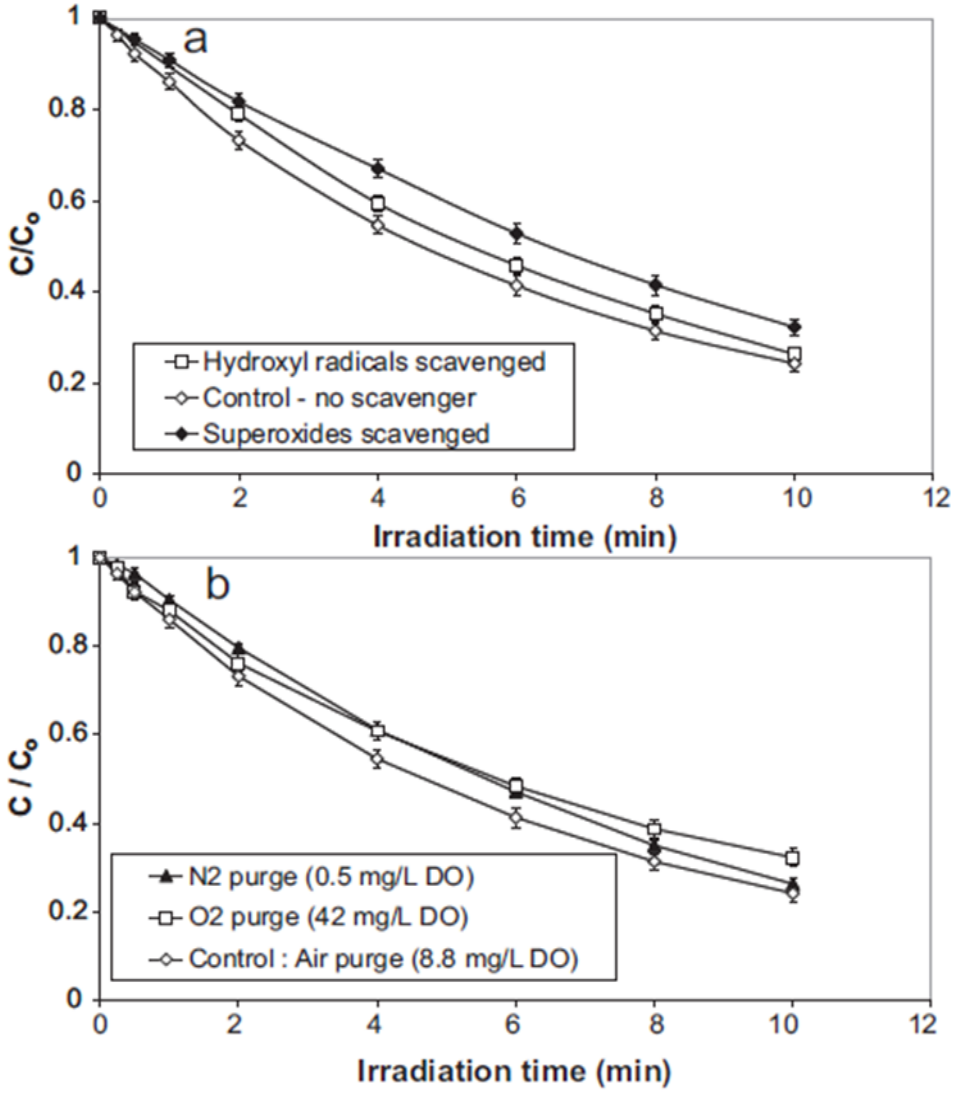

Figure 4.7- a) Effect of hydroxyl radicals and superoxides on removal of SMX during photocatalysis. b) Effect of dissolved oxygen concentration on removal of SMX during photocatalysis. Control consists of 12 mg/L of SMX and 0.05 g/L of TiO_2 under air purge.

4.5.4 Evolution of photoproducts formed and degree of COD removal

The persistence of the degradation products was determined by running irradiation experiments for longer times (up to 6 hours) under photolytic and

photocatalytic conditions. The initial SMX concentration for both cases was 12 mg/L and the TiO_2 concentration was 0.05 g/L for photocatalysis. Photoproducts with similar retention times were observed for both types of photodegradation methods studied as seen in chromatograms in Figure 4.8 suggesting that the mechanism of degradation of SMX is not altered by the presence of TiO_2. The intermediates formed during the initial stages of photolysis are resistant to further decomposition (Figure 4.8a). In contrast when TiO_2 is present, SMX is removed at a much slower rate; however the intermediates formed are removed from the system. Chemical oxygen demand (COD) can be used as an indication of the extent of mineralization. The hypothesis that photoproducts are more resistant to UV but are easily removed during photocatalysis can also be supported by analyzing the evolution of COD removal during the two treatment methods, as presented in Figure 4.9. Approximately 87 % of the initial COD is removed during photocatalysis; however only about 24 % reduction was observed for photolysis even after 360 minutes of irradiation.

Two of the five major photoproducts observed (Figure 4.8) were identified by comparing them with standards corresponding to products previously identified by Zhou and Moore [140]: sulfanilic acid and 3-amino-5-methylisoxazole. As a first confirmation, HPLC chromatograms of UVC treated SMX were compared to HPLC chromatograms of the two standards. Matching retention times were observed, as shown in Figure 4.10. Mass spectrums, presented in Figure 4.11 a and b with matching MRM transitions selected for the standards (Table 4.1), confirmed the nature of these two products.

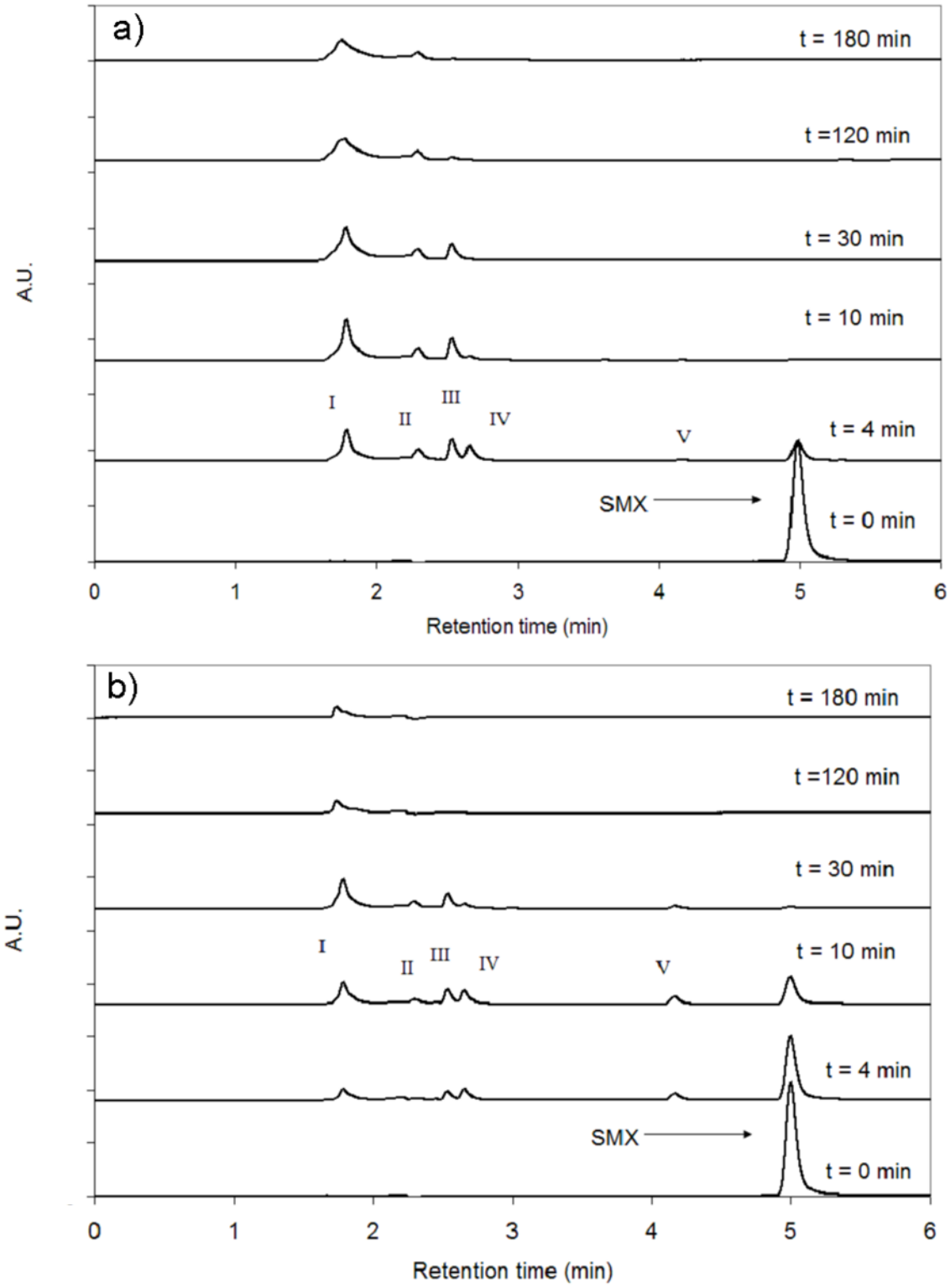

Figure 4.8- HPLC chromatograms showing the evolution of intermediates with increasing irradiation time during removal of 12 mg/L SMX by a) UVC b) UVC + TiO2.

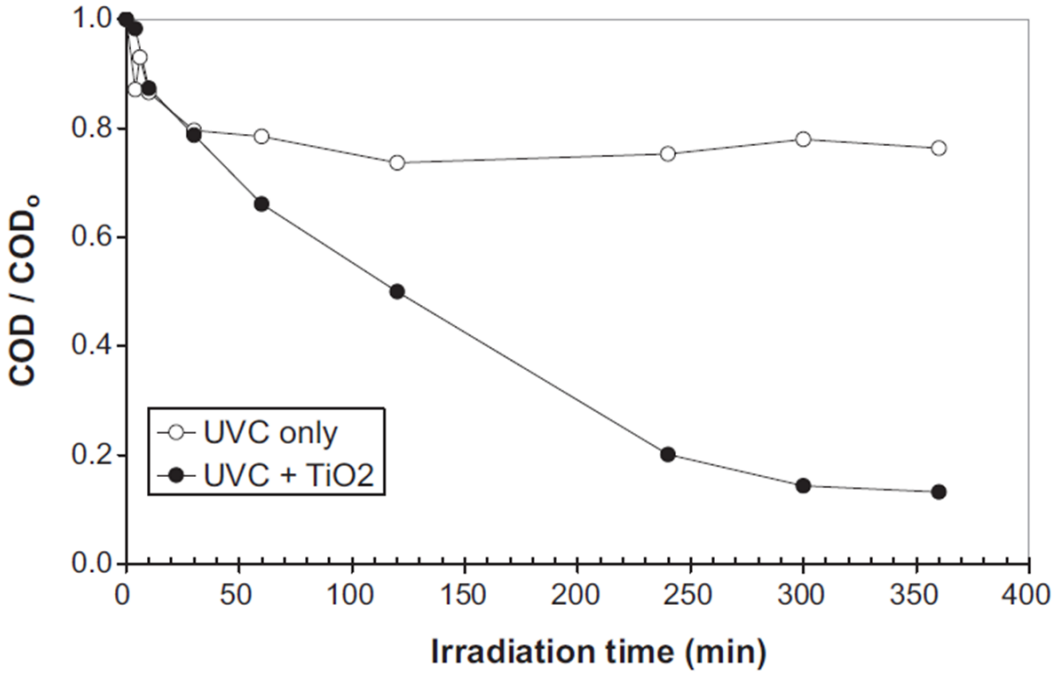

Figure 4.9- COD removal during UVC and UVC + TiO_2 treatments of 12 mg/L SMX

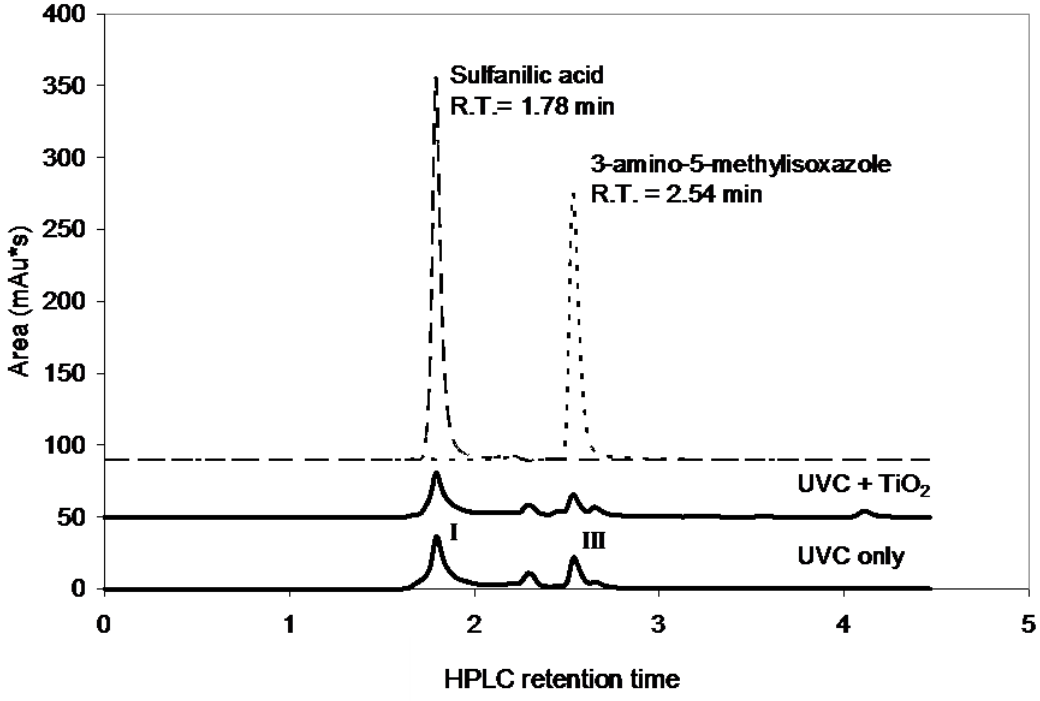

Figure 4.10- HPLC chromatograms of UVC , UVC + TiO_2 treated SMX , untreated solutions of 40 mg/L sulfanilic acid and 25 mg/L 3-amino-5-methylisoxazole.

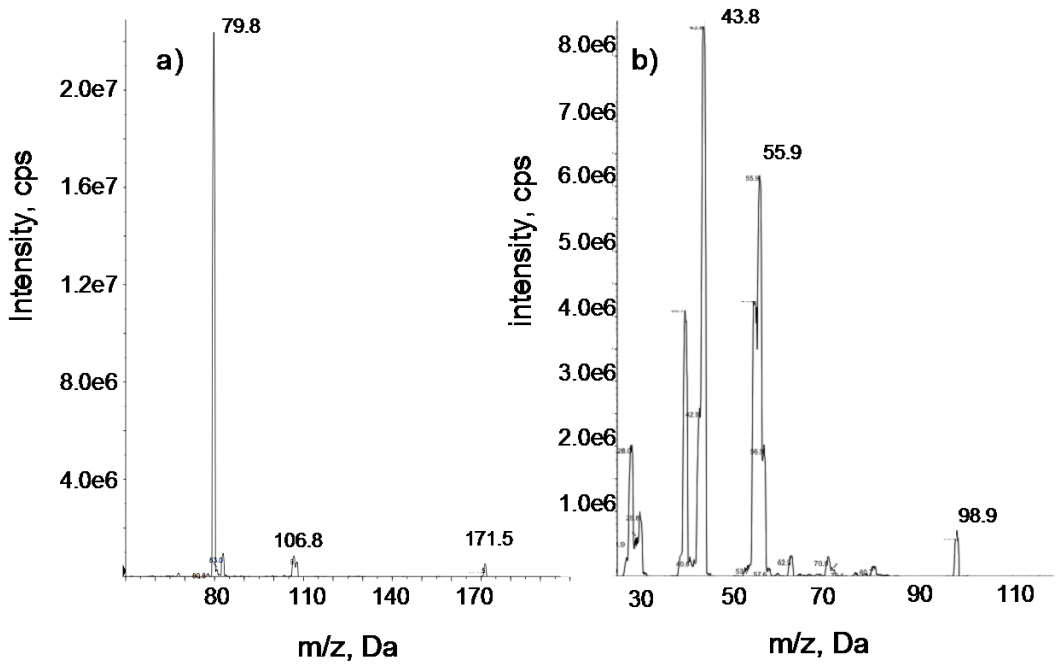

Figure 4.11- Mass spectra of a) Compound I and b) Compound III obtained in negative ionization mode

4.5.5 Ecotoxicological effect of photolysis and photocatalysis of SMX on *Daphnia magna*

The majority of the photolytic and photocatalytic experiments were performed with an initial SMX concentration of 12 mg/L. At this initial concentration of SMX with a dilution of 3 to 1 (sample to SFW), it was difficult to obtain reproducible values of mortality. At the high concentration of 60 mg/L untreated SMX with a 3 to 1 ratio (sample to SFW), all three replicates showed reproducible values of mortality of daphnia, approximately 30 % and 50% at 24 and 48 hours of exposure, respectively. Toxicity of UV treated solutions of SMX with an initial concentration of 60 mg/L with a 3 to 1 dilution ratio (sample to SFW) are presented in Figure 4.12 below. According to results not presented here, 30 minutes and 60 minutes of irradiation are required to completely remove 60 mg/L SMX with photolysis and photocatalysis, respectively. Therefore, the toxicity data presented in Figure 4.12 are solely due to the presence of photoproducts rather than the presence of the target compound. The increase in toxicity with increasing irradiation time is observed for both UVC only (i.e. photolysis) and

UVC coupled with TiO_2 oxidation (i.e. photocatalysis) processes. Such effects were expected for the initial stages of irradiation however, the persistence of toxicity after longer irradiation times during photocatalysis was not expected since further removal of the products were achieved in the presence of TiO_2; in fact, a decrease in toxicity would be the more probable expectation for the photocatalytic treatment.

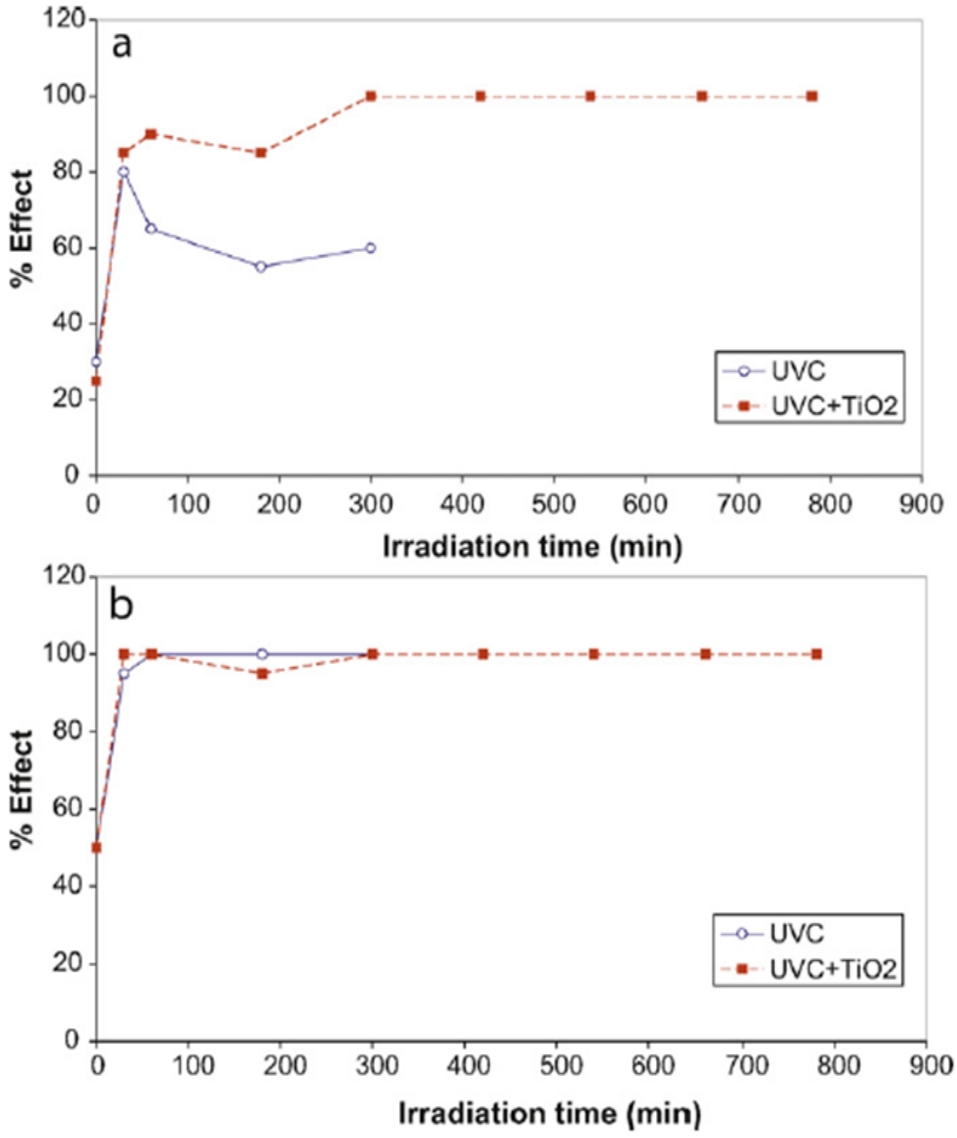

Figure 4.12- Toxicity of SMX (C_o = 60 mg/L) towards *Daphnia magna* during UVC and UVC + TiO_2 treatments: a) after 24 hours of exposure. b) after 48 hours of exposure

Figure 4.13 and Figure 4.14 show the evolution of COD and evolution of the HPLC peak areas of major photoproducts detected, respectively. The detected products for both types of treatment (UV and UV/TiO_2) are present at high concentrations but their removal starts by photocatalysis only after 360 minutes of irradiation (Figure 4.14). The major photoproducts are removed during photocatalysis and only a small portion of compound I (i.e. sulfanilic acid) remains after 13 hours of irradiation. The lack of reduction of toxicity can be attributed to two reasons; either the presence of sulfanilic acid even at the concentrations encountered at the end of 13 hours of photocatalytic treatment are still too high for *Daphnia magna* or there are photoproducts present which are highly toxic but are not detected by the HPLC method. From the data currently presented here, treating SMX with UVC or UVC/TiO_2 processes leads to formation of more toxic species than the original target compound.

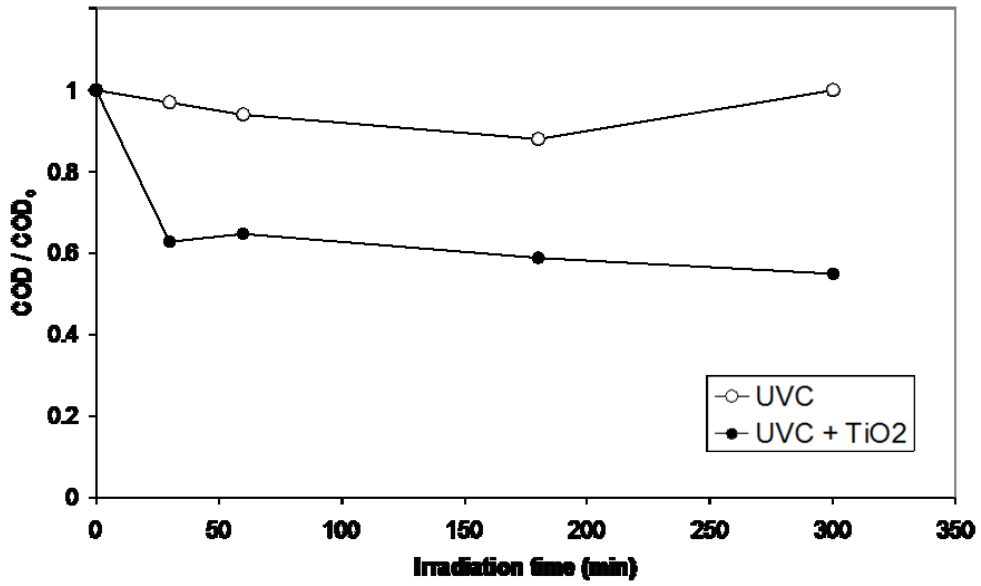

Figure 4.13- Evolution of COD for SMX (C_o = 60 mg/L) during UVC and UVC + TiO_2 treatments

4.6 Conclusions

Removal of the antibiotic sulfamethoxazole (SMX) by photolytic and photocatalytic processes was investigated in a pure water matrix. Following conclusions can be drawn from the work presented here:

- Photolysis is the dominant mode of removal of SMX during photocatalysis when operated under UV-C radiation.
- Two of the major photoproducts formed during UV-C treatment were identified as sulfanilic acid and 3-amino-5-methylisoxazole by LC-MS analysis. Especially sulfanilic acid is persistent against further removal by UVC however; it is susceptible to removal by oxidizing species generated during photocatalysis due to the presence of TiO_2.

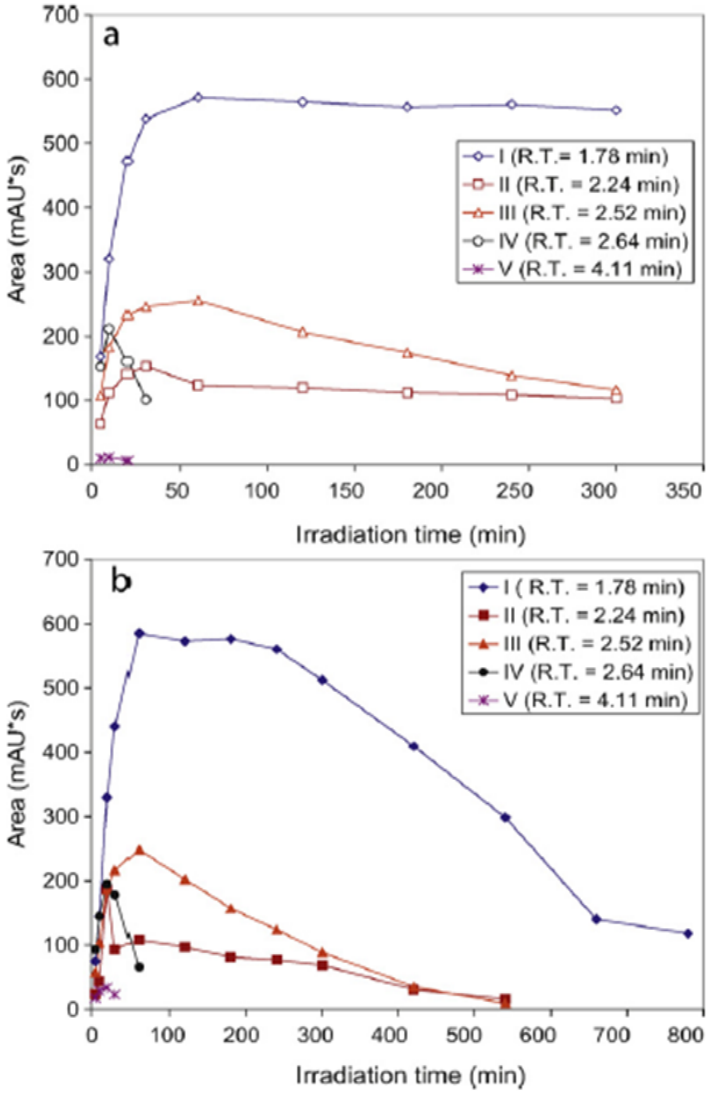

Figure 4.14- Evolution of major products of SMX (C_o = 60 mg/L) detected by HPLC during a) UVC and b)UVC + TiO_2 treatments

- Considerably higher COD removal is observed during photocatalysis. Even though with photolysis SMX is removed faster, presence of TiO_2 leads to removal of the persistent products formed.
- Both UVC treatments of SMX lead to generation of products which are more toxic than the parent compound towards *D. Magna*. These results underline the fact that analysis of toxicity of treated samples is necessary in any research that deals with treatment of contaminants.

4.7 Acknowledgements

Authors would like to acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC) and Eugenie Ulmer Lamothe Chemical Engineering Fund (McGill University) for the financial support provided for this work.

5. REMOVAL OF THE ANTIBIOTIC LEVOFLOXACIN (LEVO) IN WATER BY OZONATION AND TiO_2 PHOTOCATALYSIS

5.1 Preface

In contrast to SMX, LEVO is a more recently developed synthetic fluoroquinolone antibiotic and the prescription trend has been shifting towards LEVO in the recent years. The data presented in this manuscript which is about the photocatalytic removal of LEVO would allow the development of alternative methods of treatment before its occurrence and environmental impact becomes of greater concern, since a shift in the prescription trend is already reported.

Similar experimental procedure was used as described in the previous manuscript to evaluate the potential of photocatalysis as a treatment method. Unlike the previous manuscript, the photolytic removal of LEVO (i.e. absence of TiO_2) was not as significant as the photolytic removal of SMX because LEVO has a maximum absorption in the UVB range in contrast to the maximum absorption for SMX in the UVC range. These results supported the hypothesis that photolysis is significant for compounds which have a maximum absorbance in the wavelength of operation. Since the photolytic removal of LEVO was already low, the hypothesis that photocatalysis leads to higher mineralization efficiency was already verified. The degree of mineralization during photocatalysis was determined by COD measurements. Changes in dissolved oxygen concentration and scavenging of hydroxyl radicals influenced the photocatalytic removal rate of LEVO significantly. These experiments allowed us to conclude that the major mode of removal of LEVO is due to the presence of hydroxyl radicals and the synergistic effect of photolysis towards removal of LEVO under UVC radiation is low.

In this manuscript, ozonation of LEVO was additionally investigated to evaluate the possibility of applying photocatalysis as a treatment system by comparing its performance to another widely used advanced oxidation process. COD measurements and evolution of degradation products showed that excessive ozonation leads to generation of products that are resistant to further oxidation and remain in the reaction mixture whereas COD was continuously removed in photocatalytic treatment. Also by

treating ozonated LEVO samples by photocatalysis further reduction in COD removal was achieved. These results supplied significant evidence that photocatalysis had higher oxidation potential and that it can be used as a treatment technology.

Since one of the major concerns related to occurrence of antibiotic in the environment, is the fact that they can lead to antibiotic resistance in pathogenic bacteria, instead of toxicity experiments residual antibiotic activity was reported in this manuscript. Complete removal of antibiotic activity after photocatalytic treatment was reported to clearly indicate that this type of treatment can be used to mitigate the concern for increase in antibiotic resistance due to the presence of LEVO.

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Removal of the antibiotic levofloxacin (LEVO) in water by ozonation and TiO_2 photocatalysis

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5.2 Abstract

Removal of the fluoroquinolone antibiotic levofloxacin (LEVO) was studied in two oxidation processes: photocatalysis (UVC lamp (254 nm), TiO_2) and ozonation. LEVO (C_o = 20 mg/L) was no longer detected after an ozone dose of 20.5 mg/L and after 180 minutes of photocatalytic oxidation. COD removals of 59 % and 70 % were measured for 270 mg/L of transferred ozone dose and 300 minutes of photocatalytic oxidation, respectively. Extensive treatment with ozone did not result in further reduction in COD levels reaching a plateau at the above mentioned value, however increased irradiation time led to increased COD removal during photocatalytic treatment. Both treatment methods proved to be effective ways of removing antibacterial activity. From agar diffusion test with *E. coli*, it was observed that a transferred ozone dose of at least 20.5 mg/L and 180 minutes of irradiation were enough to completely remove antibacterial activity. Both treatments methods were shown to efficiently remove LEVO and its antibacterial activity and show promising results as possible applications for removal of antibiotics in wastewater.

Key words: Levofloxacin, photolysis, photocatalysis, antibacterial activity

5.3 Introduction

The risk associated with pharmaceuticals in the environment is a rising issue of global concern as significant amounts have been detected up to µg/L levels in the aquatic environment [8]. After their use, these substances are usually excreted only partially

metabolized and end up in the sewage system. A great portion of these compounds is not removed by conventional sewage and wastewater treatment plants and is eventually discharged into receiving water [183]. Several of them are antibiotics that were demonstrated to lead to increased antibiotic-resistant pathogens in wastewater [12, 13] and potentially in receiving streams. Recent work also demonstrated that mixtures of various antibacterial classes can exert unexpectedly high levels of algal growth inhibition at individual concentration levels on the order of 1 μg/L [203].

Levofloxacin (LEVO) is a more recently developed antibiotic belonging to the fluoroquinolones (FQs) which are synthetic broad spectrum antibiotics. The first and second generation quinolones are active against gram-negative bacteria whereas the third and fourth generation quinolones have extended activity against gram-positive bacteria as well. Ciprofloxacin belonging to the 2^{nd} generation was the mostly prescribed quinolone in Europe in 2003. Currently the prescription trend is shifting towards levofloxacin and moxifloxacin both of which are 3^{rd} generation quinolones [142]. There are limited reports on the presence of LEVO in aquatic environments; however other FQs such as ofloxacin and ciprofloxacin were commonly detected in effluents of hospital wastewaters, sewage and wastewater treatments plants at μg/L levels [10, 145, 146]. The biodegradability of quinolones were shown to be very low [148] thus making conventional biological treatment methods ineffective for their removal. Due to the fact that multiple FQs are commonly found to occur within wastewater matrices [146, 203] the total biological activity associated with all such compounds is considerably higher than the activity attributable to a single compound. Within a typical wastewater treatment facility, conventional wastewater treatment will result in prolonged exposure of wastewater-borne bacteria to significantly higher FQ concentrations than are present in wastewater effluents. Prolonged exposure of bacterial communities to an antibacterial compound is a condition which can result in evolution of low-level antibacterial resistance in affected bacterial communities [72, 73]. Therefore, it is necessary to propose efficient treatment methods to transform these antibiotic agents to non-toxic, pharmaceutically less active or more biodegradable species.

Advanced oxidation processes (AOPs) have received great interest in the recent years as alternative or complementary methods to conventional wastewater treatment to

prevent the release of these compounds into aquatic environments. Among these AOPs; ozonation [133, 134, 189]; fenton and photo-fenton oxidation [135]; photolysis and H_2O_2 enhanced photolysis [192, 193]; heterogenous photocatalysis [41, 44] were frequently studied. During advanced oxidation of pharmaceutical compounds, intermediate compounds are formed that might show more toxic effects than the parent compound [204]; therefore overall goal of treatment processes should be the transformation into non-toxic or biologically less active products rather than just the removal of the parent compounds.

Reports on the advanced oxidation of relatively older FQs such as ofloxacin and ciprofloxacin are abundant [18, 27, 37, 42, 43, 49, 152-154]; however advanced oxidation of LEVO has seldom been investigated. The main results on ozonation of LEVO were very recently published [155] and only few other papers report the fate of this compound during UV radiation under close to sunlight conditions [156, 157]. There is only one study in literature on photocatalytic removal of LEVO under UVA radiation [19] reporting reaction rate constants for reactions of LEVO with hydroxyl radicals and hydrated electrons and proposing reaction pathways. There are no reports on photocatalytic degradation of this compound under UVC radiation and no data on residual antibacterial activity during the any type of photocatalytic treatment of LEVO. Considering that UVC radiation is commonly used as a disinfection method for a variety of water treatment facilities, that possible synergistic effects can arise from coupling UVC radiation with TiO_2 and that absorption spectra of LEVO [156] show significant absorption in the UVC range, it is essential to fill that knowledge gap and determine the fate of LEVO during photocatalytic treatment based on UVC radiation.

The focus of the work presented here was to show the applicability of ozonation and UVC photocatalysis towards removal of LEVO in aqueous systems. The simultaneous study of performances of ozonation and UVC photocatalysis towards the removal of LEVO also allowed for the first time to compare the efficiency of this these oxidation techniques for the removal of this compound. During both advanced oxidation methods the evolution of generated products was monitored and the corresponding residual antibacterial activity was investigated. COD removal during treatment was used as a way of comparing mineralization capacity of both processes. Finally combination of

ozonation followed by photocatalysis treatment was used to investigate the resistance of transformation products towards reaction with ozone and other oxidizing species formed during photocatalysis (e.g. hydroxyl radicals, superoxides or holes). In addition to providing knowledge on the fate of LEVO during ozonation and photocatalysis, this work also provided insight on the applicability of this type of treatment as a pretreatment step to industrial or hospital wastewater before their discharge to municipal treatment facilities to minimize the exposure of bacteria community to antibiotics and mitigate the risks of developing resistant bacteria.

5.4 Materials and Methods

5.4.1 Reagents

Levofloxacin ($C_{18}H_{20}N_3O_4F$, > 98%) and sodium dihydrogen phosphate were obtained from Sigma–Aldrich, Canada. Aqueous stock solution (20 mg/L LEVO) was prepared in reverse osmosis (RO) water and kept at 4°C in the dark until the time of treatment (maximum time of storage was one week). Initial pH of the solution was 6.5 and no buffer was added during the treatment of samples. Commercial TiO_2 Degussa P25 (70% anatase and 30% rutile) was used as catalyst with an average particle size of 30 nm and BET surface area of 50 $m^2 g^{-1}$, according to the manufacturer. HPLC grade methanol, acetonitrile and isopropanol were purchased from Fisher Scientific, Canada. All the chemicals were used as received without purification.

5.4.2 Ozonation setup

Ozonation experiments were carried out in a 2-L acrylic reactor (600 mm height, 70 mm diameter) with continuous supply of an ozone-oxygen gas mixture to the bottom of the reactor containing 500 mL of LEVO solutions at a concentration of 20 mg/L. Ozone was produced by an OZO-4VTT (Ozomax) at a rate of 3.3 g/h, using oxygen as a feed gas, and the O_3/O_2 gas was bubbled through a porous stainless steel diffusion plate (Mott Corporation, 2μm) located at the bottom center of the ozonation column. Ozonation experiments were run for various times so that different doses could be applied (and thus transferred). The amounts of ozone fed to the system and leaving the system per unit of time were measured using the standard iodometric titration (Standard

Method # 2350 E). The rate of ozone transfer into the solution was calculated as the difference between the amounts fed and leaving the system. All ozone doses reported here correspond to the ozone transferred to solution during experiments and are referred to as ozone dose (mg/L). It was observed that the presence of LEVO did not influence the rate of ozone transfer when compared to pure water. This indicated that the transfer of ozone to the solution was not affected by the reaction of ozone with dissolved constituents as observed in previous research [132]. Prior to ozonation all solutions were adjusted to a temperature of 17°C which corresponds to the average annual values for a typical wastewater treatment plant effluent in Quebec.

5.4.3 Photocatalysis setup

Irradiation experiments were carried out in 2 L capacity cylindrical acrylic photoreactor (215 mm height, 108 mm diameter). The reactor walls were covered with aluminum foil to avoid exposure to UV radiation. 1.6 L of an aqueous solution of LEVO (20 mg/L) was charged to the reactor for each experiment. The solution was irradiated by an Hg-Ar (Germicidal UV-C) lamp (Atlantic Ultraviolet Corp. GPH212T5L) located in the center of the reactor and protected in a quartz sleeve (maximum output at 254 nm) and mixing was achieved by magnetic stirring. Light intensity inside the reactor was measured by azoxybenzene actinometry. It was previously shown that light intensity inside the reactor varies highly with respect to the position inside the reactor and that the maximum intensity of incident radiation per unit volume measured was $1.3 \times 10^{-3} \pm 0.3$ Einstein/min/L at a radial distance of 0.7 cm from the lamp and 10 cm from the bottom of the reactor [40]. For photocatalytic degradation experiments, a wide range of titanium dioxide concentrations of 0.05, 0.2 and 0.5 g/L was chosen to be studied based on our previous work using the same reactor [40]. Before each experiment the required amount of titanium dioxide particles were suspended in RO water and sonicated for 30 minutes to reduce agglomeration and create a more stable suspension. In photolytic experiments no titanium dioxide was added to the reaction mixture.

In order to determine if the hydroxyl radicals contribute significantly to the removal of LEVO or if other oxidizing species generated during photocatalysis play a more important role, the effect of dissolved oxygen concentration and scavenging of

hydroxyl radicals was investigated. Different concentrations of dissolved oxygen were obtained by continuously bubbling air (8.8 mg/L) or pure oxygen (42 mg/L) into the reaction mixture through a sparger located at the bottom of the reactor. For close to anoxic conditions, the dissolved oxygen in the reaction mixture was purged off by continuously bubbling nitrogen for 2 hours before turning on the lamp and maintaining the flow of nitrogen through out the reaction time to obtain low dissolved oxygen levels (0.5 mg/L).

The method of scavenging and concentrations of scavenging compounds were based on results reported by Palominos et al. [41]. Isopropanol, described as one of the best hydroxyl radical quencher due to its high reaction rate constant with the radical (1.9 x 10^9 mol/L/s) [201], was used in this work to scavenge the hydroxyl radicals. Isopropanol was added to the reaction mixture (10 mg/L LEVO and 0.2 g/L TiO_2) at a molar concentration which was three orders of magnitude larger than the initial concentration of LEVO.

5.4.4 Analytical methods

Prior to analysis, the collected samples were filtered using 0.22 μm syringe filters. LEVO concentration was monitored by HPLC (Agilent 1200 series) equipped with a diode array detector set at a wavelength of 294 nm. The ozonation and photocatalysis products were monitored at a wavelength of 225 nm. The column used was Agilent Zorbax Eclipse Plus C-8 (4.6 x 150 mm, 3.5 microns). The eluents used were 15 mM sodium dihydrogen phosphate at pH 2.6 (A) and acetonitrile (B). A gradient from 20% B to 30% over 10 minutes at a constant flow rate of 0.8 ml min^{-1} was employed. COD levels were monitored using a HACH Digital Reactor Block (DRB 200), a HACH spectrophotometer (DR/2500) and ultra low range (0-40 mg/L) COD digestion vials (HACH).

Agar diffusion tests were performed to evaluate the remaining antibacterial activity of LEVO after treatment by ozonation and photocatalysis. Strains of *Escherichia coli* (ATCC 1303) and *Pseudomonas fluorescens* (ATCC 13525) were inoculated onto nutrient agar. Ten ml aliquots were withdrawn from both the ozonation and/or the photocatalytic reactors at various reaction times. Filter papers (diameter of 8 mm,

Millipore 0.20 μm GNWP04700) were soaked in these aliquots for 30 seconds and were placed in duplicates on to the bacteria inoculated agar plates. The plates were incubated for 24 hours at 26°C. Residual antibacterial activity of the samples was determined by measuring the inhibition diameter produced around the filter papers. The magnitude of the inhibition zone diameter was used as a semi-quantitative way to compare the efficiency of the AOPs studied at removing the antibiotic activity.

5.5 Results and Discussion

5.5.1 Removal of levofloxacin (LEVO) during photocatalysis

Preliminary experiments were performed at an initial concentration of LEVO of 10 mg/L to determine the amount of photocatalyst that would lead to highest LEVO removal. Highest removal of LEVO after 10 minutes was achieved when the reaction mixture contained 0.2 g/L of TiO_2. Percentages of LEVO removed after 10 minutes of irradiation were 53.1 ± 2.4%, 72.1 ± 2.7% and 52.5 ± 2.1% for TiO_2 concentrations of 0.05, 0.2 and 0.5 g/L, respectively. The concentration of TiO_2 observed for maximum removal of LEVO in the work presented here is lower when compared to other photocatalytic systems [27, 37, 41-44]; this can be explained by the larger diameter of the reactor employed. From our previous work [40], significant portion of the reactor was found to operate under UV dark indicating that only part of the volume of reactor participates in removal of the target compound since the TiO_2 particles away from the lamp do not receive enough light. Increasing catalyst concentration above 0.2 g/L leads to reduced removal rates since these high catalyst loadings lead to unfavourable light scattering and reduction of light penetration [196, 205]. For the remainder of the photocatalytic experiments a concentration of 0.2 g/L of catalyst was used.

Figure 5.1 compares the performances of photolytic and photocatalytic removal of LEVO (C_o = 20 mg/L) for longer irradiation times. After 120 minutes of irradiation under photolysis (UVC) 65% of LEVO still remains in solution, however during photocatalysis after 120 minutes of reaction 97% of LEVO is removed and it is no longer detected at 180 minutes of irradiation. These results indicate that the photolytic removal of LEVO is not significant under UVC radiation. From the UV-Vis absorption

spectrum of LEVO (Supplementary Material, Figure 5.10) it is evident that this compound absorbs the most in the UVB range ($280 < \lambda < 315$ nm) with moderate absorption in the UVC ($\lambda < 280$ nm) and UVA ($315 < \lambda < 400$ nm) ranges. Since the lamp used in the experiments operates in the UVC range, there is only slight photolytic removal of LEVO but the removal is enhanced when TiO_2 is present due to generation of other oxidizing species. When using light sources operating at higher wavelengths ($\lambda > 280$ nm), direct photolysis of LEVO may be more pronounced. Lam and Mabury [156] reported that 50% of LEVO was removed within 20 minutes of irradiation when using a Xe lamp as the UV radiation source and radiation below 290 nm was cut off with filters. Significantly higher removal observed when working at higher wavelengths for LEVO suggests that its photolytic removal in the environment is possible; however the nature of the transformation products should be examined carefully to assess any remaining antibacterial activity.

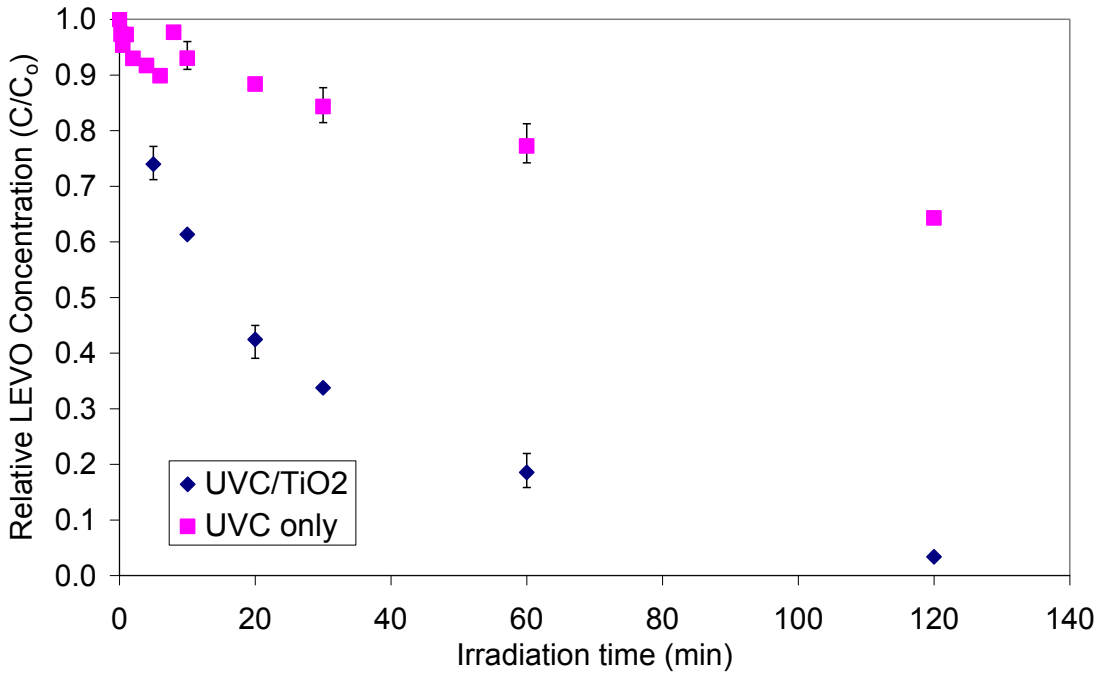

Figure 5.1- Removal profiles of LEVO (C_o = 20 mg/L) with photolysis (C_{TiO2} = 0 g/L) and photocatalysis (C_{TiO2} = 0.2 g/L) for long irradiation times

5.5.2 Removal of levofloxacin (LEVO) by ozonation

Removal profile of LEVO by ozonation for an initial concentration of 20 mg/L is presented in Figure 5.2. The rate of ozone transferred into the solution was determined from iodometric titrations to be 0.45 mg/L/s. It was found that after 20.5 mg/L of ozone was transferred into the solution, LEVO was no longer detected (corresponds to 45 seconds of continuous ozone bubbling). Furthermore, in order to remove 50 % of the initial LEVO, 2.65 mg/L of O_3 needs to be transferred into the solution, which corresponds to a half-life time for LEVO of 6 seconds. When compared with the only data available on ozonation of LEVO in literature by Witte et al. [155], the half-life time observed here is about 80 times shorter than the reported value of 12.8 minutes (initial concentration of LEVO was 16.4 mg/L). Witte et al. [155] reported an ozone consumption value of 0.61 mmol during 60 minutes of reaction for a reaction volume of 1.75 L. This value corresponds to an ozone transfer rate of 4.7 μg/L/s. This lower rate of ozone transfer, compared to our of 0.45 mg/L/s, explains the discrepancy in the half-life values reported here (6 s) and in the work of Witte et al. (12.8 min) [155]. However, the calculated ozone dose corresponding to their reported half-life time is 3.6 mg/L, which is in the same order of magnitude as the value of 2.65 mg/L reported above. The lower transfer rate they observed might be explained by the higher temperature they used, 27.5 °C, compared to the temperature of 17 °C used here. The difference in temperature greatly influences the solubility of ozone leading to a reduction in transfer rate and higher half-lives. Other mass transfer limitation factors, such as gas retention time and bubble size may also explain this difference. Our results showed that ozonation can remove the target compound from the solution and emphasized the importance of mass transfer limitations in semi-continuous systems.

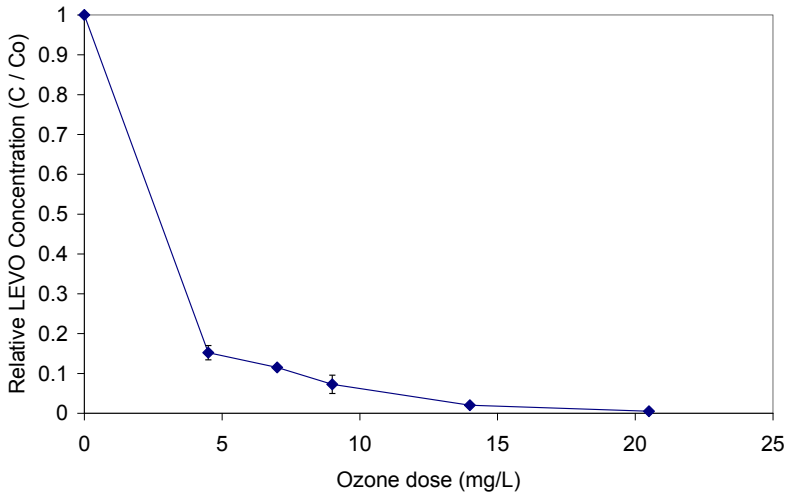

Figure 5.2- Removal of LEVO (C_o = 20 mg/L) as a function of ozone dose transferred. Error bars = ± 1 standard deviation

5.5.3 Effect of hydroxyl radicals and dissolved oxygen concentration on removal of LEVO

Participation of hydroxyl radicals on oxidation of LEVO during ozonation and photocatalysis was studied by addition of isopropanol. Results associated to scavenging of hydroxyl radicals are presented in Figure 5.3 a and b for photocatalysis and ozonation, respectively. Because previous work in our research group (unpublished) showed that addition of isopropanol during the ozone bubbling enhanced removal of pharmaceuticals due to increased mass transfer of ozone, scavenging experiments were carried out in batch mode. This phenomenon was also observed by De Witte et al. [153], where adding t-butanol as a scavenger for hydroxyl radicals during continuous ozonation led to formation of smaller gas bubbles and increased mass transfer coefficient due to increased interfacial area. The stock solution of ozone was prepared by bubbling ozone in a glass-washing bottle containing reverse osmosis water to obtain a solution having an ozone concentration of 11 mg/L. Required volume of the stock ozone solution was added to the LEVO-containing water to obtain three different ozone doses (1.1 mg/L, 2.2 mg/L and 4.4 mg/L). For scavenging experiments, isopropanol was added to the LEVO (C_o = 10 mg/L = 2.8 x 10^{-5} M) containing water (at a concentration three orders

of magnitude larger than the initial molar concentration of LEVO, 2.8 x 10^{-2} M) prior to dosing with the ozone solution.

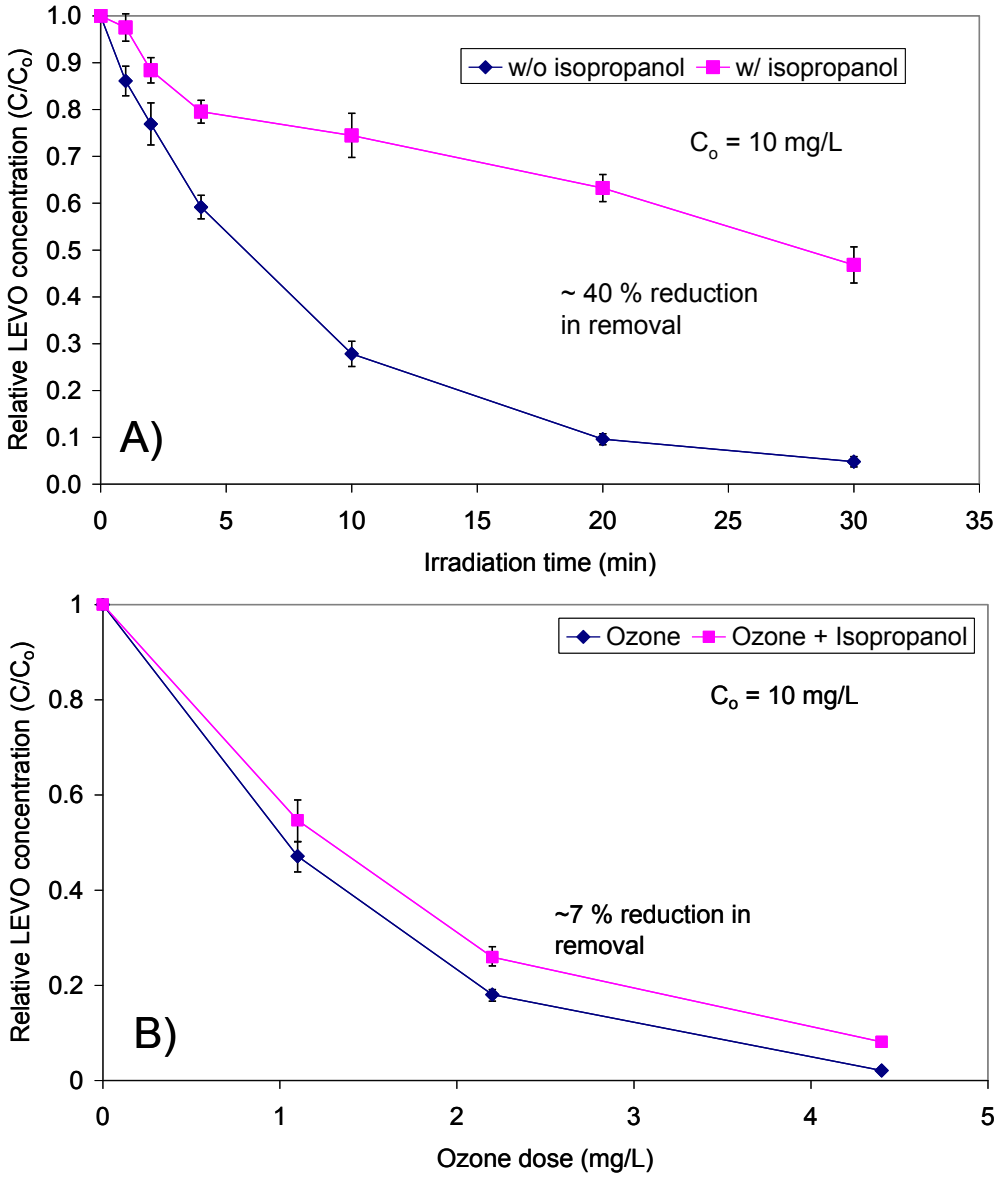

Figure 5.3- Effect of the presence of hydroxyl radicals during a) photocatalysis and b) ozonation on removal of LEVO (C_o = 10 mg/L, C_{TiO2}= 0.2 g/L) is studied by scavenging the hydroxyl radicals by addition of isopropanol . Errors bars = ± 1 standard deviation

As evident from Figure 5.3a addition of a scavenger during photocatalysis significantly inhibited the removal of LEVO. When no scavenger was present, 95% of

LEVO was removed after 30 minutes of irradiation compared to 53% when the scavenger was present. This result suggests that the hydroxyl radicals contribute significantly to the removal of LEVO. Significant contribution of hydroxyl radicals on photocatalytic removal of fluoroquinolones under UVA radiation was demonstrated by An et al. [18, 19], and Van Doorslaer et al. [206]. The work of Van Doorslaer et al. [206] also showed that contribution of photogenerated holes towards removal of moxifloxacin was more significant than hydroxyl radicals under UVA radiation. However, this could not be verified in our work due to the fact that potassium iodide also absorbs considerable amount of radiation in the UVC radiation and distinction between oxidizing species scavenging effect and light reduction effect would not be possible. On the other hand, during ozonation (Figure 5.3b) no significant inhibitory effects were observed when the scavenger was present (only 7% reduction in final removal). This result suggests that the main removal mechanism of LEVO during ozonation is due to direct reaction with ozone and that hydroxyl radicals play only a minor role in removal of the target compound. Dodd et al. [207] demonstrated that for fluoroquinolones the second reaction rate constants with hydroxyl radicals are significantly larger than rate constants with molecular ozone ($> 2.2 \times 10^5$ times). Based on the fact that scavenging of hydroxyl radicals do not lead to considerable deviation in removal of LEVO during ozonation, it can be hypothesized that either the ozone has higher reaction rate constant with LEVO compared to hydroxyl radicals or that there is limited generation of hydroxyl radicals during the ozonation experiments. This can also be supported by the fact that initial pH is 6.5 and after instantaneous reaction with ozone, pH drops to 4.8, at this point generation of hydroxyl radicals would be limited. It is commonly shown that hydroxyl radicals are generated more at higher pH during ozonation [153].

The effect of the presence of hydroxyl radicals on removal of total organic content during photocatalysis was also studied by analyzing the UV-Vis absorption spectra of samples with and without the addition of the scavenger. The results are shown in Figure 5.4 a and b as absorption spectra observed for various irradiation times. As irradiation time was increased up to 60 minutes treated samples showed considerably less UV absorption when no scavenger was present. Thus, it can be concluded that

hydroxyl radicals are not only responsible for the removal of the target compound but also of the products generated during oxidation of LEVO during photocatalysis.

Effect of dissolved oxygen concentration on photocatalytic removal of LEVO was also studied. Removal of LEVO after 10 minutes of irradiation were measured for three different types of gases bubbled in the solution in order to vary the dissolved oxygen concentration. As expected, enhanced removal was achieved when pure oxygen was introduced into the system; removal of 91.6 ± 3.3% of LEVO over 10 minutes of irradiation compared to 72.1 ± 2.7% and 42.6 ± 4.3% for air and nitrogen, respectively. The presence of oxygen reduces the recombination of electrons and holes generated upon irradiation of TiO_2, thus allowing holes to either directly react with the target molecule or lead to generation of hydroxyl radicals as well as making it possible for super oxide molecules to be formed. When the system is purged with nitrogen, recombination of electrons and holes are enhanced thus leading to lower concentrations of oxidizing species to remove the target compound. The considerable reduction in photocatalytic removal of another fluoroquinolone, moxifloxacin, when the system is sparged with pure nitrogen (instead of air or oxygen) was also demonstrated by Van Doorslaer et al. [206].

5.5.4 Evolution of products and chemical oxygen demand (COD) removal

During both photocatalysis and ozonation, the pH of the solution decreases with increased irradiation time or increased applied ozone dose (from pH 6.5 to 4.8). This suggests the generation and accumulation of acidic products. The evolution of the HPLC peak areas and the retention times of the major products detected are presented in Figure 5.5 a and b for photocatalysis and ozonation, respectively. During photocatalysis three of the five major products were removed from the system within 3 hours of irradiation. For ozonation four of the six major products were removed when 55 mg/L of ozone were transferred into solution (2 minutes). However, two products of ozonation were found at higher HPLC peak areas and were persistent towards further removal by ozone. These ozonation products were found to exist even after 10 minutes of ozonation (270 mg/L of applied ozone). Even though considerably high peak areas were also detected for two of

the products generated during photocatalysis, the evolution over time of their peak areas indicates that they are continuously being removed with increased irradiation time.

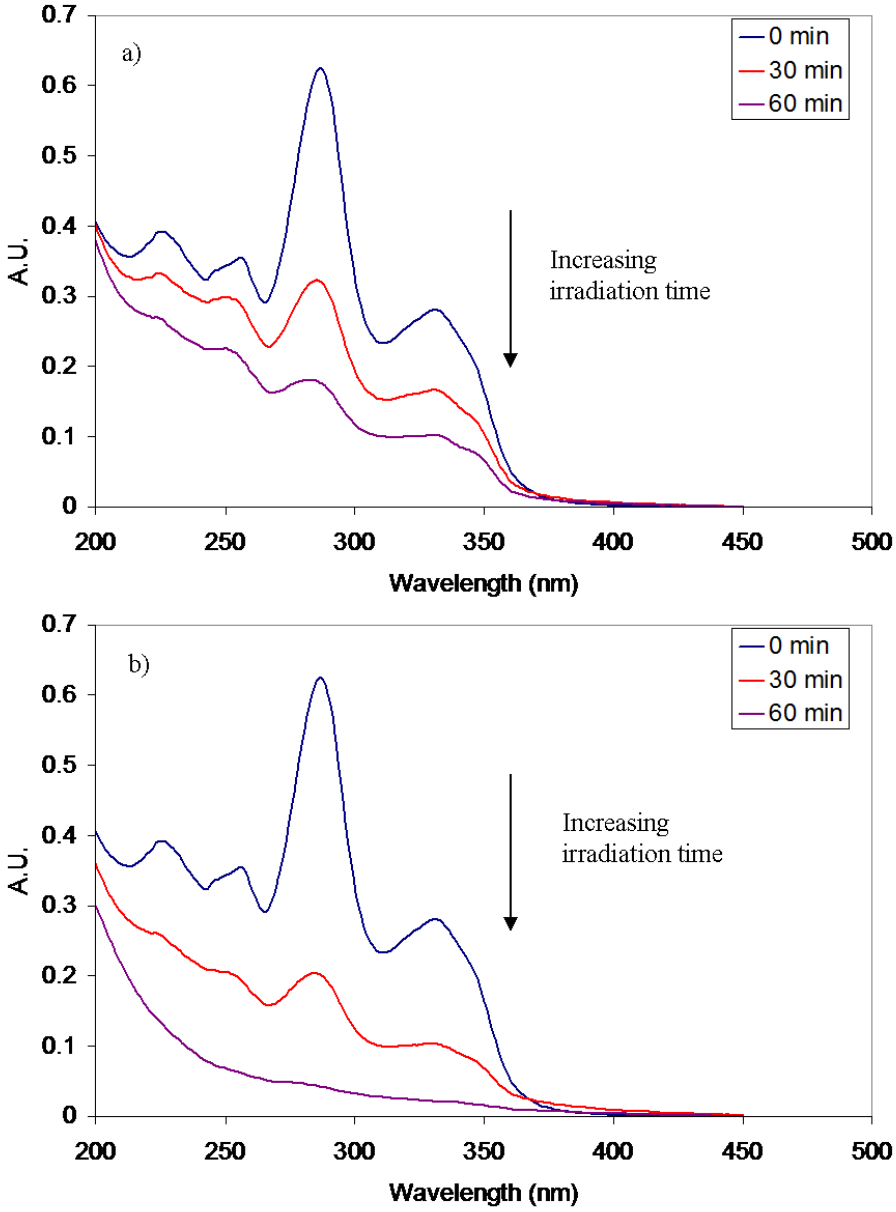

Figure 5.4- UV-Vis spectra during photocatalytic treatment of LEVO a) with or b) without the addition of hydroxyl radical scavenger isopropanol for various irradiation times (C_o = 10 mg/L, C_{TiO2} = 0.2 g/L)

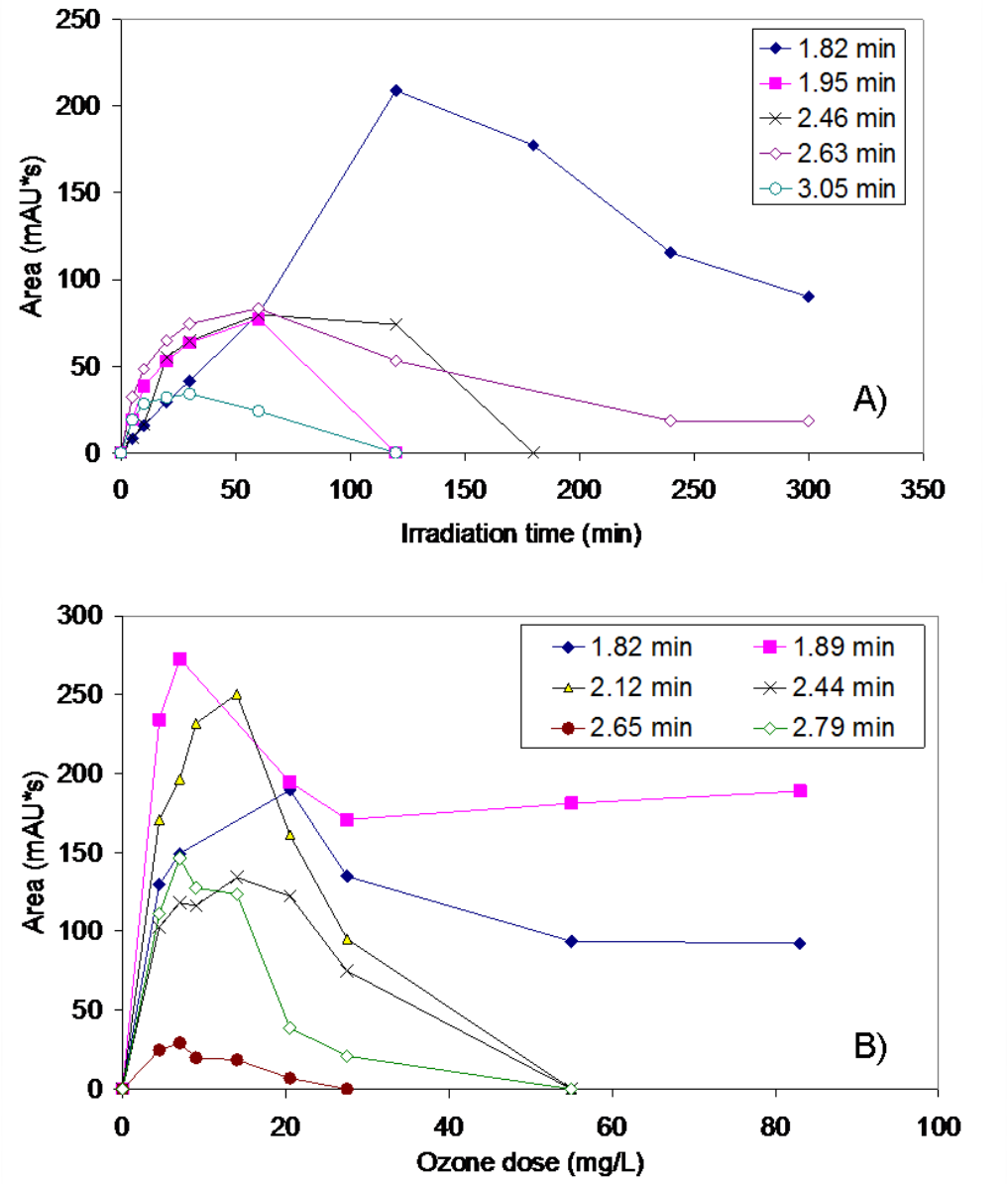

Figure 5.5- HPLC peak area evolution of major product peaks detected at various retention times during a) photocatalytic treatment b) ozonation of LEVO (C_o = 20 mg/L, C_{TiO2}= 0.2 g/L)

These observations are in accordance with the chemical oxygen demand (COD) evolution results presented in Figure 5.6a and b. For ozonation (Figure 5.6a), the COD reduction is halted after an applied ozone dose of 55 mg/L, corresponding to a maximum COD removal of 46% to 59% and suggesting that the organic matter is no longer being removed. On the other hand, the residual COD during the photocatalytic process started to monotonically decrease after 120 minutes of irradiation and reached 70% removal at

300 minutes (Figure 5.6b). Hapeshi et al. [27] showed that for 20 mg/L of ofloxacin (racemic mixture of levofloxacin and its stereoisomer) UVA photocatalysis led to about 70% reduction in dissolved organic content (DOC) after 120 minutes whereas here UVC photocatalytic led to a reduction in COD of about 45% after 120 minutes. The discrepancy can be attributed to the fact that Hapeshi et al. [27] used a much smaller reactor volume (350 ml) and the distribution of light was more homogenous leading to a larger portion of the reactor to be illuminated (i.e. more fraction of catalyst particles are illuminated leading to higher number of reactive radical species). Additionally, lower COD removal during ozonation compared to photocatalysis of another fluoroquinolone, ciprofloxacin, in hospital wastewater was also demonstrated by Vasconcelos et al. [50]. They showed that a maximum of 40% of initial COD was removed during heterogenous photocatalysis whereas this value was limited to only 10% for ozonation and enhanced ozonation did not lead to further decrease. Based on the previously published data for other compounds and on our results, it can be hypothesized that the active species generated during photocatalysis (e.g. hydroxyl radicals, superoxide radicals and holes) are less selective than ozone and react better with the transformation products. In order to investigate this hypothesis, ozonated samples of LEVO, collected after a transferred ozone dose of 20.5 mg/L to obtain a solution containing no residual LEVO but containing the ozonation transformation products were subjected to photocatalysis. The COD removal for the combined ozonation/photocatalysis process is also presented in Figure 5.6b. Since the LEVO was already removed by ozone when the photocatalytic treatment was started, the initial COD value was 80%. With increased irradiation time, more COD was removed and again after 120 minutes of irradiation in the presence of TiO_2, COD started to monotonically decrease. This indicates that the products generated during ozonation, which cannot be removed by additional amounts of ozone, can be removed by reaction with the oxidizing species present during photocatalysis. These results confirm that oxidative species formed during photocatalysis are less selective than ozone for oxidation of organic content and can then enhance the removal of the transformation products.

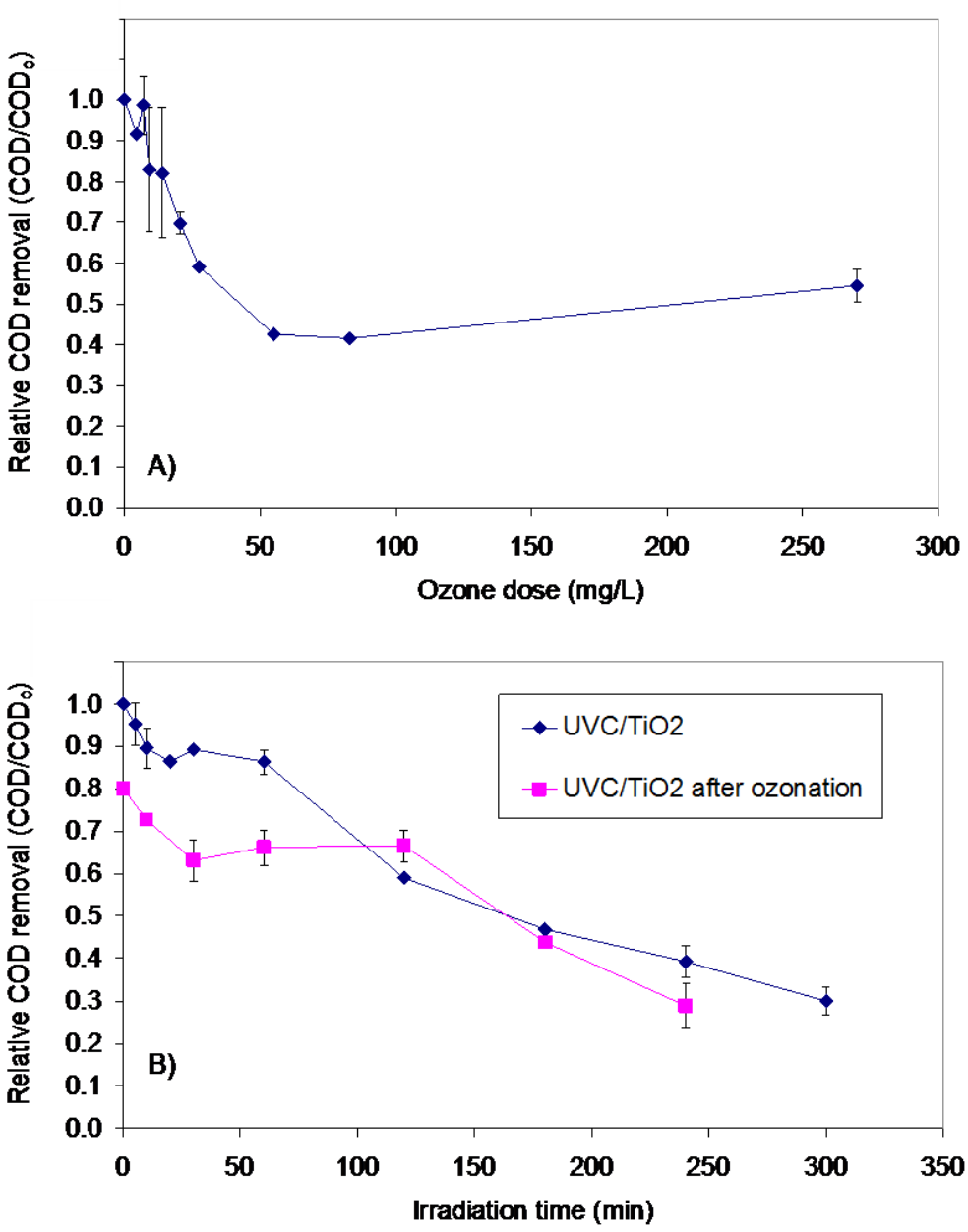

Figure 5.6- COD removal during a) ozonation b) photocatalysis and photocatalysis after ozonation. Error bars = ± 1 standard deviation

The products of ozonation and UVC photocatalysis for LEVO were not identified; however published data on removal of fluoroquinolones demonstrate that during ozonation, molecular ozone leads to degradation in the piperazinyl substituent [50, 153-

155]. It is also hypothesized that due to hydroxyl radical mediated reaction mechanism during ozonation (at pH 7) removal of carbonyl and carboxyl groups at the quinolone moiety, essential for antibacterial activity, is achieved [154, 155]. The scavenging results presented here for ozonation show that there is limited generation of hydroxyl radicals thus degradation at quinolone moiety is not expected for ozonation at the conditions studied. The major products generated from the photodegradation under UVA radiation of fluoroquinolones are due to the substitution of the fluorine substituent, the piperazine ring cleavage or the opening of quinolone ring following reactions with holes or hydroxyl radicals [18, 19, 42]. There is a need for further investigation into the nature of the UVC photocatalysis products of LEVO; however based on results obtained for other quinolones subjected to ozonation and UVA radiation, ozonation products would most probably maintain an intact quinolone moiety while for products of UVC photocatalysis the quinolone ring might be inactivated.

5.5.5 Residual antibacterial activity in treated solutions

Even though both treatment methods were shown to completely remove LEVO, formation of organic species was observed. In order to assess the residual antibacterial nature of LEVO and its transformation products for each treatment type, agar diffusion tests were performed for *E. coli* and *P. fluorescens*. The inhibition zone diameters measured as a semi-quantitative indication of residual antibacterial activity are presented in Figure 5.7a and b for photocatalysis and ozonation, respectively. For both photocatalysis and ozonation, the antibacterial activity was removed with increasing irradiation time and ozone dose, respectively. For treated solutions in which LEVO was no longer detected, the inhibition ring was barely measurable and reliable values were hardly obtained. As a result, the solid lines representing the data on Figure 5.7 end when the minimum measurable zone diameter was observed. Pass that point, the dashed lines indicate that there might still be slight antibacterial activity whose magnitude couldn't be determined accurately. This observation can also be viewed on the photographs of the agar diffusion test plates shown in Figure 5.8 for the photocatalytic treatment of LEVO. Similar observations were made for ozonation (images not presented). As observed on

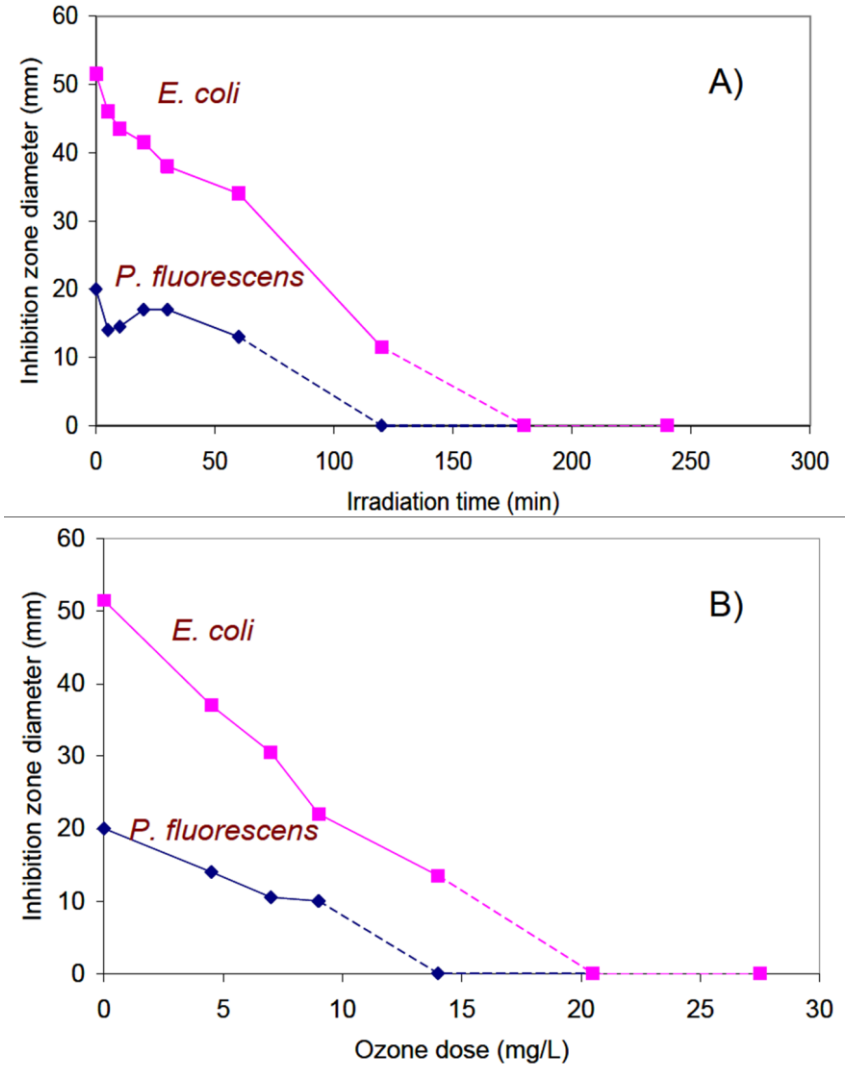

Figure 5.7- Antibacterial activity measured as inhibition zone diameters from Agar diffusion tests during a) photocatalytic treatment b)ozonation of LEVO (C_o = 20 mg/L) for *E. coli* and *P. fluorescens*

the *P. fluorescens* plate exposed to the solution after 120 minutes of irradiation, the slight inhibition observed is not measurable. However, for both organisms the antibacterial activity is completely removed after 180 minutes of irradiation since no inhibition halo is observed and the organisms clearly grew over the filter paper. The absence of antibacterial activity was considered obtained only when the microorganisms clearly grew over the placed filter papers. Considering that Paul et al. [42] showed that the antibacterial activity removal followed closely the fluoroquinolone removal even

though a large number of photocatalysis products having an intact quinolone moiety were present, the complete removal of the antibacterial activity as observed here, cannot be interpreted as an inactivation of the core quinolone structure of levofloxacin.

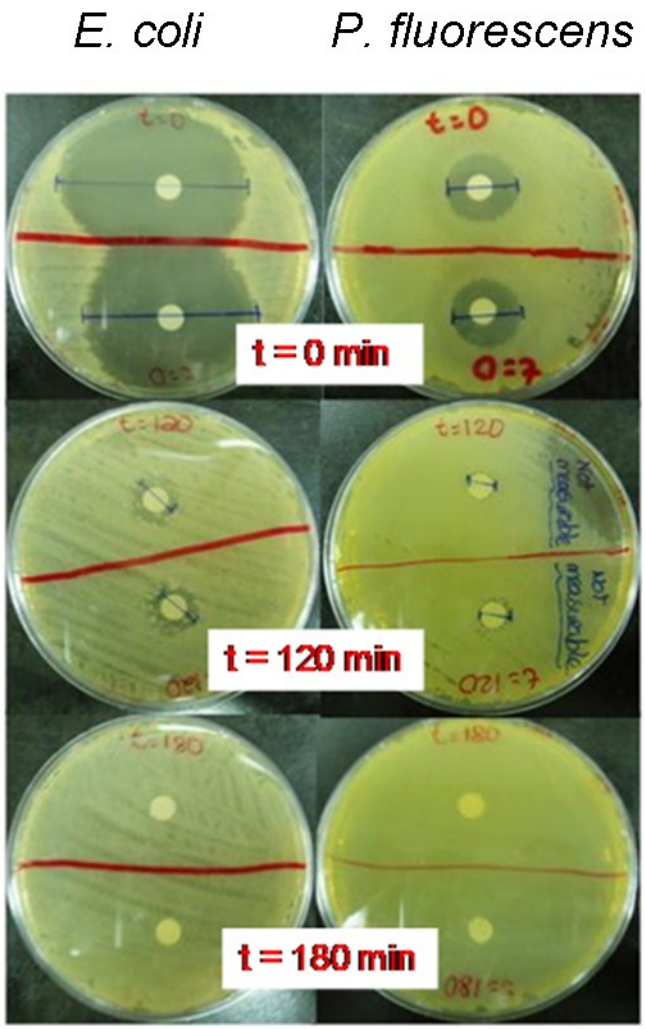

Figure 5.8- Photographs of agar diffusion test plates during photocatalytic treatment of LEVO (C_o = 20 mg/L, C_{TiO2} = 0.2 g/L) at various irradiation times. Left hand side: *E. coli* Right hand side: *P. fluorescens*

As observed from the inhibition zone diameters, *E. coli* shows more sensitivity to the presence of LEVO than *P. fluorescens*. For an initial concentration of 20 mg/L LEVO, there is at least 3 cm of difference in the inhibition zone diameters between

E.coli and *P. fluorescens*. Looking at the plates of *E. coli*, transferred ozone dose of at least 20.5 mg/L and 180 minutes of irradiation were sufficient to remove completely antibacterial activity. In order to distinguish the antibacterial activity of products from the antibacterial activity of the parent compound, the agar diffusion tests were also performed on dilutions of LEVO with varying concentrations corresponding to the residual concentrations of LEVO in the treated samples. The measured inhibition zone diameters were normalized by the initial zone diameter observed for the untreated 20 mg/L of LEVO and defined as normalized inhibition zone diameter (d/d_o). The values obtained were compared to those obtained for ozonation and photocatalysis in Figure 5.9 but this time presented as a function of residual LEVO concentration as the abscissa to facilitate comparison.

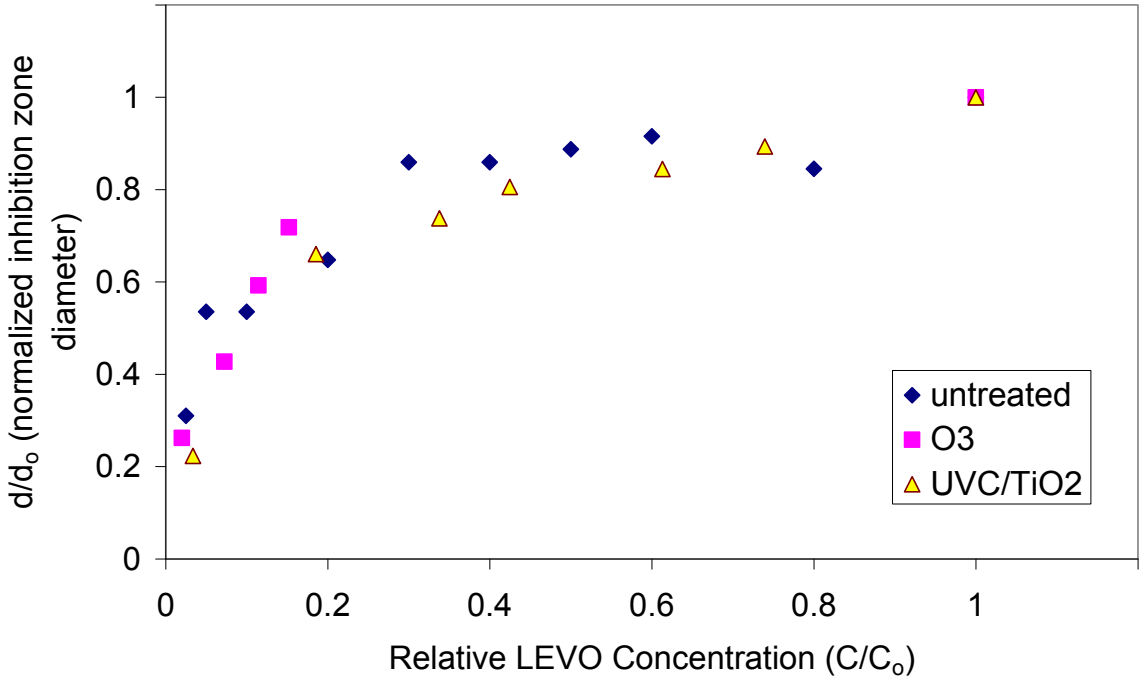

Figure 5.9- Comparison of antibacterial activity effect of treated and untreated solutions. Untreated case contains only LEVO in solution. Ozone and UVC/TiO_2 treated cases also

The untreated case refers to the samples that contain only LEVO at varying concentrations. All the data points for both treatments and for the untreated case scatter

around a single curve indicating no observable difference between the treatment methods and the diluted LEVO samples. This suggests that for the two organisms studied the antibacterial activity is only related to the parent compound (LEVO) and that the generated products (transient or persistent) do not show any antibacterial activity. These observations were also demonstrated by De Witte et al. [154] for ozonation and by Paul et al. [42] for UVA photocatalysis. De Witte et al.[154] showed that residual antibacterial activity of ciprofloxacin after ozonation towards *E. coli* and *P. Fluorescens* to be mainly determined by the target compound degradation rate. Paul et al. [42] showed that antibacterial activity correlated well with the residual ciprofloxacin concentration and antibacterial activity of reaction products to appear to be insignificant against *E. coli* during UVA photocatalysis.

5.6 Conclusion

Comparison of performances of ozonation and photocatalysis under UVC radiation for removal of LEVO was reported for the first time. Both methods ensured removal of the target compound below the detection limit (200 µg/L). A transferred ozone dose of 20.5 mg/L was found to completely remove LEVO corresponding to a half-life time of only 6 seconds. Only 35% of LEVO was removed by direct photolysis after 120 minutes of UVC radiation (254nm). The removal of LEVO was enhanced in presence of TiO_2, suggesting that the oxidizing species generated during photocatalysis are effective towards oxidation of LEVO. At 120 minutes of irradiation 97% of LEVO was removed and it was no longer detected at 180 minutes of irradiation. Ozonation products detected by HPLC were shown to be more persistent than the products observed during photocatalysis as indicated by the products HPLC peak area profiles and the respective COD removals obtained. Hydroxyl radicals were shown to play a significant role in the removal of LEVO during photocatalysis but to have a minor influence of removal of LEVO during ozonation. The hydroxyl radicals were also shown to play a significant role in removing the transformation products generated during each treatment. This observation was also supported by the COD removal results obtained for a two-step process combining ozone and photocatalytic treatments during which the

persistent ozonation products were removed during the second step treatment by photocatalysis.

Antibacterial activity was removed completely both by ozonation and photocatalysis indicating no formation of transformation products having antibacterial properties. These results indicate that both ozonation and photocatalysis are effective in removing LEVO however, COD results indicate that photocatalysis seems to have a higher mineralization efficiency. The results presented here provide strong evidence that removal of LEVO and its antibacterial activity in pure water is possible by ozonation and photocatalysis. Further investigation on removal of this compound in more complex matrices is necessary to assess the applicability of ozonation and photocatalysis as potential pre-treatment methods of hospital or industrial wastewaters, prior to their discharge into sewage systems and to evaluate the residual toxicity associated with transformation products. This approach would also help minimize the risk of increasing antibacterial resistance of microorganisms found in biological treatment systems. Also, since UVC radiation is commonly used as a disinfection method in various treatment facilities, this research also allows estimating the UV doses that would be required for elimination of antibacterial activity of pharmaceuticals during disinfection and confirm the possibility of enhanced benefits when coupled with TiO_2 photocatalysis.

5.7 Acknowledgements

Authors would like to acknowledge the Natural Sciences and Engineering Research Council of Canada (NSERC) and Eugenie Ulmer Lamothe Chemical Engineering Fund (McGill University) for the financial support provided for this work.

5.8 Supplementary Material

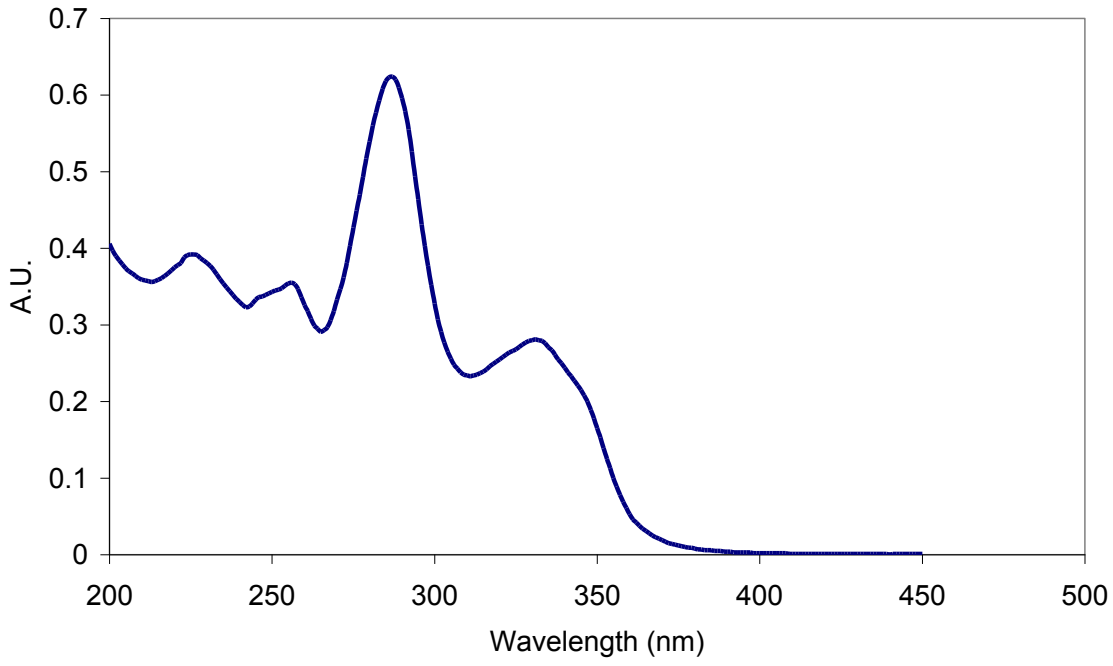

Figure 5.10- UV-Vis absorption spectra of LEVO (C_o = 10 mg/L) showing that the maximum absorption is around 290 nm

6. PHOTOCATALYTIC REMOVAL OF 17α-ETHINYLESTRADIOL (EE2) AND LEVONORGESTREL (LNG) FROM CONTRACEPTIVE PILL MANUFACTURING PLANT WASTEWATER UNDER UVC RADIATION

6.1 Preface

The major objective of thesis was to show that photocatalysis can be applied for removal of two major classes of pharmaceuticals antibiotics and hormones from water. In the first two manuscripts the applicability of photocatalysis and photolysis towards the removal of two antibiotics was investigated. In this manuscript the performance of photocatalysis towards removal of two hormones 17α-ethinylestradiol (EE2) and levonorgestrel (LNG) is presented. These two compounds are chosen since they are used in combination in most oral contraceptive formulations. EE2 is commonly detected in wastewater effluents and in surface waters. The endocrine disrupting effects of EE2 is frequently reported and is a major concern for reproduction of aquatic organisms at environmentally relevant concentrations. Little information on occurrence of the progestin LNG is reported and currently not much information on its environmental effects is available.

Another major objective of this thesis was to investigate the performance of photocatalysis in real wastewater samples from a pharmaceutical manufacturing plant. Investigating the removal of target compounds in industrial wastewater allows a more realistic evaluation of the applicability of this advanced oxidation process. In order to evaluate the effect of wastewater constituents on removal of EE2 and LNG during photolysis and photocatalysis, experiments were carried out as single contaminants, in mixtures as co-contaminants, in simulated synthetic wastewater consisting of major components of the real wastewater and finally in the real wastewater sample. This manuscript provides for the first time in literature quantification of hormones in a real wastewater obtained from the first wash of vessels used to produce contraceptive pill. Significantly high concentrations of EE2 and LNG well above their solubility limits (in

suspension) were detected in the wastewater also a coloring agent, tartrazine (TART) was found in the wastewater. The total organic carbon (TOC) content of the wastewater was determined to be approximately 30,000 mg/L, this high value was attributed to other unknown organic species present in the wastewater and mineralization efficiency measurements were omitted since changes in TOC would not be easily monitored. Other objectives such as effect of TiO_2 and dissolved oxygen concentration were also omitted due to time constraints. Additional objectives of effect of light availability in complex matrices on removal of EE2 and LNG and applicability of photocatalysis towards color removal were studied instead.

Individual photo-removal experiments showed that since LNG absorbs significantly in the UVC range compared to EE2, its direct photolytic removal was significantly more pronounced. Scavenging of hydroxyl radicals during photocatalytic treatment of EE2 and LNG as individual contaminants showed that for both compounds hydroxyl radicals play major roles in their photocatalytic removals. The major hypothesis that as the reaction medium got more complex, photolytic removal rates and efficiencies would be diminished was verified. Also during photocatalysis in complex matrices, competition for hydroxyl radicals by the presence of other organic species retarded removals, especially for LNG, since its aqueous phase solubility was significantly lower than EE2 and it was shown to experience competition at a higher level. Simultaneous removal of EE2 and LNG in the real wastewater during photocatalysis was observed verifying that even in such a complex reaction medium, this type of advanced oxidation process is capable of reducing hormone content and conditions can be further optimized to satisfactorily apply photocatalysis as a pre-treatment system in similar pharmaceutical manufacturing plants instead of incinerating their waste.

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plant wastewater under UVC radiation, Chemical Engineering Journal, 185 – 186, 52 - 60.
(doi: 10.1016/j.cej.2012.01.012)

Photocatalytic removal of 17α-ethinylestradiol (EE2) and levonorgestrel (LNG) from contraceptive pill manufacturing plant wastewater under UVC radiation

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6.2 Abstract

Photolytic and photocatalytic removals of 17α-ethinylestradiol (EE2) and levonorgestrel (LNG) in pharmaceutical wastewater were investigated under UVC radiation. Wastewater collected from WYETH, St-Laurent, Canada contained high concentrations of EE2 and LNG in suspension and coloring agent tartrazine in solution. Aqueous phase removals of EE2 and LNG were studied as individual contaminants in water and in complex matrices including: co-contaminants in water, in simulated synthetic wastewater and in the wastewater. After 30 minutes of UVC photocatalysis of the individual contaminants, removal efficiencies of EE2 and LNG were 92% and 97%, respectively, while higher photolytic removal was observed for LNG (94%) compared to EE2 (60%). Hydroxyl radicals were shown to contribute significantly to the removal of both compounds in water. In contrast to EE2, photolytic removal of LNG was higher than its photocatalytic removal efficiencies in all complex matrices. Higher photolytic removal of LNG was attributed to the fact that it absorbs UVC radiation considerably more than EE2. Lower photocatalytic removals of LNG in complex matrices compared to its photocatalytic removal as an individual contaminant was due to the presence of EE2 at concentrations up to five times larger than LNG in water, thus leading to increased competition for hydroxyl radicals and retarding LNG removal. In the wastewater matrix photocatalytic removals for EE2 and LNG were similar at 48%,

whereas the photolytic removal of LNG (76%) was higher than EE2 (29%). The applicability of UVC processes for reduction of hormone content in similar wastewaters was demonstrated.

Key words: 17α-ethinylestradiol, levonorgestrel, photocatalysis, photolysis, pharmaceutical wastewater

6.3 Introduction

The occurrence of natural and synthetic chemicals in the aquatic environment has been reported regularly in the recent years [160, 208] and the observed adverse effects of these compounds on human and aquatic wildlife by interfering with the endocrine system is now an issue of global concern [74, 75]. These compounds are referred to as endocrine disrupting chemicals (EDCs). The U.S. Environmental Protection Agency (EPA) defines environmental EDCs as xenobiotics that interfere with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour [209]. Common EDCs include natural estrogens such as estrone (E1), 17β-estradiol (E2) and estriol (E3), synthetic estrogens such as 17α-ethinylestradiol (EE2) and industrial chemicals such as bisphenol A, DDT, alkylphenols, PCBs and phtalic esters.

A major source of aquatic contamination by EDCs is the effluent of sewage treatment plants (STPs) associated with domestic and hospital wastewater as well as with manufacturing plant wastewater. Estrogenic hormones have been commonly detected in the effluents of STPs, surface waters and even in treated drinking waters [158-160, 210]. Among these, EE2 is a synthetic estrogen widely used in oral contraceptives and hormone replacement therapy. A whole lake addition study performed in Canada showed that spiking a lake with EE2 concentrations of 5-6 ng/L resulted in extinction of whole fish populations [211]. EE2, even at ng/L concentration levels, was also shown to induce the expression of vitellogenin in male fish, cause sex differentiation, and lead to the reduction in fish fertility [162, 163]. LNG is a synthetic

progestogen (i.e. progestin), used either alone or in combination with EE2 in a variety of hormonal formulations [173, 176]. Occurrence of progestins only recently received attention, and currently there are very few data on the presence of these compounds in aquatic environments [177, 178]. LNG was found to affect male fertility and exposure to high levels of LNG (500 μg/day over 10 weeks) was shown to lead to azoospermia (no measurable sperm in semen) by Bebb et al [179]. Due to the undesirable affects of EDCs on the environment and low removal in wastewater treatment plants, more effective treatment methods are necessary to mitigate their impact on the environment and public health.

Advanced oxidation processes (AOPs) have been recently investigated as complementary or alternative methods to conventional wastewater treatment. AOPs such as ozonation [165, 166, 168], fenton and photo-fenton oxidation [169, 170], photocatalysis and photolysis [26, 29, 30, 32, 34-36, 45-47, 172] have been investigated as effective methods for elimination of estrogenic activity of EDCs such as EE2. Few photo-removal studies for EE2 were performed in complex matrices as mixtures of estrogens [32], with co-pollutants in complex water matrices [29, 30] and in natural waters [172]. Generally there is lack of data on the photo-removal of EE2 in a wastewater matrix, especially no information on removal of mixture estrogens and progestins in manufacturing plant wastewater is available. Additionally, there are no reported data on the photolytic or photocatalytic removal of LNG.

Based on the previously mentioned gap of knowledge, the objective of this work was to evaluate the applicability of UVC photolysis and photocatalysis to remove simultaneously EE2 and LNG in pure water matrix, and more importantly in a complex wastewater matrix produced at a pharmaceutical processing plant. The wastewater selected for this study was the highly concentrated wastewater generated from the oral contraceptive production facilities of WYETH, St-Laurent production plant. The current method of disposal of the first wash of the mixing vessels used for production is segregation followed by incineration. Incineration of large quantities of diluted aqueous solutions is costly. Therefore studying the applicability of a photolytic or a photocatalytic process as a wastewater treatment alternative is necessary to help reduce (or completely remove) the concentrations of the hormonally active compounds from the

first wash, which might then be safely mixed with the rest of the wastewater generated at the plant.

6.4 Materials and Methods

6.4.1 Reagents

17α-ethinylestradiol (EE2, $C_{20}H_{24}O_2$, ≥ 98%), 17β-estradiol (E2, $C_{18}H_{24}O_2$ ≥ 98%), levonorgestrel (LNG, $C_{21}H_{28}O_2$, ≥ 99%), 19-norethindrone (NOR, $C_{20}H_{26}O_2$ ≥ 99%), tartrazine (TART, $C_{16}H_9N_4Na_3O_9S_2$, ≥ 98%) and ammonium acetate (≥ 98%) were obtained from Sigma–Aldrich, Canada. Commercial TiO_2 Degussa P25 (70% anatase and 30% rutile) was used as catalyst with an average particle size of 30 nm and a BET surface area of 50 $m^2 g^{-1}$, according to the manufacturer. HPLC grade methanol, acetonitrile, chloroform and isopropanol as well as KOH pellets were purchased from Fisher Scientific, Canada. 95 % ethanol was obtained from (Commercial Alcohols Inc, Boucherville). All the chemicals were used as received without purification.

6.4.2 Wastewater collection and quantification of pharmaceutical compounds

Industrial wastewater samples were collected at the WYETH production plant located in St-Laurent, Canada on November 11th, 2008 by taking a grab sample (10 L) from the first wash of the vessels used for production of oral contraceptive pills. The sample was collected in amber bottles and frozen within two hours following sampling. The wastewater was stored at -30°C until the time of treatment (12 – 18 months). The only information disclosed by the company about the constituents used in the production of the pills was the presence of EE2, LNG and dye FD&C YELLOW 5 LAKE 960 (i.e. tartrazine). Visual observations indicated that a considerable amount of material was in suspension. The low solubility of EE2 and LNG suggests that a significant portion of the hormones were in suspension.

Aqueous phase EE2 and LNG concentration in the wastewater was determined by HPLC analysis of syringe filtered wastewater samples. In order to quantify the total hormone concentration (in suspension and in solution), a chloroform extraction method was developed. The optimum volumetric ratio of sample to chloroform was determined by sequentially adding 50 ml chloroform to a 100 ml of wastewater sample in a 250 ml

separatory funnel. After each 50 ml chloroform addition, UV-Vis absorbance spectrum of the organic portion was determined (Thermo Scientific Evolution 300). The optimum ratio was determined when negligible UV absorption in the 200 – 400 nm range was measured. This value was determined to be 5:1 (chloroform volume : sample volume). For the determination of extraction recoveries of EE2 and LNG, E2 and NOR were chosen as internal standards, respectively. In order to validate the extraction method and choice of internal standards, 250 ml pure reverse osmosis (RO) water samples were spiked with EE2, E2, NOR and LNG stock solutions in methanol at concentrations of 10 mg/L for each compound. Six 10 ml samples were extracted with 50 ml of chloroform in a 125 ml separatory funnel. 4 ml samples from the organic phase were withdrawn, chloroform was evaporated from the extracted samples by Thermo Scientific Savant SPD 131 DDA Speedvac Concentrator equipped with RVT 4104 refrigerated vapor trap, and the samples were reconstituted in 4 ml methanol to be analyzed by HPLC. The extraction recoveries of internal standards and respective pharmaceuticals of interest were shown to be satisfactorily close (Table 6.1), validating the extraction method and choice of internal standards. The described extraction method was applied to wastewater for the determination of the total concentrations of EE2 and LNG in the wastewater (as well as their total concentrations in pure suspensions). The concentration of coloring agent in the wastewater, tartrazine (TART) was quantified by UV-Vis absorbance at 428 nm. Since TART is strongly hydrophilic, transfer of this compound to the organic phase during extraction was not a concern. The aqueous portion of the wastewater sample was directly compared to standards of TART in RO water to quantify its concentration.

Table 6.1- Extraction recoveries of compounds of interest and their respective internal standards

Extraction recoveries (%)			
E2	**EE2**	**NOR**	**LNG**
97 ± 4	98 ± 4	101 ± 5	102 ± 5

6.4.3 Preparation of hormone stock solutions and types of matrices

Stock solutions of EE2, E2, LNG and NOR (1000 mg/L) were prepared in methanol. EE2 and LNG stock solutions in methanol were used to prepare HPLC standards and solutions in RO water. Stock solutions of E2 and NOR, used as

performance surrogates, were used to spike samples prior to chloroform extraction. All solutions were kept at 4°C in the dark until the time of analysis (maximum time of storage was one week).

Photolytic and photocatalytic removal of EE2 and LNG were studied in four different matrices: 1) individual contaminants in RO water (**Pure**), 2) co-contaminants in RO water (**MIX**), 3) multi-component mixture in tap water along with TART to obtain a simplified synthetic wastewater (**SWW**) and 4) the real wastewater from WYETH (**WW**). In order to compare the removal of EE2 and LNG in the four matrices studied, similar initial concentrations of compounds in all matrices were required. The total concentration of LNG in the WW was found to be above 500 mg/L. Therefore, to ease the analysis and to reduce reaction times and the significantly high costs associated with hormonal compounds at the relevant wastewater concentrations, the degradation experiments were performed using 10 times diluted wastewater (first wash) and corresponding total concentrations in the other matrices (EE2 ~ 5 mg/L and LNG ~ 50 mg/L). The concentrations of LNG and EE2 in the matrices studied are tabulated in Table 6.2. The working volumes were stirred overnight and sonicated for 30 minutes prior to treatment in order to obtain a well-mixed suspension and avoid agglomeration.

6.4.4 Photolysis and photocatalysis setup

Irradiation experiments were carried out in 2-L cylindrical water-cooled jacketed pyrex photoreactor (215 mm height, 108 mm diameter). The reactor walls were covered with aluminum foil to avoid exposure to UV radiation. 1.6 L of working solution was charged to the reactor for each experiment. The solution was irradiated by an Hg-Ar (Germicidal UV-C) lamp (Atlantic Ultraviolet Corp. GPH212T5L) located in the center of the reactor and protected in a quartz sleeve (maximum output at 254 nm). It was previously shown that light intensity inside the reactor highly varied by position and the maximum intensity of incident radiation per unit volume was $1.3 \times 10^{-3} \pm 0.3$ Einstein min^{-1} L^{-1} [40]. Mixing was achieved by magnetic stirring, and oxygen was supplied via bubbling air through a sparger located at the bottom of the reactor. The concentration of TiO_2 was fixed at 0.2 g/L for all photocatalytic experiments. For photocatalytic experiments, TiO_2 suspensions were sonicated for 30 minutes prior to addition to the

reaction mixture to avoid agglomeration and subsequent reduction in active surface area. The initial pH for all the matrices studied is provided in Table 2. EE2 and LNG have high pKa values of 10.4 and 19.3, respectively [212]. At the studied ambient pH values they are not charged therefore slight changes in pH is expected not to affect their removal.

It was found that both photolytic and photocatalytic removal of EE2 and LNG in each matrix followed pseudo-first order reaction kinetics in the first ten minutes of removal. Therefore the rate constants where determined from

$$\ln(C / C_o) = -k\,t \qquad (1)$$

where, C is the concentration of compound of interest (mg L^{-1}), C_o is initial concentration (mg L^{-1}), t is time (min) and k is the pseudo-first order reaction rate constant (min^{-1}). Pseudo-first order reaction rate constants were calculated from the slope of the plots of -ln (C/C_o) against time.

Adsorption and dark control experiments were performed in triplicates to make sure that removal of compounds was not due to adsorption of compounds to either TiO_2 particles or to the walls of the reactor. Suspensions of EE2 (5 mg/L) and LNG (50 mg/L) as well as aqueous solutions of TART (0.7 mg/L) were tested for adsorption onto TiO_2 at a fixed concentration of 0.2 g/L. Erlenmeyer flasks were filled with TiO_2 and the compounds of interest were placed inside an incubator shaker set at 25 °C. After 24 h, samples were analyzed by HPLC and compared to the controls containing only the compounds. Dark control experiments for all compounds were performed inside the reactor with the lamp turned off. Samples were taken over a period of 2 hours and analyzed for EE2 and LNG content to see if any removal was due to sampling or normal operation of the reactor. Samples were analyzed for concentrations of LNG and EE2, both as total and in solution.

Evidence for hydroxyl radical participation in removal of both EE2 and LNG in aqueous phase was evaluated by scavenging experiments. The method of scavenging and the concentrations of scavenging compounds were based on the results reported by Palominos et al [41]. Isopropanol has been described as one of the best hydroxyl radical quencher due to its high reaction rate constant with the radical (1.9×10^9 mol L^{-1} s^{-1}) [201]. In this work, scavenging of hydroxyl radicals was achieved by adding isopropanol

to the reaction mixture at a molar concentration which was three orders of magnitude larger than the initial total molar concentration of EE2 and LNG.

6.4.5 Light intensity determination and fraction of light absorbed

In order to quantify the intensity of incident radiation, azoxybenzene was used as a chemical actinometer. The method was modified from the technique developed by Bunce et al [202]. The detailed description of the actinometric method employed here can be found in a previous study [40]. All the actinometric experiments were performed at a single location in the reactor (radial distance of 2 cm away from the lamp, at height of 10 cm from the bottom of the reactor). Maximum available light intensity (I_{max}) was determined from the amount of light absorbed by the actinometric solution when the reaction system is only composed of pure RO water (no hormones etc.) The actinometric solution was irradiated in each matrix (i.e. pure EE2, pure LNG, MIX, SWW and WW) and the associated light intensity was measured (I_a). The differences between I_{max} and I_a were normalized by I_{max} ($[I_{max} - I_a] / I_{max}$) to give the respective fractions of light absorbed by the additional constituents of the matrices studied.

6.4.6 Analytical methods

Prior to analysis, samples were filtered using 0.22 μm syringe filters. EE2 and LNG concentrations were monitored by a HPLC system (Agilent 1200 series) equipped with a Zorbax Eclipse Plus C-8 (Agilent, 4.6 x 150 mm, 3.5 microns) and two detectors (fluorescence and diode array) with an injection volume of 20 μL. Starting from initial conditions of acetonitrile/5mM ammonium acetate 30/70 (v/v), the mobile-phase gradient linearly increased to 60/40 (v/v) over 20 minutes. The flow rate was set at 0.8 ml/min. The same HPLC method was used for samples in water and in methanol. The excitation and emission wavelengths of the fluorescence detector were respectively set at 280 nm and 310 nm to quantify EE2 concentration. For the LNG concentration the diode array detector was set at a wavelength of 244 nm. Chemical oxygen demand (COD) of the wastewater sample was measured by using a HACH Digital Reactor Block (DRB 200), a HACH spectrophotometer (DR/2500) and low range (3 – 150 mg/L) COD

digestion vials (HACH). Total organic content (TOC) determination of the wastewater was achieved via Shimadzu TOC-VCPH total organic carbon analyzer.

6.5 Results and Discussion

6.5.1 Quantification of the pharmaceuticals in the wastewater

The method of determining the required volumetric ratio of chloroform to sample was described in Section 6.4.2. The evolution of UV-Vis chromatograms associated to the described procedure is presented in Figure 6.7 (Supplementary Figure). The volumetric ratio of sample to chloroform was determined to be 1:5 to maximise the recovery and obtain an accurate measurement of the initial concentration of EE2 and LNG in the wastewater.

The total (suspended and dissolved) concentrations of EE2 and LNG in the wastewater were determined to be 55.1 ± 7.5 and 567 ± 25 mg/L, respectively. The concentration of the coloring agent, TART, in the wastewater was 7.2 ± 0.5 mg/L by UV-Vis absorption analysis at 428 nm. COD and TOC measurements were made in a 1000 times diluted version of the wastewater due to its highly concentrated state. Initial COD and TOC values were 78600 ± 1600 mg/L and 28600 ± 7100 mg/L, respectively.

The characterization results suggest that the wastewater sample obtained from WYETH is highly concentrated both in hormones and other undisclosed constituents. The high COD and TOC values can be attributed to the presence of organic compounds used in the preparation of the contraceptive pills that also end up in the first wash of the production vessels. As mentioned previously the degradation experiments were performed using 10 times diluted wastewater (first wash), the resulting corresponding total and aqueous concentrations are tabulated in Table 6.2. All adsorption and degradation experiments were performed at these initial total and respective aqueous phase concentrations. Where applicable the total concentration refers to the total hormone content including both in suspension and in solution, as measured after chloroform extraction (described in Section 6.4.2).

Table 6.2- Concentration of EE2 and LNG in the matrices studied

Type of Matrices	Total Conc. (mg/L)		Aqueous Conc. (mg/L)	
	EE2	LNG	EE2	LNG
Pure EE2	~ 5	-	3.5 - 5	-
Pure LNG	-	~ 50	-	0.8 - 1.6
MIX	~ 5	~ 50	3.5 - 5	0.8 - 1.6
SWW [a]	~ 5	~ 50	3.5 - 5	0.8 - 1.6
WW [b]	~ 5	~ 50	1.6 - 2.4	1.5 - 2.5

[a] *In tap water, contains TART at 0.7 mg/L*

[b] *Wastewater diluted with tap water contains TART at 0.7 mg/L and other unknown organic material*

6.5.2 Adsorption and control experiments

After 24 hours of mixing, adsorption of 14.8 ± 4.7%, 2.1 ± 0.5% and 4.4 ± 1.2% were observed for TART, EE2 and LNG, respectively. The total concentrations of EE2 and LNG obtained from experiments performed in the reactor in the absence of light, with or without TiO_2, are shown in Figure 6.1. No measurable loss of EE2 was observed suggesting that loss due to adsorption to the reactor wall is negligible (Figure 6.1). Although the LNG soluble concentration did not change (results not shown), the total concentration of LNG obtained by extraction was highly variable (Figure 6.1b) and some of the concentrations measured were even higher than the initial total LNG concentration. These unexpected results are due to the non-homogeneity of the solution in terms of LNG concentration because of its high concentration in solids. Deposition of suspended solids was also visually confirmed from the formation of a deposit on the quartz sleeve and the sides of the pyrex reactor. Because of these limitations, the LNG concentration was monitored only in the aqueous phase, which was stable throughout the experiments.

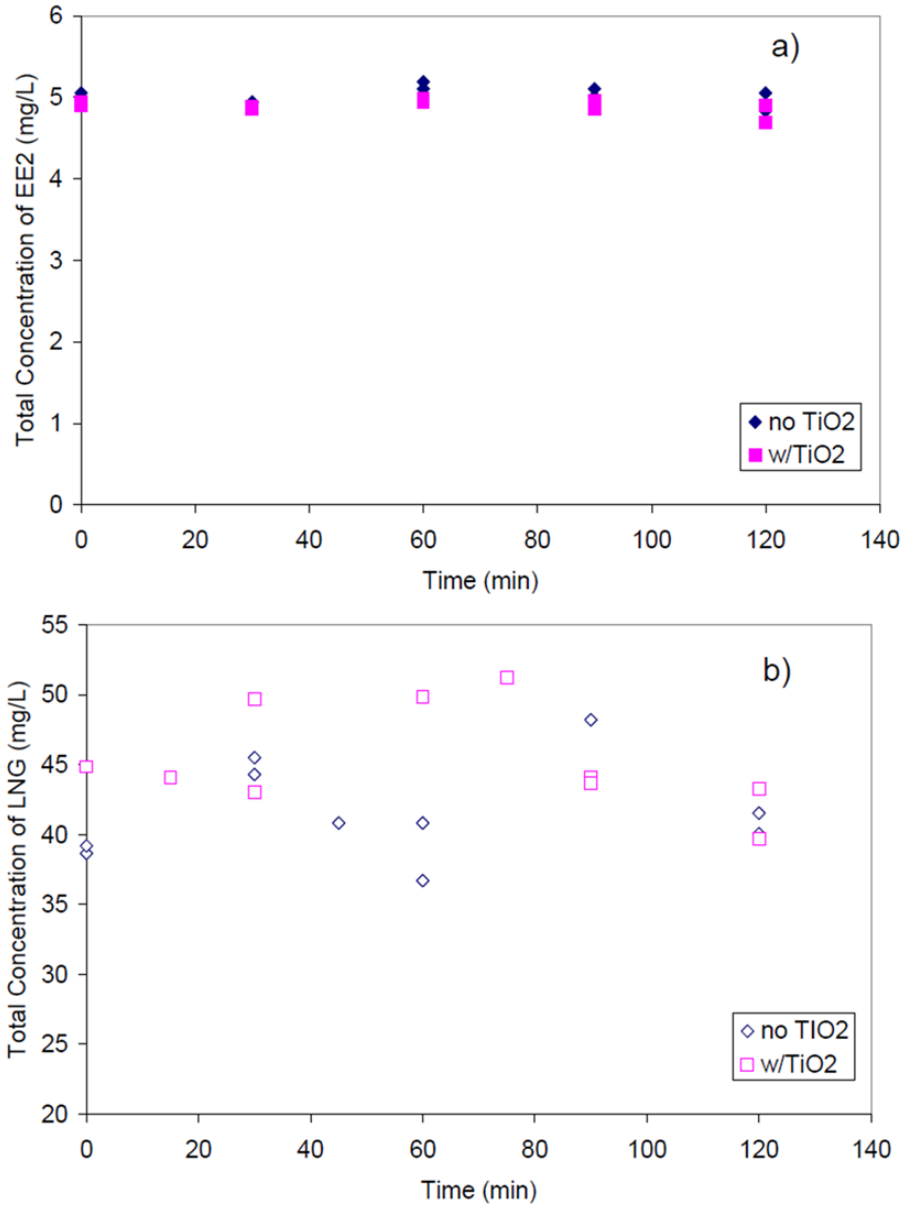

Figure 6.1- Evolution of total concentration of a) EE2 and b) LNG under dark conditions in the reactor in a pure RO water matrix

6.5.3 Photolytic and photocatalytic removal of EE2, LNG and TART as pure compounds in RO water

The removal data of TART in RO water is presented in Figure 6.2a. Photocatalytic removal of TART is considerably higher than its photolytic removal. Complete elimination of color was achieved during photocatalytic treatment after 45

minutes while only 3% was removed by photolysis. A recently published study by Gupta et al [213] also confirms the slower removal rate under photolytic conditions. In the work of Gupta et al [213], the pseudo-first order reaction rate constant for photocatalysis at 0.2 g/L TiO_2 concentration was estimated to be approximately 4.5 x 10^{-2} min^{-1} compared to 7.8 ± 1.7 x 10^{-2} min^{-1} (± 1 standard deviation) calculated in the work presented here. Even though the pseudo-first order reaction rate constant values are of the same order of magnitude, the discrepancy can be associated to various factors such as: the initial concentration of TART used by Gupta et al [213] was two orders of magnitude higher than what was used here, the photocatalyst used was pure anatase TiO_2 instead of Degussa P25 (anatase/rutile) powder and a smaller reactor was employed by Gupta et al. [213] (150 ml), leading to differences in light intensity distribution.

The removal data of EE2 as in RO water is presented in Figure 6.2b (aqueous). EE2 removal is considerably enhanced in the presence of TiO_2. After 30 minutes of irradiation, 92 ± 7 % of initial EE2 in the aqueous phase is removed during photocatalysis compared to 60 ± 12 % photolytic removal. The work by Liu et al. [34] for the photolytic removal under UVC radiation of EE2 resulted in 50 % removal over 30 minutes which is in accordance with the value presented here. From another study by Mazellier et al [36], 40 % photolytic removal over 30 minutes was calculated from their reported data. This slightly lower removal can be attributed to the fact their working volume (4L) was more than twice the working volume used in this work. Higher reactor volume would lead to larger variations in light distribution inside the reactor, resulting in decrease in light availability and thus inefficient removal. A more recent study, investigating the removal of estrogenic compounds in multi-component estrogen mixtures by Li Puma et al [32], also confirmed faster removal of EE2 during photocatalysis than photolysis. They obtained a value of 86 % photocatalytic removal of EE2 in a mixture of E1, E2 and E3 over 30 minutes, which lies within the removal efficiency reported here. However, they obtained a far lower removal efficiency of about 22 % of EE2 under photolysis over 30 minutes compared to the photolytic removal presented here. The slower EE2 removal is most likely due to the presence of other estrogens in the multi-component mixture leading to enhanced competition and reducing

light availability. The removal trend of total EE2 was shown to closely follow the trend observed for aqueous removal. Similar pseudo-first order reaction rate constants are calculated for total and aqueous phase removal of EE2 during both treatments (Table 6.3). This suggests that the system is mass-transfer limited, i.e. the removal rates in the aqueous phase are higher than the dissolution rate of EE2 for both photolysis and photocatalysis.

Preliminary experiments indicated that the total amount of LNG removed during treatment lies in the range of the error of measurement of the total concentration of LNG. Considering this limitation, only aqueous phase degradation of LNG was considered here. Removal data of LNG in RO water are presented in Figure 6.2c. Similarly to EE2, removal of LNG during photocatalytic treatment was faster than during photolysis. Over 10 minutes of irradiation, 93 ± 2 % of initial LNG present in solution is removed during photocatalysis compared to 82 ± 4 % photolytic removal. However, in both cases LNG is no longer detected after 30 minutes. The fact that more than 80 % of LNG removed within 10 minutes compared to about 25 % removal observed for EE2 for the same time period strongly suggests that LNG is far more sensitive to UVC radiation than EE2. Molar absorption coefficients (ε) at 254 nm of EE2 and LNG (ε_{EE2} = 216 ± 40 L mol^{-1} cm^{-1}, ε_{LNG} = 8617 ± 210 L mol^{-1} cm^{-1}) calculated from UV-Vis spectra shown for both compounds in Figure 6.8 (Supplementary Figure) also support this observation. Additionally, the inhibition of photocatalytic removal for both compounds was observed when hydroxyl radicals were scavenged (Figure 6.2b and c). After 30 minutes of irradiation, 62% and 88% reduction in photocatalytic removal efficiencies for EE2 and LNG, respectively. This observation suggests that the hydroxyl radicals contribute strongly towards the degradation of compounds during photocatalysis.

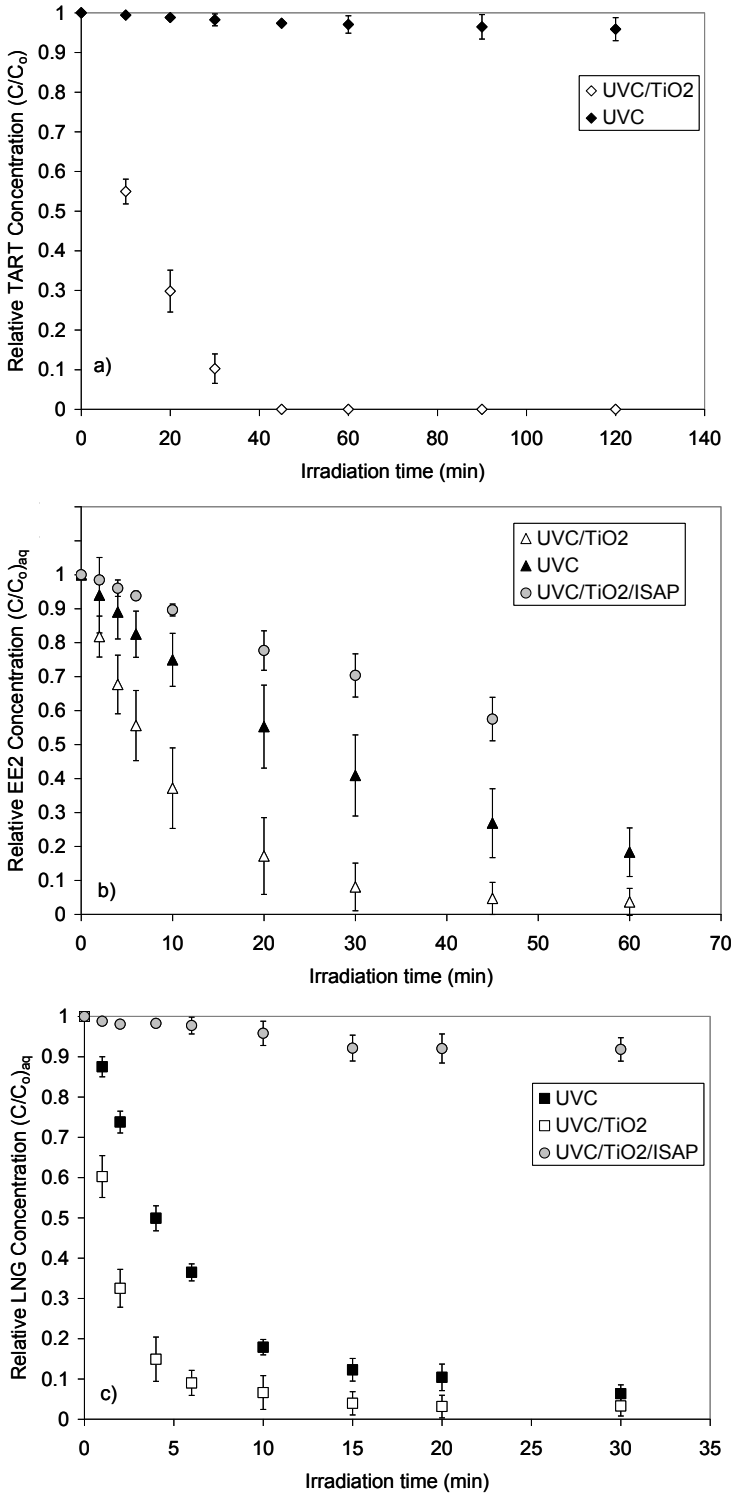

Figure 6. 2- Evolution of relative concentration of a) TART b) EE2 and c) LNG during photolysis (UVC) and photocatalysis (UVC/TiO_2) in pure RO water matrix (based on aqueous concentrations). Effect of the presence of hydroxyl radicals during photocatalysis on removal of b) EE2 and c) LNG is also studied by scavenging the hydroxyl radicals by addition of isopropanol. Errors bars = ± 1 standard deviation

Table 6.3- Pseudo-first order reaction rate constants of EE2 based on total and aqueous concentration for UVC photolysis and photocatalysis

	Pseudo first order reaction rate constant , k x 10^{-2} (min^{-1})	
Treatment	**Based on aqueous concentration**	**Based on total concentration**
UVC	3.0 ± 1.3	2.8 ± 1.1
UVC/TiO2	10.6 ± 5.6	9.5 ± 3.2

6.5.4 Removal of EE2 and LNG in complex matrices

Figure 6.3, shows the fractions of light absorbed by each compound and by each matrix. Compared to LNG and TART, the presence of EE2 did not lead to any measurable decrease in the available light, suggesting that EE2 has minimal absorption of radiation at 254 nm (also confirmed from UV-Vis spectrum for EE2 in Figure 6.8, Supplementary Figure). LNG and TART alone absorb 22 and 15 % of the max light available, respectively. The fact that WW matrix absorbs higher fraction of light when compared to that by SWW confirms the possibility of other unidentified species contributing to the light absorption and resulting decrease in light available for EE2 and LNG to undergo direct photolysis in complex WW matrix. When 0.2 g/L of TiO_2 is present in the system, 97 % of the light is absorbed. Thus, for the photocatalytic experiments the availability of light is already diminished and the contribution of photolysis to the removal of any compound during photocatalytic treatment can be considered minimal.

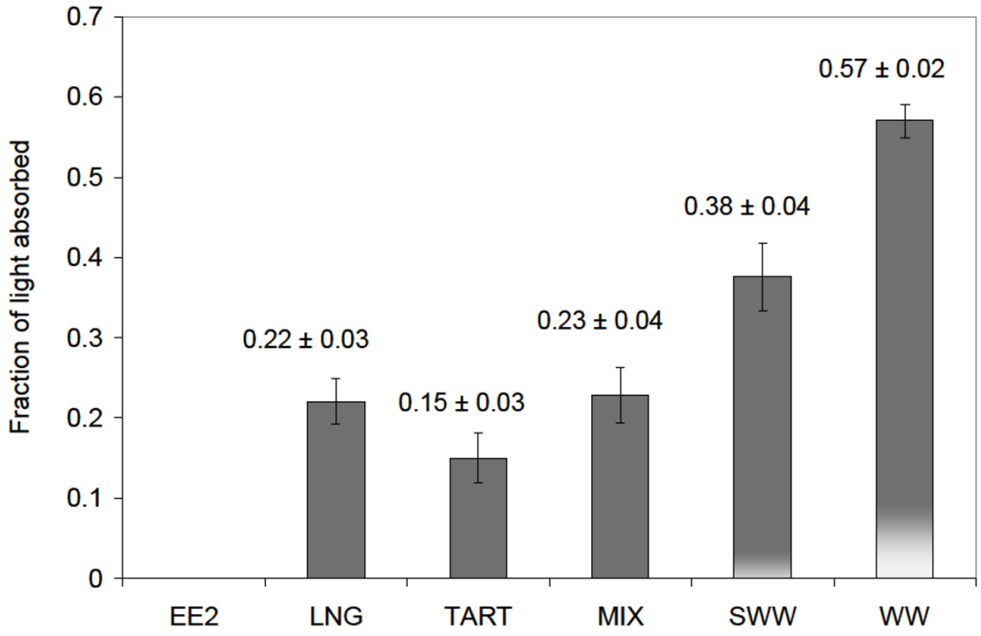

Figure 6.3- Fraction of light absorbed by each compound and by each matrix determined using azoxybenzene actinometry. Errors bars = ± 1 standard deviation

Figure 6.4 show the photolytic removals of EE2 (Figure 6.4a) and LNG (Figure 6.4b) in all three matrices (Pure, MIX and SWW). Pseudo-first order reaction rate constants calculated are tabulated in Table 6.4. As the complexity of the matrix increases (from Pure to SWW), the general trend for both compounds is that the removal efficiency decreases. At 30 minutes of irradiation, EE2 the photolytic removal efficiency was 60% as pure compound, where as this value is decreased to 41% and 21%, for MIX and SWW, respectively. For LNG, in pure and MIX systems photolytic removal efficiency after 30 minutes was measured to be 94%, however this value reduced to 87% for the SWW system. This reduction of removal efficiency and rate is expected, since presence of other organic species and suspended material can compete for the photons or lead to scattering of light both resulting in the reduction of available light for the compound of interest to go through direct photolysis.

In all three matrices photolytic removal efficiencies and rates are higher for LNG compared to EE2. For EE2, going from pure to MIX, about 40 % reduction in removal rate is observed, however for LNG the removal rates and efficiencies are not reduced in the MIX system. The presence of EE2 does not have an impact on the photolytic

removal of LNG, but presence of LNG has a large impact on EE2 removal. This behaviour can be attributed to two aspects. First, in the MIX, the total LNG concentration is 40 – 60 mg/L where as EE2 concentration is only about 5 mg/L. Since LNG is present at a considerably higher concentration than EE2, its presence will have a greater impact. Second and most importantly, LNG absorbs significantly higher than EE2 in the UVC range as observed from their respective UV-Vis spectra (Figure 6.8, Supplementary Figure) and from the fraction of light absorbed by pure LNG (Figure 6.3). The amount of light available for EE2 removal in pure is considerably reduced by the addition of a high concentration compound with high UVC absorption due to scattering and competition effects. High photolytic removal rate associated to LNG in pure systems is not hindered by the presence of EE2 due to its lower total concentration and considerably lower UVC absorption. The fact that the fraction of light absorbed by the MIX is almost entirely due to the presence of LNG also supports this observation. In all matrices, photolytic removal of LNG is slower after 30 minutes of irradiation compared to its initial removal rate. This can due to the accumulation of degradation products at prolonged irradiation resulting in enhanced absorption or scattering of light. Removal rates in the SWW are further reduced by 38% and 56% for EE2 and LNG, respectively compared to their removal rates in MIX. In SWW, the reduction in removal rate of LNG is more pronounced than that of EE2. As evidenced from Figure 6.3, an additional 15% of light is absorbed in the SWW compared to MIX due to the presence of TART. Since LNG is more sensitive to direct photolysis than EE2 under UVC radiation, reduction in light availability affect the photolytic removal of LNG more than it does EE2.

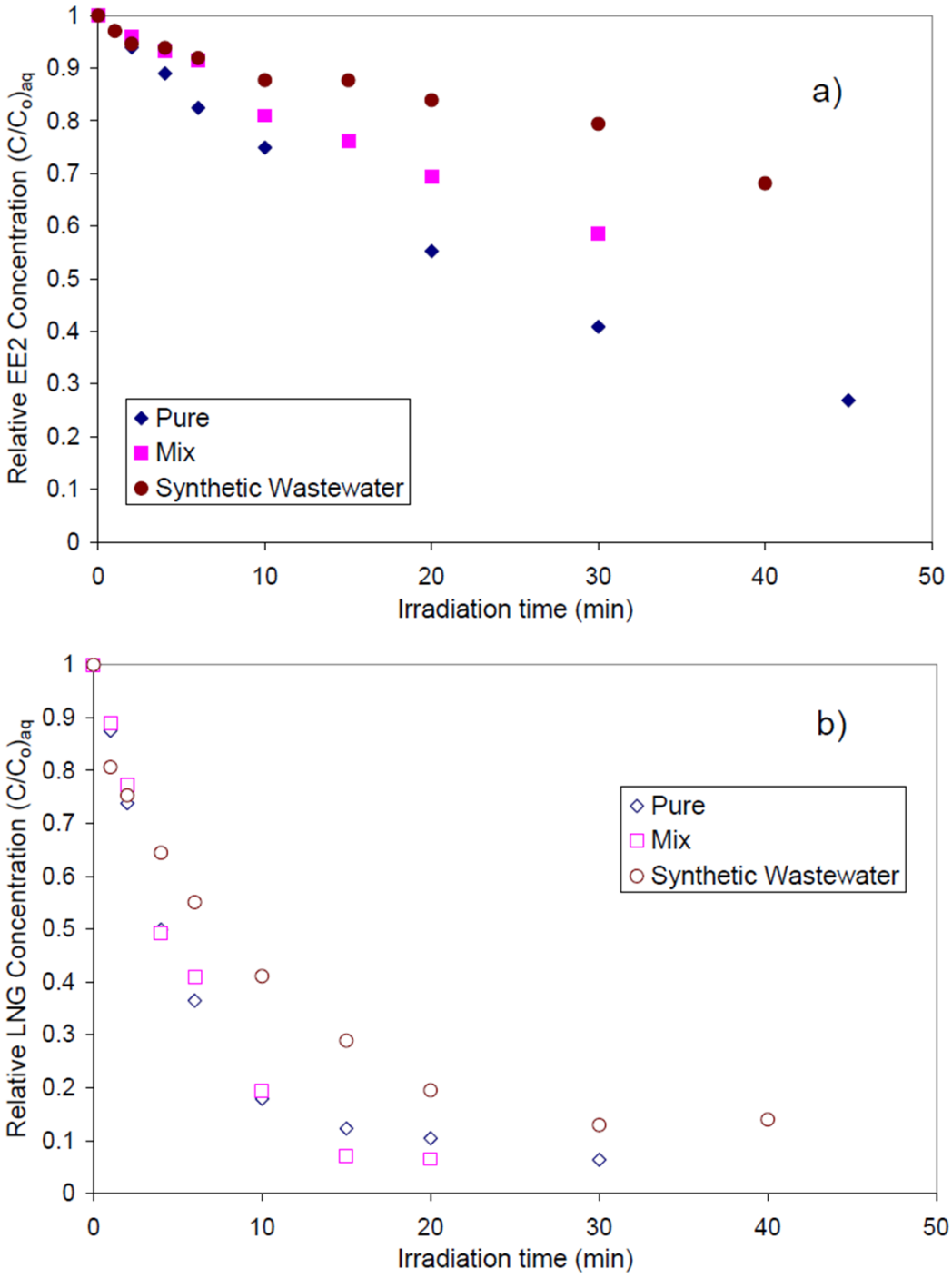

Figure 6.4- Evolution of relative EE2 (a) and relative LNG (b) concentration during photolysis (based on aqueous concentrations) in Pure, MIX and SWW systems

Figure 6.5 shows the photocatalytic removals of EE2 (Figure 6.5a) and LNG (Figure 6.5b) in three matrices (Pure, MIX and SWW). The calculated pseudo-first order

reaction rate constants are tabulated in Table 6.4. Similar to the trends observed for the photolytic removals of these compounds, their photocatalytic removals tend to decrease as the matrix gets more complex. At 30 minutes of irradiation, the photocatalytic removal efficiency of EE2 was 97% as pure compound, whereas this value is decreased to 92% and 85% for MIX and SWW, respectively. For pure LNG, photocatalytic removal efficiency after 30 minutes was measured to be 97%; however this value reduced to 71% and 47% for MIX and SWW, respectively. Hydroxyl radicals generated during photocatalysis have high oxidizing potential and they are considered to be less selective than a variety of oxidizing species. Therefore, overall reduction in removal efficiencies can be associated to the enhanced competition for hydroxyl radicals due to the presence of other organic species in more complex systems. This reduced photocatalytic removal of EE2 in a complex reaction medium was also reported by Karpova et al. [30] in presence of saccharose and urine. As mentioned earlier the total concentration of LNG (40 – 60 mg/L) is considerably larger than the total concentration of EE2 (5 mg/L); thus in the MIX system high reduction in removal rate would be expected for EE2 compared to the removal rate observed as pure compound. In contrast to our expectation, the photocatalytic removal rate of EE2 in the MIX system was not reduced whereas the LNG rate decreased considerably by 80% when compared to their respective pure compound photocatalytic removal rates. The explanation lies in the aqueous phase concentrations of the compounds. EE2 being more soluble than LNG, its aqueous concentration is up to five times higher than the LNG aqueous concentration (3.5-5 mg/L EE2 compared to 0.8-1.3 mg/L LNG). It is therefore more likely for hydroxyl radicals to encounter EE2 molecules than LNG. The slight increase in the aqueous concentration in the MIX due to the addition of LNG then had no considerable influence on the removal rate of EE2. However for LNG, in the MIX system the addition of a compound (EE2) at higher concentration induces more competition; thus the removal rate was extensively reduced. The previous observations also support the hypothesis that most of the photocatalytic reactions occur in the aqueous phase and that the suspended particles are not attacked by hydroxyl radicals within the irradiation time frame investigated here.

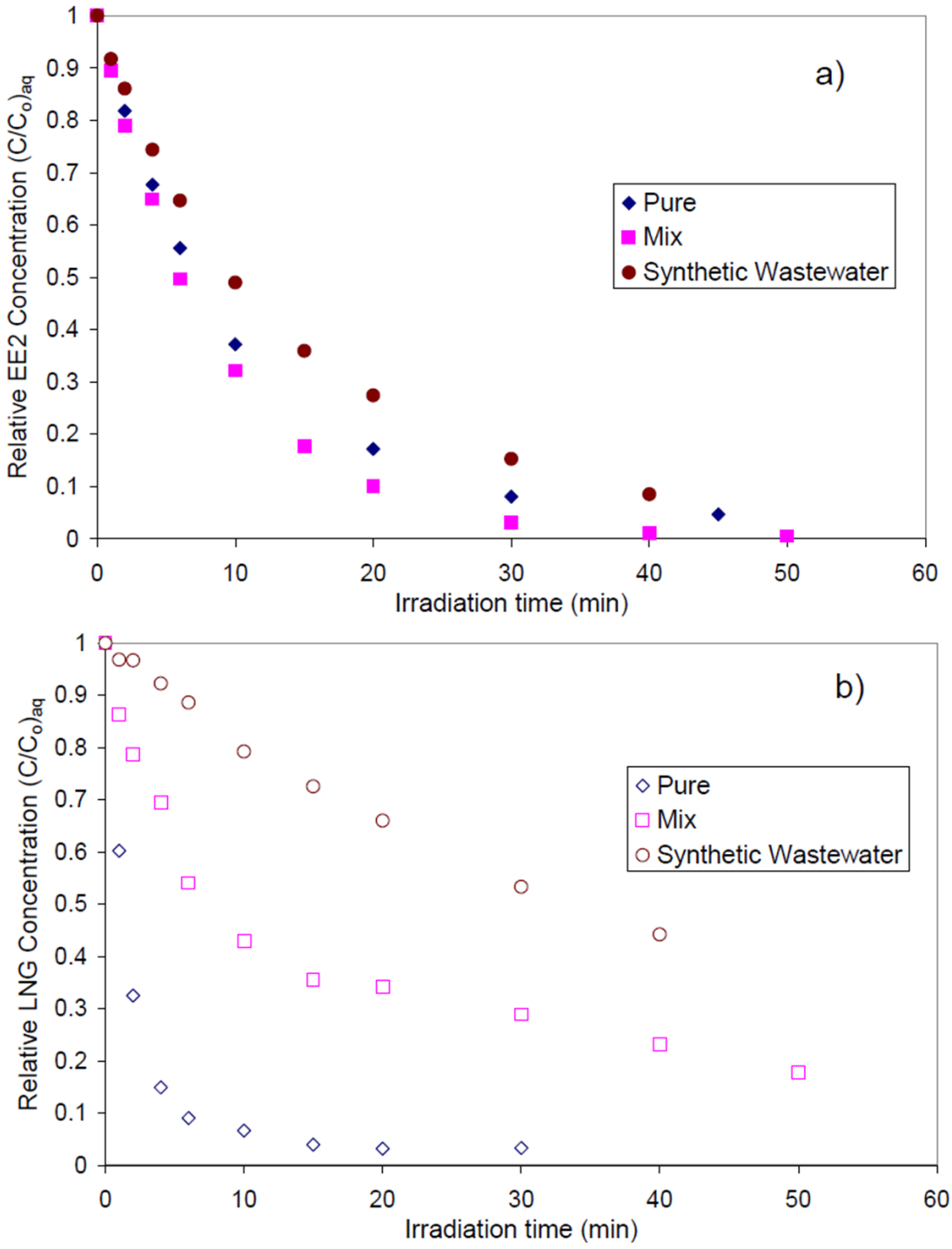

Figure 6.5- Evolution of relative EE2 (a) and relative LNG (b) concentration during photocatalysis (based on aqueous concentrations) in Pure, MIX and SWW systems

It was previously shown that removal of TART is mainly due to the generation of hydroxyl radicals during photocatalysis while removal by direct photolysis was

minimal (Figure 6.2). This implies that the presence of this compound would contribute to consumption of hydroxyl radicals when present in a mixture. Due to the presence of TART, reduction of removal rates by 44% and 72% from MIX to SWW systems was observed for EE2 and LNG, respectively. EE2 still has the highest aqueous concentration in SWW; therefore it experiences competition of hydroxyl radicals to a lesser extent.

The WW samples used for UVC irradiation experiments had different aqueous phase concentrations from the samples used for quantification of hormones. The expected aqueous phase concentration of EE2 from the characterization tests was 5 mg/L; however this value was 1.6 – 2.4 mg/L in the WW system. The expected aqueous phase concentration of LNG was 0.8 – 1.3 mg/L, but the samples used for degradation experiments contained 1.5 – 2.5 mg/L of LNG. Even though, the real concentrations are of the same order of magnitude with the expected values, the ratio of aqueous phase concentrations of EE2 to LNG are different. Therefore, removals of EE2 and LNG in the WW system were studied separately, as comparison of removals in WW to removals in other matrices would not be possible. Removal data of EE2 and LNG in the WW system are presented in Figure 6.6. The corresponding pseudo-first order reaction rate constants are tabulated in Table 6.4. After 40 minutes of irradiation time, the removal efficiencies of EE2 in the WW system were 36% and 59% during photolysis and photocatalysis, respectively. On the other hand, photolytic removal efficiency of LNG (76%) in the WW system was larger than its photocatalytic removal efficiency (55%). Given similar initial aqueous phase concentrations of EE2 and LNG, as is the case in the treated WW, photocatalytic removal efficiencies and rates of these compounds are similar since hydroxyl radicals are known to be non-selective oxidizing species. The higher sensitivity of LNG to direct degradation by UVC radiation leads to higher removal of this compound compared to EE2 during photolytic treatment.

Estrogenic activity of EE2 was shown to be completely removed by Coleman et al [26] under both UVA photolysis and UVA photocatalysis with the latter being faster. Also Mazellier et al [36] showed that the products generated from UVA photolysis of EE2 were not different than UVC photolysis. Conversely, Rosenfeldt et al [45] demonstrated that during UVC photolysis estrogenic activity of EE2 was not reduced. In

addition to these results, the mechanism of removal of EE2 during UVA photocatalysis is not expected to be different from that of UVC photocatalysis since for both treatment methods the generation of hydroxyl radicals is mainly responsible for the removal of the parent compound as also demonstrated here in Figures 2b and c. Therefore, during photocatalysis of EE2 the estrogenic activity is expected to be reduced. However for UVC photolysis more investigation about estrogenic activity removal is necessary to completely evaluate the possibility of using this as an alternative treatment method. Based on our results, if the objective of a treatment system is to remove the estrogenic activity due to EE2 in similar wastewaters, photocatalytic treatment would be recommended over photolytic treatment. For LNG, photolytic removal rates are higher in all complex matrices (MIX, SWW and WW). However, photolytic and photocatalytic degradation products of LNG might possess estrogenic activity. Therefore, future experiments should include the identification of degradation products of this compound and assess the hormonal activity of treated samples in order to compare the performances of photolysis and photocatalysis for removal of LNG.

Table 6.4- Pseudo-first order reaction rate constants of EE2 and LNG during their photolytic and photocatalytic removals in all matrices

	Pseudo-first order reaction rate constant k x 10^{-2} (min^{-1})			
	EE2		**LNG**	
Matrix	UVC	UVC/TiO2	UVC	UVC/TiO2
PURE	3.0 ± 1.3	10.8 ± 5.6	17.4 ± 1.1	40.3 ± 4.1
MIX	1.8 ± 0.4	10.9 ± 3.1	17.6 ± 0.9	8.4 ± 0.9
SWW	1.1 ± 0.2	6.1 ± 1.7	7.8 ± 0.4	2.3 ± 0.6
WW [a]	0.8 ± 0.2	2.2 ± 1.1	7.2 ± 0.3	2.1 ± 0.5

[a] *Initial aqueous phase concentrations of EE2 and LNG in WW are not the same as in the other matrices*

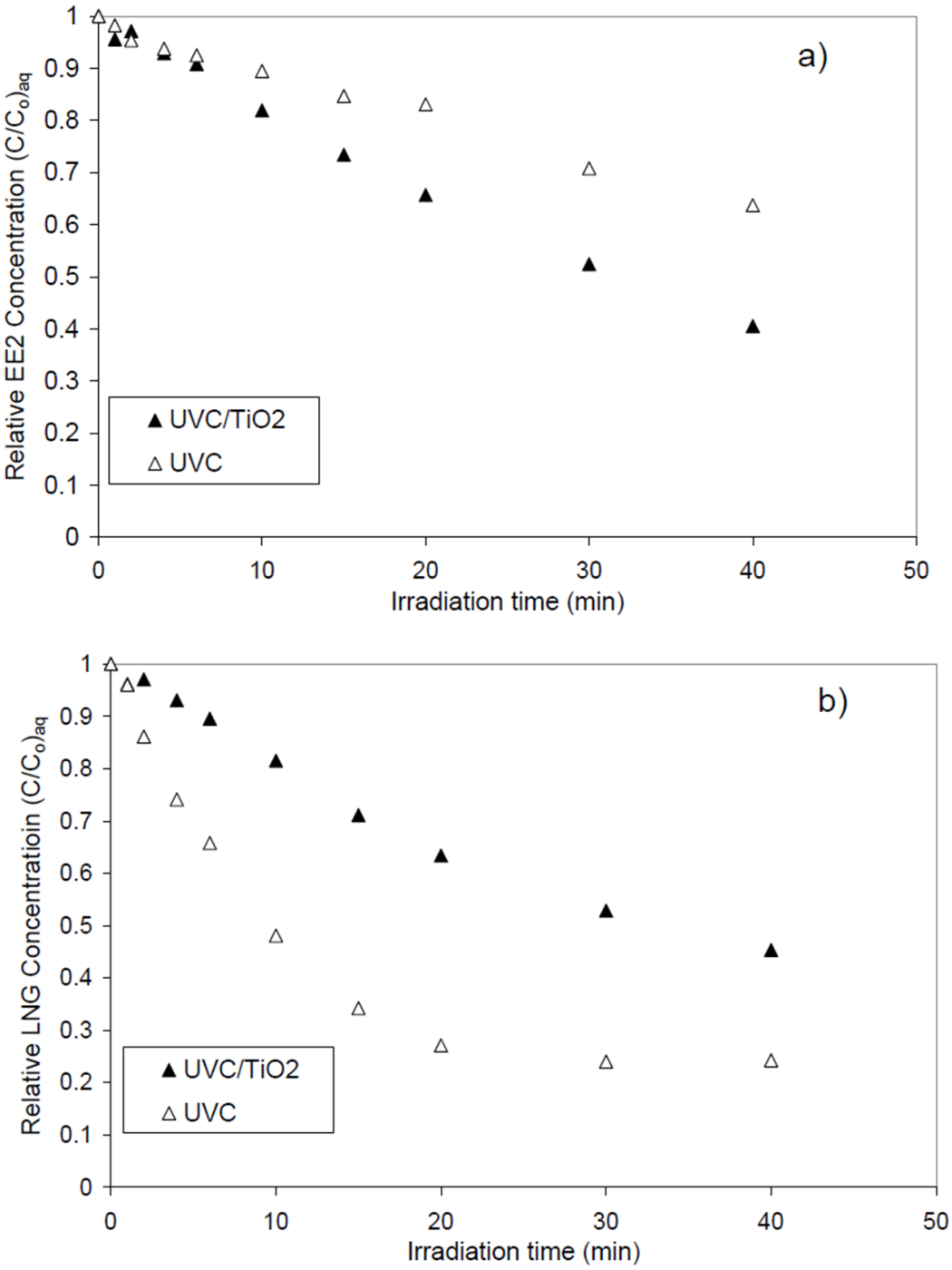

Figure 6. 6- Evolution of relative EE2 (a) and relative LNG (b) concentrations (based on aqueous concentrations) during photolysis (UVC) and photocatalysis (UVC/TiO2) in the WW system.

6.6 Conclusions

The applicability of UVC photolysis and photocatalysis to industrial pharmaceutical wastewater was demonstrated for the removal of EE2 and LNG. Results related to photolytic and photocatalytic removals of LNG and its mixtures with EE2 and other wastewater components are presented here for the first time in the literature. Only LNG was shown to be completely removed by photolysis within the irradiation time frame studied here. Complete photocatalytic removals of both compounds as individual contaminants indicated that, UVC photocatalysis can be applied satisfactorily to similar wastewaters especially if these types of wastewaters are further diluted to solubility limits of hormonally active compounds to avoid mass transfer limitations. Similar removal efficiencies and removal rates were determined for EE2 and LNG in the WW system after 40 minutes of photocatalytic treatment. This suggested that simultaneous removal of both compounds in a complex matrix is possible during UVC photocatalysis. Only very recently, researchers started investigating the occurrence and environmental impact of progestins, especially LNG. Therefore, the results presented here provide researchers with strong evidence for applying UVC induced photodegradation processes to mitigate possible adverse environmental effects of this compound.

6.7 Acknowledgements

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6.8 Supplementary Figures

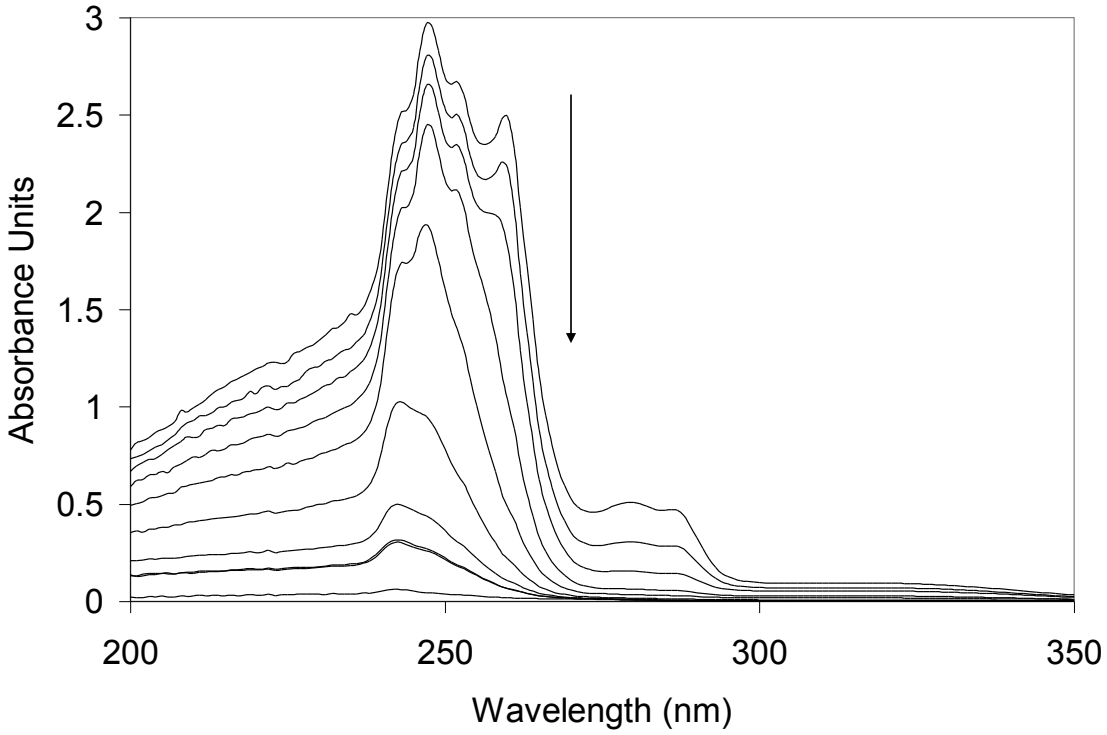

Figure 6. 7- Evolution of UV-Vis spectra of organic phase after each 50 ml chloroform addition to an initial wastewater sample of 100 ml. As chloroform volume (50 ml increment per scan) is increased, less absorption is observed. Final scan corresponded to a total chloroform volume of 500 ml (ratio 5:1, chloroform : wastewater).

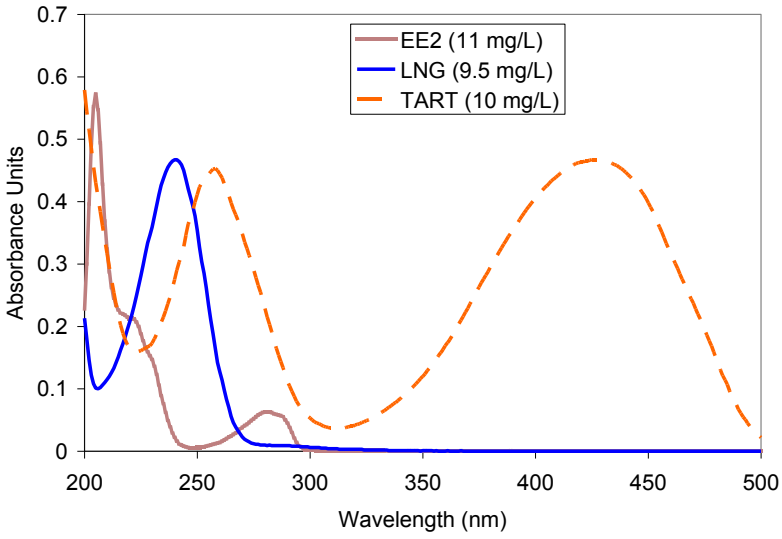

Figure 6. 8- UV-Vis absorbance spectra of EE2, LNG and TART.

7. ORIGINAL CONTRIBUTIONS

The original contributions of this PhD thesis can be divided into three areas.

I. Photo-removal of pharmaceutical compounds:

I contributed new knowledge about the removal of antibiotics and synthetic hormones during their photolytic and photocatalytic treatment under UVC radiation.

i. In this thesis, the photocatalytic and photolytic removal of LNG and LEVO under UVC radiation was investigated for the first time. Also additional new data were provided on the removal of SMX and EE2 during UVC mediated photodegradation methods. The participation of hydroxyl radicals towards the removal of these compounds during photocatalysis was confirmed. Complete removal of antibacterial activity with photocatalytic treatment with LEVO was also demonstrated.

ii. For the first time, ozonation and photocatalysis were evaluated and compared for the removal of LEVO and their effectiveness for its mineralization. Even though combined ozonation and UV photocatalytic systems were studied for the removal of pharmaceuticals by other research groups, the results of this work showed for the first time that persistent ozonation products can be removed if ozonated samples are further subjected to photocatalysis. This result further demonstrated the high oxidation potential of photocatalytic systems.

II. Photo-removal in complex matrices and in real wastewater

I applied UVC photolysis and photocatalysis to a real wastewater sample from a manufacturing plant to investigate the applicability of these treatments and help our industrial partner find an alternative disposal method of their waste.

i. Characterization of the industrial WYETH wastewater, generated from the first wash of oral contraceptives manufacturing vessels yielded data

regarding the concentrations of hormones in their respective industrial wastewaters, for which no information was published previously.

ii. Photolytic and photocatalytic treatment was applied for the first time to pure suspensions of hormones.

iii. UVC mediated photolytic and photocatalytic removals of EE2 and LNG in mixtures, synthetic and real manufacturing wastewater samples were investigated. The results showed that as the matrix became more complex the removal rate of the compounds of interest diminished. The differences in photolytic removal rates were related to the availability of light in each matrix and reduction in availability of hydroxyl radicals by competition.

III. Development of new analytical techniques:

I developed new analytical methods or modified existing ones to provide future researchers with tools necessary for investigation of advanced oxidation of pollutants of emerging concern:

i. Actinometric method developed by Bunce et al. [202] was modified and used for the first time for characterizing the light intensity distribution in large photocatalytic reactors and for quantifying the fraction of light absorbed by TiO_2, emerging pollutants and other wastewater constituents.

ii. The *Daphnia magna* toxicity test was modified for the evaluation of toxicity of treated samples in pure unbuffered systems. In majority of the published literature *Daphnia magna* toxicity kit DAPHTOXKIT F is used to evaluate product toxicity. In the methodology sections of these reports, only the standard toxicity method associated to determination of EC 50 values of known concentrations of compounds are included. However in advanced oxidation treatment systems, following the oxidation of the parent compound, the treated sample contains mixtures of unknown compounds at unknown concentrations. The published reports do not contain information to how these samples were treated prior to being subjected to *Daphnia magna*. In this thesis we also developed a method to provide the necessary ratio of synthetic freshwater to sample for the survival of the daphnids.

iii. An extraction method with high reproducibility was developed to monitor the total (suspended + in solution) and aqueous (in solution) concentrations of hormones EE2 and LNG.

8. CONCLUSIONS

The general conclusion of the work presented here is that the UVC photocatalysis is effective towards the removal of four pharmaceutical compounds: SMX, LEVO, EE2 and LNG.

The products of SMX degradation had a higher toxicity to *Daphnia magna* than the parent compound. This result underlines the fact that the toxicity evaluation should accompany removal and mineralization determination during advanced oxidation processes of pharmaceutical compounds to assess the possibility of application of treatment methods more realistically. For wastewaters containing contaminants producing toxic photolysis products, such as SMX, UVC mediated photodegradation processes would not be beneficial. However, for other contaminants such as LEVO, increased mineralization efficiency compared to that of ozonation and complete removal of antibacterial activity, demonstrated that UVC photocatalysis is an interesting advanced oxidation treatment method for the removal of emerging pollutants.

Observed photolytic and photocatalytic removals of hormones (EE2 and LNG) in pure and complex matrices such as industrial wastewater, extend the applicability of these treatments to a wider range of compounds and especially underline the non-selective nature of hydroxyl radicals towards removal of organic pollutants. In light of the results presented in this thesis, UVC mediated photodegradation processes (especially photocatalysis) would be more applicable to point sources with relatively higher pharmaceutical concentrations (e.g. hospital and industrial pharmaceutical wastewater).

The specific conclusions drawn from the results presented here are summarized below:

- Considerably faster removal of SMX was observed during UVC photolysis compared to photocatalysis. The photodegradation products of SMX were shown to be resistant to further removal by photolysis; however higher mineralization efficiencies were reported during photocatalytic treatment of SMX. The main mechanism of the removal of SMX during photocatalysis was

confirmed to be photolysis with photocatalysis being more effective towards removal of products. Both treatment methods lead to the generation of more toxic products towards *Daphnia magna* than the parent compound.

- UVC induced photolytic contribution to the removal of LEVO was insignificant; however high photolytic removal efficiencies were observed for SMX, EE2 and LNG.
- For all compounds, except SMX, the generated hydroxyl radicals were shown to influence significantly photocatalytic degradation rates. For SMX, the presence of hydroxyl radicals mostly contributed to the removal of products rather than the parent compound.
- Higher mineralization effectiveness was observed for the photocatalytic removal of LEVO compared to the ozone treatment. Ozonation products were shown to be more resistant to further degradation by ozone; however during photocatalysis, these ozonation products were continuously removed. Both processes showed complete removal of antibacterial activity towards *E. coli* (ATCC 1303) and *P. fluorescens* (ATCC 13525). *E. coli* was shown to be the more sensitive microorganisms towards the presence of LEVO.
- Compared to the only other ozonation study of LEVO, significantly faster removal of the compound was shown here. The discrepancy in removal rates was largely attributed to the difference in operating temperatures used in both studies, affecting the solubility of ozone. These results stressed again the importance of considering mass transfer limitations in the design of ozonation units and being careful in using data obtained in the lab for large-scale applications.
- For EE2, the total and aqueous photolytic and photocatalytic removal rates were not significantly different from each other. This result confirmed that the reactions predominantly occur in the aqueous phase and that the dissolution rate of EE2 is significantly lower than the reaction rate in the aqueous phase.
- LNG was more sensitive to photolytic removal than EE2 as suggested by its high absorption bands close to the operation wavelength of the lamp. Overall,

as pure substances LNG was removed considerably faster than EE2 during both UVC mediated processes.

- In mixtures of EE2 and LNG, the presence of LNG leads to a reduction of removal of EE2 by photolysis but the presence of EE2 does not significantly alter the photolytic reaction rate of LNG, since absorption of UVC irradiation of EE2 is significantly less than that of LNG.
- For photocatalytic systems, presence of EE2 significantly reduces LNG removal rates, due to the higher solubility associated to EE2, it is always found at higher aqueous concentrations than LNG and the likeliness of hydroxyl radical attack is higher for EE2. This leads to consumption of hydroxyl radicals that would be otherwise available if LNG was treated as a single contaminant.
- Tartrazine which is the coloring agent present in the industrial pharmaceutical wastewater was completely removed by photocatalysis. There was no significant removal by photolytic treatment. The presence of this compound retarded the photolytic and photocatalytic removals of EE2 and LNG by reducing the availability of light and by increasing the competition for hydroxyl radicals.

9. RECOMMENDATIONS

- EE2 and LNG was shown here to be efficiently removed by photolytic and photocatalytic processes. Estrogenic activity removal of EE2 by photolytic and photocatalytic processes were confirmed by previous researchers however; LNG degradation products might contain estrogenic activity which needs evaluation.
- Occurrence, ecotoxicity and removal by advanced oxidation processes is lacking for relatively newer FQs and progestins. Therefore, these types of investigations should be extended to these compounds and the research should follow prescription trends in order to find solutions prior to encountering major problems in the environment.
- Ecotoxicity, antibacterial, estrogenic and/or androgenic activity of pharmaceutical compounds should not only be evaluated as pure compounds but also in mixtures. It is of great importance to evaluate the additive or synergistic environmental effects of the mixtures of contaminants of emerging concern on aquatic wildlife since mixtures can exert enhanced toxicity or bioactivity.
- Sequential combination of AOPs should be further investigated for removal of contaminants of emerging concern since the degradation products generated by one method could be more susceptible to other types of treatment than the parent compound. As it was shown here that the ozonation products of LEVO were resistant to further ozonation but were easily removed by photocatalysis.
- There is limited data on the constituents of wastewater generated from manufacturing plants. There should be incentives to push pharmaceutical manufacturers to collaborate with research groups to analyze their wastewater to quantify pharmaceutical discharges and to potentially implement advanced treatment methods on site to reduce their contribution to emerging pollutant contamination of aquatic ecosystems.
- Major problem associated to using TiO_2 suspensions for photocatalytic treatment of organic pollutants is the difficulty associated to the separation of the TiO_2 particles afterwards and there is concern for possible toxicity of TiO_2

nanoparticles towards aquatic organisms. Efficient and economical methods of separation of nanoparticles should also be researched in order to make photocatalysis a viable option for treatment of wastewaters. One way of avoiding costly separation methods is immobilizing the TiO_2. Even though, there is extensive research on developing new methods of immobilizing TiO_2, the following decrease in activity due to reduction in surface area is still a major problem. Doping TiO_2 with certain metals seems to provide higher activity and in some cases increasing the photo-activity of the TiO_2 into the visible spectrum of solar irradiation. Research on incorporating advanced materials such as carbon-nanotubes with TiO_2 could also provide higher activity resulting from charge separation (reducing electron and hole recombination). It could be interesting to start collaborating research groups involved in synthesis of advanced materials with research groups involved in advanced oxidation of emerging pollutants to evaluate the photocatalytic removal of emerging pollutants by newer advanced material TiO_2 composites.

- As shown here, in certain cases, removal of a compound does not necessarily lead to generation of less toxic compounds (as in the case of SMX). Therefore, advanced oxidation studies should include information on residual bioactivity of treated solutions. However, this requires closer collaboration between disciplines in order to develop proper toxicity evaluation platforms essential to assessing the risk associated with such a large number of contaminants of emerging concern and their transformation products.

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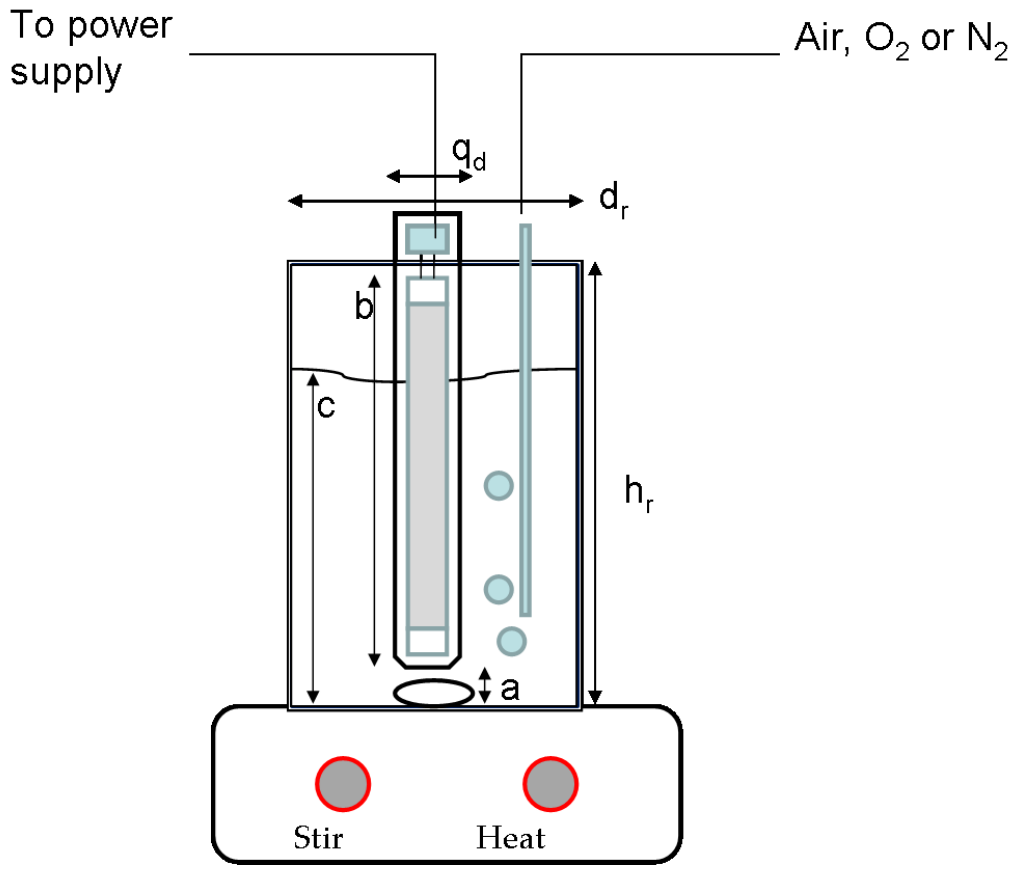
APPENDIX I: Key findings of related research

Compound	Treatment Conditions	Matrix	Key Findings	Reference
SMX	λ_{max} = 366 nm TiO2 conc = 2.5 g/L	Pure water	Initial SMX solutions resistant to biodegradation and toxic towards Chlorella vulgaris. Photocatalytic products more biodegradable and less toxic than parent compound.	Baran et al. [21]
SMX	UV-ABC (λ > 235 nm) UV-A (λ > 300 nm) TiO2 conc = 0 -0.5 g/L	Pure water	UV-ABC radiation more efficient than UV-A during photocatalysis. Mechanism or toxicity not studied.	Bayarri et al. [22]
SMX	UV-A (λ > 300 nm) TiO2 conc = 1.5 g/L	Pure water	Complete removal of 10 mg/L SMX after 15 min under UV-A photocatalysis. Ecotoxicity determined by *Daphnia magna* for products are higher than the parent compound.	Beltran et al. [24]
SMX	UV-A (λ > 300 nm) TiO2 conc = 0.1 - 0.25 g/L	Pure water Ground water Wastewater effluent	Complete mineralization of 10 mg/L SMX after 120 minutes at 250 mg/L TiO2 loading. Kinetic studies were conducted but no removal mechanism.	Xekoukoulotakis et al. [52]
SMX	UV-A (λ > 300 nm) TiO2 conc = 0.1 - 0.25 g/L	Pure water water containing natural organic matter	Presence of natural organic matter inhibits photocatalytic degradation of SMX. Hydroxyl radical mechanisms are discussed.	Hu et al. [28]

LEVO	Solar simulator (λ > 300 nm) No TiO_2	Pure and five different synthetic field water	Half-lives of LEVO in SFW (17 - 67 min) are generally longer than its half-life in pure water (19 min) . Structure of photoproducts are identified.	Lam and Mabury [156]
LEVO	λ_{max} = 365 nm TiO_2 conc = 2 g/L	Pure water	Absolute rate constants for hydroxyl radicals and hydrated electrons are reported. Photocatalytic degradation pathways were proposed. After 180 min >95% TOC was removed.	An et al. [19]
EE2	UV-A (λ > 300 nm) Immobilized TiO_2	Pure water	Estrogenic activity was removed by both UVA photolysis and photocatalysis. Photocatalytic removal rates were higher than photolytic removal rates.	Coleman et al. [26]
EE2	UV-A (λ > 365 nm) UV-C (λ = 254 nm No TiO_2	Pure water	More than 95% removal after 90 min during UVC photolysis (no removal during UVA photolysis).	Liu et al. [34]
EE2	UV-A (λ = 300 -420 nm) UV-C (λ = 254 nm 0.4 g/L TiO_2	Mixture of estrogens in pure water	Faster degradation of estrogens under UVC photolysis compared to UVA photolysis. Maximum removal rate observed for UVC photocatalysis. UVA photocatalysis was faster than photolytic removal methods. Radiation field was modeled and new kinetic models were proposed independent or reactor geometry.	Li Puma et al. [32]
EE2	λ_{max} = 365 nm TiO_2 conc = 2 g/L	Pure water Urea, sacchrose and urine as co-pollutants	0.5 mg/L EE2 removed by UV-A photocatalysis after 30 min. Presence of urine and sacchrose inhibited the removal of EE2.	Karpova et al. [29,30]

APPENDIX II: Reactor Setup and dimensions



Dimensions

h_r	height of reactor	21.5 cm
d_r	diameter of reactor	10.8 cm
q_d	diameter of quartz tube	3.0 cm
a	distance from the bottom of reactor to the quartz tube	1.9 cm
b	height of UVC lamp	19.6 cm
c	liquid height	17.5 cm

APPENDIX III: Daphnia magna test procedure and control results

The procedure to determine the acute toxicity was conducted following the commercial test kit DAPHTOXKIT F™ (MicroBioTests Inc, Gent, Belgium). Ephippia were first activated by rinsing with tap water. They were then transferred into petri dishes in standard freshwater (prepared with supplied salts $NaHCO_3$, $CaCl_2$, $MgSO_4$ and KCl) and placed in an incubator to hatch (72 – 90 hours) at 20 – 22 °C under continuous illumination of at least 6000 lux. The hatched neonates were fed with *Spiruluna* two hours prior to testing with sample media to avoid mortality caused by starvation. No food was provided for test organisms during 48 hours of exposure time. At least 120 neonates were required to perform a single test. Five daphnids were exposed to each sample to be tested in quadruplicate in specific test wells (total of 20 daphnids per each sample). The control consisted of only standard freshwater (SFW). The test plate then was covered and incubated at 20 °C under dark. After 24 and 48 hours of exposure, dead or immobilized daphnids were counted and results were tabulated as % Effect (percentage of immobilized organisms). Species were considered immobilized if they did not move freely after gentle tapping of the test plates, even if they did move their antennas. When the percentage of immobilization is less than or equal 10% at the end of the test (48 h), it can be considered that the solution does not show acute toxicity to *D. magna*. In order to determine EC_{50} values of a known compound; varying concentrations of the compound in SFW are placed in the test wells, the concentrations are plotted against % effect and the points are connected by straight lines, finally the 2 most adjacent points on the plot which are separated by the 50 % effect line are located and the value corresponding to % 50 effect is directly read from the plot. Potassium dichromate ($K_2Cr_2O_7$) was the reference chemical used. An EC_{50} 24 h of 1.23 mg L^{-1} was obtained for the reference compound which is within the range of the 0.6–2.1 mg L^{-1} stipulated in the ISO 6341 to ensure test validity (International Organization for Standardisation, 1996). No swimming inhibition was observed in the controls exposed in each plate.

DAPHTOXKIT F^{TM} MAGNA is a relatively easy and effective kit to use to determine toxicity of variety of compounds, especially if the concern is to determine

EC_{50} values of a specific compound. However problem arises when determining toxicity of treated solutions where the identity of the constituents and their concentrations in the sample are unknown. In this case one is required to dilute the samples with varying dilutions of the SFW and determine the dilution ratio where 50 % effect is observed. As it can be imagined, for each treatment time if one decided to employ this method of finding the dilution ratio that gave 50 % effect, at least one whole test plate would be sacrificed for only one treatment time resulting in a very expensive method of analysis for the whole range of treatment times and methods used.

It was found difficult to replicate some of the values reported in literature. For example, for an untreated SMX solution of 30 mg/L Beltrán, Aguinaco et al. 2008 reported a value of 35% and 60 % for 24 hours and 48 hours of exposure respectively. However, when solutions of SMX in RO water only were exposed to daphnia at various concentrations (2.4 mg/L to 60 mg/L) all the daphnids were immobilized even at the lowest concentration. Also, when solutions of SMX were prepared in standard fresh water (SFW) almost no immobilization was observed even at concentrations as high as 60 mg/L. These results suggested that it was necessary to obtain a certain ratio of SMX in RO water to SFW to obtain a reliable basis for assessment of treatment in regards to removal of toxicity. All the dilution ratios mentioned in this section are by volume. Conventionally, having a value close to 50 % effect for the untreated case would allow better assessment of treatment methods. Therefore, initially it was necessary to determine the amount of RO water that the daphnids can handle without the presence of SMX. Due to absence of salts in RO water, the osmotic pressure and pH were expected to influence the survival of the daphnids. Dilutions of RO water up to 5 to 1 (RO water to SFW) showed no inhibition however after dilutions of 7 to 1, daphnids started to get swimming inhibition and in pure RO water conditions, no survival was observed. For most of the toxicity testing of samples containing SMX a dilution ratio of 3 to 1 (RO water to SFW) was used. For samples containing treated or untreated SMX, the dilution ratio is between the sample and the SFW. The pH of the SFW was measured to be 7.60. In order to evaluate the effect of pH on daphnia in presence of SMX, SMX solutions of 12 mg/L and 30 mg/L in pure RO water were adjusted to 7.6 and daphnids were exposed to these samples. Both adjusted and not adjusted samples at the two concentrations

studied showed complete inhibition after 48 hours of exposure, suggesting that at the concentrations of SMX studied, pH is not the significant factor in survival of the daphnids but change in osmotic pressure is. As another control experiment, effect of TiO_2 on survival of daphnids was investigated. Even though TiO_2 is filtered by 0.22 μm syringe filters prior to any analytical analysis, due to their nanoscale nature, they cannot be completely removed. 0.5 g/L of TiO_2 suspensions in RO water were filtered and diluted with SFW to have a ratio of sample to SFW of 3 to 1. Three replicates were exposed to D. Magna and no inhibition was observed up to 48 hours.