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Trimethoprim degradation using high frequency ultrasonication

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Micropollutants such as antibiotics are becoming a challenge. One of the most used antibiotics is Trimethoprim. Conventional wastewater treatment plants are not efficient in removing these contaminants due to their poor biodegradability. This environmental problem requires new technologies able to eliminate its presence. High frequency ultrasonication, a technology focused on the generation of hydroxyl radicals, is a real option for water treatment for the removal of micropollutants. In this work, the efficiency of treating contaminated water with ultrasounds was studied after overcoming experimental and measurement challenges. Experiments were carried out using a High multifrequency system working at two different frequencies: 580 kHz and 865 kHz. Varying diverse parameters like treatment time, Trimethoprim concentration or power amplitude, it was observed that at concentrations of 5 ppm of Trimethoprim in synthetic solutions, the treatment was more efficient working at 580 kHz than at 865 kHz. This efficiency grew as power amplitude and treatment time increased. For others concentrations between 6 ppm and 10 ppm, the behavior was very similar but the levels of effectiveness were not that high. These results mean that the studied treatment has high efficiencies for low concentrations whose values are very close to the ones found in real wastewater.

Keywords High Frequency, micropollutants, antibiotics, ultrasounds, environmentally friendly

Table of contents

Preface

I want to thank Supervisor Anna Mikola and Advisor Raed Al-Juboori for their guidance and their kindness during my stage in Finland; I have learned a lot of them and their methods and I will always remember these months there. I want to thank Aino, Marina, Heikki and Antti for always being ready to help me in the Lab and to teach me about any doubt I had.

Otaniemi, 19 July 2021
Guillem Horrach

Abbreviations

AMR	Antimicrobial Resistance
AOPs	Advanced Oxidation Processes
APIs	Active pharmaceutical ingredients
CAS	Conventional activated sludge systems
DBPs	Disinfection By-Products
EWW	Effluent wastewater
IWW	Influent wastewater
MBR	Membrane Biological Reactor
ppb	Parts-per billion
ppm	parts-per million
RO	Reverse Osmosis
SOCs	Synthetic Organic Compounds
TOC	Total Organic Carbon
US	Ultrasound
UV	Ultraviolet
WWTPs	Wastewater Treatment Plants

1. Introduction

1.1 Background

Water is one of the principal components for human life. In recent decades, human-related actions have deteriorated water sources quality. In order to reverse this deterioration, many treatments can be applied. In fact, water treatment is an essential part of the cycle of water. Wastewater treatment plants (WWTPs) play a major role in this cycle as they treat the wastewater produced from domestic and industrial activities and; subsequently; reinstate it in the water network. To ensure a safe use and consumption, WWTPs aim to remove contaminating compounds potentially dangerous for the environment and human health. The treated water by WWTPs is used for agricultural, industrial and non-potable uses, such as watering green areas in big cities. This is one of the reasons why municipal water recycling is progressively becoming a crucial point of water resources management practices all over the world. (Le Minh et al., 2010). The principal advantages of recycling wastewater are to extend water supplies, reduce discharge and costs and also, save energy. By 2005, over 35% of the world's inhabitants resided countries with moderate to high water stress (Kummu et al., 2010). Moreover, this percent will increase until 52% by 2050 due to the increment in the population (Gupta and Gupta, 2021). The critical issue is centered on the expensive price for acquiring water with the adequate quality. This limitation involves the necessity for developed countries to take care of those who cannot afford the required infrastructure. Even more so considering that the rise in temperatures from climate change is drying lakes and rivers in less developed areas. Nonetheless, it has the same importance to ensure water supply as having the adequate quality to consume it.

Despite not being directly hazardous, the presence of pharmaceuticals in wastewater effluents coming from WWTPs is becoming a public health problem, an environmental controversy and a problem for the pharmaceutical manufacturing. (Sbardella et al. 2018). WWTPs are normally designed to remove expected pollutants like nutrient, but they are inefficient in eliminating micropollutants such as antibiotics. In a study conducted by Verlicchi et al. (2012) on the removal of 39 distinct antibiotics in WWTPs, it was found that there was a high variability in the concentration of these micropollutants in the effluent stream, and for some antibiotics negative removal efficiencies were found. This is the reason why the effluents from WWTPs continue to be the origin of pharmaceuticals in aquatic environment (Daughton and Ruhoy, 2009). Antibiotics are one of the most used type of pharmaceuticals. Normally, they are present in wastewater in slight concentrations range of µg L^{-1} - ng L^{-1} and that is why they are referred to as micropollutant. The chemical structure of antibiotics can be modified by non-biotic transformation such as photo-transformation, oxidation or hydrolysis assuming a decreased toxicity (Kümmerer, 2009).

There is no data accessible related to total worldwide use of antibiotics. Nonetheless, there is a disparity between countries in the use of these products. Due to changing regulations, some countries allow the use of determined antibiotics that other countries ban. Also, there is an increased concern due to the lack of legislations for determining discharge limitations of treated water from WWTPs (Verlicchi et al., 2012).

The adverse effects of antibiotics' presence in wastewater on different life aspects can be summarized in the following points:

- Public health problem: Antibiotics could become less efficient due to the consumption of water with traces in our day-to-day activities. Humans would consume these remains, which renders medicines used for treatment purposes ineffective due to the development of microbial resistance to pharmaceuticals.

- Environmental issue: WWTPs normally recycle treated water into rivers, lakes, sea or maybe, directly to the human consumption. This water is used in the agricultural sector and, is part of the aquatic biosphere. This means all the environment is being contaminated by the presence of pharmaceuticals. This contamination implies that bacteria develop resistance to these compounds. For example, bacteria such as fecal coliforms and E.coli detected in wastewater influent and effluent have shown resistance to some pharmaceuticals such as ciprofloxacin, trimethoprim, sulfamethoxazole and vancomycin (Nagulapally er al., 2009). This phenomenon is named antimicrobial resistance (AMR) and its development is being accelerated by over-consumption of pharmaceuticals as antibiotics are being prescribed over the real necessities. Without strategies to cease the disturbing expand of AMR, today's already large 700,000 annual deaths would turn into an extremely frightening 10 million every year (O'neill, 2016).

- Pharmaceutical industry challenges: The development of bacteria resistance negatively affects the efficacy of medications. This in turn necessitates the development of new medication formulas which requires extensive efforts and resources. Also, the elevated risk for an accelerated resistance rises from the expel of antibiotics could cause standard requirements well above local legislations (Larsson and Fick, 2008).

The synthetic nature of pharmaceuticals makes these compounds much harder to remove. There is a wide range of pharmaceuticals that are prevalent in wastewater streams. The growing evolution of the presence of these compounds must be accompanied by effective technologies for their removal. Recent years have witnessed the growing application of advanced oxidation processes (AOPs) for antibiotics removal. AOPs degradation mechanisms rely on hydroxyl radicals (HO·) generation. Hydroxyl radicals are highly reactive class of oxidants characterized by their non-selectivity and capacity for achieving full mineralization of complex pollutants structures. Hydroxyl radicals' generation with AOPs does not necessitate specific temperature or pressure making them easier to monitor. These processes are environmentally green under these specific conditions. (Khan et al., 2020a).

1.2 Gap on the research

The selected antibiotic in this study was Trimethoprim as it is one of the most commonly found antibiotics in wastewater (Gros et al., 2009), and it is one of the safest antibiotics to deal with from an experimental point of view. The use of common AOPs for Trimethoprim degradation was reviewed and the number of studies reported on individual AOPs is summarized in Table 1. Web of Science database was used for obtaining information on the number of studies conducted with the different treatments and selecting as subject areas "Chemistry" and "Chemical Engineering". It can be seen that there is a very limited number of studies on the use of ultrasounds (US) for Trimethoprim degradation compared to the other AOPs. Hence, this study focuses on addressing this research gap.

Table 1. Summary of the number of studies available on the use of common AOPs for Trimethoprim

	AOPs	Number of studies
Combinations of AOPs	UV	795
	H_2O_2	325
	O_3	363
	Ultrasounds (US)	157

1.3 Objectives of the research

The principal objective of the research is to investigate the most efficient way of removing targeted pharmaceuticals compounds from wastewater using AOPs. Considering all the AOPs, this study was focused on the use of high frequency ultrasound systems for the degradation of trimethoprim as a model for pharmaceuticals. Trimethoprim degradation with US alone will be optimized with regards to treatment time, frequency, power density, and initial concentration. This research could help water treatment enterprises to find the practical efficient way to remove pharmaceuticals from wastewater.

Based on the use of US systems, the principal research questions which are going to be answered during this study are:

1. Is high frequency US efficient for degrading Trimethoprim in synthetic samples?
2. What are the optimum conditions for Trimethoprim degradation using US? What is the optimum working frequency? 580 kHz or 865 kHz?
3. Does initial Trimethoprim concentration have an influence on treatment's efficiency?
4. Does Hydrogen Peroxide have a positive effect on Trimethoprim's degradation?

2. Literature review

2.1 Pharmaceuticals' presence in wastewater

The development of new formulas for treating diseases using drugs has made people more dependent on their consumption. This evolution has resulted in an increase of production of medications in the last few decades. Biotechnology and the pharmaceutical industry are very important for investigating new ways for healing people from their illness. Making progress in the creation of pharmaceuticals is essential to prolong life and increase the level of well-being. However, sometimes people use medication for unnecessary non-medical causes. This behavior can generate two principal problems. First, a dependence on the use of these drugs. Second, it can create environmental problems because those pharmaceuticals are consumed by humans and then expelled through toilet waters. Moreover, some of the pharmaceuticals found in aquatic environment are flushed in toilets or sinks. Due to the expiration of these products, they are thrown away which increases their concentration in wastewater treatment plants' influent (Mpatani et al., 2021). As it is shown in Figure 1, there are several pathways that contribute to aquatic environment contamination.

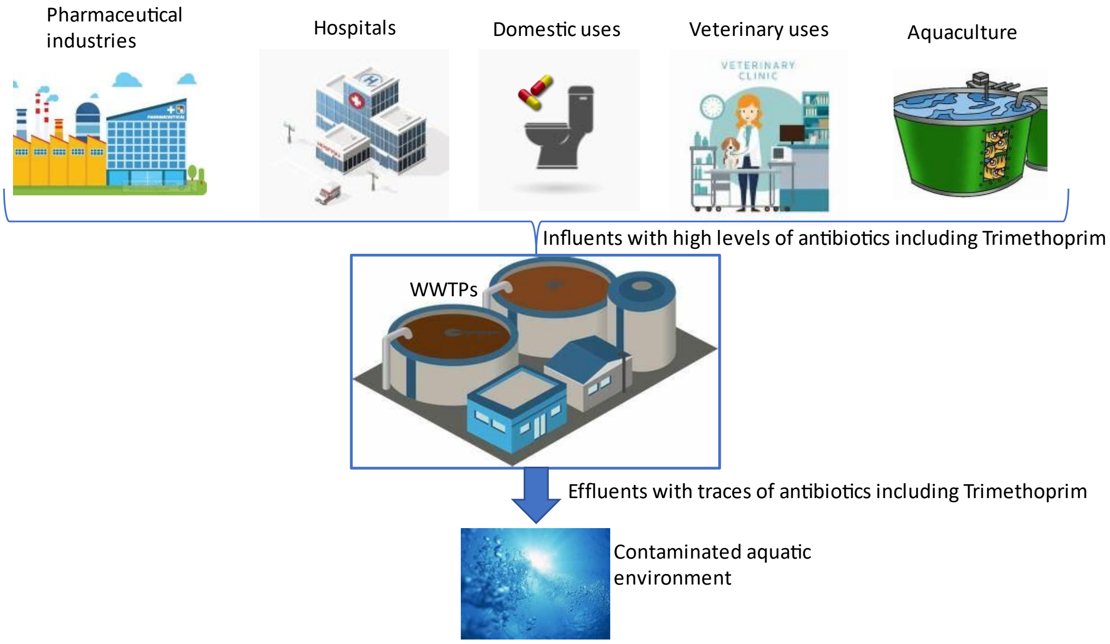


Figure 1. Common pathways contributing to aquatic environment contamination

Between 2000 and 2010, consumption of antibiotics raised by 35%. Asian countries and other heavy populated nations like Brazil or Russia caused three quarters of this increase (Van Boeckel et al., 2014). This diversity of countries emphasizes that the problem of over-consumption of pharmaceuticals is global. Some of the pharmaceuticals found in aquatic environment are flushed in toilets or sinks. Due to the expiration of this products, they are thrown away which increases their appearancesampl in wastewater treatment plants' influent

(Mpatani et al., 2021). As it is shown in Figure 2, Trimethoprim was the fifth consumed antibiotic worldwide between 2000 and 2010.

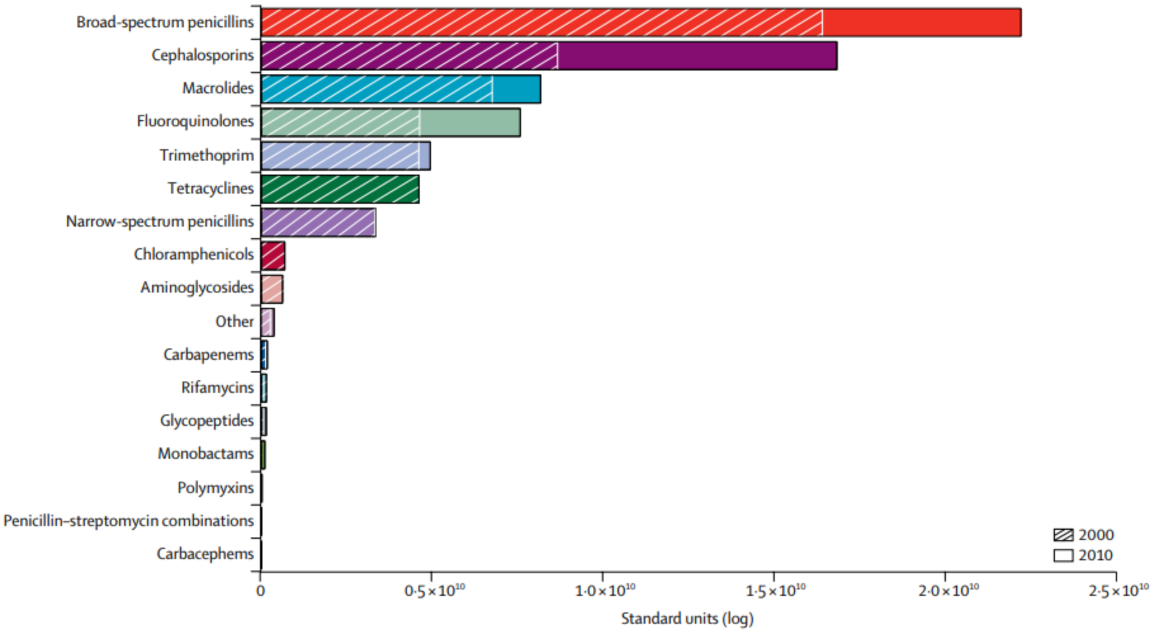


Figure 2. Global antibiotic consumption by class in 2000 and 2010 (Van Boeckel et al., 2014)

Looking from a more specific point of view, Martínez Bueno et al. 2012 conducted a study financed by the Spanish Government obtaining information during almost two years of monitoring samples in order to analyze the removal efficiency of 5 sewage treatment plants of Spain. Also, they analyzed the presence of chemicals in WWTP effluents leaked to the environment. During this study, Trimethoprim was detected in concentrations of 0,07 to 1 µg/L for output waste.

2.2 Trimethoprim

Trimethoprim is utilized to treat bladder infections; this antibiotic is available as a generic medication. It is commonly used in combination with sulfamethoxazole for effective treatment of urinary tract problems. In fact, the first time it was used for this purpose was in Finland in 1972 (Huovinen, 2001). Acting alone, it can stop the proliferation of a big quantity of bacteria. Trimethoprim chemical formula is $C_{14}H_{18}N_4O_3$ and it is formed by two principal rings as it is shown in Figure 3.

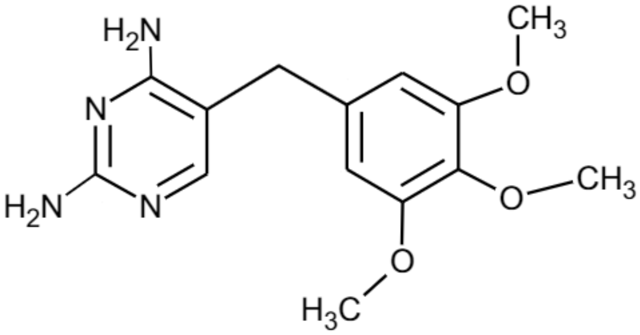


Figure 3. Structure of trimethoprim (Silva et al., 2020)

The components of Trimethoprim are carbon, hydrogen, nitrogen, and oxygen. The biggest particularity of this drug is that it contains 2-aminopyrimidine scaffold and that its two rings allow for the structural changes which may result in diverse resistance forms (Rashid, et al., 2016).

Regardless of the antibiotic applications of Trimethoprim for aquaculture, medical and veterinary purposes, it is badly metabolized by humans and animals (Dan et al., 2013). In aquaculture, Trimethoprim is generally used for the prophylaxis and cure of infectious illnesses of fish and shrim via the live food (Chair et al, 1996). Moreover, in the veterinary field, this antibiotic is used as feed additive in most countries (Raich-Montiu et al, 2007). Due to this poor metabilization, almost 80% of this drug is expelled by consumers through the feces and urine (Yang et al, 2011). The increase in Trimethoprim’s global consumption leads to an increasing resistance to this antibiotic. This resistance is caused by a reduction in membrane’s permeability, an overproduction of the target enzyme or by modifying the structure of chromosomally encoded dihydrofolate reductase. (Huovinen, 2001).

2.3 Advanced Oxidation Processes

Advanced Oxidation Processes (AOPs) offer interesting assets for efficient remediation of emerging pollutants, such as antibiotics (Taoufik et al., 2021). AOPs are on site oxidation processes generating highly reactive agents such as hydroxyl radical, at standard conditions without having to modify temperature or pressure. AOPs are capable of completely degrading of pharmaceuticals compounds at trace levels (Capodaglio et al., 2018). The past two decades have witnessed a significant increase in AOPs progress due to two reasons: the variety of technologies implicated and the large number of areas of potential application. Water and wastewater being the most usual area for investigation where AOPs could help (Klavarioti et al., 2008)

Normally, the effectiveness of AOPs is based on the generation of highly reactive free radical groups such as hydroxyl radicals leading to the degradation of complex pollutants to simpler structure (Taoufik et al., 2021). Hydroxyl radicals have a solitary electron in their exterior layer giving them the ability to oxidize a vast range of organic compounds unselectively. The formulation of hydroxyl radicals is shown in Figure 4.

Figure 4. Form of the Hydroxyl Radicals (Odorox)

Due to their non-selective character, the reaction rates for hydroxyl radicals are much quicker than the reaction rates for conventional oxidants. Also, their high grade of reaction entails a short life because they quickly develop into hydroxy groups.

Depending on the procedure for the generation of hydroxyl radicals, AOPs have three types of processes: photochemical processes, non-photochemical processes and hybrid or combined processes. They can also be assembled as heterogeneous and homogeneous processes. Heterogeneous processes are described by the use of solid materials as catalysts like metals aided on carbon materials or semiconductors such as TiO_2 or ZnO. In the other hand, homogeneous processes need only one phase to generate hydroxyl radicals from ozone, hydrogen peroxide or other oxidants (Taoufik et al., 2021). Moreover, homogeneous processes can be also divided in two groups depending on energy requirement as shown in Figure 5.

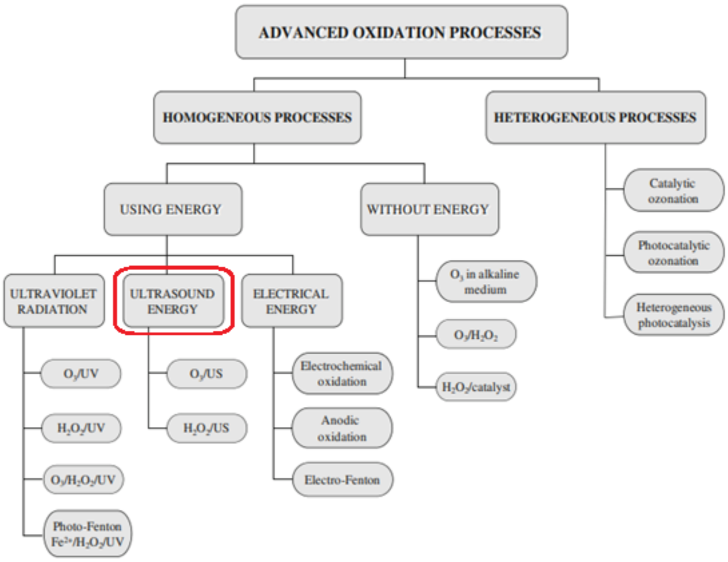


Figure 5. Classification of AOPs (Poyatos et al., 2009)

There are others oxidation processes that are capable of oxidizing organic compounds using hydroxyl radicals like gas-phase combustion or wet oxidation. However, these processes require more effort as need to work in specific conditions of temperature and pressure making the process more complicated. For example, one of the significant difficulties wet oxidation processes has is that high temperatures (and pressures) are commonly required to reach a high degree of oxidation of many organic compounds in an acceptable period of time (Bhargava et al., 2006).

2.2.1. Ozonation

The ozonation process consists of adding ozone (O_3) gas to an aqueous solution. Ozone is extremely unstable. Due to its volatile nature, ozone is normally generated on site. The elevated reactivity of ozone toward in reference to organic and inorganic pollutants, affordability and self-decomposition of ozone to oxygen make it more appropiate than many other oxidants (Loeb et al., 2012). The oxidation of a determined compound by ozone can take place through two methods. The direct reaction occurs when the reaction is via molecular ozone. Meanwhile, the indirect reaction happens when ozone reacts with hydrogen and hydroxyl ions forming hydroxyl radicals (von Gunten, 2009). The direct reaction has a more selective behavior. Functional groups containing sulfur, phosphorous, nitrogen, oxygen and compounds with C=C bonds are among the organic and inorganic compounds that are affected by the direct reaction. The indirect reaction conducted by hydroxyl radicals is more unselective reacting through hydrogen abstraction, radical-radical reactions, electrophilic addition and electron transfer (Khuntia et al., 2015). The generation of hydroxyl radicals in an acid-neutral medium directly from O_3 follows the following succession of reactions (Gardoni et al.,2012):

$$OH^- + O_3 \rightarrow HO_2^- + O_2 \tag{1}$$
$$HO_2^- + O_3 \rightarrow O_3^- \cdot + HO_2 \cdot \tag{2}$$
$$HO_2 \cdot \leftrightarrow O_2^- \cdot + H^+ \tag{3}$$
$$O_2^- \cdot + O_3 \rightarrow O_3^- \cdot + O_2 \tag{4}$$
$$O_3^- \cdot + H^+ \rightarrow HO_3 \cdot \tag{5}$$
$$HO_3 \cdot \rightarrow HO \cdot + O_2 \tag{6}$$
$$HO \cdot + O_3^- \cdot \rightarrow OH^- + O_3 \tag{7}$$

Resuming this sequence, following overall stoichiometry is obtained:

$$3\,O_3 + OH^- + H^+ \rightarrow 4\,O_2 + 2\,HO \cdot \tag{8}$$

As it is seen, 1,5 mol of ozone are needed to produce 1 mol of hydroxyl radical, noting the inefficiency of the process. In addition, the speed of the initial step of the succession is proportional to the pH. The higher the pH, the faster the reaction. However, high pH values have a bad influence in the generation of hydroxyl radical. The stability of ozone in water is based on pH, ozone concentration, temperature, fluid-dynamic conditions, the presence of UV radiation and inorganic or organic compounds that can act as promoters (Gardoni, et al., 2012). The principal benefit of ozone-based AOPs is a shorter reaction time allowing the administration of higher ozone doses without having overabundance of ozone in the effluent of the reactor (von Gunten, 2009). Most of the times ozonation is not enough for creating the required amount of hydroxyl radical for degrading targeted hazardous compounds. Hence, ozonation is combined with other AOPs in some cases. In the case of our study, US systems will be combined with ozonation in order to examine the synergistic effect of ozone on ultrasonic degradation of antibiotics.

2.2.2 Hydrogen Peroxide

The family of the peroxide is characterized by an oxygen-oxygen single bond with the following chemical formula: R-O-O-R. The “R” represents any element. The hydrogen peroxide is the simplest member of this family because both Rs are substituted by a hydrogen molecule as it is shown in Figure 6.

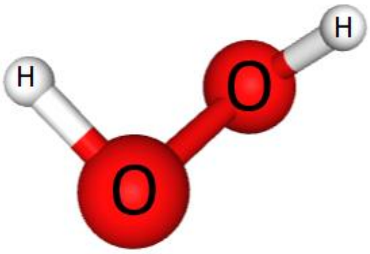


Figure 6. Form of the Hydrogen Peroxide (PubChem)

In chemical terms, hydrogen peroxide is poorly reactive. It can operate as a moderate oxidizing or as a moderate reducing agent, but it does not oxidize most molecules quickly, including lipids, DNA and proteins. Nonetheless, the oxidation potential of hydrogen peroxide comes from its transformation into hydroxyl radical. There are two very known ways of conversion from hydrogen peroxide into hydroxyl radical:

- The presence of certain transition metal ions such as Fe^{2+} and Ti^{3+}. In fact, the decomposition of hydrogen peroxide with the presence of Fe^{2+} is named Fenton’s reagent and creates reactive HO· (Tokumura et al., 2021).

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + \mathrm{HO} \cdot + OH^- \quad (9)$$

- The presence of UV light decomposes hydrogen peroxide creating hydroxyl radical.

$$H_2O_2 \rightarrow 2\ \mathrm{HO} \cdot \quad (10)$$

Residual compounds in water can become a problem. In this case, it is even more important to monitor the residual traces that can be generated because of the stability of hydrogen peroxide. This stability lengthens over time favoring the presence of hazardous traces after the treatment in acidic environment. Residual hydrogen peroxide can be destroyed by adding catalase enzyme (Andreozzi et al., 2004).

One of the principal problems of using O_3 or H_2O_2 is the appearance of dangerous by-products. DBPs appear after chemical reactions between inorganic and organic compounds present in water (Ates and Argun, 2021). The principal and more perilous are those who show up after ozonation processes. Reactions of hydroxyl radicals and ozone along with bromide and its oxidation products promote the appearance of bromate (BrO_3^-) which is considered as a potential human carcinogen (Bourgin et al., 2017). Consequently, US technology for water treatment is used to avoid appearing DBPs that could potentially be more noxious than the presence of pharmaceuticals.

2.2.3 UV light

UV light is characterized by having wavelength between 10 nm and 400 nm. UV-driven AOPs belong to the family of photochemical technologies which are easy to handle, clean and, also, deliver a twofold profit of disinfection and environmental contaminant treatment. There is an increased number of studies carried out using these technologies due to their minor impact in the environment.

The first law of photochemistry affirms that only light absorbed by a molecule can be effective in generating a photochemical alteration in that molecule. The absorption spectrum traits are subjected to the molecular structure of the absorber, and on the dealings between this compound and the solvent. Most of the UV light absorbers consist of double bonds or conjugated double bonds, involving carbon, nitrogen or oxygen atoms. Once absorbers attract enough light, their state changes to an excited one. This new state caused by light absorption is very energetic and can experience thermal chemical reactions. The bond scission through thermal chemical reactions is the prevailing chemical direction inducing the degradation of some water pollutants (Stefan, 2004).

The principal way of application of UV-light in aqueous solutions is through low-pressure Hg lamps. This technology emits light at wavelengths of 254 nm most of the time (85-90%) as well as wavelengths of 185 nm (7-10%). Both are important for AOPs application because they are part of the absorption spectra of most of pollutants. Low-pressure Hg lamps have life time up to 25000 operating hours but due to the effect of electrode failure, lamp current or solarization, their initial efficiency decays over the operation time (Stefan, 2004).

2.2.4 Ultrasounds

2.2.4.1 Introduction

Sound waves generated by US systems have a frequency that exceeds humans’ hearing limit (>16kHz). This technology has many applications in the engineering field depending on the range of frequencies in which it is used. Either alone or combined with other technologies. One of the examples of utilization is the production of useful gases like hydrogen, offering a green way to generate hydrogen by introducing ultrasound in liquid water

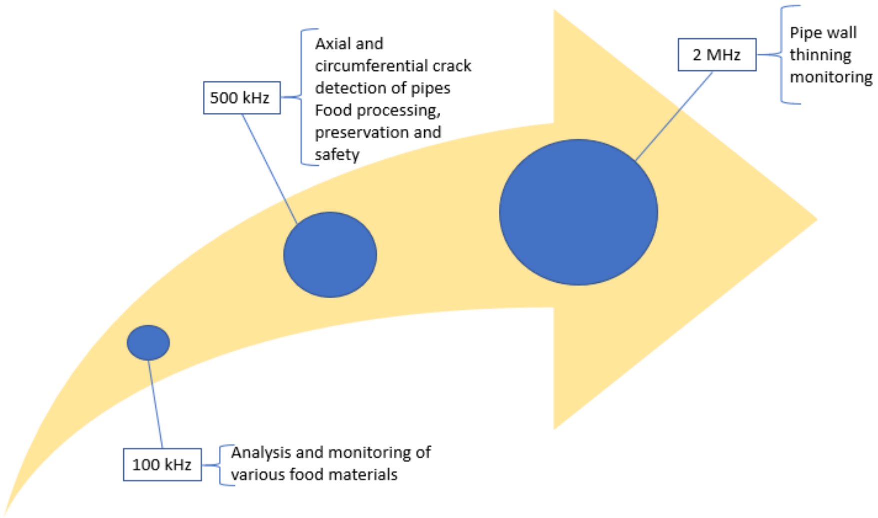


Figure 7. Summary of sonochemistry applications (Rashwan et al., 2019)

Sonochemistry is a section of chemical investigation dealing with the chemical effects derived from the application of ultrasonics (Schiel, et al., 2015). Sonochemistry is considered in the range of 100 kHz and 2 MHz. There are several benefits that we can extract from sonochemistry like creating a determined chemical environment. Typical applications for high-intensity ultrasound on liquid medium are heat transfer, atomization or oil recovery (Yao, et al., 2020). In Figure 7, a summary describing sonochemistry applications is shown. However, the principal focus of this study is the enablement of chemical reactions. The main advantage of ultrasound is the generation of free radicals without the need for chemicals addition. When chemicals added to water, they create hazardous by-products that are potentially dangerous for the environment. The generation of hazardous by-products with ultrasound is none existent or minimal making the technology an eco-friendly alternative to chemical treatments.

2.2.4.2 Sonochemistry in the 21st century

The general interest in sonochemistry from the developed world has increased since the beginning of the 21st century. The aforementioned big quantity of applications and its environmentally sustainable nature are the main reasons for the growth of interest that has been generated. In fact, the percentage of papers linking green chemistry and eco-friendly processes based on ultrasound has raised from 4.8% in 2000 to 33.3% in 2016 as it is shown in Figure 8 (Chatel, 2017). The advantages of sonochemistry in terms of efficiency and eco-friendliness have led more investigators to learn, research and write about its possible applications.

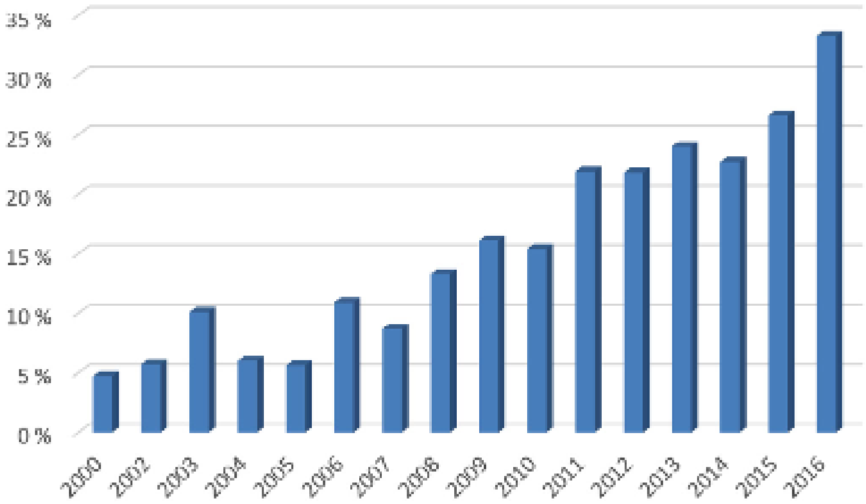


Figure 8. Progression of the proportion of annual papers in Ultrasonics Sonochemistry associated to green chemistry and eco-friendly processes (title, abstract, keywords and manuscript) (Chatel, 2017).

2.2.4.3 Basics of sonochemistry

The fundamental cause of the study of sonochemistry is the use of ultrasonic radiation for diverse applications. For example, ultrasonic radiation, also named sonolysis, is recognized as an efficient process for removing environmentally hazardous contaminants, such as pesticides, fuel additives, aromatic compounds or atrazine (Schramm and Hua, 2001). The ultrasonic radiation is created by applying ultrasounds waves to a targeted medium. Generating sound waves at high frequencies, molecules present in the environment vibrate and transmit energy along the way. If the medium by which vibrations move is water, acoustic cavitation appears. This phenomenon happens in a very short period during which microbubbles are formed, subsequently grow and collapse as it is shown in Figure 9.

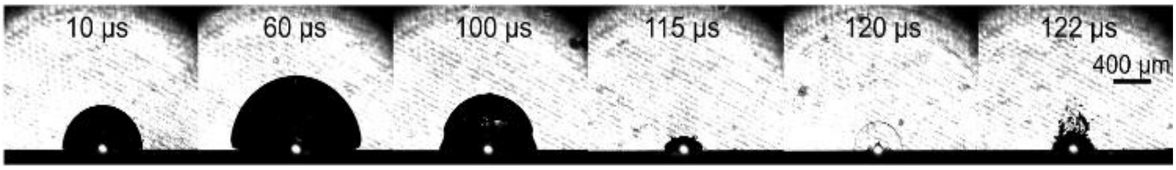


Figure 9. Temporal evolution of laser-induced cavitation bubbles (Long et al., 2020)

During this process, the fast implosion creates a focus of energy in which the approximated temperatures are between 4000 and 10.000 K and the pressure range can go higher than 975 bar (Hoffmann et al., 1996). When this collapse occurs, extreme conditions create three critical zones where high energy chemical reactions take place:

- The inner region of the bubble. Pyrolytic reactions occur in this region due to extreme temperatures inside the bubble degrading volatile and hydrophobic molecules. In a smaller scale, hydroxyl radicals formed during sonolysis participate also in reactions (Hoffmann et al., 1996).

- The interface between bubble and liquid: The concentrations of hydroxyl radical in this region can be $4 \cdot 10^{-3}$ mol/L obtaining values much higher than other AOPs (Hoffmann et al., 1996).

- The bulk zone. The voyage of free radicals from the liquid-bubble interface to the liquid generates chemical reactions driven by the radicals and their recombination products (e.g. H_2O_2) (Hoffmann et al., 1996).

The energy generated during the process enable pyrolysis reactions which is one of ultrasound mechanisms to eliminate undesirable organic compounds present in water. During cavitational bubble disruption, water molecules go through thermal dissociation within the vapor phase resulting in hydroxyl radicals and hydrogen atoms as presented in Eq. (11). The thermal separation is not the only reaction that take place due to ultrasound irradiations. As can be seen in eqs (12) and (13), by-products are generated directly from the incidence of ultrasounds on pollutants and indirectly from the reaction between pollutants and hydroxyl radicals (Adityosulindro et al., 2017).

$$H_2\mathrm{O} +))) \rightarrow HO\cdot + \mathrm{H}\cdot \tag{11}$$

$$Pollutant +))) \rightarrow \text{byproducts} \tag{12}$$

$$Pollutant + HO\cdot \rightarrow \text{byproducts} \tag{13}$$

where))) refers to ultrasound irradiations.

2.2.4.5 Ultrasounds generation’s principal parameters

Ultrasonic irradiation technology is very powerful and has a lot of different working methods. Having the capacity of vary parameters allow seeing their influence in the technology’s effectiveness. There are many factors that affect ultrasound performance such as medium characteristics (viscosity, pressure, temperature, and contents of solid and gas impurities), functioning parameters of ultrasonic machinery (power, frequency, treatment time, mode of operation, and shape of the exciting waves) and design-related aspects (reactor shape and liquid height) (Al-Juboori and Bowtell, 2019)

Between all these parameters, some of them are very relevant for the generation of hydroxyl radicals in the case of study. This variables are: pressure amplitude, operational mode, frequency, pH and medium nature.

Generally, an increment in pressure amplitude is directly related to the power transferred to the liquid. The quantity of cavitation bubbles, the temperature of collapse and the sonochemical yield are proportional to a rise in the power transmitted (Kanthale et al., 2007). Nevertheless, pressure amplitude has upper and lower limits of functioning in which the behavior of bubble growth is unusual. Under the lower limit, bubble expansion and nucleation can not be developped. Bubbles succumb to surface tension effects and limit cavitational activity. Above the upper limit, degassing and coalescence occurs (Wood et al., 2017). The phenomenon of coalescence is a merging of bubbles as it is shown in Figure 10. However, if pressure amplitude stays inside the limits, increasing his value will suppose a growth in sonochemical activity and, consequently in the generation of hydroxyl radicals.

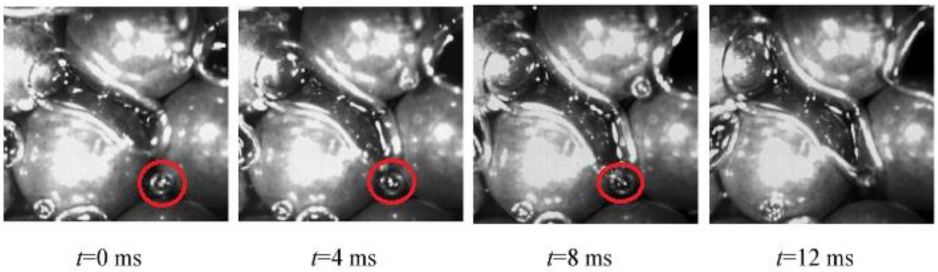


Figure 10. Process of coalescence of a bubble (Xu et al., 2021)

The second parameter crucially important for the effectiveness of US systems that can be controlled is the frequency of the sound field. The quantity of bubbles, their size, their distribution and the chemical activity are some of the variables in which frequency has a high influence. Normally, higher frequencies create a higher quantity of nucleations and bubbles. Also, the size of the bubbles is inversely proportional to the frequency because the quantityincre of vapour entering each bubble diminish due to the lack of time between each expansion phase. As in the previous case concerning the pressure amplitude, the influence of frequency is restricted with upper and lower limits. At low frequencies, bubbles are large and when they collapse, they generate surface instabilities. Also, coalescence can appear because bubbles have longer expansion times and, consequently, bigger sizes. In the other hand, high frequencies entail smaller bubbles. Their reduced size suppose two big problems: coalescence due to the short period between bubble generation and also, the shorter the growth time, the lower the production of reactive species (Wood et al., 2017). So, intermediate frequencies are the best option for generating good chemical conditions for the surge of chemical reactivity. Some authors propose frequencies in the range of 300 kHz to 500 kHz (Koda et al., 2003). However, some other investigations recommend values closer to 1 MHz (Entezari and Kruus, 1994).

The third important parameter that can be modified in the US systems is the operational mode. The principal doubt is whether is more efficient to use pulsed or continuous signals. Pulsed signals consist on having a power on time and a power off time. So, during a determined time the power signal is on as well as the bubble generation. Nevertheless, during another immediate period of time, the power signal is off.

During this time before another power on phase, bubbles must have enough time to grow and collapse, generating hydroxyl radical as it was explained in previous paragraphs. The critical point is based on how much time does the power off time need for achieving the desired sonochemical activity (Wood et al., 2017). Continuous signals are characterized by a constant input of ultrasonic power. In this case, coalescence tend to appear due to the constant generation of bubbles. Also, there is a problem with the excessive growth of bubbles that exceeds the region of active cavitation (Tuziuti et al., 2008). These two problems just mentioned are the most important reasons why pulsed signals can rise active cavitation compared to continuous signals (Henglein, 1994).

For comparing the chemical activity created by both pulsed and continuous irradiation experiments, the same quantity of acoustic energy has to be irradiated to the system. In this case, the time needed in pulsed mode for matching the energy irradiated in continuous mode is expressed through the following equation:

$$t_{TOT} = t_c \cdot (1+1/R) \qquad (14)$$

in which t_{TOT} is the time needed in pulsed mode, t_c is the time in continuous mode and R is the ratio between the pulse lenght and the interval lenght (Henglein, 1994). In Figure 11, it is represented how the pulse mode is working during a period of time without having to continously apply energy to the system.

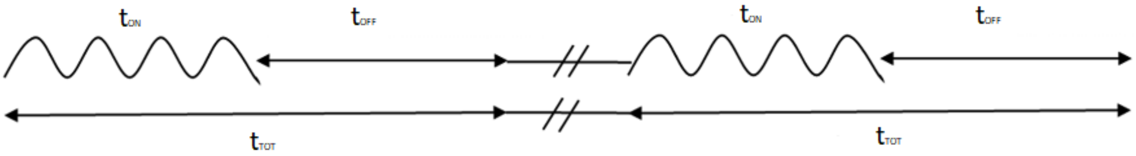


Figure 11. Graphic of pulse mode (Delacour, 2019)

The following parameters to analyse are pH and medium nature. It is well known that pH of solutions notably affect the sonochemical decay of organic pollutants (Méndez-Arriaga et al., 2008). Villaroel et al. studied the effect of this parameter on sonochemical degradation rate for ACP (N-acetyl-p-aminophenol or paracetamol), one of the most common pharmaceutical compounds used in natural and drinking water. Different initial pH values ranged between 3 and 12 were tested. Results showed that ultrasonic degradation in acidic medium is higher than those obtained in basic aqueous systems.

Medium peculiarities such as viscosity, pressure and temperature can affect the intensity of ultrasound effects. Viscosity affects negatively the generation and collapse of microbubbles due to high cohesion forces in the liquid. Pressure’s influence is only significative when dealing with closed system treatment chambers. In the case of study, the chamber will be a openned one (Al-Juboori and Bowtell, 2019).

3. Research material and methods

3.1 Synthetic sample preparation

The first step for the conduct of experiments is preparing samples for measurements. As it is said in previous chapters, medium characteristics and pH are parameters influencing the generation of hydroxyl radicals. An acid pH and a medium with low viscosity and low presence of suspended particles are important characteristics to ensure the generation of hydroxyl radicals. The selected medium for the realization of the experiments is Reverse Osmosis (RO) water avoiding the presence of impurities. Synthetic sample preparation was carried out with the following steps:

1) Fill 1-liter volumetric flask with RO water.
2) Prepare the quantity of Trimethoprim that is going to be diluted in RO water.
3) Take a 1-liter bottle
4) Introduce Trimethoprim pouring RO water from the volumetric flask to the tray with pharmaceutical and then into the 1-liter bottle filling it halfway.
5) Mix it with a magnetic mixer as it is shown in Figure 12.

Figure 12. Mixers for the dilution of pharmaceuticals

6) Add the remaining water from the 1-liter volumetric flask inside the bottle after ten minutes of mixing.
7) Mix during 10 more minutes.
8) Using a pH measurer, add NaCl drops until the pH of the liquid is 5.
9) Keep the bottle in a fridge until you use it for the treatment.

3.2 Experimental setup

The principal components of the experimental setup can be divided in three: Ultrasonic multifrequency system, Reactor and External cooling system. Each one of them has its purpose and subcomponents that are going to be analyzed in this chapter. It is essential to explain the different part that make up the final assembly. In order to illustrate the description, in Figure 13, a photo of the assembly of elements is shown. Additionally, a scheme of the experimental setup is also shown in Figure 14 to explain what corresponds to each of the components seen in Figure 13.

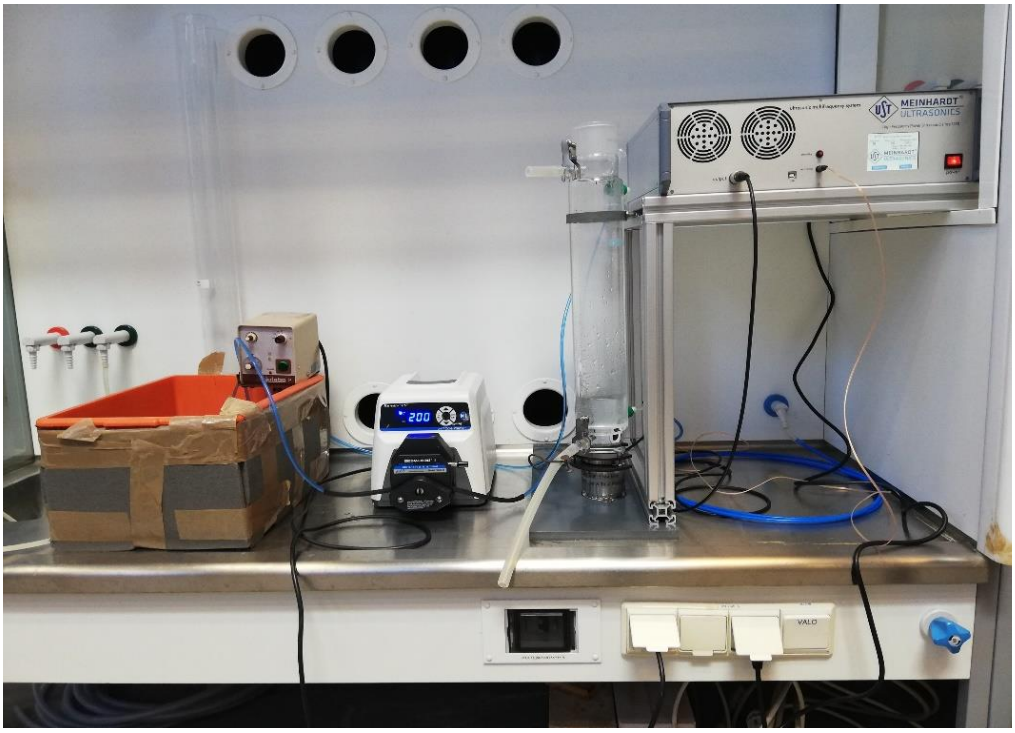


Figure 13. Real experimental setup

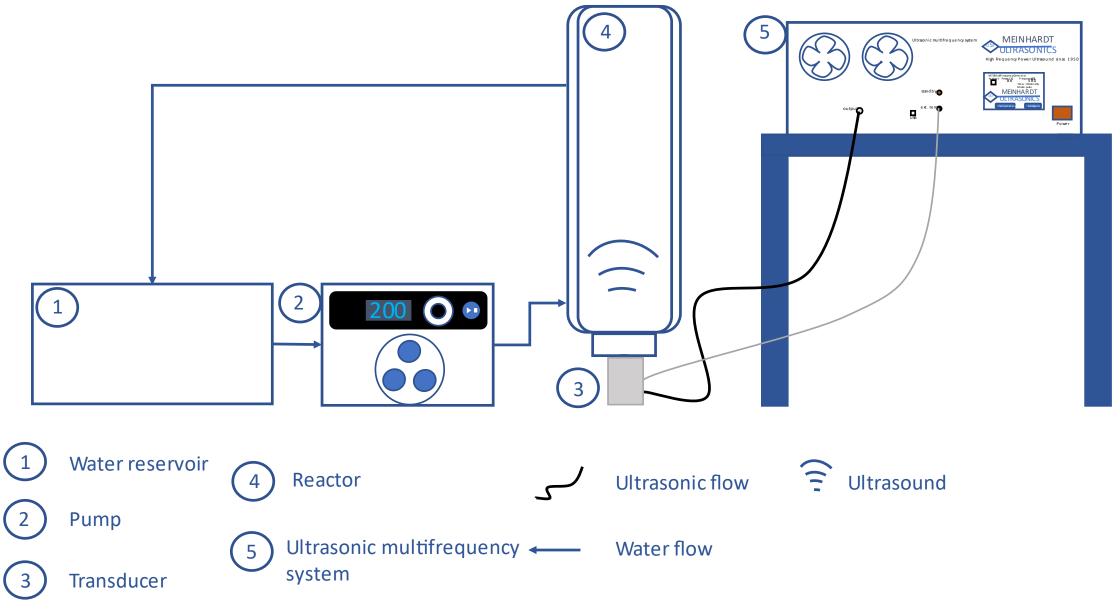


Figure 14. Scheme of the experimental setup

Ultrasonic multifrequency system

The generation of ultrasounds is elemental for moving on with the experimentation for finding the better solution for the degradation of traces of pharmaceuticals in water. For this purpose, a Multifrequency high power system can provide us with an ultimate way of generating US signals. The selected system is from Meinhardt Ultrasonics and gives the possibility to apply ultrasound signals at different conditions. The principal applications of this technology are permitting to work at 3 different frequencies (0.58; 0.865; 1.15 MHz) with only 1 transducer. As the frequency is modifiable, we can see the influence of this variable on the degradation of targeted compounds. This machinery at a high power and with a very short impulse mode is giving us a more accurate image of how ultrasounds work on the traces of pharmaceuticals. This multifrequency ultrasound supplier has a good hardware part. For example, we can find a digital display for time and scale frequency as well as a touch panel. It is important to mention that we can exchange full data using USB and memory function. So, we can extract the necessary data to work on interpretating the results.

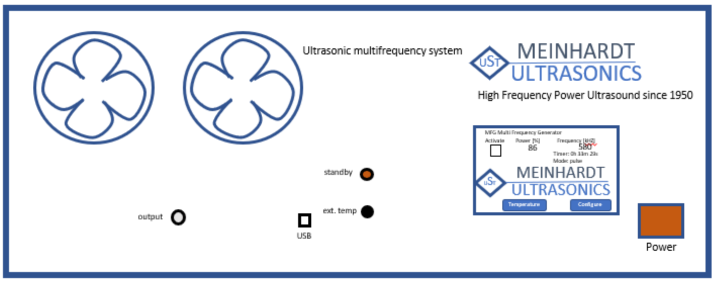


Figure 15. Ultrasonic multifrequency system scheme

In Figure 15 it is possible to observe closely the scheme of the Ultrasonic multifrequency system. The fans located in the upper left part of the device are part of the internal cooling system. Its function is to cool the device while it is running. The effectiveness of these pieces is essential because the device cannot operate at temperatures above 40ºC. At the bottom of the ultrasonic multifrequency system, there are two basic parts that connect the device with the reactor. The first one is the output signal whose mission is to transport the electric signal to the transducer for the generation of ultrasounds. The second one is the thermometer that measures the temperature of the liquid inside the reactor. As it is said before, the digital display plays an important role in device's operation.

Reactor and transducer

The reactor is the part of experimental setup that contains the treated aqueous solution. The capacity of this glass laboratory reactor is 1500 ml. As shown in Figure 14, the reactor has an outer layer creating a space around itself for introducing water in order to cool the liquid inside. This space is called "jacket". This jacket will allow us to keep the temperature of the liquid under 40 ºC using an external cooling system. The other important piece going along the reactor is the transducer. This metallic device made of Titan is in charge of transforming electrical impulses into vibration to generate ultrasounds. As shown in Figure 16, the geometry of the high-performance transducer makes it possible to "flange" it to vessels, pipes, reactors and boilers. Also, this part of the experimental setup is connected to the Ultrasonic multifrequency system with two cables. One of them is used to monitor temperature and the other one transports electrical impulses to the transducer.

Figure 16. Transducer

External cooling system

One important factor for the operation of the Ultrasonic multifrequency system is to keep temperature under 40ºC. For this purpose, the external cooling system was added to the installation. This system is composed by a water reservoir and a pump. Using the jacket surrounding the reactor, the pump introduces water to stabilize the inner temperature. This water comes from the reservoir in which ice is introduced manually to cool the system and maintain temperature under 40 ºC. Water circulation is constant during the treatment in order to ensure that the temperature is stable. The reactor has different points through which water can be introduced. In this case, water is introduced in a point located at the bottom of the reactor and extracted from a point at the top of it as shown in Figure 17.

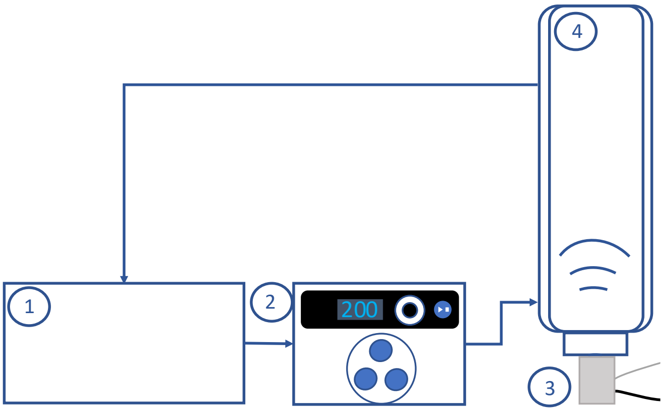


Figure 17. External cooling system scheme

3.3 Experimental design

The experimental design has been carried out with the software for response surface methodology (RSM). This class of designs is aimed at process optimization. This software is very practical for performing experimental activities with a minimal number of experiments to economize resources and time as long as obtaining convincing results (Lessoued et al., 2017). Central Composite Design (CCD) was used to determine the number of runs needed to develop a good RSM. This practical tool is so powerful that allows studying the behavior of factors on responses based on defined ranges. In the case of study, four factors were chosen. CCD gives two kinds of factors for the experimental design: categoric and numeric. The categoric factors doesn't change in the design. However, the numeric factor behaves differently. Giving two different values, CCD establishes them as a range and then, uses 3 different values from inside this range for generating the experimental design.

In the case of study, we selected four factors. The only categoric factor is the frequency and the numeric factors are power, treatment time and concentration of Trimethoprim. The chosen values are:

- Frequency: 580 kHz and 865 kHz
- Power: 70% and 90% of amplitude
- Treatment time: 60 min and 120 min
- Concentration: 5 ppm and 10 ppm

Then, for power, treatment time and concentration, CCD chose three intermediate values that are suitable for further statistical analysis. These values are:

- Power: 74%, 80% and 86%
- Treatment time: 72 min, 90 min and 108 min
- Concentration: 6 ppm, 7.5 ppm and 9 ppm

With all these values, we obtained an experimental design based on 66 different experiments. This number of experiments are enough for RSM to optimize the model.

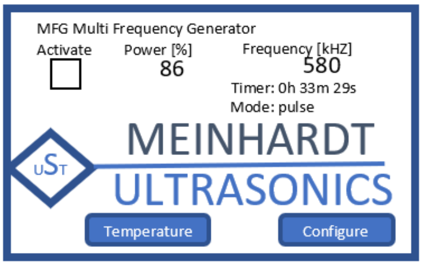
3.4 Experimental procedure

In this chapter, the experimental procedure will be explained step by step. This procedure includes the use of the ultrasonic multifrequency system for the application of determined settings.

1) With the reactor clean, 400 ml of the synthetic sample are introduced into the reactor. As it is said in previous chapters, this solution is stored in a fridge in bottles of one litter.

2) The next step is to extract a sample before applying treatment using a syringe. This extraction will be used as a zero for comparing it with the sample that will be extracted after the treatment.

3) Then, it is time to press the button "Configure" placed in the right bottom of the principal page of the interface of the Ultrasonic Multifrequency system as it is shown in Figure 18.



MFG Multi Frequency Generator

Activate ☐

Power [%] 86

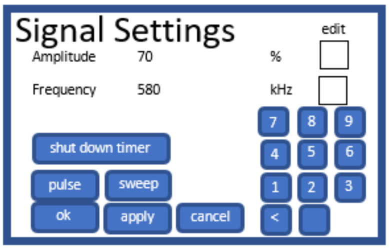
Frequency [kHZ] 580

Timer: 0h 33m 29s

Mode: pulse

Figure 18. Principal page

4) The next step is to apply the settings for the treatment. The first configuration to be applied are the amplitude of the signal and the frequency as it is shown in Figure 19. If the previous treatment has been done at the same frequency as the next one, changes don't have to be done. If amplitude changes are required, the new value has to be configured.



Signal Settings

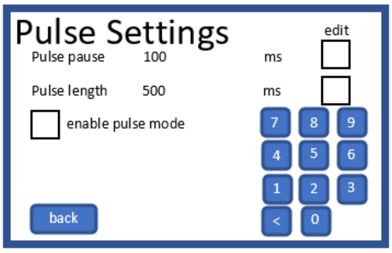
Amplitude 70 % ☐

Frequency 580 kHz ☐

Figure 19. Signal settings

5) Pulse settings have to be configured now. Pressing the button "pulse" found in Figure 19, the interface gives the possibility to activate pulse mode. As we work in pulse mode, pulse pause and pulse length need to be set. In the case of study, the pulse

mode will be enabled during all treatments. The pulse pause lasts 100 ms and the pulse length is 500 ms as it is shown in Figure 20.



Pulse Settings

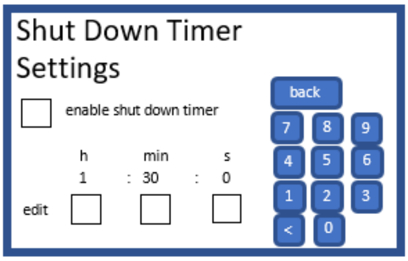
Pulse pause 100 ms

Pulse length 500 ms

☐ enable pulse mode

Figure 20. Pulse settings

6) The next parameter to configure is the treatment time. Pressing the button “shut down timer” found in Figure 19, the interface allows the enablement of a shutdown timer and the edition of this time as it is required. As it is shown in Figure 21, the precision with which time can be set is seconds.



Shut Down Timer Settings

☐ enable shut down timer

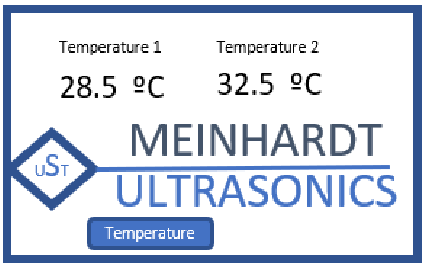
h : min : s

1 : 30 : 0

Figure 21. Shut down timer settings

7) After shut down timer configurations, the treatment is activated by coming to the principal page of the interface and press the cage under the word “Active” shown in Figure 18.

8) While treatment is applied, the temperature has to be monitored. Pressing the button “Temperature” of the principal page, Temperature 1 (temperature of the transducer) and Temperature 2 (temperature of the device) can be monitored easily as it is shown in Figure 22.



Temperature 1: 28.5 ºC

Temperature 2: 32.5 ºC

Figure 22. Temperatures

9) Finally, once the treatment is over, samples used for analysis can be extracted with a syringe. After extracting them directly from to reactor, they are kept in small bottles and introduced in a fridge before measurements.

3.5 Analytical measurements

3.5.1 Calorimetric measurements

Calorimetric measurements are very interesting from an energetic point of view. Calculating the power applied to the liquid, it is easier to find out the most energetical efficient point of work. Setting determined treatment conditions on the Ultrasonic multifrequency system and adding 500 ml of RO water to the reactor, temperatures were calculated before starting treatment and after 1-, 2-, 3-, 4- and 5-min. The conditions under which RO water was treated were 70%, 74%, 80%, 86% and 90% of power amplitude for the two different frequencies: 580 kHz and 865 kHz. These conditions are the same under which treatments were developed during the experimental part. For the realization of the temperature measurements, the used tool was a Testo 925 thermocouple. This thermocouple is directly measuring the temperature of the liquid inside the reactor.

The bases of this measurements are focused on the equation found in Equation 1. As it is shown in Equation X, the calorimetric power transferred from the device to the liquid (P) is equal to the product of the mass of water (m), the specific heat of the water (C) and the differential of temperature over the differential of time (dT/dt).

$$P\ (W) = m \cdot C \cdot \frac{dT}{dt}$$

Equation 1. Calorimetric power equation

Knowing that the density of water is 1 g/ml and that 500 ml of RO water are introduced into the reactor, the value of the mass of water (m) is 500 grams. The specific heat of water (C) is a constant whose value is 4,18 J/g·ºC. The differential of temperature over the differential of time is the obtained value from the calorimetric measurements. As we calculate how temperature increases over time, a curve describing the evolution of this value is drawn for each condition. The obtained slope of each of these curves is the differential of temperature over the differential of time. This parameter is added to the equation and the calorimetric power can be obtained for further calculations.

3.5.2 Hydroxyl radical measurements

One of the experiments carried out was conducted with the aim of measuring the generation of hydroxyl radicals. The principal objective of this part is to rely the generation of hydroxyl radicals with the degradation of the pharmaceuticals in the treated solutions. The generation of OH· is calculated by KI dosimetry. Ultrasonic irradiation of KI aqueous solution leads to the oxidation of iodine ions by the generated hydroxyl radicals into iodine as it is shown in (15), (16) and (17).

$$HO\cdot + I^- \rightarrow \mathrm{I} + OH^- \tag{15}$$
$$\mathrm{I} + I^- \rightarrow I_2^- \tag{16}$$
$$2\,I_2^- \rightarrow I_2 + 2\,I^- \tag{17}$$

Then, iodine reacts with the excess of I^- ions generating triiodide ions as it is shown in (18).

$$I^- + I_2 \rightarrow I_3^- \tag{18}$$

This I_3^- in the solution are measured spectrophotometrically at a wavelength of 355 nm with molar absorptivity ($\mathcal{E}$) = 26,303 L/mol·cm. For the spectrophotometric analysis, the used cuvette has a path length of 1 cm. So, the calculations are based on dividing the value obtained at a wavelength of 355 nm by the product of molar absorptivity and 1 cm. The obtained result is the concentration of hydroxyl radicals in the aqueous solution.

The potassium iodide solution is prepared by dissolving 0.1 mole of KI in 1 L deionized water. Then, the solution is treated with ultrasounds in order to see the evolution of the generation of hydroxyl radicals. Triiodide generated during the reactions has a yellowish color whose intensity grows as concentration increases. As it is shown in Figure 23, the presence of triiodide ions is escalating during the treatment.

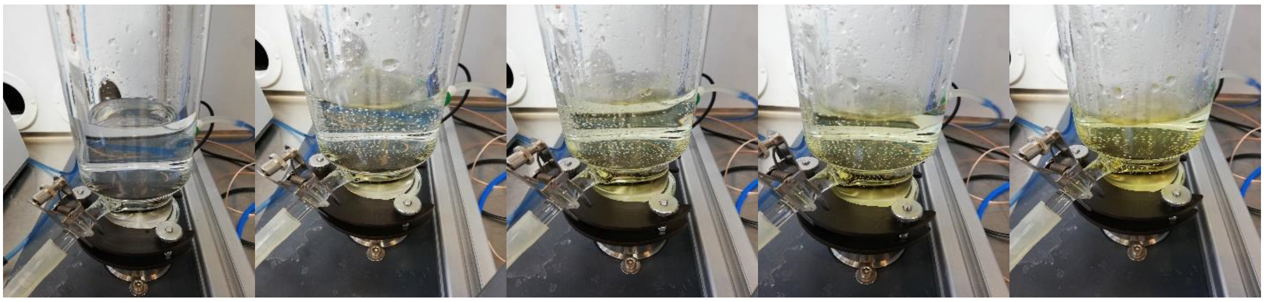


Figure 23. Potassium iodide solution’s change of color during treatment

3.5.3 Degradation quantification with UV absorbance

The measurement of the UV absorbance was conducted using a spectrophotometer. Due to the complexity of Trimethoprim’s decomposition pathways, the measurement method was simplified to track the degradation based on the decay of the area under the curve of the compound spectrum. The limits for the wavelengths are 258 nm and 300 nm for all measurements. From left to right of the Figure 24, the area under the curve before treatment, after 60 min, 90 min and 120 min decreases showing changes in Trimethoprim’s structure and also, how degradation evolves over time.

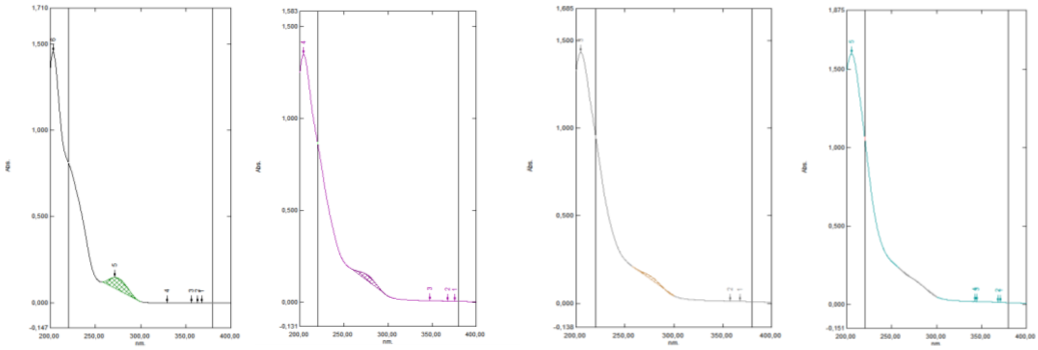


Figure 24. Evolution of area under the curve

The spectrophotometer gives the possibility to calculate automatically the area under the curve for each measured sample. Placing two vertical bars on the two aforementioned wavelengths, the software associated to the spectrophotometer measures the area under the curve. This tool gives the possibility to quantify the degradation of Trimethoprim using UV absorbance. This measuring method is much more effective, easy and waste-less than calculations made with COD, that require chemicals. For this study, the influence of calorimetric power on area degradation has been studied dividing every degradation are result obtained by the calorimetric power results. This division was named area degradation efficiency. This value shows how many percentage points the ultrasound system eliminates for each watt applied to the liquid.

3.5.4 TOC measurements

This measurement was performed to follow the complete mineralization of TMP using Shimadzu TOC-V-analyzer shown in Figure 25. TOC is the acronym of Total Organic Carbon. This parameter measures the quantity of organic carbons presents in the sample before and after every treatment. It is another way to quantify degradation of Trimethoprim and also, to measure the efficiency of the treatment. This tool is used to obtain quick results for TOC in every analyzed sample.

Figure 25. Shimadzu TOC-V-analyzer

3.5.5 ANOVA (Analysis of Variance)

ANOVA is a selection of statistical bases and their related estimation methodologies used to examine the differences among means. RSM generates an analysis for the variance of obtained results. This further study gives a statistical test of whether two or more population means are equal, generating a t-test beyond two means.

4. Results & Discussion

4.1 Calorimetric results

In Graphic 1, the obtained results for calorimetric power are shown. These values were calculated according to the calorimetric measurement method explained in the point 3.5.1. These results reflect that higher the power amplitude, higher the calorimetric power applied to the liquid. This fact is logical because the efficiency of the transducer is the same in each one of the power amplitudes ranges. Also, for low amplitudes, calorimetric power is lower for 580 kHz than for 865 kHz. Nonetheless, for high amplitudes, the generation of calorimetric power is higher for 580 kHz than for 865 kHz.

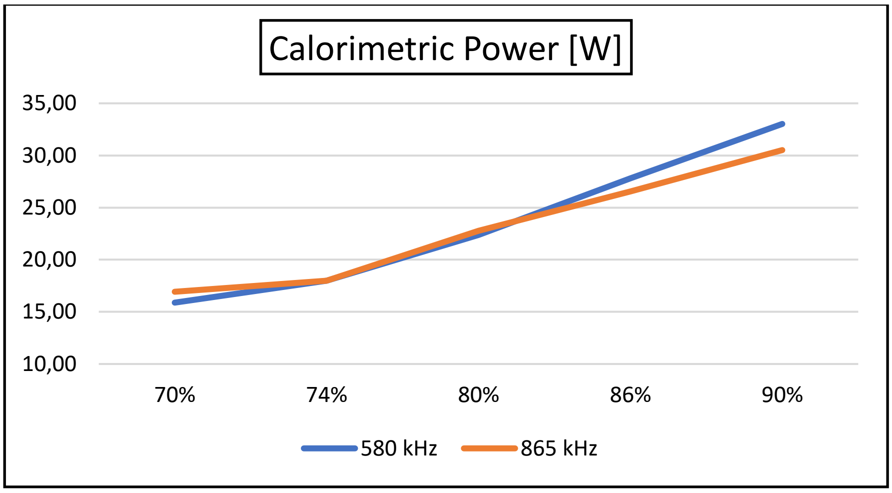


Figure 26. Calorimetric power results' representation

Then, in Table 2, the average of the division explained in chapter 3.5.3 has been calculated for every configuration of power amplitude and frequency.

Table 2. Calorimetric degradation efficiency

Power amplitude	Area degradation [%/W]	
	580 kHz	865 kHz
70%	3,98%	2,45%
74%	3,48%	2,82%
80%	3,61%	2,24%
86%	3,14%	2,71%
90%	2,88%	2,38%

As it is shown in Table 2, area degradation values in [%/W] are higher for 580 kHz in every power amplitude studied. These results mean that if the same power is applied for both frequencies, the degradation obtained will be higher working with a frequency of 580 kHz. Looking for the results obtained for every frequency in particular, for 865 kHz, they are inconsistent. But, for 580 kHz, the higher power amplitude, the lower quantity of area percentage points.

4.2 Hydroxyl radical results

The amount of hydroxyl radicals was calculated following the methods indicated in the point 3.5.2. As it is shown in Table 3, the concentration of hydroxyl radicals generated during the process is significantly higher for a frequency of 580 kHz than for 865 kHz.

Table 3. Hydroxyl radical concentration

Power [%]	Treatment time [min]	Hydroxyl radical concentration [mol/L]	
		Frequency 865 kHz	Frequency 580 kHz
70	0	0,0003	0,0003
	5	0,0033	0,0042
	10	0,0075	0,0115
	15	0,0129	0,0203
	20	0,0188	0,0240
74	0	0,0003	0,0004
	5	0,0036	0,0055
	10	0,0080	0,0131
	15	0,0136	0,0219
	20	0,0204	0,0268
80	0	0,0003	0,0003
	5	0,0043	0,0080
	10	0,0100	0,0193
	15	0,0180	0,0277
	20	0,0257	0,0394
86	0	0,0003	0,0007
	5	0,0045	0,0109
	10	0,0120	0,0252
	15	0,0204	0,0335
	20	0,0283	0,0487
90	0	0,0004	0,0005
	5	0,0064	0,0122
	10	0,0163	0,0260
	15	0,0269	0,0360
	20	0,0361	0,0536

The greater differences are seen in the middle times where the variation between 865 kHz and 580 kHz can be more than the 50%. Specifically, after 5 and 10 min the differences are accentuated much more.

4.3 UV Absorbance results

4.3.1 Diagnostic

Table 4. Degradation results

Power amplitude	Treatment Time [min]	Concentration [ppm]	Degradation Frequency 865 kHz [%]	Degradation Frequency 580 kHz [%]
90%	90	7,5	60,44%	94,78%
	90	7,5	84,87%	94,95%
86%	108	6	87,31%	100,00%
	108	6	100,00%	100,00%
	72	6	59,76%	77,21%
	72	6	70,69%	81,93%
	108	9	73,60%	98,20%
	108	9	73,60%	100,00%
	72	9	50,93%	63,22%
	72	9	59,81%	78,43%
80%	120	7,5	62,49%	100,00%
	120	7,5	63,33%	100,00%
	90	10	45,31%	76,21%
	90	10	52,26%	71,46%
	90	7,5	46,48%	86,50%
	90	7,5	56,16%	81,21%
	90	7,5	47,58%	80,20%
	90	7,5	46,22%	81,11%
	90	7,5	56,57%	78,82%
	90	5	62,27%	94,66%
	90	5	62,73%	87,30%
	60	7,5	32,34%	56,42%
	60	7,5	30,02%	55,79%
74%	108	6	62,57%	65,86%
	108	6	65,11%	88,68%
	72	6	44,87%	41,21%
	72	6	42,22%	52,28%
	108	9	61,91%	75,18%
	108	9	50,73%	76,16%
	72	9	34,43%	50,08%
	72	9	44,31%	51,45%
70%	90	7,5	35,42%	70,28%
	90	7,5	47,37%	56,05%

Table 4 shows the obtained results for peak area's degradation observed with the spectrophotometer. These results are separated in two different ways: by groups of rows and by columns depending on the frequency at which the treatment was made. Comparing both columns of results, it is clear that for the same treatment time, power amplitude and concentration of Trimethoprim, working at 580 kHz is more effective than doing it at 865 kHz.

Analyzing deeply the results, it is noted that higher power amplitudes imply higher degradations. Then, results have been plotted in graphics in order to see its behavior. These graphics are shown in Figure 27.

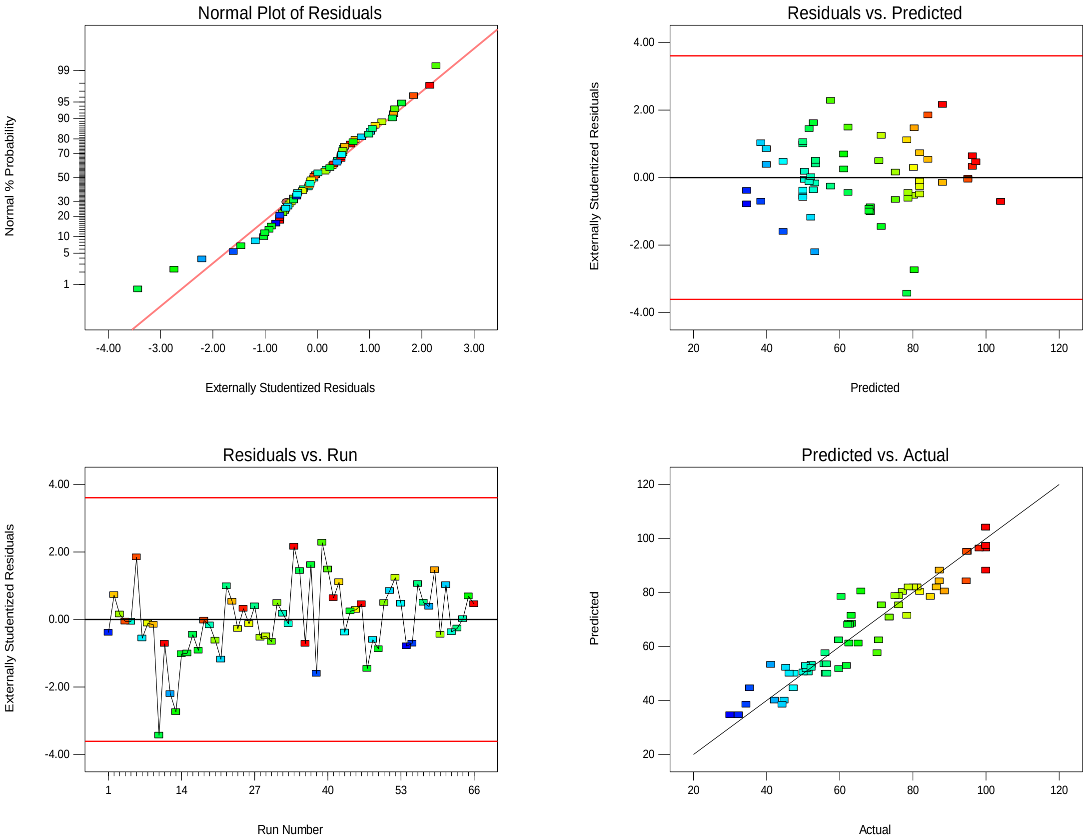


Figure 27. Diagnostic results for area degradation

In Figure 27, 4 graphics are shown: Normal Plot of Residuals, Residuals vs. Predicted, Residuals vs. Run and Predicted vs. Actual. In the Normal Plot of Residuals graphic, it is seen that none of the degradation points has a significant difference with the reference line. This graphic is normally used to check for normality of residuals. In both Residuals vs. Predicted and Residuals vs. Run, the externally studentized residuals limits are not overcome. In their case, for Residuals vs. Predicted, the finality is to check if there is a constant error in data and Residuals vs. Runs is used for outliers such as influential values. Finally, the Predicted vs. Actual graphic shows no meaningful deviations. The fact that the obtained results stay into the limits and without any disruption is important because it shows the quality of the data.

4.3.2 Analysis of Variance (ANOVA) for area degradation

The ANOVA results are shown in Table 5.

Table 5. Analysis of Variance for area degradation results

ANOVA for Response Surface Reduced Cubic model
Analysis of variance table [Partial sum of squares - Type III]

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	22469.03	19	1182.58	24.31	< 0.0001	significant
A-Power	*6145.59*	*1*	*6145.59*	*126.35*	*< 0.0001*	
B-Treatment time	*7275.99*	*1*	*7275.99*	*149.59*	*< 0.0001*	
C-[TMP]	*742.96*	*1*	*742.96*	*15.28*	*0.0003*	
D-Frequency	*2563.55*	*1*	*2563.55*	*52.71*	*< 0.0001*	
AB	*0.90*	*1*	*0.90*	*0.019*	*0.8921*	
AC	*114.61*	*1*	*114.61*	*2.36*	*0.1316*	
AD	*16.50*	*1*	*16.50*	*0.34*	*0.5631*	
BC	*16.02*	*1*	*16.02*	*0.33*	*0.5689*	
BD	*121.23*	*1*	*121.23*	*2.49*	*0.1212*	
CD	*59.94*	*1*	*59.94*	*1.23*	*0.2727*	
A^2	*45.84*	*1*	*45.84*	*0.94*	*0.3367*	
B^2	*32.37*	*1*	*32.37*	*0.67*	*0.4188*	
C^2	*82.07*	*1*	*82.07*	*1.69*	*0.2004*	
ABD	*32.56*	*1*	*32.56*	*0.67*	*0.4175*	
ACD	*4.73*	*1*	*4.73*	*0.097*	*0.7566*	
BCD	*31.09*	*1*	*31.09*	*0.64*	*0.4281*	
A^2D	*379.32*	*1*	*379.32*	*7.80*	*0.0076*	
B^2D	*84.79*	*1*	*84.79*	*1.74*	*0.1933*	
C^2D	*197.10*	*1*	*197.10*	*4.05*	*0.0500*	
Residual	2237.38	46	48.64			
Lack of Fit	*805.30*	*10*	*80.53*	*2.02*	*0.0596*	*not significant*
Pure Error	*1432.08*	*36*	*39.78*			
Cor Total	24706.41	65				

The Model F-value of 24.31 implies the model is significant. There is only a 0.01% chance that and F-value this large could happen due to interferences. Values of “Prob>F” less than 0.05 indicate model terms are significant. In this case, A, B, C, D, A^2D and C^2D are significant model terms. These terms are located in the first column of Table 5. As it is shown, A, B, C and D correspond, respectively, to Power, Treatment time, Concentration of Trimethoprim and Frequency. The other labels correspond to the interaction between the first ones. Values bigger than 0.1 mean the model terms are not significant. If there are lot of insignificant model terms, model reduction may improve the model. Then “Lack of Fit F-value” of 2.02 implies there is a 5.96% chance that a “Lack of Fit F-value” this large could occur due to noise. Lack of fit is bad. This relatively low probability (<10%) is troubling.

Table 6. Statistical values for area degradation

Std. Dev.	6.97	R-Squared	0.9094
Mean	67.26	Adj R-Squared	0.8720
C.V. %	10.37	Pred R-Squared	0.8073
PRESS	4760.16	Adeq Precision	18.094

In Table 6, some statistical values are shown. The “Pred R-Squared” of 0.8073 is in reasonable agreement with the “Adj R-Squared” of 0.872 because their disparity is less than 0.2. “Adeq Precision” measures the signal to noise ratio. A ratio greater than 4 is desirable. The obtained ratio of 18.094 means an adequate signal. This model can be used to navigate the design space.

4.3.3 Response Surface Method for area degradation

Response Surface Method gives the possibility to plot results in 3D. In Figure 28 and Figure 29, three different plots are representing how area degradation behaves depending on two parameters: treatment time and power amplitude. Both figures differ on the frequency used for the treatment of the aqueous solutions. In Figure 28, the working frequency is 580 kHz and in Figure 29, the frequency is 865 kHz. The difference between the three graphics is the concentration of Trimethoprim studied in each one. From left to right, the graphics show the surface for 5 ppm, 7.5 ppm and 10 ppm.

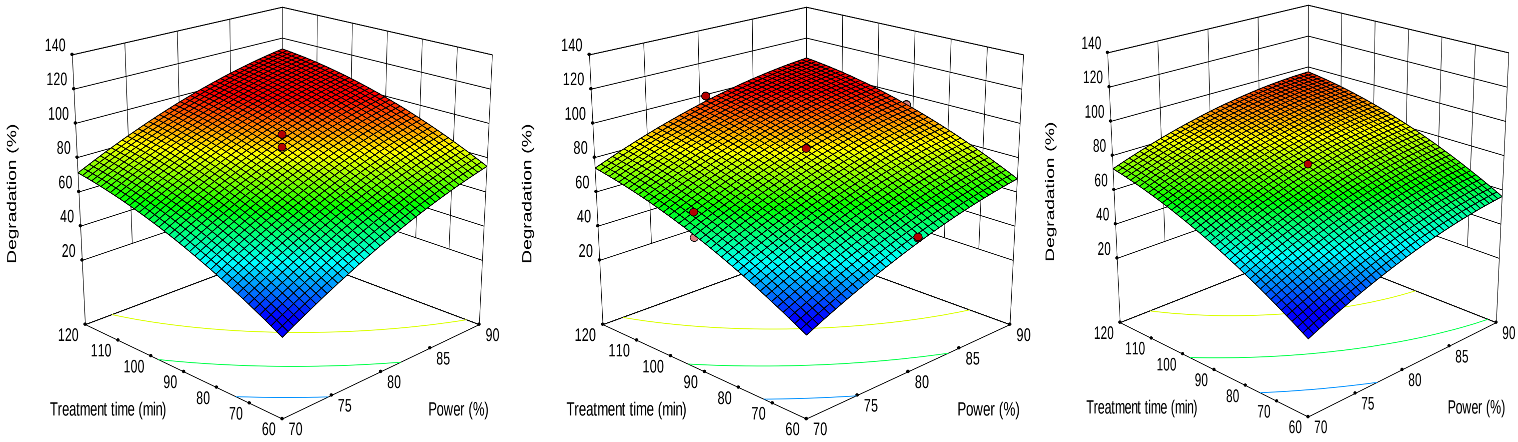


Figure 28. Plotted results showing the response surface of area degradation for 5 ppm, 7.5 ppm and 10 ppm for 580 kHz

Analysing Figure 28, as treatment time and power amplitude increase, percentage of degraded area increases too. This trend is observed for every concentration. No big differences are shown between 7.5 ppm and 10 ppm graphics. For 5 ppm, the general value of percentage of degraded area is higher.

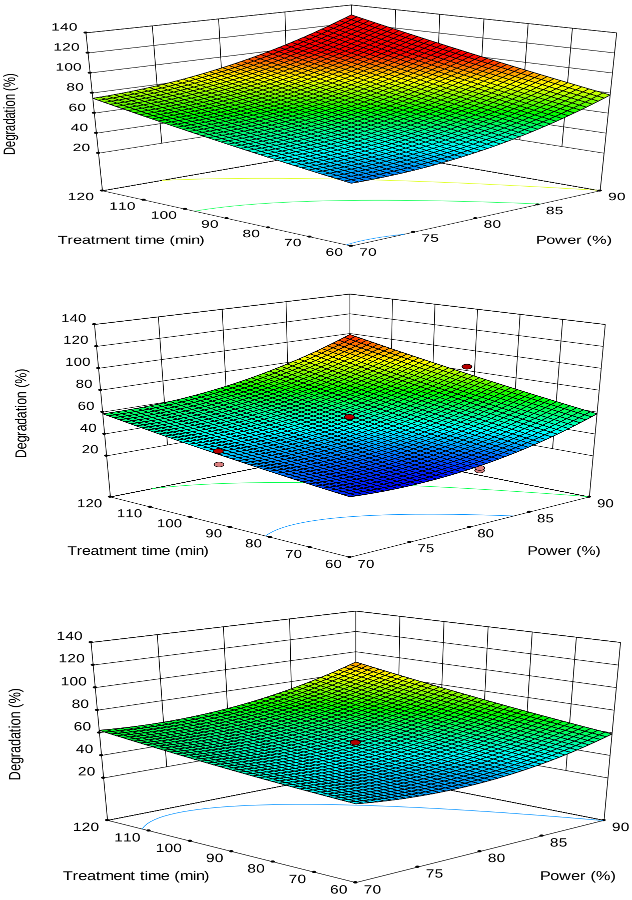


Figure 29. Plotted results showing the response surface of area degradation for 5 ppm, 7.5 ppm and 10 ppm for 865 kHz

In the case of Figure 29, the behavior for the three graphics is very similar to the trend observed in Figure 28. Nonetheless, the results obtained in the case of 580 kHz of frequency tend to non-linearity and those obtained in 865 kHz are linear. This difference in the behavior shows that higher degradation values are achieved with less treatment time and power amplitude for every antibiotic concentration when the treatment is developed at 580 kHz.

4.4 TOC results

4.4.1 Diagnostic

Table 7. TOC removal results

Principal values				
Power	Treatment Time [min]	Concentration [ppm]	TOC removal Frequency 865 kHz [%]	TOC removal Frequency 580 kHz [%]
90%	90	7,5	5,85	3,06
	90	7,5	5,57	6,07
86%	108	6	16,09	0,00
	108	6	2,38	0,00
	72	6	14,88	7,85
	72	6	0,00	0,00
	108	9	0,00	9,84
	108	9	10,59	5,28
	72	9	0,00	7,28
	72	9	5,13	3,36
80%	120	7,5	0,00	4,88
	120	7,5	0,00	0,00
	90	10	4,50	9,41
	90	10	3,07	0,00
	90	7,5	1,47	0,00
	90	7,5	13,16	0,00
	90	7,5	0,00	2,33
	90	7,5	0,00	0,00
	90	7,5	1,50	3,45
	90	5	3,70	0,00
	90	5	5,66	7,36
	60	7,5	1,36	0,46
	60	7,5	0,00	7,11
74%	108	6	0,47	9,10
	108	6	9,11	3,75
	72	6	0,00	4,14
	72	6	10,38	1,74
	108	9	2,80	0,00
	108	9	0,97	0,35
	72	9	0,00	1,12
	72	9	3,14	5,00
70%	90	7,5	1,63	6,41
	90	7,5	0,00	2,98

Table 7 shows the results obtained for TOC removal with Shimadzu TOC-V-analyzer. These results are separated also by columns of working frequency and by rows depending on the power amplitude. Observing deeply the data, it is noted that results are more inconsistent than they were for peak area degradation. Also, it is not assured that high power amplitudes give higher TOC removal values.

Then, these results were plotted as it is shown in Figure 30.

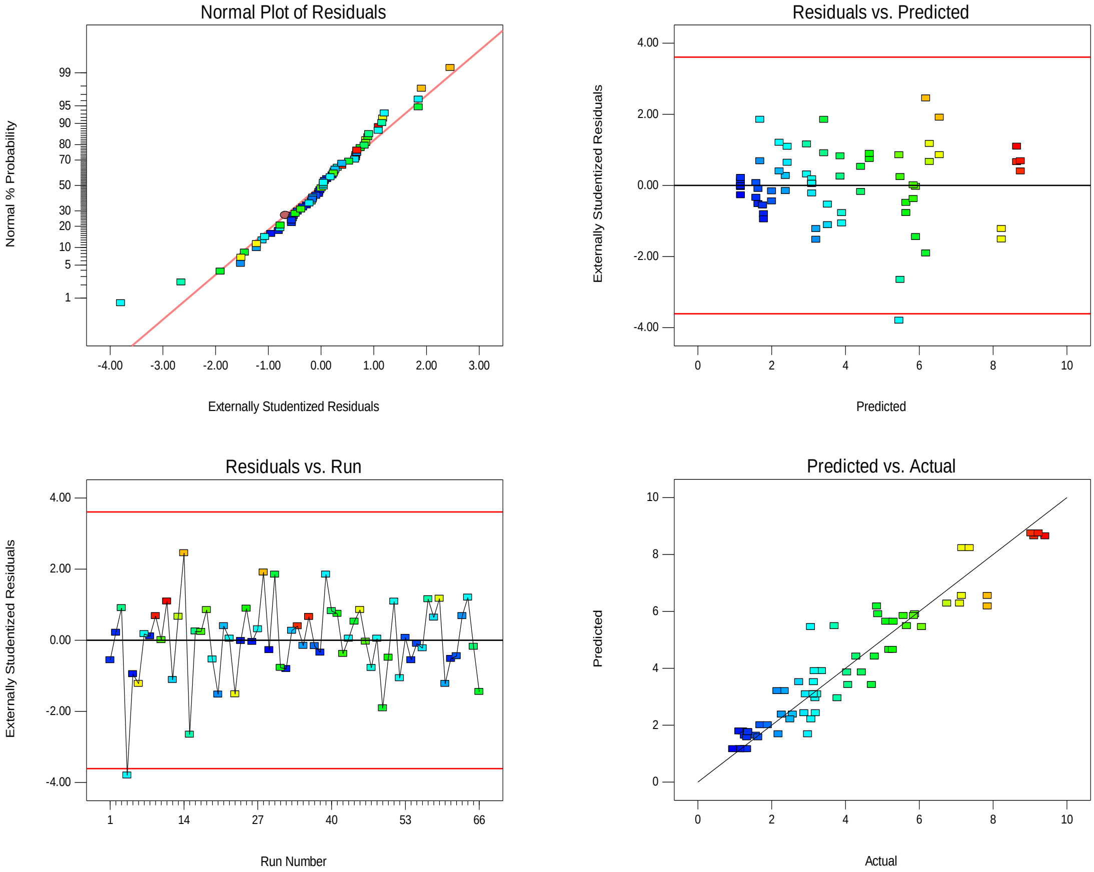


Figure 30. Diagnostic results for TOC removal

In Figure 30, diagnostic results for TOC removal shown the aforementioned inconsistency. For the Residuals vs. Predicted and Residuals vs. Run graphics, one result obtained is outside recommendable limits. Also, in the graphic Predicted vs. Actual, the dispersity of results is shown but there is no presence of a constant error. Despite the deviation of some results, the quality of data is assured observing the graphics and analyzing them.

4.4.2 ANOVA (Analysis of Variance) for TOC removal

Table 8. Analysis of Variance for TOC removal results

ANOVA for Response Surface Reduced Cubic model
Analysis of variance table [Partial sum of squares - Type III]

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	**p-value Prob > F**	
Model	313.26	19	16.49	22.33	< 0.0001	significant
A-Power	*77.93*	*1*	*77.93*	*105.53*	*< 0.0001*	
B-Treatment time	*65.58*	*1*	*65.58*	*88.82*	*< 0.0001*	
C-[TMP]	*78.99*	*1*	*78.99*	*106.97*	*< 0.0001*	
D-Frequency	*9.36*	*1*	*9.36*	*12.67*	*0.0009*	
AB	*2.49*	*1*	*2.49*	*3.37*	*0.0730*	
AC	*1.29*	*1*	*1.29*	*1.74*	*0.1931*	
AD	*0.30*	*1*	*0.30*	*0.40*	*0.5287*	
BC	*4.41*	*1*	*4.41*	*5.97*	*0.0184*	
BD	*2.59*	*1*	*2.59*	*3.51*	*0.0675*	
CD	*2.85*	*1*	*2.85*	*3.86*	*0.0555*	
A^2	*11.90*	*1*	*11.90*	*16.11*	*0.0002*	
B^2	*22.29*	*1*	*22.29*	*30.19*	*< 0.0001*	
C^2	*38.07*	*1*	*38.07*	*51.56*	*< 0.0001*	
ABD	*6.35*	*1*	*6.35*	*8.61*	*0.0052*	
ACD	*0.024*	*1*	*0.024*	*0.033*	*0.8571*	
BCD	*0.038*	*1*	*0.038*	*0.051*	*0.8220*	
A^2D	*4.16*	*1*	*4.16*	*5.63*	*0.0219*	
B^2D	*7.39*	*1*	*7.39*	*10.01*	*0.0028*	
C^2D	*19.77*	*1*	*19.77*	*26.78*	*< 0.0001*	
Residual	33.97	46	0.74			
Lack of Fit	*20.53*	*10*	*2.05*	*5.50*	*< 0.0001*	*significant*
Pure Error	*13.44*	*36*	*0.37*			
Cor Total	347.23	65				

Now, the results obtained in Table 8 are going to be analyzed. The Model F-Value of 22.33 implies the model is significant. There is only a 0.01% chance that an F-Value this large could occur due to noise. Values of "Prob>F" less than 0.05 indicate model terms are significant. In this case, A, B, C, D, BD, A^2, B^2, C^2, ABD, A^2D, B^2D, C^2D are significant model terms. Values greater than 0.1 indicate the model term are not significant. If there are mane insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. The "Lack of Fit F-value" of 5.50 implies the Lack of Fit is significant. There is only a 0.01% chance that a "Lack of Fit F-value" this large could occur due to noise. Significant lack of fit is bad.

Table 9. Statistical values for area degradation

Std. Dev.	0.86	R-Squared	0.9022
Mean	3.97	Adj R-Squared	0.8618
C.V. %	21.65	Pred R-Squared	0.7921
PRESS	72.17	Adeq Precision	16.037

In Table 9, the “Pred R-Squared” of 0.7921 is in reasonable agreement with the “Adj R-Squared” of 0.8618; the difference is less than 0.2. “Adeq Precision” ratio of 16.037 indicates an adequate signal because it is greater than 4.

4.4.3 Response Surface Method for TOC removal

Response Surface Method plotted the results obtained for TOC removal. In Figure 31 and Figure 32, the observed results are shown for working frequencies of 580 kHz and 865 kHz, respectively. As it happened for area degradation in previous chapters, the three graphics of each figure correspond to different concentrations of Trimethoprim in samples. The concentrations are, from up to bottom, 5 ppm, 7.5 ppm and 10 ppm.

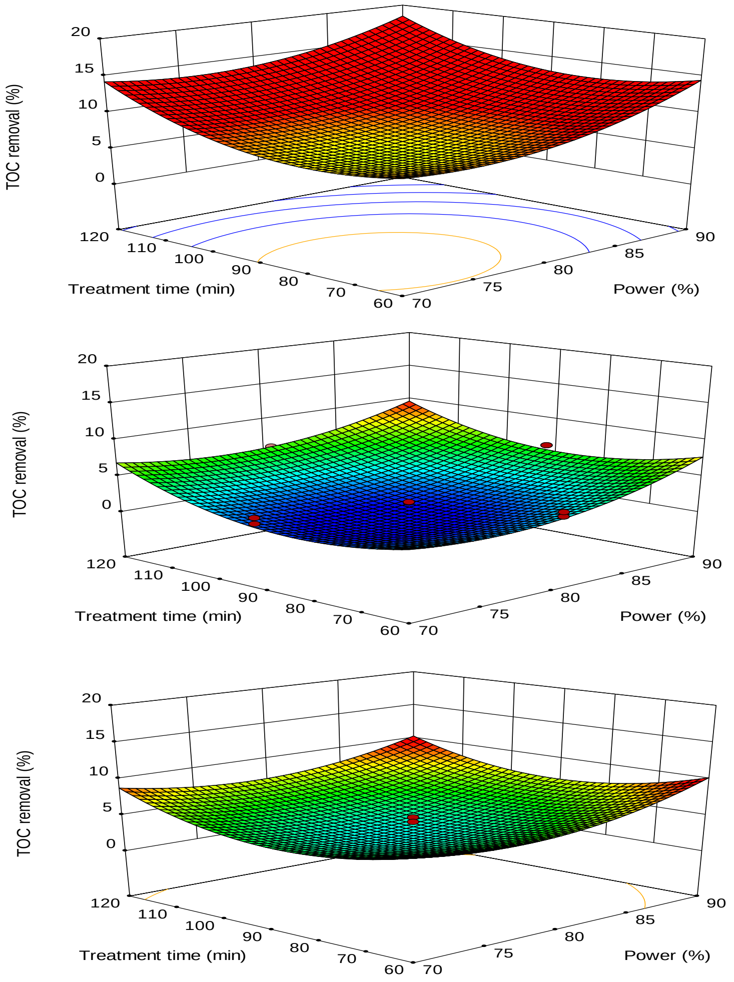


Figure 31. Plotted results showing the response surface of TOC removal for 5 ppm, 7.5 ppm and 10 ppm for 580 kHz

In Figure 31, it is noted that TOC removal is higher and much more important when the initial concentration is 5 ppm. Then, the observed results for 7.5 ppm and 10 ppm have not a significant disparity. Only when treatment time and power amplitude are high, TOC removal reaches 10% for 7.5 ppm and 10 ppm samples.

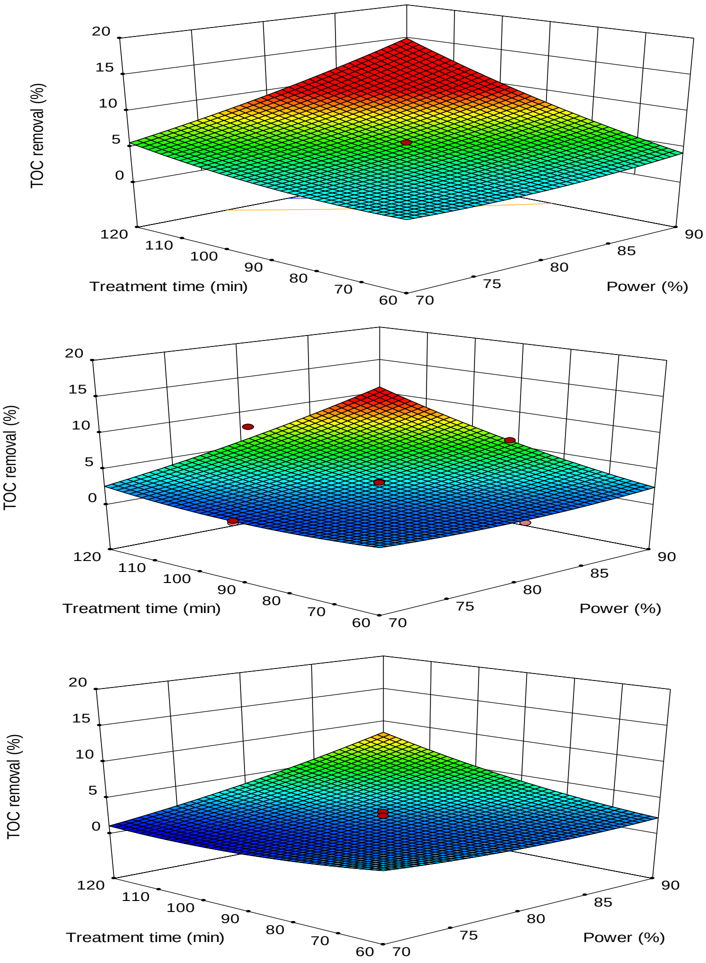


Figure 32. Plotted results showing the response surface of TOC removal for 5 ppm, 7.5 ppm and 10 ppm for 865 kHz

In the case of Figure 32, the effectiveness of treatment for TOC removal is not very high comparing it to the results obtained in Figure 31. The only difference residing in the working frequency, it is noted that it is more efficient to work with 580 kHz than with 865 kHz. As it happened with area degradation, the behavior of data in the case of working at 580 kHz is non-linear, while working at 865 kHz gives a more linear output.

4.5 Optimization

The desirability option given by Response Surface Method allows to analyze the optimum working point by setting conditions to the software. In this case, the proposed conditions were:

- For 5 ppm Trimethoprim:
    - Treatment time to minimum
    - Power to minimum
    - TOC removal and area degradation to maximum with the highest-ranking importance

- For 7.5 and 10 ppm Trimethoprim, the same conditions as above except that power was set to be in range.

Then, the software gave power amplitude and treatment time for each combination of Trimethoprim concentration and working frequency. With these parameters set, the next step of experimentation was to carry out each test. The objective was to check that there were no significant differences between the expected results coming from the desirability option and the obtained results during experimentation. In Table 10, there is a summary of the results.

Table 10. Summary table for desirability results

	Concentration	Expected Area degradation - Desirability	Expected TOC removal - Desirability	Area degradation	TOC removal
580 kHz	5 ppm	91%	9%	89,99%	9,80%
	7,5 ppm	69%	8%	61,91%	6,25%
	10 ppm	66%	8%	59,47%	2,53%
865 kHz	5 ppm	88%	9%	87,92%	5,25%
	7,5 ppm	88%	8%	80,81%	5,15%
	10 ppm	74%	4%	63,86%	0,00%

The obtained results during experimentation don’t show big differences from the expected results from desirability. In fact, the maximum difference is seen in the case of 10 ppm Trimethoprim concentration and 865 kHz where the difference is more than the 10%. This model is accepted if there is no more than a 20% of deviation between the expected and obtained results. It is noted that there are bigger differences in the results for area degradation than for TOC removal. Also, the disparity of the results is higher when Trimethoprim’s concentration on samples is higher as it is shown in Table 11. The data observed in this table are extracted from subtracting the obtained result from the expected one. A fact that attracts the attention is that the value of the real results is often lower than the expected one. Only in the case of 5 ppm Trimethoprim concentration and 580 kHz the result from experimentation is higher than the one predicted by the model.

Table 11. Comparative table between expected and real results

	Concentration	Delta between expected and real results for Area degradation	Delta between expected and real results for TOC removal
580 kHz	5 ppm	0,72%	-0,67%
	7,5 ppm	7,12%	1,46%
	10 ppm	6,81%	5,80%
865 kHz	5 ppm	0,54%	3,36%
	7,5 ppm	7,07%	2,79%
	10 ppm	10,30%	4,47%

After analyzing the results, the model proposed by the desirability is accepted because it ensures a predictability in the experimental results.

4.6 Combination with Hydrogen Peroxide

As it is explained in previous chapters, hydrogen peroxide is an AOP that often needs to be combined with other processes in order to be effective. In the case of study, the influence of the addition of hydrogen peroxide to the sample was tested. Establishing the optimum treatment conditions, hydrogen peroxide was added to the aqueous solution. In Table 12, there is a summary of the obtained results after the addition of H_2O_2.

Table 12. Summary table for the influence of Hydrogen Peroxide

Frequency [kHz]	H2O2 [ppm]	Area degradation	TOC removal
865	50	43,14%	6,31%
	100	39,15%	1,26%
580	50	67,10%	5,42%
	100	66,87%	6,46%

Data from Table 12 don’t show any big difference between adding 50 ppm or 100 ppm to the aqueous solution. In the case of working at 865 kHz, the area degradation decreases if there is more H_2O_2 present in the solution. In addition, TOC removal also decreases. For 580 kHz, results are similar for area degradation. But it is observed an increase in TOC removal. This increase is 1% therefore cannot be considered as relevant.

4.7 Discussion

Calorimetric and hydroxyl radical results discussion

In study conducted by Lee et al., 2011, results showed that bubbles appear at a lower acoustic power for 448 kHz than for 726 kHz. As it has been explained previously, the presence of bubbles implies a higher generation of hydroxyl radicals and higher pharmaceuticals' degradation rates. Comparing these results to those obtained in chapter 4.1, there is a similarity between both because it is noted that less calorimetric power is needed for the generation of the same number of bubbles in Lee et al., 2011. As the degradation is directly proportional to bubble and hydroxyl radical generation, obtained results in chapter 4.2 were expected. In the case of working at medium-high frequencies (between 400 and 600 kHz) is more effective compared to work at very high frequencies (between 600 kHz and 900 kHz) based on results obtained in the analysis. Hydroxyl radical generation and calorimetric efficiency are higher both in Lee et al., 2011 and in this study when working at medium-high frequencies (580 kHz in this study and 448 kHz in Lee et al, 2011).

UV absorbance results

In this study, results obtained with the spectrophotometer show acceptable outcomes working at 865 kHz and excellent results at 580 kHz. In Serna-Galvis et al., 2019, the results obtained show a percentage of degradation of 69,44% after 90 minutes of treatment. Comparing these results with the ones obtained in this study, it is noted that almost every treatment lasting 90 minutes and working at 580 kHz has higher degradation results. However, in the case of working at 865 kHz, degradation values are in the range of 40% and 50%. In this regard, it could be said that there is a decay in the effectiveness of Trimethoprim's degradation if frequency used in water treatment increases indefinitely. The fact that Trimethoprim's concentration in Serna-Galvis et al., 2019 is smaller than the ones used in this study is interesting because it shows that very low concentrations of pharmaceuticals are easier to remove. This last statement matches with the obtained results in the Response Surface Method in previous chapters.

Comparison of area degradation results

Another interesting study realized by Alfonso-Muniozguren et al., 2021, shows its degradation results working at different frequencies for such pharmaceuticals as Diclofenac, Levodopa and Paracetamol. As it happens in this study for Trimethoprim, medium-high frequencies treatments are more effective in degrading pharmaceuticals than very high frequencies as it is seen in Table 13.

Table 13. Frequency performance on the degradation of different pharmaceuticals (Alfonso-Muniozguren et al., 2021)

Pharmaceuticals	Frequencies				
	<300	300-500	500-700	700-900	>900
Diclofenac	-	92%	95%	25%	-
Levodopa	-	-	91%	90%	66%
Paracetamol	-	-	95%	92%	67%

So, according to these results, it is noted that there is a decreasing trend in the degradation effectivity of very high frequency values.

TOC removal results

As observed previously, the obtained results from TOC removal have lack of consistency. However, analyzing results with Response Surface Method, it is noted that lower initial concentrations of Trimethoprim give higher rates of TOC removal. This data could be compared to the obtained one in Martini et al., 2018 where different treatments are analyzed for diverse pharmaceuticals. One of these pharmaceuticals is Sulfamethoxazole, very often used alongside Trimethoprim for dealing against infections. Results in Martini et al., 2018 show high TOC removal for treatments like ozonation, ozonation combined with hydrogen peroxide or photolysis combined with ozonation. All these results are much better than the ones obtained in this study. The only treatments with similar outcomes are photolysis and photolysis combined with hydrogen peroxide.

The inconsistency of the obtained results shows that ultrasounds have potential for improving its TOC removal efficiency. Maybe an available option could be combining it with other advanced oxidation processes.

Combination with hydrogen peroxide results

In Martini et al., 2018, an improvement in TOC removal is shown after adding hydrogen peroxide to the aqueous solution. In the results obtained in this study, inconsistency is still a problem with unconclusive results. However, the good results from Martini et al., 2018 are promising for keeping with the investigation on new technologies and treatments more environmentally friendly.

5. Conclusions

In this study, the lack of response to the challenge proposed by the presence of micropollutants such as Trimethoprim in wastewater was tried to be answered. Due to the low concentration of this compounds in the influent of wastewater treatment plants and also due to their poor biodegradability, actual treatments are not prepared for their complete elimination. This deficiency is causing environmental problems for the society.

The biggest challenges of this investigation have been the assembly of the experimental setup and the realization of reliable measurements for obtaining quality data that supports the study. Finally, the use of UV absorbance and TOC measurements helped to develop a good framework on which to base the analysis.

The main objective of the study was to find out if high frequency ultrasonication was effective for the elimination of traces of Trimethoprim in synthetic samples. The base of high frequency ultrasonication treatments is the generation of hydroxyl radicals. These highly reactive compounds destroy molecular bonds present in the micropollutants. It was noted during the analysis that this degradation system was efficiently removing the targeted pharmaceutical from synthetic samples. In addition, a relation between the generation of hydroxyl radicals and the degradation of pharmaceuticals was established. It was shown that higher the generation of hydroxyl radicals, higher the degradation of micropollutants present in synthetic samples.

After analyzing the obtained results, it can be concluded that high frequency ultrasonication is an efficient method for the elimination of low concentrations of pharmaceuticals in synthetic samples. Two generation frequencies were studied: 580 kHz and 865 kHz. All the obtained data showed that the most efficient one was 580 kHz. In addition, after calorimetric measurements, it was shown that with the same amount of calorimetric power, working at 580 kHz gave better degradation results. So, it is more energetically efficient to work at 580 kHz. It was observed that the behavior of TOC removal and Area degradation was non-linear working with 580 kHz. While, operating at 865 kHz gave more linear results. Working with low concentrations of micropollutants was also a challenge. Initial concentrations of Trimethoprim have an influence on treatment’s efficiency because working with 5 ppm gave best Area degradation and TOC removal results than doing it with 7.5 ppm or 10 ppm. Answering to the principal research questions, the optimum conditions to work for degrading Trimethoprim are with frequencies in between 500 kHz and 700 kHz.

Finally, combination of hydrogen peroxide and high frequency ultrasonication was tested. The inconsistency of the obtained results doesn’t allow to conclude anything about this study. However, it keeps a door open for further and more detailed studies. There is scope to continue working on totally eliminating the presence of micropollutants in the environment. The next step is to investigate the use of ultrasounds with real samples and to analyze the most environmentally-friendly way to do it.

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