MEASUREMENT OF THE PURIFICATION EFFICIENCY OF PHARMACEUTICAL WASTEWATER

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Bachelor´s thesis
November 2011
Degree Programme in Laboratory Sciences
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ABSTRACT

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POHJA, JUKKA:
Measurement of the Purification Efficiency of Pharmaceutical Wastewater

Bachelor´s Thesis 47 pages, appendices 4 pages
November 2011

Advanced technology has created a medicine production which has spread all over the world. Medicine factories produce huge amounts of pharmaceuticals and also form industrial pharmaceutical wastewater during the manufacturing process. Some pharmaceuticals in the wastewater are not eliminated in the sewage treatment plants because of their huge molecular size so they end up to the nature intact. The purpose of this study was to find the best way to mineralize pharmaceuticals in the wastewater sample from medicine factory in Kikuyu, Kenya.

Sample water contained Sulfamethaxazole, Ciprofloxacin, Ibuprofen, Paracetamol and Metronidazole. Ozonation, UV-radiation, ozone/UV and flocculation methods were used to the sample water. Sample water was treated with one method at a time and the best way was selected.

Ozonation, UV-radiation and ozone/UV decayed pharmaceuticals almost at the same rate. But there were differences between the decaying rates of each pharmaceutical. In each method, Sulfamethaxazole was the fastest to mineralize and Ciprofloxacin was the slowest. Because of the slow mineralization of Ciprofloxacin, doses needed for decaying were too high for each method. Flocculation was the key to proper and reasonable elimination of pharmaceuticals. Flocculation eliminated over half of Ciprofloxacin in the sample water.

According to the results, flocculation has to be done before mineralization of the pharmaceuticals. This lowers the doses needed to the reasonable range so that the method can be used in the medicine factory. There were no notable differences between the used methods. Further studies are needed to analyze the effect of the flocculation in the factory. After that, calculations have to be made to the needed doses to the ozone generator or UV-lamp.

Keywords: pharmaceuticals in the nature, ozonation, UV-radiation, ozone/UV, flocculation

TIIVISTELMÄ

Tampereen Ammattikorkeakoulu
Laboratorioalan koulutusohjelma

POHJA, JUKKA:
Lääkeaineita sisältävän jäteveden puhdistusmenetelmien tehokkuuden määrittäminen

Opinnäytetyö 47 s., liitteet 4 s.
Marraskuu 2011

Teknologian kehittyminen on synnyttänyt lääkeaineita valmistavia lääketehtaita, joita on jo joka puolella maapalloa. Nämä lääketehtaat tuottavat valtavia määriä lääkeaineita, joiden tekovaiheessa syntyy myös lääkeaineita sisältävää teollisuusjätevettä. Lääkeaineiden suuren molekyylikoon vuoksi jotkin lääkeaineet jätevedessä eivät tuhoudu jätevedenpuhdistuslaitosten käsittelyprosesseissa. Näin ne voivat päästä takaisin luontoon muuttumattomina. Tämän työn tarkoituksena oli löytää paras keino saada Kikuyussa, Keniassa, sijaitsevan lääkeainetehtaan teollisuusjätevedessä olevat lääkeaineet hajoamaan.

Näytevesi sisälsi sulfametoksatsolia, siprofloksasiinia, ibuprofeenia, parasetamolia ja metronidatsolia. Näihin lääkeaineisiin kokeiltiin puhdistusmenetelminä otsonointia, UV-säteilyä, otsonointia ja UV-säteilyä yhdessä sekä flokkulaatiota. Näyteveteen käytettiin yhtä puhdistusmenetelmää kerrallaan ja lopulta paras menetelmä määritettiin.

Otsonointi, UV-säteily ja otsoni/UV hajottivat lääkeaineita melkein samalla nopeudella. Jokaisen lääkeaineen hajoamisnopeuksissa oli kuitenkin eroja. Jokaisella menetelmällä sulfametoksatsoli oli nopein hajoamaan ja siprofloksasiini hitain. Siprofloksasiinin hitaan hajoamisen vuoksi menetelmien käyttämät annokset olivat liian suuria. Flokkulaatio oli ratkaisu kunnolliseen ja järkevään lääkeaineiden hajottamiseen. Flokkulaatio poisti yli puolet näyteveden siprofloksasiinista.

Tulosten perusteella flokkulaatio pitää tehdä ennen lääkeaineiden hajottamista. Se alentaa tarvittavia annosmääriä järkeviin lukemiin, jotta menetelmää voidaan käyttää lääkeainetehtaassa. Käytetyissä hajotusmenetelmissä ei ollut ratkaisevia eroja. Lisätutkimuksia tarvitaan flokkulaation onnistumisessa tehdasmittakaavassa. Sen jälkeen täytyy tehdä laskelmia tarvittaville otsonigeneraattorin tai UV-lampun annosmäärille.

Avainsanat: lääkeaineet luonnossa, otsonointi, UV-säteily, otsoni/UV, flokkulaatio

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1 INTRODUCTION

Pharmaceuticals are used and produced all around the world. Significant concentrations of pharmaceuticals and their metabolites have been found in the environment. Researchers have thought that the use and disposal of pharmaceuticals is the main source of such high concentrations but in recent years there have been studies which indicate that industrial waste water from medicine factories can also be a major source.

When reaching the wastewater treatment plants, some pharmaceuticals in the industrial wastewater aren't eliminated. Pharmaceuticals have been found in effluents from the wastewater plants after the treatments. This is a consequence of the poor biodegradability of some pharmaceuticals. Eventually, treated but still pharmaceutical containing effluent is released into nature. In the environment pharmaceuticals can affect the cells of organisms and even get to human drinking water.

Pre-treatment processes are recommended for industrial wastewater containing pharmaceuticals to get into a more biodegradable form. When it is biodegradable it is easy to treat in the wastewater treatment plant. One of the successful pre-treatments is ozonation. Ozonation is a simple and effective way to mineralize pharmaceuticals.

This bachelor thesis was done at the Tampere University of Technology in the Department of Chemistry and Biotechnology Laboratory of Bio- and Environment Technology.

The purpose of this study was to test purification methods for the pharmaceutical wastewater samples from medicine factory in Kenya. First the sample water was characterized to get the original concentrations of the known pharmaceuticals. Then purification methods were tested. The purification methods were ozonation, UV-radiation, UV/ozone and flocculation. The aim of this study was to find the best purification method which can be used in the medicine factory.

2 BACKGROUND

2.1 Pharmaceutical industry

Pharmaceuticals are produced in factories worldwide for human and animal needs. The quantity of these produced pharmaceuticals is huge and has been growing since the beginning of the 1980´s. (WHO 2004.) In the year 2009, the global pharmaceutical market was 808 billion US dollars (URCH Publishing 2011). The manufacture of pharmaceuticals is concentrated to industrialized countries. The largest manufacturing countries are the USA, Japan, France, Germany and the UK which account for two thirds of the value of all medicines produced. In recent years, highly competitive markets have formed in India and China. They have rapidly grown their pharmaceuticals biotechnological market. In these kinds of lands, large volume markets of lower-price medicines exist. (WHO 2004.)

2.2 Pharmaceuticals in the environment

Complex molecules with different medicinal functionalities, physicochemical and biological properties are called pharmaceuticals (Kümmerer 2004, 3). Their molecular weight is typically below 500 Daltons and they are bioavailable and biologically active, because they are water soluble and lipophilic (Ikehata & Co 2006, 354). Pharmaceuticals are often characterized by their ionic nature and under environmental conditions they can be neutral, cationic or zwitterionic (Kümmerer 2004, 3).

Pharmaceuticals can be made by synthetic reactions by chemists or they can also be found in nature from plants. People have used pharmaceuticals for a long time and nowadays the production is huge. (Ternes 1998, 3245.) Every year the consumption is increasing and so is the concern of the effects of pharmaceuticals on the environment (Vieno & Co 2005, 8220).

Interest about the pharmaceuticals in the environment first occurred in the 1970s. Also during 1980s there was little curiosity, but not until the mid nineties awareness increased and researchers started to study the effects of the pharmaceuticals on the

environment. (Kümmerer 2004, 6.) Since then, according to Ternes & Co (2003), huge amounts of pharmaceuticals have been found in treated wastewater, rivers, lakes and even in drinking water in Europe, Brazil and North America.

Although some pharmaceuticals and their metabolites can be partially removed by absorption or adsorption and biotic or abiotic degradation in the environment, they can still reach drinking water. Several studies indicate that traditional water and wastewater treatment processes don't eliminate some pharmaceuticals completely. (Ikehata & Co 2006, 353.)

2.2.1 Exposure routes

Current environmental legislations for different regions concentrate on the release of active ingredients from municipal sewage plants. Although production facilities present another possible way of pharmaceuticals entering the environment. (Larsson & Co 2007, 751.) Municipal wastewater and the wastewater from industrial processes are very different from each other. Municipal wastewater is formed from rain and household waters whereas industrial wastewater consists of water used in production processes or water used in cleaning the equipment used in production. The industrial wastewater contains mainly the chemical and solvent residues that are used or produced in the process. Therefore it is important to know the production processes and the chemicals used in it. (Woodard 2001, 29-30.)

Pharmaceutical industrial wastewater is mainly generated by washing of the equipment in pharmaceutical industries. Wastewater discharged is mainly small in volume but it is highly polluted because of the large number of organic pollutants in it. That is also the reason for really high results in the chemical oxygen demand (COD) and total organic carbon (TOC). (Imran 2005, 995.)

In addition to pharmaceutical industrial wastewater from medicine factories, there are other routes how pharmaceuticals can get to the nature. Humans and animals are the two main users of pharmaceuticals, although they use almost the same drugs (Jørgensen & Co 2000, 691.) Human pharmaceuticals are used orally or intravenously in hospitals or at home. After consumption, the drug is absorbed, distributed, metabolized or non-

metabolised, and finally excreted from the body as urine or faeces to sewage. Many of the new drugs are designed so that they are easy to metabolize in the body. Metabolization changes the composition of pharmaceuticals to more non-toxic, polar and hydrophilic form by eliminating the excess toxic molecules. This happens in the liver or kidney with various biochemical reactions. But there are lots of drugs that are not metabolized, for example non-therapeutic medical agents and xenobiotics are excreted to the environment intact. (Ikehata & Co 2006, 354.)

Lots of pharmaceuticals are also disposed to household drains when they are outdated. In 1994 the EU made legislation that permitted discarding of unused drugs to household waste. There they will end up in landfill sites intact and enter the landfill effluent. (Kümmerer 2004, 5.) People have discarded drugs even to the toilet, although it is not recommended today (Ikehata & Co 2006, 355). If the drugs that are thrown in the sewage are not eliminated during sewage treatment, they may enter the aquatic environment and eventually even to our drinking water. Disinfectants that are used in the glue factories and in the food industry can enter the environment through different routes than sewage (figure 1.). (Kümmerer 2004, 4.)

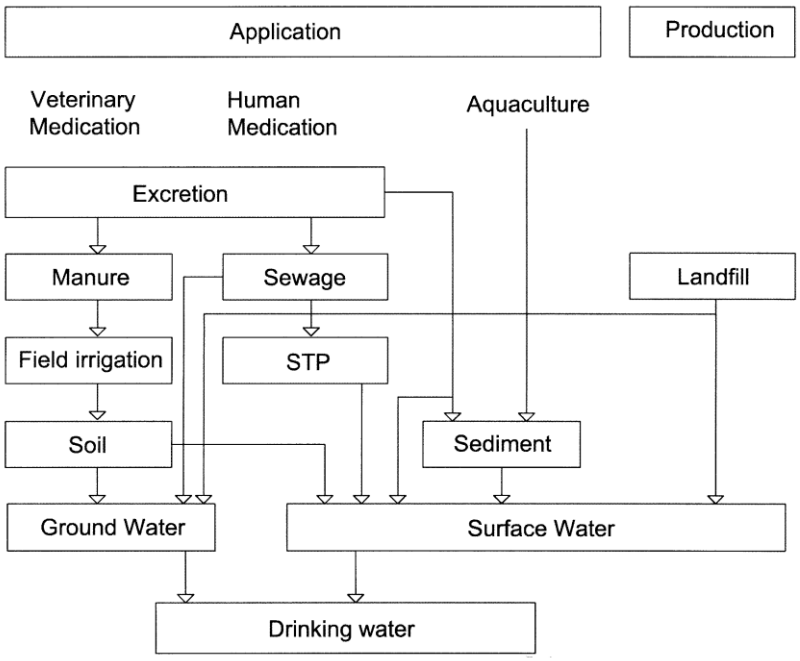


FIGURE 1. Possible exposure routes of pharmaceuticals to the aquatic environment (Hirsch & Co 1999, 111)

Pharmaceuticals are also used in veterinary purposes, animal husbandry and as growth promoters for large-scale animal farming. Animals' metabolites are excreted as manure and farmers use this manure and also sewage sludge to fertilize fields, so the soil is contaminated with pharmaceuticals. After heavy rain, veterinary pharmaceuticals can be washed off from the soil into surface water. (Kümmerer 2004, 5.) The aquatic system can also be contaminated from pharmaceuticals directly as fish farms use drugs as a feed additive (Jørgensen & Co 2000, 693).

2.2.2 Occurrence

Studies made at the turn of the 21st century indicates that several pharmaceuticals have been found in sewage treatment plant (STP) effluents and also in the surface water in Germany, the Netherlands, Switzerland, Canada, Brazil, Italy, Spain and the United States. Pharmaceuticals found were antibiotics, anticonvulsants, painkillers, cytostatic drugs, hormones, lipid regulators, beta-blockers, antihistamines and X-ray contrast media. The concentrations were from the ng/l to µg/l range and also polar compounds and metabolites were detected from groundwater samples at concentrations up to 1 ng/l. (Ikehata & Co 2006, 355.)

Larsson & Co (2007) measured pharmaceutical concentrations from the effluent of a wastewater treatment plant that receives medicine factory process waters in India. They found twenty one pharmaceuticals that were present in concentrations above 1 µg/l and eleven of them were detected in concentrations above 100 µg/l. Ciprofloxacin was detected in concentrations (up to 31 000 µg/l) which exceeded EC_{50} toxicity levels for two water micro-organisms.

Compared to the most widely used chemicals, the amount of pharmaceuticals in the environment is relatively low. But drug concentrations over 10 – 100 ng/l can cause serious problems to the environment. This can happen if drugs are discharged locally, for example hospital wastewater or medicine factories wastewater is discharged into a small STP and drugs are not eliminated there. Then the effluent is released into small rivers or lakes, which raises the local concentrations of pharmaceuticals high. (Jørgensen & Co 2000, 693.)

2.2.3 Effects

Pharmaceuticals medicinal properties are based on biological activity which means that they can penetrate through cell tissues so the pharmaceutical can have an effect. Pharmaceuticals biological activity is very crucial to the environment, because even if the pharmaceuticals have medicinal effects in certain dosages, they can also have deadly effects on the organisms if the dose is too high or the target organism is wrong. (Kümmerer 2004, 3, 7.)

In the aquatic environment some compounds in the mg/l range have been found to produce effects in environmental organisms. But also low concentrations of antibiotics have been found to affect algae and bacteria, because those can become resistant to the antibiotic. This results in natural selection of more harmful bacteria and may be a reason for an increasing number of pathogenic bacteria resistant to antibiotics. (Kümmerer 2004, 7-8.)

In addition to antibiotic resistance, Jørgensen & Co (2000) categorize the effects of the pharmaceuticals and their metabolites to the environment to two more groups. The first group contains the normal toxic effect of all xenobiotics on the cell, organ and organism level. The second group consists of so called endocrine disrupters which disturb the normal balance of hormones by replacing the hormone with a pharmaceutical. It has been found that this disturbance of normal hormonal balance happens in a few nanograms of drug per liter scale.

Drug concentrations found in drinking water are so small that the immediate adverse effect to humans is basically insignificant compared to the doses given in therapy. But in therapy, the doses are high and the effect is short. So it has not yet been tested how the long term small concentration ingestion from drinking water really affects the human body. (Kümmerer 2004, 8-9.)

Wastewater treatment is the key step to remove pharmaceuticals, especially human drugs. Because current systems cannot remove drugs effectively, improvements and modifications are needed. (Ikehata & Co 2006, 357.) One option is to use pre-treatment processes for example ozonation which makes pharmaceuticals more biodegradable in wastewater treatment (Gottschalk & Co 2010, 27).

2.3 Ozone in waste water treatment

Ozone is created naturally by the discharge of lightning and it can also be produced artificially by the discharge of electricity in the presence of oxygen. Ozone was discovered in 1839 by Christian Friedrich Schönbein who noticed a strange smell at the anode of an electrolytic cell. He postulated it as a new substance “ozein” which was Greek meaning the word smell. This discovery started intensive research towards ozone. In 1845 Schönbein discovered the oxidizing properties of ozone and also its danger to living beings. (Gottschalk & Co 2010, 27-28.)

In 1886 it was discovered that ozone can be used to clean and sterilize drinking water and wastewater. At the turn of the nineteenth century the first ozone units were installed to drinking water treatment plants. The technology spread quickly, reaching 50 installations in 1915. Ozonation was first used as a last stage of the treatment for disinfection. But in the 1960s, in order to utilize ozone's ability to oxidize manganese and iron in wastewater treatment, its coagulating effects and even more recently micropollutant oxidation, it was moved up in the treatment chain. The concept of pre-ozonation was formed. The idea of pre-ozonation is that the ozone breaks down non-biodegrable compounds into a more biodegradable form which is easier to remove in the wastewater treatment process. (Gottschalk & Co 2010, 28.)

Ozonation is a particularly good treatment for industrial wastewater containing recalcitrant organic pollutants such as pharmaceuticals because of ozone's oxidation capability which makes pharmaceutical molecules mineralize (Ikehata & Co 2006, 358). Oxidation does not usually result in complete mineralization, but it forms degradation products which usually have a much lower biological activity than the original compounds (Zimmermann & Co 2010, 606). These products are easier to eliminate in biological treatment with activated sludge in wastewater treatment plant (WWTP). They are also less harmful to humans and to the environment (Ikehata & Co 2006, 358).

2.3.1 Physical and chemical properties

Ozone molecule consists of three oxygen atoms and it is often called an allotropic form of oxygen. These three oxygen atoms are bound to each other with two oxygen-oxygen bonds in the angle of 116,49 ° (figure 2.). This structure is inherently unstable which is the reason for ozone's strong oxidizing ability. Oxidizing ability makes ozone also a harmful chemical so it must be handled carefully. It acts as a primary irritant, affecting mainly the eyes, lungs and upper respiratory tract. (Loeb 2009, 16.)

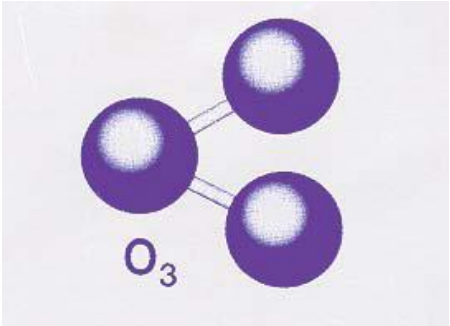


FIGURE 2. Ozone atom. (Loeb 2009, 16)

Ozone is a gas which has a pungent odor (table 1). This odor can be detected from concentrations of about 0,04 mg/m^3 by humans, so fortunately people can notice when there is ozone in the air. The recommended maximum exposure time for ozone is eight hours in 0,2 mg/m^3. Ozone gas has a pale blue color and in -119 °C it turns into dark blue liquid. This liquid can explode easily if suitable initiators are present, for example shocks, catalysts or changes in temperature or pressure. Because ozone is so unstable, it has to be produced at the site of the application. (Loeb 2009, 16.)

TABLE 1. Some constants of pure ozone. (Longley 1986, 99)

Molecular weight	48 g/mol
Boiling point	- 111.9 ºC
Melting point	- 192.5 ºC
Density (liquid)	1.572 g/ml at – 183 ºC
Density (gas)	2.154 g/l at 0 ºC, 1 atm
Heat capacity, (gas)	33.5 J/K · mol at -111.9 ºC

Ozone is produced in nature by coronal discharge (lightning strikes) or by UV-radiation. Oxygen near the ground in the presence of corona discharge creates ozone. And when oxygen from the atmosphere is affected by UV-light, ozone is formed. Ozone can be formed also with ozone generators. These generators use the same idea as creation in nature. (Ozonesolutions, Inc. 2011.) A typical ozone generator is a coronal discharge generator which works when oxygen gas flows to the discharge gap and there a high voltage creates collisions between electrons and oxygen molecules. In the result of these reactions ozone is formed. (Loeb 2009, 17.)

Ozone dissolves sparingly in water. That is the reason why the main limitation in ozonation applications is the low mass transfer rate of ozone from the gas phase to the liquid phase. The mass transfer efficiency depends on the mixing abilities used, the kinetics of ozone degradation in the water, and the number and size of bubbles produced. (Shin & Co 1999, 272.) In order to use ozone in water phase, it has to be transferred directly into the water using ozone generator (Gottschalk & Co 2010, 13).

2.3.2 Reactions of ozone

In water, ozone can react with substances in two different ways: directly and indirectly. It reacts directly with a compound using ozone molecule (O_3) or indirectly by producing hydroxyl radical (OH^o) that then reacts with a compound. These two different reaction ways produce different oxidation products and also use different kinds of oxidation kinetics. (Gottschalk & Co 2010, 13.)

The direct oxidation of organic compounds, for example pharmaceuticals, by ozone is a selective reaction with relatively slow rate constants. Typically the rate is in the range of $k = 1.0\ M^{-1}s^{-1} – 10^6\ M^{-1}s^{-1}$. In direct oxidation, the ozone molecule (O_3) reacts with the unsaturated bond due to its dipolar structure (figure 3). This leads to a splitting of the bond. Ozone reacts faster with compounds that have higher electron density so their nucleophilicity is also higher. For example, ozone reacts fast with aromatic compounds that have electron-supplying substituent such as hydroxyl or amine groups. Contrarily, aromatic compounds that have no substituent groups react slower to ozone. (Gottschalk & Co 2010, 17.)

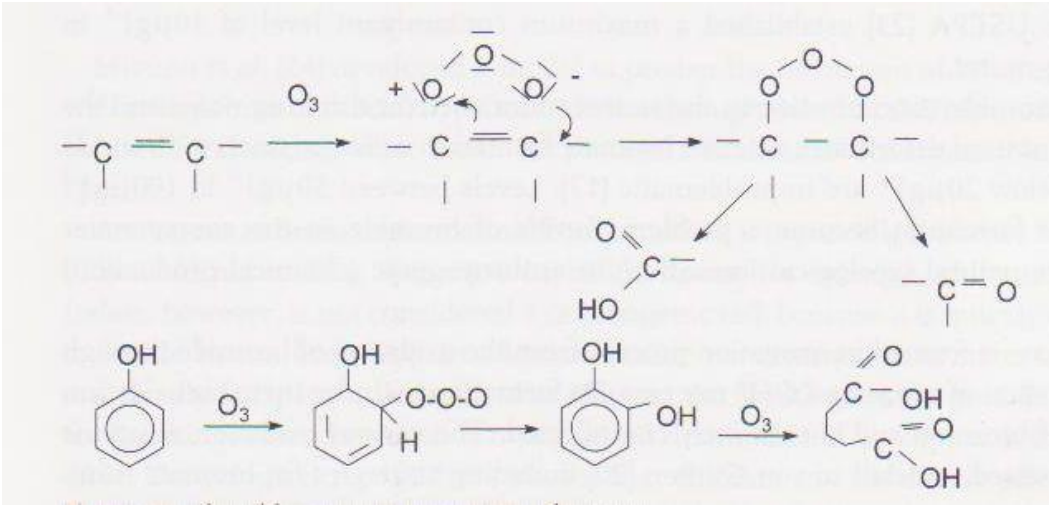


FIGURE 3. Plausible aqueous reactions with ozone (Gottschalk & Co 2010, 17)

The indirect reaction of ozone bases on radical formation. Radicals are molecules that have an unpaired electron and because of that electron they are highly unstable. Radicals immediately undergo a reaction with another molecule in order to obtain the missing electron. The ozone radical chain mechanism consists of three different steps: initiation, chain reaction and termination. (Gottschalk & Co 2010, 13.)

At first, the ozone molecule is decayed into secondary oxidants such as hydroxyl radicals (OH^{o}). This decaying is accelerated by initiators, for example OH^{-}. Secondary oxidants react non-selectively and immediately ($k = 10^{8}$-$10^{10} M^{-1}s^{-1}$) with target molecules. For example, hydroxyl radical regains its missing electron by taking away a hydrogen electron from the target molecule to form a water molecule. Now target molecule has lost an electron, so it becomes a radical itself and will react further, propagating the chain reaction. But if a radical reacts with another radical, they will neutralize each other. This happens, because both radicals' unpaired electrons are paired and no further radicals are formed. (Gottschalk & Co 2010, 13-14.)

Reactions in the initiation step are between hydroxide ions and ozone molecules. This reaction leads to the formation of one superoxide anion O_2^{-} and one hydroperoxyl radical HO_2^{o} in acid – base equilibrium (table 2 reactions 1 & 2). In the radical chain reaction, the superoxide anion (O_2^{o-}) formed in the initiation step reacts with ozone to form an ozonide anion (O_3^{o-}) (table 2 reactions 3 & 4). The ozonide anion then decomposes immediately via hydrogen trioxide (HO_3^{o}) to an OH^{o} radical (table 2 reaction 5). OH^{o}-radical can react with ozone (table 2 reactions 6 & 7) forming finally

oxygen and hydroperoxyl radical. These substances were used in the initiation step, so a chain reaction is formed. (Gottshalck & Co 2010, 14-15.)

TABLE 2. Ozone decomposition process and rate constants (Gottschalk & Co 2010, 14-15)

Reaction		
$O_3 + OH^- \rightarrow O_2^{o-} + HO_2^o$	$k = 70\ M^{-1}s^{-1}$	(1)
$HO_2^o \leftrightarrow O_2^{o-} + H^+$	$pK_a = 4.8$	(2)
$^oO_2^- + O_3 \rightarrow O_3^{o-} + O_2$	$k = 1.6 \times 10^9\ M^{-1}s^{-1}$	(3)
$O_3^{o-} + H^+ \leftrightarrow HO_3^o$	$pK_a = 6.2$	(4)
$HO_3^o \rightarrow OH^o + O_2$	$k = 1.1 \times 10^5\ s^{-1}$	(5)
$OH^o + O_3 \rightarrow HO_4^o$	$k = 2.0 \times 10^9\ M^{-1}s^{-1}$	(6)
$HO_4^o \rightarrow HO_2^o + O_2$	$k = 2.8 \times 10^4\ s^{-1}$	(7)
$3\ O_3 + OH^- + H^+ \rightarrow 2\ OH^\circ + 4\ O_2$		(8)

The decay of the ozone molecule, initiated by the hydroxide ion leads to a chain reaction where fast-reacting and non-selective OH^o-radicals are produced. The overall reaction (table 2 reaction 8) shows that three ozone molecules produce two OH^o-radicals. These radicals react with target molecules which are in the position of the highest electron density due to their electrophilic properties. OH^o-radicals are extremely reactive and for example the reaction rate constants for OH^o radicals and aromatic compounds are close to the diffusion limit which means that they react as soon as they come into contact with each other. (Gottshalck & Co 2010, 16.)

2.3.3 Initiators, promoters and inhibitors

Decaying of ozone and thereby the forming of radicals can be initiated, promoted or inhibited by many substances (Gottschalck & Co 2010, 16). Initiation can be accelerated for example by adjusting the pH and the addition of hydroxyl peroxide or ferrous ions and the use of UV radiation (Nissinen 2002, 23).

Promoters keep the chain reaction going. They are substances that convert OH^o radicals into superoxide radicals O_2^{o-}/HO_2^o so they promote the chain reaction. Organic

molecules can also act as promoters. Some of them contain functional groups, like many pharmaceuticals, that react with OH^o-radicals and form organic radicals. And if oxygen is present, organic radicals can react into organic peroxy radicals, which can further react and enter again into the chain reaction. (Gottschalk & Co 2010, 15.)

When some organic and inorganic substances react with OH^o-radicals, they form secondary radicals that do not produce superoxide radicals O_2^{o-}/HO_2^o. The chain reaction is generally terminated by these inhibitors. And when the chain reaction is stopped, also the ozone decomposition is inhibited. Some of the important inhibitors in natural water systems are bicarbonates and carbonates. The reaction rate constants are relatively low, but the concentrations of these substances are high. (Gottschalk & Co 2010, 15, 17.)

2.3.4 Advanced oxidation process

Advanced oxidation process (AOP) is a process where OH^o-radical formation affects on water purification. AOPs are used to generate OH^o-radicals, because OH^o-radicals have higher oxidation potentials than molecular ozone and they can affect organic and inorganic molecules extremely fast and non-selectively. The most common AOPs are O_3/H_2O_2, O_3/UV and H_2O_2/ UV. (Gottschalk & Co 2010, 20.)

UV-radiation itself can change the composition of the organic chemicals, for example pharmaceuticals, in water even in disinfection doses (Von Gunten & Co 2006, 1865). In the advanced process with ozone/UV-radiation, UV-radiation at 254 nm starts the photolysis of ozone. Then ozone photodecays into hydrogen peroxide which reacts with ozone and in the end forms 2 OH^o-radicals (reaction 9 & 10). (Gottschalk & Co 2010, 21.)

$$O_3 + H_2O \rightarrow H_2O_2 + O_2 \quad \text{(in the presence of UV-light)} \qquad (9)$$

$$2\ O_3 + H_2O_2 \rightarrow 2\ OH^o + 3\ O_2 \qquad (10)$$

Because of the radical formation, the removal efficiency of ozone/UV-radiation is usually higher than ozone or UV-treatment alone. Ozone/ UV-treatment does not use any chemicals, but OH^o-radical formation is not as high as when using hydroxyl

peroxide/ozone or hydroxyl peroxide/UV, because of ozone's bad mass transfer ability to water. That is also the reason why operational costs are high if the water is highly contaminated. Also turbidity affects the efficiency of UV-radiation. The higher the turbidity, the weaker the UV-radiations efficiency range. (Spartan Environmental Technologies 2011.)

2.4 The order of a reaction

In chemical kinetics, the order of a reaction is defined as the power to which its concentration term is raised to in the rate equation. The reaction order is the change of concentration of any of the substances per time (formula 1).

$$\frac{-d[A]}{dt} = k[A]^m[B]^n = R, \quad (1)$$

Where [i] = concentration of component i
k = reaction rate constant
R = reaction rate mol / l · s

Order of reaction is the sum of exponent's m + n. The order of the reaction can be 0, 1, 2 or more (table 3). There are also reactions whose order is a fractional number or whose order seems to change. (Gottshalck & Co 2010, 206.)

TABLE 3. Reaction rates in reaction order 0, 1, 2 and 3 (Gottshalck & Co 2010, 206)

Order	Reaction rate
0	$R = k[A]^0 = k$
1	$R = k[A]$
2	$R = k[A]^2$
3	$R = k[A]^2[B]$

Zero order reaction rates don't depend on the concentrations of reactants (formula 2).

$$R = \frac{-d[A]}{dt} = k \qquad \text{and integrated form is } [A]_0 - [A] = kt \qquad (2)$$

First order reaction rates are linearly affected by one of the reactant concentrations (formula 3). Reactions that follow first order kinetics are for instance single reactant reactions like radioactive breakdown.

$$R = \frac{-d[A]}{dt} = k[A] \qquad \text{and integrated form is } \ln(\frac{[A]_0}{[A]}) = kt \qquad (3)$$

Second order reaction rates are influenced by concentrations of two reactants (formula 4). Usually chemical reaction rates are affected by all the reactant concentrations and in reactions with two reactants, second order is the most common case.

$$R = \frac{-d[A]}{dt} = k[A]^2 \qquad \text{integrated form is } \frac{1}{[A]} - \frac{1}{[A]_0} = kt \qquad (4)$$

If the concentration of the other reactant stays constant, then the reaction is called a pseudo first-rate constant. Pseudo first-rate constants use the reaction rate order of a first order reaction. (Gottshalck & Co 2010, 207, 212.)

2.5. Flocculation

Flocculation is used in wastewater treatment to separate suspended solids from water. Suspended solids often have a negative charge on their surface causing them to repel each other. Since this prevents them from colloiding with each other they can't form larger masses, flocs, and they do not settle. Chemical flocculation is required to assist the removal of colloidal particles from the suspension. Usually a positively charged flocculation chemical is mixed with the wastewater to promote the aggregation of the negatively charged suspended solids into particles large enough to settle or be removed. (Water Spesialists Technologies LLC. 2009.)

2.6 Pharmaceuticals used in research

Sulfamethaxazole (figure 4) is a bacteriostatic antibacterial agent that interferes with the life-maintaining folic acid syntheses in vulnerable bacteria (Medicinenet 2011). Ternes & Co (2003) discovered that Sulfamethaxazole could be eliminated completely from sewage treatment plant effluents by ozonation.

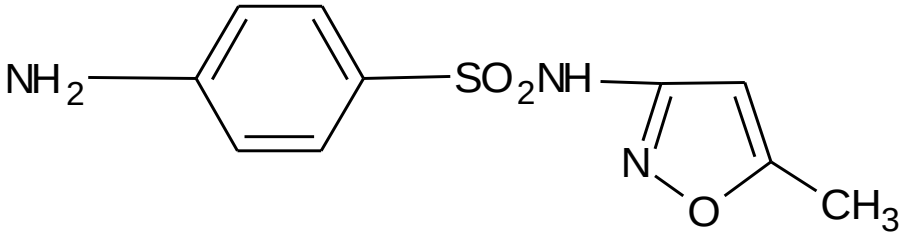


FIGURE 4. Molecular structure of Sulfamethaxazole

Ciprofloxacin (figure 5) belongs to fluoroguinolone class and it is an antibiotic which is used to treat bacterial infections. It stops the multiplication of bacteria by inhibiting the reproduction and repair of their DNA. (Medicinenet 2011.)

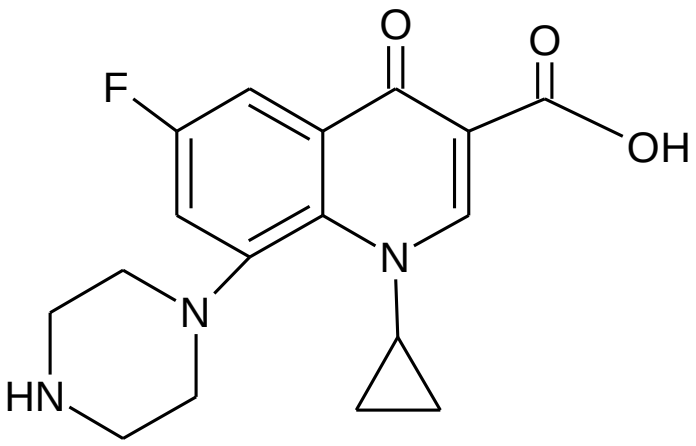


FIGURE 5. Molecular structure of Ciprofloxacin

Ibuprofen (figure 6) is non-steroidal anti-inflammatory drug which is used for the management of mild or moderate pain, fever and inflammation. It works by blocking the formation of an enzyme that makes prostaglandins which are chemicals that promote pain, fever and inflammation. (Medicinenet 2011.) Ibuprofen has been found to react moderately with ozone (Ikehata & Co 2006, 374).

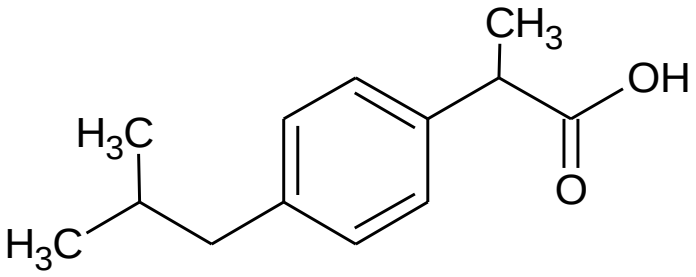


FIGURE 6. Molecular structure of Ibuprofen

Paracetamol (figure 7) belongs to a class of drugs called analgesics (pain relievers) and antipyretics (fever reducers). The action mechanism of Paracetamol is unknown but it is known that it relieves pain by elevating the pain threshold. A greater amount of pain is needed before person can feel it. Paracetamol also affects the heat-regulating center of the brains and lowers the fever. (Medicinenet 2011.) Paracetamol have been found to react well and decay fast with ozone (Ikehata & Co 2006, 375 - 376).

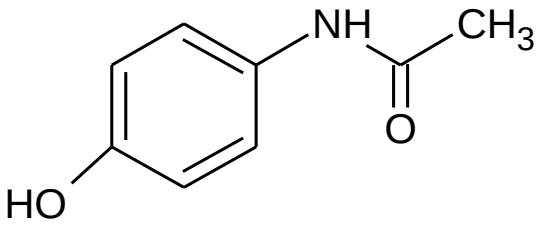


FIGURE 7. Molecular structure of Paracetamol

Metronidazole (figure 8) is an antibiotic which is used against anaerobic bacteria and certain parasites. It selectively blocks some of the functions within the bacteria cells and the parasites which in the end results in their death. (Medicinenet 2011.)

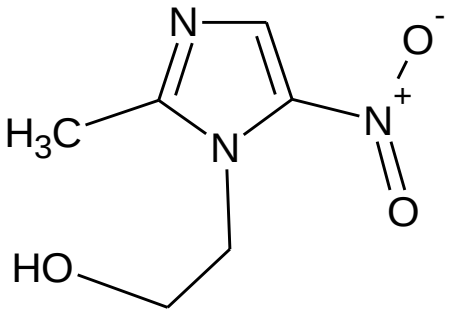


FIGURE 8. Molecular structure of Metronidazole

3 MATERIALS AND METHODS

3.1 Samples

Universal Corporation Limited has a medicine factory in Kikuyu, Kenya, which produces medicines for malaria and HIV. It also manufactures many products for managing pain, dermatology, anti-infectives, psychiatry, neurology and drugs to fight opportunistic infections. (Universal Corporation Limited 2009.) During manufacturing, medicinal dust is formed which is then rinsed with ionized water to the sewer. The sewer leads to two 10 m^3 tanks where the wastewater is supposed to be treated. (Lammi 2010.)

On the week when the samples were taken, five medicines were manufactured in the factory. These medicines were Sulfamethaxazole, Ciprofloxacin, Ibuprofen, Paracetamol and Metronidazole. One sample was taken from tank 1 on the 23.11.2010 and two samples were taken from tank 2 on the 22.11.2010. On tank 2 the different sampling places were called SP-1 and SP-2. SP-1 meant that the sample was taken from the wastewater before treatment in tank 2 and SP-2 was taken after the treatment in tank 2. This treatment consisted of precipitation with sodium hydroxide. Samples were taken after half an hour of mixing from the full tank. (Lammi 2010.)

Sample volume was two times one liter bottle. They arrived on 2.12.2010 to Tampere University of Technology where the analyses were done. The waste water was pale red, it had a lot of turbidity and there were small amounts of solids floating in the water. The red color was probably from the shell color which was used on the tablets.

3.2 Characterization of the sample water

3.2.1 pH and dissolved organic carbon

pH was measured with WTW pH 330i –pH-meter. Electrode was put into the sample water so long until the pH-reading in the pH-meter stabilized. Dissolved organic carbon

(DOC) was measured using Shimadzu TOC 5000 analyzer with ASI-5000 auto-sampler. Sample waters were filtered through 0,45 µm filter to TOC-tubes and put to the auto-sampler. Parallel samples were used. Analyzer injected sample into the total carbon combustion tube (+680 °C) filled with oxidation catalyst. Synthetic air was used as a carrier gas. The carbon compound was decomposed to CO_2 which flowed with the carrier gas into an infrared gas analyzer where CO_2 is detected.

3.2.2 High performance liquid chromatography

High performance liquid chromatography (HPLC) is a method which is widely used for analyzing organic and inorganic compounds. It can detect many substances at the same time which makes it irreplaceable in analyzing organic molecules which have large molecular weight, for example pharmaceuticals. In theory, the only condition for HPLC is that sample can be dissolved to some solvent. (Jaarinen & Co 2000, 144.)

In HPLC, a small amount of sample (10 – 20 µl) is injected to the liquid phase (mobile phase or eluent) using the injector. Liquid phase travels evenly in narrow capillaries with the help of a pump into the column. The column is packed with the stationary phase which consists of small particles. These particles divide the sample into its components which stay in the particles for different time. At each turn, components come out from the column and into the detector which signals a peak onto the chromatogram. (Jaarinen & Co 2000, 145.)

The equipment used was a Hewlett Packard 1100 –series HPLC (figure 9.), the column was Phenomenex Gemini-NX 5 um C18 110 A and eluent was combination of 0,02 M KH2PO4 and acetonitril. It was adjusted in the method how many percent of eluent was 0,02 M KH2PO4 and how many acetonitril, because this method was adjusted so that first comes out the more hydrophilic, polar substances and then hydrophobic, non-polar substances (appendix 1.). Injection volume was 20 µl, flow rate 1,5 ml and detector was UV/Vis-detector which used four different detection wavelengths. Those wavelengths were 210 nm, 240 nm, 254 nm and 265 nm where always the best and clearest signal was used for further analyzes.

FIGURE 9. High Performance Liquid Chromatography system used (Photo: Jukka Pohja 2011)

Calibration was performed for five pharmaceuticals which were supposed to be in the sample. Pure forms of Sulfamethaxazole, Ciprofloxacin, Ibuprofen, Paracetamol and Metronidazole was used. Each pharmaceutical was weight 0,1 g to 100 ml volumetric flask so the concentration was 1 mg /ml. Paracetamol was only diluted in water, others were diluted with acetone and milliQ-water mixture. Stock solution 1 of 0,01 mg/ml and stock solution 2 0,1 mg/ml were made from the original 1 mg/ml solution. Six calibration solutions were directly prepared to the vials (appendix 1.). Calibration solutions also informed the retention time for each pharmaceutical.

The untreated sample waters were filtered through the 0,45 µm filter to the vials and analyzed with HPLC. Parallel samples were used. MilliQ-water and acetonitril + milliQ-water mixture were used as blank samples.

3.3 Ozonation

Ozonation was made in fume hood using the coronal discharge Red Sea Fishpharm ltd. Redox plus ozone generator. It transformed oxygen from the oxygen gas bottle to ozone using electric discharge. Ozone generator was adjusted to produce 150 mg ozone in one hour (2,5 mg/ml) and the air flow was adjusted to 1 l/min with a rotameter. Always at first ozone generator was switched on and left to heat up for 10 minutes. The efficiency of ozone was increased by using bubble producing sinters in the sample and washing bottles (figure 10).

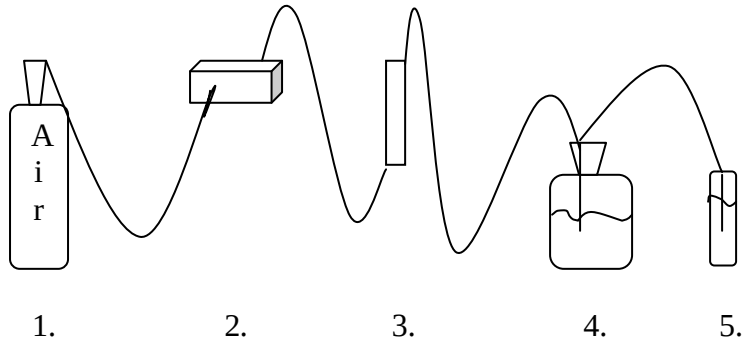


FIGURE 10. The ozone generator system (1. Air bottle 2. Ozone generator 3. A rotameter 4. Sample flask 5. Washing flask)

Although the ozone generator was set to produce 2,5 mg/ml, the real gas flow of ozone was determined in the beginning and at the end of the ozonation. All of the ozone didn't react with the sample. The overflow of ozone from the sample flask that not reacted with the sample was determined with iodine metric titration. The amount of non-reacted ozone was reduced from the original ozone amount.

For these analyses two liters of potassium iodine buffer solution was prepared. It consisted of 40 g of potassium iodine (KI), 11,65 g of sodium hydrogen phosphate (Na_2HPO_4) and 7,0 g of potassium phosphate (KH_2PO_4) which were diluted with milliQ-water. This buffer solution had to be prepared many times. Already prepared were 0,1 M of sodium thiosulfate, 4,5 M sulfuric acid and starch solution.

Concentration of sodium thiosulfate was verified according to SFS 3036 standard. 25 ml of milliQ-water, 2 ml of 4 M sulfuric acid and 1 ml of potassium iodide solution was measured into beaker. Mixture was mixed up and 5 ml of potassium iodate was added.

Solution was titrated instantly with sodium thiosulfate and when light yellow color was seen, starch solution was added. Titration was continued until pale blue color was turned into transparent. Titration was done three times and the average of these three results (formula 5) was used.

$$c_{sodiumthiosulfate} = \frac{30 \cdot c_{potassiumiodatesolution}}{V} \quad (5)$$

3.3.1 Determination of ozone flow

Determination of ozone flow was also iodine metric titration. First 200 ml of KI buffer solution was measured to the flask. A bottle was attached to the ozone generator system and the air flow was set to 1 l/min through the ozone generator. The flask was ozonated for 10 minutes.

After ten minutes the bottle was taken out from the system and a new bottle was attached so that no ozone was released to the air. Six milliliters of 4,5 M sulfuric acid was added to the ozonated flask. Flask was titrated to bright yellow with 0,1 M sodium thiosulfate and then a few drops of starch solution were added to the flask. Eventually it was titrated to clear transparent. Ozone oxidizes iodide ions to yellow iodine (reaction 11) and thiosulfate restores them back to colorless ions (reaction 12).

$$O_3 + 2\ I^- \rightarrow I_2 + O_2 + O^{2-} \quad (11)$$

$$I_2 + 2\ S_2O_3^{2-} \rightarrow 2\ I^- + S_4O_6 \quad (12)$$

The mass of reacted ozone is half of the volume of thiosulfate:

$$\text{Ozone concentration of the gas (mg/l)} = \frac{1}{2} \cdot c_{thio} \cdot \frac{V_{thio}}{V_{air}} \cdot 48 g / mol O_3 \quad (6)$$

This ozone concentration measurement (formula 6) was made in the beginning and at the end of each ozonation process.

3.3.2 Determination of ozone that reacted with the sample

When a sample was being ozonated, all the ozone didn't react with the sample, so washing flasks were used to collect the overflow ozone. This had two beneficial actions. First, the amount of overflow ozone could be calculated and reduced it from the original amount of ozone to get the exact amount of ozone that has reacted with the sample. Also at the same time the irritating ozone was collected from the air to washing flasks.

Washing flasks consisted of 100 milliliters of KI buffer solution and they were changed every tenth minute during ozonation. After changing, a new flask was immediately attached to the ozonation system. Three milliliters of 4,5 M sulfuric acid was added to the ozonated washing flask and titrated to bright yellow with 0,1 M sodium thiosulfate. A few drops of starch solution were added and eventually it was titrated to clear transparent. Same reactions and calculations occurred in this determination as in the ozone flow determination.

At the end all of the washing flasks ozone doses were calculated (formula 5) and reduced from the total amount of ozone. Thus the amount of ozone which reacted with the sample was calculated.

3.3.3 Ozonation of the sample

Tank 2 SP 1 sample was used for the ozonation because it was the most contaminated with pharmaceuticals (figure 15). So it was easy to see if there occurred any decrease in the concentrations of the studied five pharmaceuticals. 500 milliliters of sample water was diluted to 1000 milliliters with milliQ-water and put to 1000 ml glass flask.

At first, ozone flow was determined and then the diluted 1000 milliliters of sample water was ozonated for 280 min. Washing flask was changed in every tenth minute and titrated immediately. A sample from the sample water was taken with syringe and filtrated through 0,45 µl filter to a vial every twentieth minute. Parallel samples were taken. After 280 minutes, ozonation was stopped and samples were analyzed with HPLC. DOC samples were taken to see if there was any change in the amount of

organic matter. The amount of ozone was calculated for 90 % elimination of each pharmaceutical.

Finally the reaction rate constant and half-life of each pharmaceutical were calculated using pseudo first reaction order because the concentration of ozone was constant. The amount of ozone which was needed to eliminate 90 % of pharmaceuticals was calculated to sample volume and 10 m^3 tanks used in medicine factory. Also the amount of how many milligrams of ozone were needed to eliminate one milligram of pharmaceutical was calculated. And the total percentage number of eliminated for each pharmaceutical was determined.

3.4 Ozone and UV-radiation

For the ozone/UV experiments, a Pyrex glass reactor of five liters was used. It was filled up (about 4,5 liters) with sample water which consisted of Tank 1: 1000 ml, Tank 2 SP 1: 620 ml, Tank 2 SP 2: 880 ml = 2500 ml which was diluted to 5000 ml with milliQ-water. According to my earlier experiments, ciprofloxacin was the hardest to mineralize, so 0,400 g of ciprofloxacin was added to the sample water to get a high concentration and thus more obvious results. Sample water was mixed over night so all of the ciprofloxacin was diluted.

The effect of the UV-radiation to the sample water was studied first. Lamp quartz tube, 9 W Osram HNS 20 W/U low pressure mercury vapour UV-lamp and magnetic stirrer were inserted to the UV-reactor. Nitrogen flow was put between UV-lamp and quartz tube to prevent ozone formation and explosion. Stirrer was turned on and the reactor was left to stabilize for one hour. During all experiments with UV-light, the reactor was covered up with aluminum foil to block the UV radiation leak to outside the reactor. After one hour of stabilization, UV-lamp was turned on (figure 11). Sample was taken every twentieth minute using sampling hole. Parallel samples were used. (Tuhkanen 2004.)

FIGURE 11. UV-reactor with UV-lamp on (Photo: Jukka Pohja 2011)

After one hour of UV-lamp on, ozonation was started. Ozone was produced in the ozone generator and added to the reactor using a sinter (figure 12). Therefore both UV-radiation and ozone were used. Ozone flow was adjusted to 1 l/min with rotameter and the flow was determined before and after ozonation. Washing bottles were changed every tenth minute and overflow ozone was titrated. A sample was taken every twentieth minute and parallel samples were used. UV/ozonation lasted for 120 min (figure 13). After that UV-lamp was turned off, but ozone generator remained on.

Finally, the sample water was ozonated for two hours to see that ozonation in UV reactor is as efficient as in regular 1 liter glass flasks. Samples from UV, UV/ozone and ozonation were analyzed with HPLC.

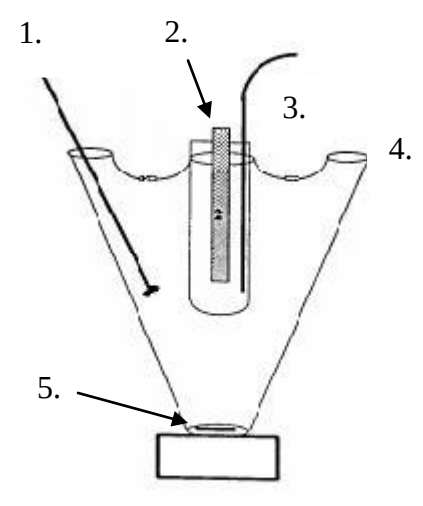


FIGURE 12. UV/ozone system. (1. Ozone sinter added to ozone generator 2. UV-lamp 3. Nitrogen flow 4. Sampling hole 5. Magnetic stirrer)

FIGURE 13. UV/ozonation in progress (Photo: Jukka Pohja 2011)

3.5 Floccualation and ozonation

Flocculation of the sample water was performed by using 2,5 g of Watermaker plus flocculate powder to about 4,5 liter of already UV/ozonated sample and mixed up (figure 14). It was done to the old sample water, because no sample water was left anymore. Sample was left to flocculate for one hour and then the precipitate was left to the bottom of the bottle and three liters of supernatant was transformed into another bottle. Supernatant was then analyzed with HPLC.

FIGURE 14. Adding of the coagulant powder (Photo: Jukka Pohja 2011)

Finally, the three liters of supernatant was ozonated for 160 minutes. Ozone flow was 1 l/min and it was determined in the beginning and at the end of ozonation. Washing bottles were titrated and samples were taken in every twentieth minute. Parallel samples were used. After 160 minutes of ozonation, the samples were analyzed with HPLC.

4 RESULTS

4.1 Characterization of the water

4.1.1 pH and dissolved organic carbon

The pH of the sample water is a little acidic but not significantly. This can affect the decomposition of ozone. DOC-ratings are quite high which is typical for pharmaceutical waste water indicating that there are organic molecules present. All the five pharmaceuticals are organic so the sample water which was the most contaminated could be concluded. pH and DOC of the sample waters are shown in the table 4.

TABLE 4. Initial pH and DOC of the sample waters

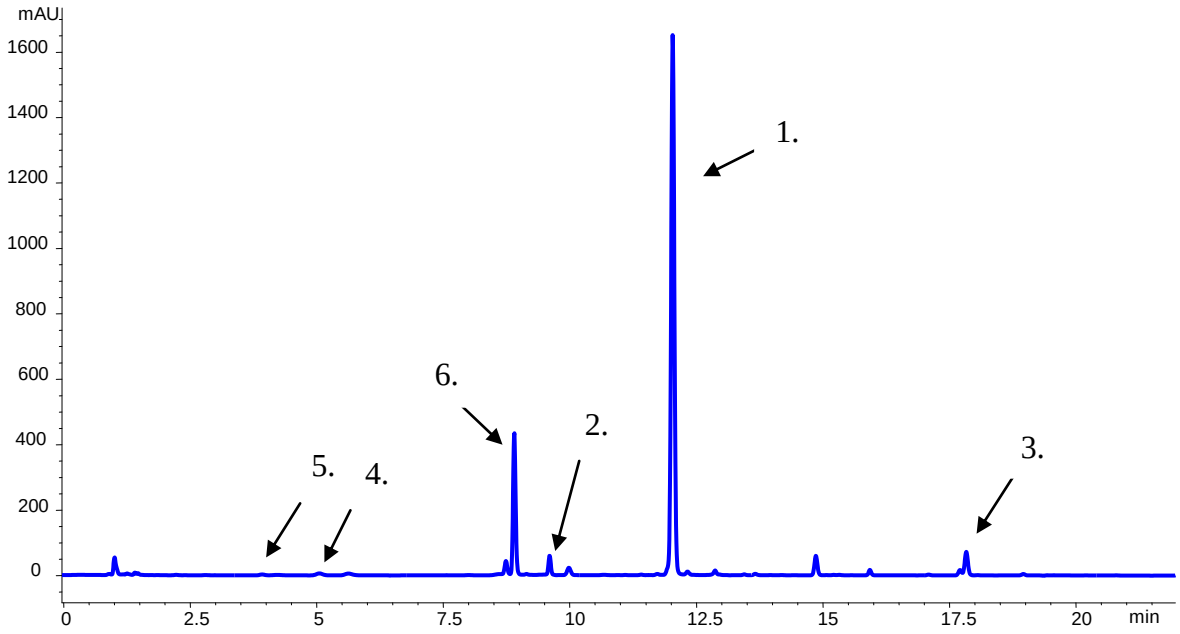
sample	**initial pH**	**DOC (mg/l)**
Tank 1	5,4	261,4
Tank 2 SP 1	5,5	378,1
Tank 2 SP 2	6,1	164,4

4.1.2 Original pharmaceutical concentrations in the sample waters

Forming of calibration curves succeeded and their results are seen in the appendix 2. All the five pharmaceuticals were clearly separated. Sample waters contained also unknown substances, but with their retention times, studied pharmaceuticals were easy to identify from the sample chromatogram (figure 15). Determination of concentrations was also successful. Parallel results were near each other and good calibration curves gave reliable results.

Sulfamethaxazole, Ibuprofen and Metronidazole gave the best results in UV-detector wavelength 211 nm. For the Paracetamol the best wavelength was 241 nm and for the ciprofloxacin the best was 264 nm. As seen in the figure 16, Tank 2 SP 1 was the most contaminated with pharmaceuticals, next was Tank 1 and the last Tank 2 SP 2. So the precipitation treatment used in the facility lowered the amount of pharmaceuticals, because Tank 2 SP 1 results are higher than Tank 2 SP 2.

Sulfamethaxazole concentration was clearly the highest in the each sample water. Ibuprofen was the second and Ciprofloxacin the third common in samples. Paracetamol and Metronidazole concentrations were so small that their concentrations didn't need to be taken into account in the later research.



FIGUR E 15. Chromatogram of the sample water Tank 2 SP 1 in wavelength 210, 8 nm (1. Sulfamethaxazole 2. Ciprofloxacin 3. Ibuprofen 4. Paracetamol 5. Metronidazole 6. Unknown)

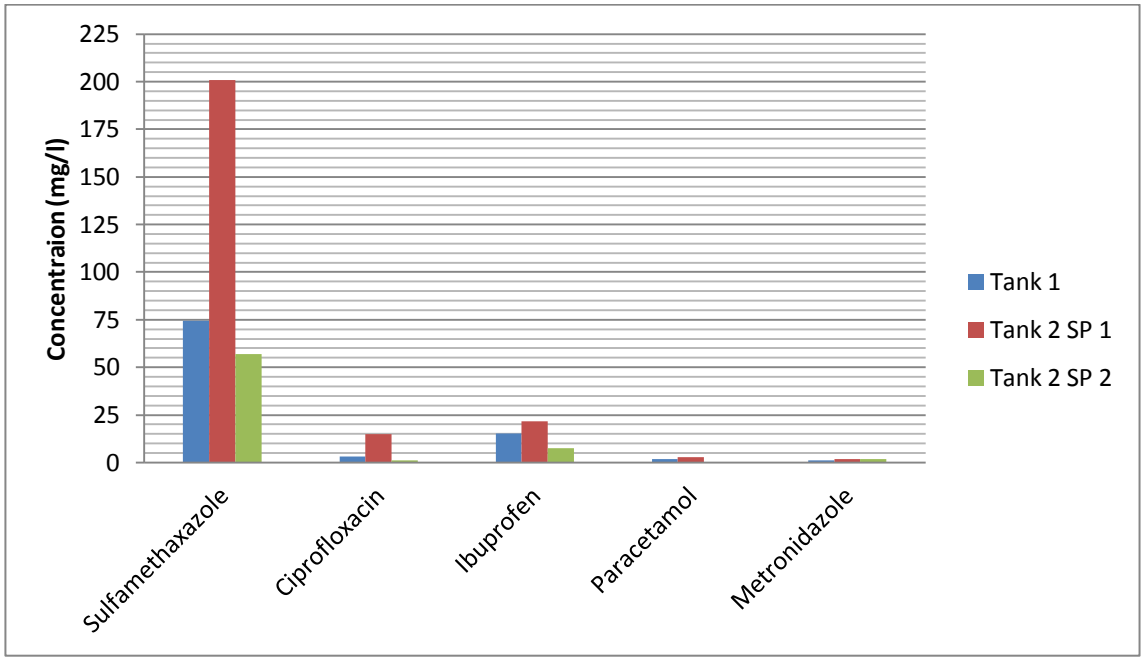


FIGURE 16. Concentrations of each pharmaceutical in Tank 1, Tank 2 SP 1 and Tank 2 SP 2

4.2 Ozonation

280 minutes of ozonation of Tank 2 SP 1 was successful. It reduced the amount of each five pharmaceuticals, but with different intensity. Although the concentration in the sample water of each pharmaceutical was different, Sulfamethaxazole was the fastest to mineralize and Ciprofloxacin was the slowest (figure 17). Paracetamol and Metronidazole were practically completely mineralized after few ten minutes so they are not involved in the results. Ozone flow was calculated to be approximately 1,4 mg/min and total ozone formation 392 mg. Overflow ozone, ozone that not reacted with sample, was calculated to be approximately 24 mg. Thus total ozone amount that reacted with the sample was 392 mg – 24 mg = 368 mg.

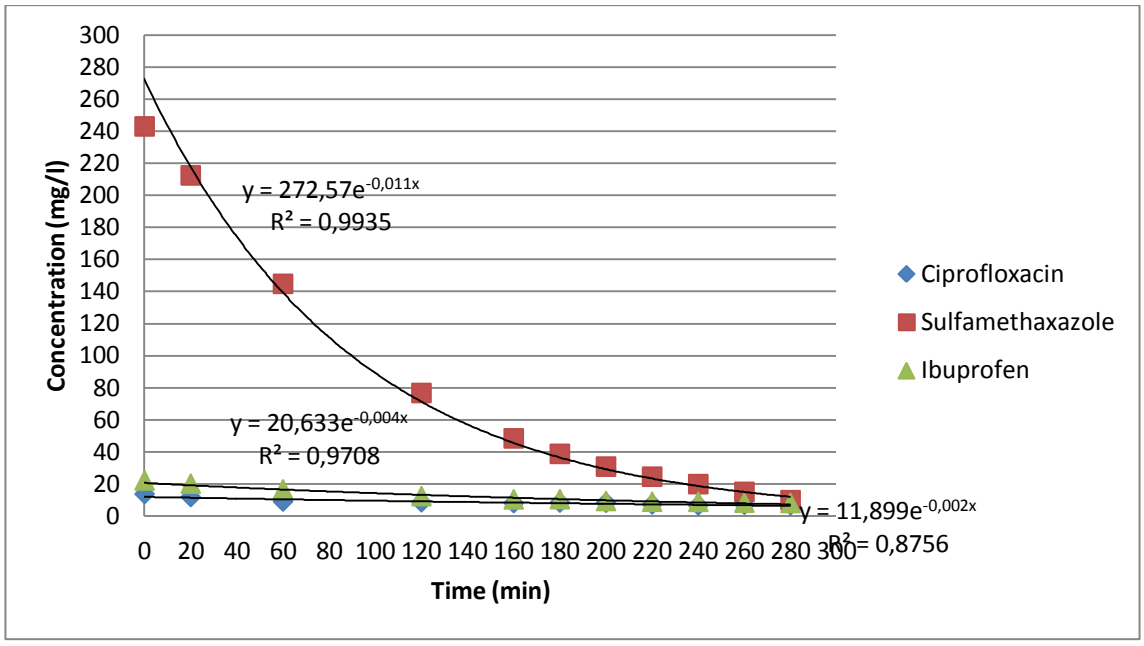


FIGURE 17. Decaying rates of Sulfamethaxazole, Ibuprofen and Ciprofloxacin after 280 min of ozonation

As seen in figure 18 peaks of the pharmaceuticals got lower and new peaks were formed to the beginning of the chromatogram. This indicates that more hydrophilic and biodegradable byproducts are formed from the pharmaceuticals.

DOC changed from 385,9 mg/l to 369,4 mg/l so the organic matter wasn´t eliminated efficiently. This showed that the molecular form of the pharmaceuticals changed into unknown, but more hydrophilic byproducts.

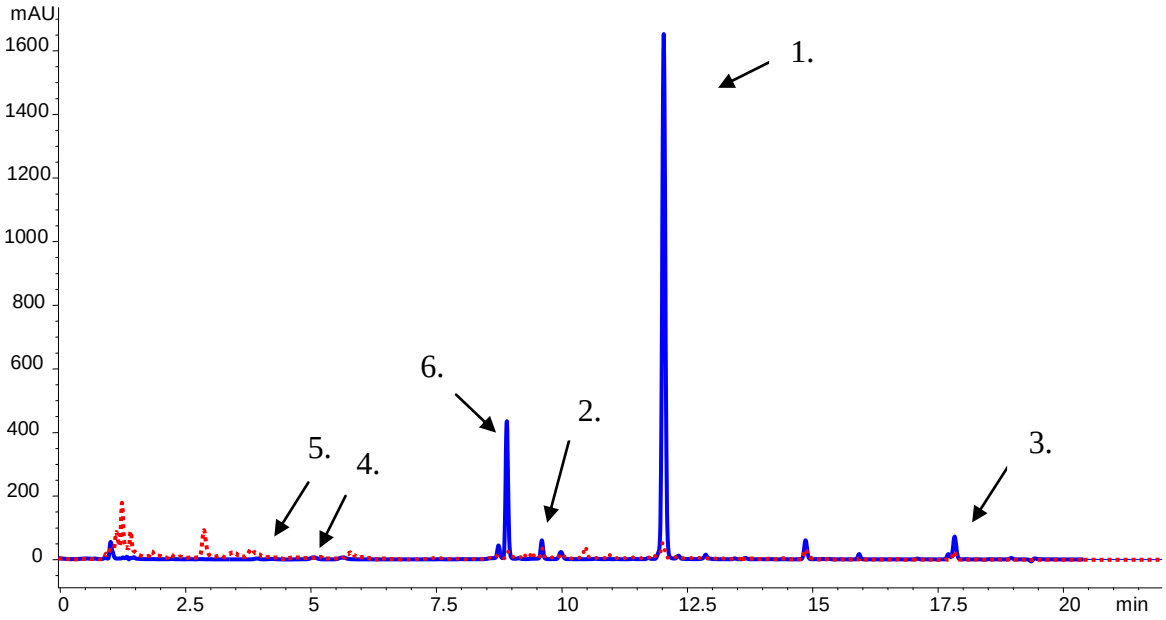


FIGURE 18. Chromatogram of Tank 2 Sp 1 before the ozonation (blue line) and after 280 min ozonation (red dash line) in wavelength 210,8 nm (1. Sulfamethaxazole 2. Ciprofloxacin 3. Ibuprofen 4. Paracetamol 5. Metronidazole 6. Unknown)

In table 5 are shown the amounts of ozone in milligrams which will be needed to eliminate 90 % of pharmaceutical and ozone dose in grams which will be needed to eliminate 90 % of pharmaceuticals in 10 m^3 tanks which are used in Union Corporate Ltd. factory. Table 5 also shows how many milligrams of ozone are needed to decay one milligram of pharmaceutical and the elimination percentage of each pharmaceutical.

TABLE 5. Elimination results for Sulfamethaxazole, Ciprofloxacin and Ibuprofen after 280 min of ozonation

	O_3 (mg)(90 % elimination)	O_3 (g) (90 % elimination in 10m^3 tanks)	mg O_3/mg drug needed	pharmaceutical eliminated (%)
Sulfamethaxazole	294	2940	1,57	94,0
Ciprofloxacin	1612	16120	52,6	51,5
Ibuprofen	806	8060	26,3	63,7

In table 6 are shown reaction rate constant and half-life for each pharmaceutical. Results were able to be calculated also for Metronidazole and Paracetamol, although the

concentrations were really low and thus the results were not very reliable. All the calculations are made by using pseudo first reaction rate.

TABLE 6. Reaction rate constant and half-time of each pharmaceutical after 280 min ozonation

	Reaction rate constant k	Half-time $t_{1/2}$ (min)
Sulfamethaxazole	0,0112	61
Ciprofloxacin	0,0022	315
Ibuprofen	0,0037	187
Metronidazole	0,0084	83
Paracetamol	0,0341	20

4.3 Ozone and UV-radiation

Bare UV-radiation also decayed the five pharmaceuticals in sample water. Although, sample volume was larger and turbidity was quite high, UV-radiation decayed pharmaceuticals almost at the same rate as ozone (figure 19). Ciprofloxacin original concentration was really high because of the addition of it but still it decayed at the same rate so the amount of original concentration is insignificant. Ibuprofen reacted really slowly to the UV-radiation.

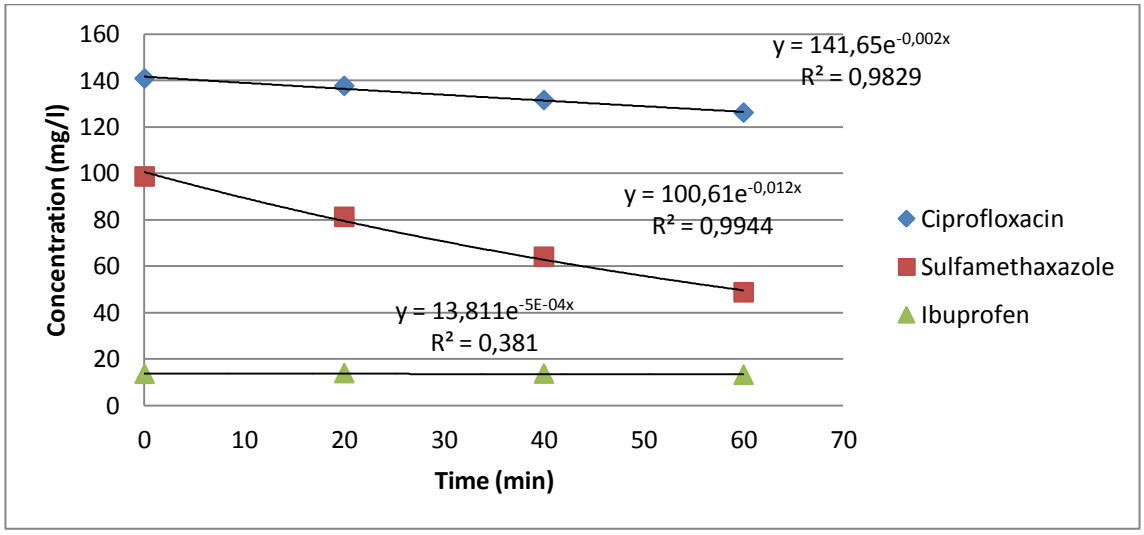


FIGURE 19. Decaying rates of Sulfamethaxazole, Ibuprofen and Ciprofloxacin after 60 min UV-radiation

Ozone/UV system also decayed the five pharmaceuticals almost at the same rate as ozone and UV (figure 20). So the OH-radical formation didn´t help the degradation process. Ciprofloxacin decayed a little faster than with UV or ozone as seen in figures 17 and 19. In ozone/UV the ozone flow was approximately 1,4 mg/min. Total ozone formation was 168 mg. Overflow ozone formation was approximately 5 mg. So the ozone amount that reacted with sample was 168 mg – 5mg = 163 mg.

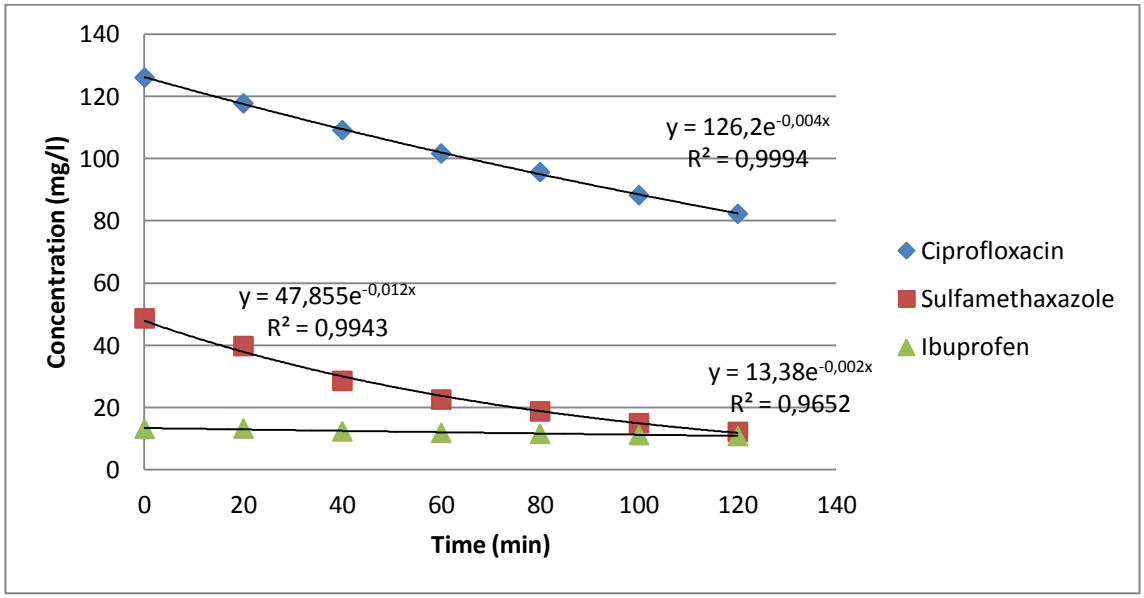


FIGURE 20. Decaying rates of Ciprofloxacin, Sulfamethaxazole and Ibuprofen after 120 min of UV/ozonation

Bare ozonation in the reactor proved that ozonation had worked in the UV-reactor. But still the results are almost identical with UV or UV/ozone (figure 21). This time also Sulfamethaxazole mineralized really slowly. Probably the really low original concentration (10 mg/l or under) affected to the ozonation results. There just wasn´t any pharmaceutical left which could mineralize. In ozonation in the 5 liters reactor the ozone flow was approximately 1,3 mg/l and total ozone production 156 mg. Ozone overflow was approximately 6 mg so total ozone amount that reacted with the sample was 156 mg – 6mg = 150 mg.

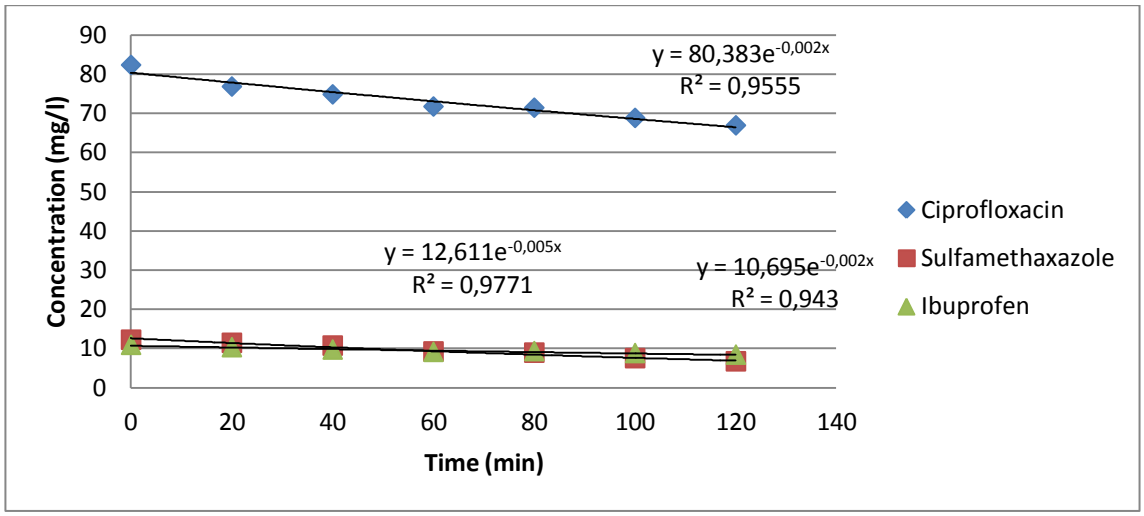


FIGURE 21. Decaying rates of Ciprofloxacin, Sulfamethaxazole and Ibuprofen after 120 min of ozonation

4.4 Flocculation and ozonation

Flocculation was easy and fast to do and it was effective in removing pharmaceuticals. Figure 22 shows how the turbidity changed and all the precipitate is located to the bottom of the flask after addition of the coagulant powder. Supernatant which can be seen in the last picture was ozonated.

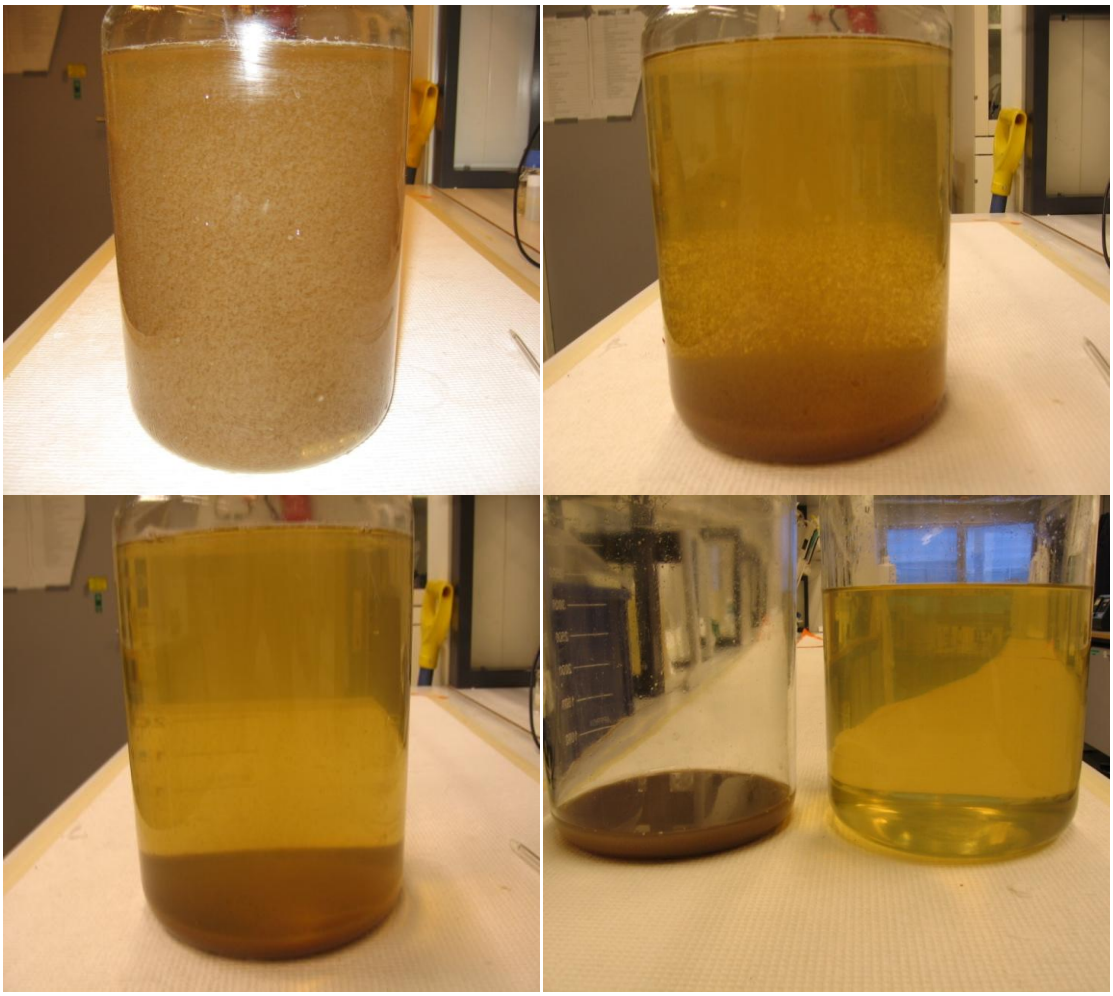


FIGURE 22. Flocculation of the sample water (Photo: Jukka Pohja 2011)

When analyzing HPLC results of the flocculation Ciprofloxacin was under research, because Sulfamethaxazole and Ibuprofen concentrations were so small (<10 mg/l) and Ciprofloxacin was the hardest to eliminate. The original Ciprofloxacin concentration in the sample water was 66,9 mg/l and after flocculation the concentration was 23,8 mg/l. So 64,4 % of the ciprofloxacin was eliminated by flocculation.

Ozonation of the supernatant for 160 minutes didn´t show any different results than before. Mineralization of Ciprofloxacin was not faster. Ciprofloxacin elimination rate was still the same (figure 23). Ozone flow was approximately 1,3 mg/l. Total ozone production was 208 mg and overflow ozone amount was 10 mg. Total ozone amount that reacted with the sample was 208 mg – 10mg = 198mg.

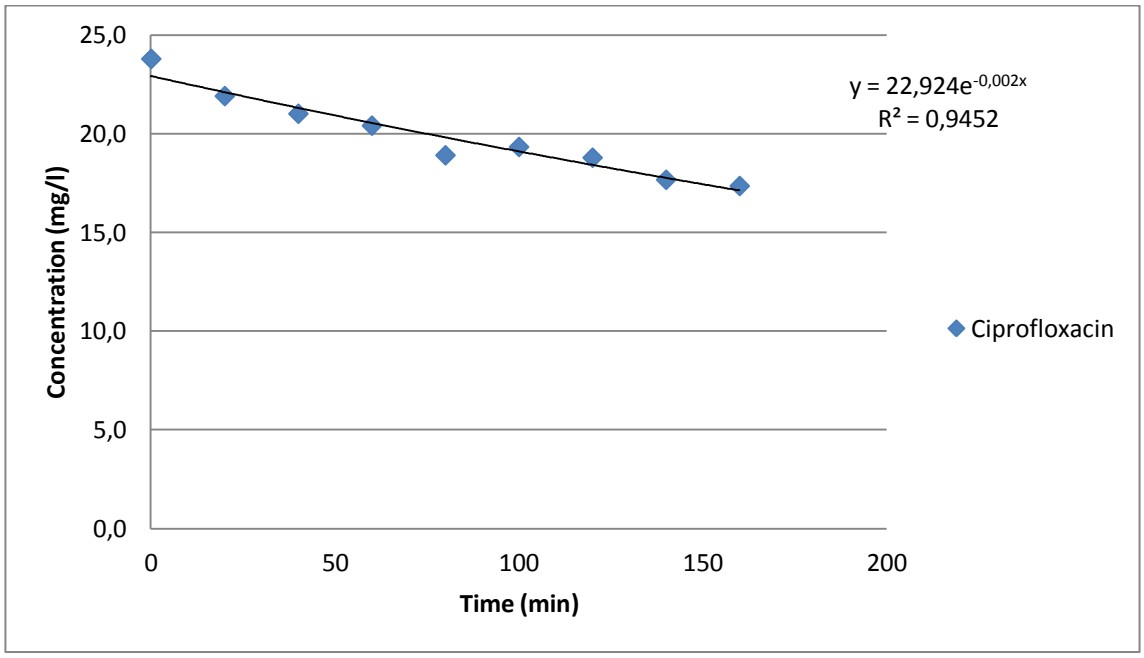


FIGURE 23. Ciprofloxacin elimination rate after 160 min ozonation

5 DISCUSSION

Sample water taken from the industrial wastewater in Union Corporation Ltd. contained lots of pharmaceuticals. Amounts were at tens of milligrams per liter which will cause damage to the environment if released untreated into nature. Precipitation treatments used in factory didn't eliminate all the pharmaceuticals which can be seen in the results from Tank 2 SP 1 and Tank 2 SP 2.

Ozonation of the sample water for 280 minutes lowered the concentrations of each studied pharmaceutical. Sulfamethaxazole was the fastest to decrease. It needed just 1,57 mg of ozone to decay one milligram although the original concentration was over 240 mg/l. Ciprofloxacin was the slowest to eliminate and although the original concentration was 13, 6 mg/l it needed 52,6 mg of ozone to eliminate one milligram of the drug. In the 10 m^3 tanks which are used in the factory, it will require 16120 grams of ozone to eliminate 90 % of ciprofloxacin.

In the factory there is about 4-6 hours time to process the two tanks. So 16120 grams of ozone should be formed in 4-6 hours. It will require a huge ozone generator which will produce 2000 - 4000 grams of ozone per hour. Alternative treatment processes were tried to lower the amount of ozone needed.

UV and ozone/UV did not fasten the elimination significantly. UV/ozone eliminated Ciprofloxacin fastest, but still it decayed really slowly. Flocculation was tried to collect the solid matter and to prevent the dissolving of pharmaceuticals to the sample water.

Flocculation succeeded to eliminate huge amounts of ciprofloxacin. 64,4 % of ciprofloxacin was eliminated during flocculation. Although ozonation after flocculation wasn´t faster, this leads to lower amounts of ozone needed, because original concentrations of pharmaceuticals will be lower. Thus insoluble, suspended and colloidal drug has to be removed by flocculation prior to ozonation. Flocculation or filtration will lower the pharmaceutical concentrations in the factories wastewater and the pharmaceuticals that are left can be eliminated with ozone, UV or ozone/UV.

These results cannot be compared to any earlier studies because the sample water was unique containing lots of pharmaceuticals. In earlier researches, elimination constants are defined by optimum circumstances and one pharmaceutical at a time. In this study all the pharmaceuticals were eliminated at the same time in the sample water so the elimination constants were really slow. Further studies are needed to test how flocculation will affect when installed to the factory and to determine the concentrations of pharmaceuticals after the flocculation and to calculate ozone doses needed to eliminate 90 % of the remaining pharmaceuticals.

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APPENDICES

FORMING OF ELUENT AND CALIBRATION SOLUTIONS APPENDIX 1

TABLE 7. The amounts of substances in eluent

Time (min)	KH2PO4 0,02 M pH 2,5 (%)	Acetonitril (%)
1	95	5
2	95	5
3	95	5
4	95	5
5	95	5
6	90	10
7	85	15
8	80	20
9	75	25
10	70	30
11	65	35
12	60	40
13	55	45
14	50	50
15	45	55
16	40	60
17	35	65
18	30	70
19	25	75
20	20	80
21	20	80
22	20	80

TABLE 8. Preparation of calibration solutions

Calibration solution concentration (mg/l)	Volume Stock Solution (µl)	Volume MQ (µl)
1	100 S1	900
2	200 S1	800
5	500 S1	500
10	1000 S1	0
25	250 S2	750
50	500 S2	500

FORMING OF CALIBRATION CURVES (HPLC) APPENDIX 2:1 (3)

TABLE 9. Sulfamethaxazole 210 nm, retention time 12,0 min:

Concentration (mg/)	Area
1	61,5
2	122,2
5	292,6
10	590,9
25	1541,4
50	3037,1

FIGURE 24. Calibration curve of Sulfamethaxazole

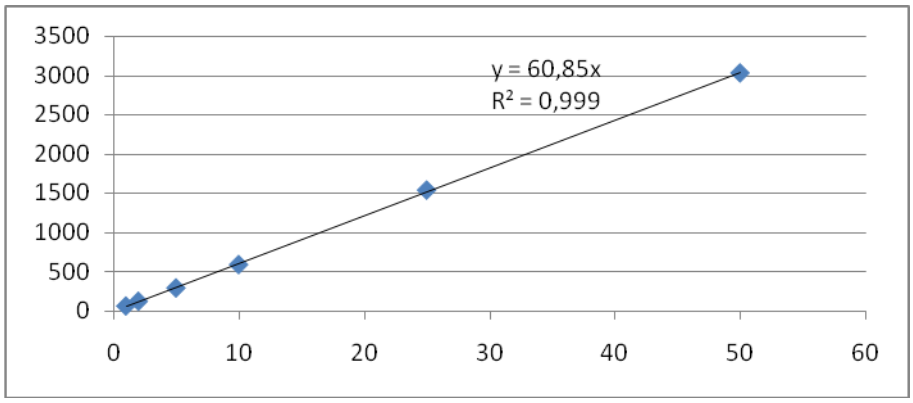
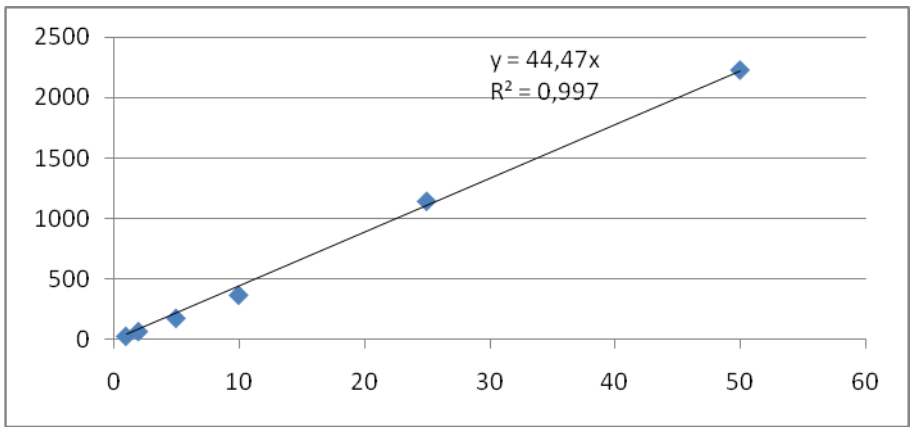


TABLE 10. Ciprofloxacin 264 nm, retention time 9,6 min:

Concentration (mg/l)	Area
1	31,5
2	69,9
5	179,8
10	370,8
25	1144,6
50	2227,3

FIGURE 25. Calibration curve of Ciprofloxacin



FORMING OF CALIBRATION CURVES (HPLC) APPENDIX 2:2 (3)

TABLE 11. Ibuprofen 211 nm, retention time17,9 min:

Concentration (mg/)	Area
1	41,3
2	89,5
5	164,3
10	314,9
25	589,8
50	1559,9

FIGURE 26. Calibration curve of Ibuprofen

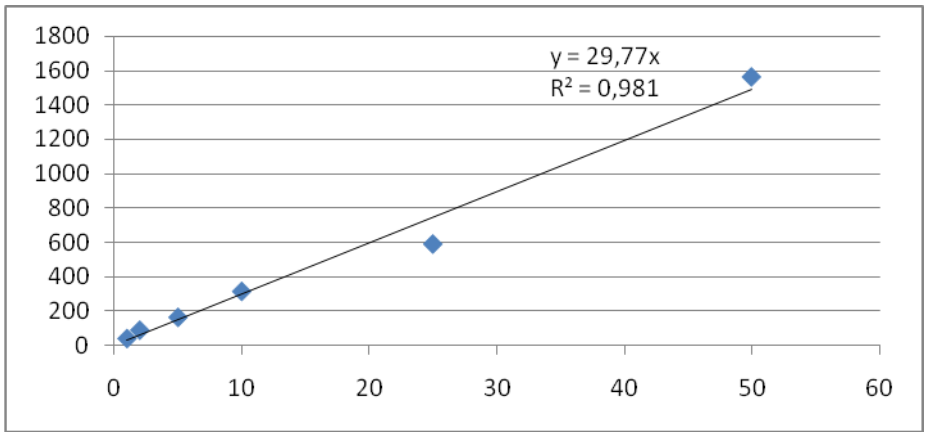
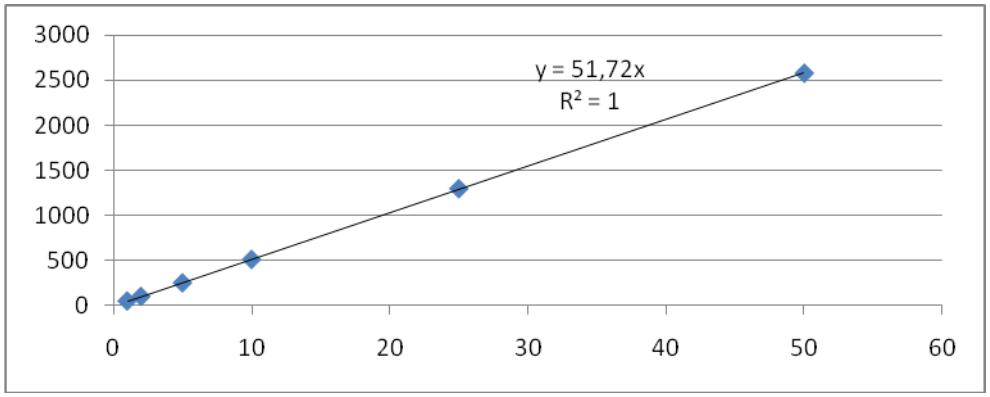


TABLE 12. Paracetamol 241 nm, retention time 5,2 min:

Concentration (mg/)	Area
1	55,6
2	106,6
5	257,8
10	516,2
25	1301,8
50	2582,2

FIGURE 27. Calibration curve of Paracetamol



FORMING OF CALIBRATION CURVES (HPLC) APPENDIX 2:3 (3)

TABLE 13. Metronidazole 211 nm, retention time 4,1 min:

Concentration (mg/)	Area
1	22,5
2	41,9
5	101,9
10	204,9
25	520,6
50	1023,2

FIGURE 28. Calibration curve of Metronidazole