

Study on the fate of pharmaceuticals in aqueous media: synthesis, characterization and detection of biotic and abiotic transformation products using electrochemical advanced oxidation processes and bioconversions

Hugo Olvera Vargas

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Joint PhD degree in Environmental Technology



Docteur de l'Université Paris-Est

Spécialité : Sciences et Techniques de l'Environnement



Dottore di Ricerca in Tecnologie Ambientali



Degree of Doctor in Environmental Technology

Thèse – Tesi di Dottorato – PhD thesis

Hugo Olvera-Vargas

Study on the Fate of Pharmaceuticals in Aqueous Media: Synthesis, Characterization and Detection of Biotic and Abiotic Transformation Products using Electrochemical Advanced Oxidation Processes and Bioconversions

To be defended on December the 17th, 2014

In front of the PhD committee

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Erasmus Joint doctorate programme in Environmental Technology for Contaminated Solids,



Soils and Sediments (ETeCoS³)





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Abstract

The present study contributes with valuable data for a better fundamental understanding on the fate of pharmaceutical residues in the environment, dealing with the main challenges concerning this increasingly worrying environmental issue.

The used Electrochemical Advanced Oxidation Processes (EAOPs), electro-Fenton (EF) and anodic oxidation (AO), showed to be a very efficient alternative for the oxidative degradation and or mineralization of acidic solutions of the pharmaceuticals ranitidine (RNTD) and furosemide (FRSM), attaining almost complete mineralization of the drugs after 6 h of electrolysis. A comparative study on the mineralization of RNTD solutions by EF and SPEF processes in a 2.5 L capacity pre-pilot flow plant demonstrated the higher oxidation capacity of SPEF, achieving very good mineralization rates, thus evidencing the potentiality of this technology at greater scale for the treatment of wastewaters containing pharmaceutical pollutants. The application of an EF pretreatment coupled with a biological process for the degradation of both drugs was also conducted. EF pre-pretreatment was capable of enhancing the solution biodegradability envisaging a biological treatment, which efficiently removed the short-chain carboxylic acids that had been formerly generated during the pre-applied electrolysis. In this way, the combination of both processes was confirmed as a very promising technology for the treatment of pharmaceuticalscontaining wastewater.

Several transformation products (TPs) were detected and identified during the electrochemical oxidation of the studied drugs. Toxicity tests, based on the inhibition of bioluminescence of the marine bacteria *V. fischeri*, evidenced that some of these oxidation by-products were more toxic than starting molecule, since the global toxicity of the solution increased on the first stages of the electrolysis. However, the abatement of the toxicity in the final stages of the electrochemical treatments, demonstrated the effectiveness of these technologies for both the mineralization and detoxification of the RNTD and FRSM solutions. The use of the fungi *Cunninghanella echinulate* for the bioconversion of FRSM led to the formation of three main biotransofrmation products: the previously identified saluamide and pyridinium, and the new detected keto-alcohol derivate. These TPs were generated by both, biological and electrochemical approches, evidencing their high probability to be found in environmental compartments as the most likely TPs of FRSM by different oxidation conditions. This study is thus presented as a very useful alternative for the assessment of the fate of pharmaceutical residues in the environment.

Résumé

Études du devenir de médicaments en milieu aqueux : synthèse, caractérisation et détection des produits de transformation abiotiques et biotiques par les procédés d'oxydation avancée et des biotransformations

La pollution des eaux superficielles et souterraines par des composés organiques est bien connue comme une préoccupation majeure de l'environnement dans de nombreux pays. Si les polluants prioritaires sont actuellement surveillés par la directive cadre européenne sur l'eau, il est désormais urgent de prendre en considération les nouveaux polluants dérivés de principes actifs des produits pharmaceutiques et d'identifier leurs produits de transformation à risque. Ce travail de thèse propose une étude globale sur l'état et l'avenir des produis pharmaceutiques dans l'environnement, sur l'exemple de deux pharmaceutiques choisis, dans le cadre de cette importante problématique environnementale.

Nous avons donc appliqué les procédés électrochimiques d'oxydation avancée, électro-Fenton (EF), oxydation anodique(OA) et photoélectro-Fenton solaire (PEFS), ainsi que le couplage électro-Fenton/traitement biologique pour une élimination effective des polluants médicamenteux furosémide et ranitidine. Les résultats obtenus confirment l'efficacité de ces technologies électrochimiques pour la minéralisation quasi-totale des produits pharmaceutiques étudiés. En outre, l'utilisation du pré-traitement par EF suivi d'un procédé biologique confirme la capacité de l'EF de transformer les polluants organiques en produits biodégradables qui peuvent être consommés par des microorganismes lors d'un traitement biologique, démontrant ainsi l'applicabilité potentiel de cette technique combinée, en termes d'une consommation énergétique réduite.

L'identification des produits de transformation (PTs) des pharmaceutiques étudiés par voie électrochimique (électro-oxydation) et biologique (bioconversion) a été effectuée par différentes techniques d'analyse physico-chimiques. La biotransformation du FRSM a conduit à la formation de trois PT principales; saluamide, pyridinium et un dérivé céto-alcool. Les deux premiers ont aussi été détectés lors du traitement électrochimique, ce qui suggère la probabilité de les trouver dans l'environnement comme les produits de transformation les plus plausibles par des différentes conditions de dégradation. Les tests de toxicité basés sur l'inhibition de la bioluminescence des bactéries marines *Vibrio fischeri* ont montré que certains PT formés lors de traitement

Résumé

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List of abbreviations

FRSM: Furosemide
RNTD: Ranitidine
AOPs: Advanced Oxidation processes
EAOPs: Electrochemical Advanced Oxidation Processes
TPs: Transformation Products
ETPs: Environmental Transformation Products
SWTP: Sewage wastewater treatment plant
AO: Anodic oxidation
EF: Electro-Fenton
SPEF: Solar Photoelectro-Fenton
BDD: Boron Doped Diamond
HPLC: High Performance Liquid Chromatography
PEC: Predicted Environmental Concentration
Pt: Platinum
DSA: Dimensionally Stable Anode
MCE: Mineralization Current Efficiency
EC _{TOC} : Energy Consumption (per unit TOC mass)
NMR: Nuclear Magnetic Resonance
MS: Mass Spectrometry



Introduction

Chapter 1 Introduction

1.1. Background

Pollution of surface and ground waters by organic compounds is now well recognized as a major environmental concern in many countries. It is primordial to screen emerging pollutants derived from active pharmaceuticals ingredients (APIs) and personal care products (PCPs). APIs and PCPs are increasingly introduced in the environment either through excretion or disposal by flushing of unused or expired medication, or directly within the sewage effluents of plants or hospitals. Metabolites of APIs are also pollutants and are mostly brought in environment through excretion. In some cases, the metabolites are active with important side effects (Bennett et al., 1996) end could be present at higher concentration than the parent molecule (Leclercq et al., 2009). These compounds end up in surface and ground waters and can even be found in drinking water (Kümmerer, 2009). They show a wide range of persistence in aquatic environments (Snyder et al., 2003), and some of them are already associated with adverse effects on aquatic organisms. The example of steroidal hormones and their disastrous effects on fishes is well documented (Jobling et al., 1998). Therefore, it seems to be reasonable to consider also the possible negative long term effects on human health (Levi and Cargouët, 2004; Casellas, 2006).

1.2. Challenges

1.2.1. Problem statement and key research questions

The presence of pharmaceuticals residues in terrestrial and aquatic systems is now well documented. Recent studies have demonstrated that despite the relatively low concentrations of pharmaceuticals in the environment (typically in sub-parts-per-billion levels), they are of ecological concern due to their potential long-term adverse effects on humans and wildlife. Furthermore, risk assessment and environmental monitoring are being performed in order to gain sufficient information that will allow prioritizing of pharmaceuticals for possible monitoring and regulation. In most of the investigations reported to date, the efficiency of pharmaceutical removal during water treatment is determined by measuring the disappearance of the parent compound but not the formation of by-products (Celiz et al., 2009). Useful data are increasingly available on APIs as

reports indicating for each pharmaceutical the predicted environmental concentration (PEC), and, when possible, the predicted no effect concentration (PNEC) (Besse and Garric, 2008).

Another source of this kind of emergent pollutants is the environmental transformation of APIs and of their mammalian metabolites. These transformation products are generated by bioconversion (micro-organisms) and abiotic degradation (chemical oxidation, photodegradation...), which occur during wastewater treatment processes and/or in environment (Celiz et al., 2009). For all these unknown compounds, no information is available about their chemical and biochemical properties, and their potential interactive effects within complex mixtures. Little attention has been given to the identification, let alone quantification, of these transformation products and similarly, available information on the environmental fate occurrence of excreted pharmaceuticals metabolites formed during drug metabolism in humans and animals are scarce.

The first problem concerning these Environmental Transformation Products (ETPs) is their detection in environment. Actually, people who are involved in monitoring the organic content of water are facing a very complex problem. Indeed, analytical methods can detect traces of compounds with a high sensitivity. Tandem mass spectrometry based methods (i.e. GC/MSⁿ or LC/MS/MS) provide further insight onto the possible structure of detected compounds increasing selectivity. However this is not enough to fully characterize ETPs in water, because they are usually present at trace levels in rather complex mixtures. It is thus necessary to have the authentic samples in order to develop an efficient analytical method (Cardoza et al., 2003; Mullot et al., 2009).

The second problem is their potential toxicity of ETPs. While pharmaceuticals and their mammalian metabolites toxicity are mandatory studied before commercialization, associated toxicity of biotic and/or abiotic transformations are not considered. However, these ETPs may be toxic and eco-toxic (Dirany et al., 2011). Some studies show that transformation of a molecule can change its biological activity and increase the toxicological risks in the environment (Tixier et al., 2000; Oturan et al., 2008; Dirany et al., 2010). Thus disappearance of chemicals after treatment of wastewater is not synonymous with decontamination.

1.3. Research objectives

The main objective of this thesis work is to bring a better fundamental understanding of the fate of organic contaminants in environment, dealing with the main challenges concerning this worrying situation: the development and application of effective technologies for their destruction and the assessment of their formed transformation products. These studies should allow to new knowledge on the structure of ETPs of the selected contaminants. This goal requires an integrated chemically and biochemically based approach to prepare and characterize metabolites and transformation products derived from target drugs. These studies will provide authentic samples from which analytical detection methods can be developed and to conduct reliable assessment of their presence in the environment.

This global study conducted in order to assess the fate of pharmaceutical organic contaminants in the environment, consists in:

- Applying different EAOPs for the mineralization of the target pharmaceuticals and identifying their transformation oxidation products. These EAOPs include electro-Fenton, anodic oxidation, solar photoelectron- Fenton and the coupling of an electro-Fenton pretreatment and a biological process.
- II. Identifying transformation products of two classes of representatives of major pharmaceuticals released in waters under laboratory conditions and testing the reactivity of the starting molecules submitted to different selective biotic and abiotic transformation processes.
- III. Implementing a predictive analysis aiming to select the most plausible ETPs among the products generated.
- IV. Conducting toxicity tests to the electrochemically treated solutions in order to evaluate the ecotoxicity of the generated TPs.

ETPs routes of formation can be used to guide chemical analysis in anticipating what products might be formed with untested chemicals today: this approach is important because it can identify potentially toxic and stable ETPs at the screening stage of environmental risk assessment. Therefore, the goal of the present proposal is to develop a method for obtaining a more comprehensive picture of the presence of ETPs in the environment through laboratory experiments associated to the most recent analytical chemistry methods.

For the majority of chemicals, including in particular those proposed in the present project, chemical legislation requiring the identification of the degradation products/metabolites has emerged only recently (EMEA, 2006). Furthermore, ETPs for these chemicals typically have to be

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mainly identified for high tonnage compounds and actual guidance on how to include them into risk assessment is weak. Additional limitations to the understanding of how much ETPs contribute to the overall presence of chemicals in the environment result from the lack of analytical reference standards for most potential ETPs, and from the fact that laboratory degradation studies may not be representative of actual environmental conditions. Therefore, the goal of this study is to develop a method for obtaining a more comprehensive picture of the presence of ETPs in the environment. Consequently, ETPs routes formation can be used to guide chemical analysis in anticipating what products might be formed with untested chemicals today so we can identify potentially toxic and stable ETPs at the screening stage of environmental risk assessment. Resources available to conduct such a task are mainly predicting biodegradation pathways tools including computational prediction systems like META (Klopman and Tu, 1997; Jaworska et al., 2002) and the University of Minnesota pathway prediction system UM-PPS (Hou et al., 2004). They all belong to the category of artificial intelligence systems and they are based on a set of transformation rules that recognize compounds' substructures and transform them into degradation substructures. However, the main drawbacks presented by their use lay in the combinatorial explosion associated with the iterative application of transformation rules to generate biodegradation pathways (Fenner et al., 2008).

The first objective of this project is the application of different EAOPs for the degradation of the target pharmaceuticals, aiming to highlight their removal efficiencies, to clarify the mechanisms and kinetic factors that are involved, and to establish the optimal operational conditions. The second main goal lies on the synthesis of the greatest possible number of biotic and abiotic transformation products of a given drug using a multidisciplinary approach; electrochemistry and bioconversion. The next phase consists in the implementation of a comparative analysis of the nature of the transformation products obtained by each technique in order to predict the most probable ETPs.

Concerning the bioconversions, we want to mimic the environmental drug metabolism using known microorganisms and produce metabolites by biotransformation of selected strains (bacteria yeast and fungi). Recently a publication in (Harms et al., 2011) pointed out that fungi were not sufficiently used in environmental degradation investigations and their synthetic potentialities are underestimated in this area. The goal is to develop a new strategy consisting in a bio-combinatorial approach: 1) simplification of the screening of active microorganisms by minimizing the number on assays and 2) preparation of the maximum of metabolites, some of them resulting from two or more steps of biotransformation which could involve one or two microorganisms.

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Literature Review

Chapter 2

Literature Review

2.1. Introduction

The presence of pharmaceuticals in the environment and its association with potential risks has become an increasingly important issue for environmental regulation and the pharmaceutical industry. This concern has emerged by widespread detection of medicines in the environment as a result of the development in analytical techniques and the increasing implementation of field surveys. Several works have reported the presence of many different classes of pharmaceuticals in surface water (Boyd et al., 2003; Calamari et al., 2003; Ashton et al., 2004; Zuccato et al., 2005) and wastewater (Andreozzi et al., 2003; Ferrari et al., 2003; Hernández et al., 2007). Active pharmaceuticals ingredients (APIs) show a wide range of persistence in aquatic environments (Zuccato et al., 2005). With advances in medical technology and growing health-care spending, the consumption and uses of pharmaceuticals has been rising consistently. The presence of pharmaceuticals in water is attributable to domestic effluents, personal hygiene products, pharmaceutical industry wastes, hospitals wastes and therapeutic drugs (Rivera-Utrilla et al., 2013). At current environmental concentrations, some of them are beginning to be associated with adverse developmental effects in aquatic organisms and on human health. It is usually accepted that some of them may cause long-term, irreversible changes to micro-organisms genome, even at low content, increasing their resistance to them. Furthermore, some drugs have been classified as endocrine disrupting compounds (Klavarioti et al., 2009). It is also important to consider the synergistic action of mixtures of pharmaceuticals and micropollutants from other sources; toxicity of these complex mixtures has seldom been predicted (Sirés and Brillas, 2012a). Despite this problem, most aquatic data and risk assessments for pharmaceuticals are based on short-term acute studies (Bound and Voulvoulis, 2004; Ferrari et al., 2004; Zuccato et al., 2005) whereby chronic aquatic toxicity test have been adopted in the draft environmental risk assessment guidance document for the human pharmaceuticals produced by the European Medicines Agency (EMEA, 2006) in support of Directive 2001/83/ED (EC, 2001), but further research is necessary (Crane et al., 2006). For the above mentioned reasons, it is an emerging issue in environmental science and engineering the development and application of effective technologies for the removal of pharmaceuticals from wastewaters.

2.2. Parent compounds and transformation products

A chemical can undergo different structural changes by a variety of biotic and abiotic processes after its introduction into the environment. Pharmaceuticals can be biotransformed in the environment by microorganisms such as fungi or bacteria. They can also undergo structural change in the body of humans and animals or during an effluent treatment. Hence the chemical structure of the active molecules can be changed by biotransformation, biodegradation and abiotic transformation such as photo transformation and hydrolysis, resulting in a change in their physico-chemical and pharmaceutical properties (Kümmerer, 2009). In mammals' organisms, organic molecules undergo two principal metabolic biotransformation phases. Phase I transformations proceed by oxidative, reductive and hydrolytic pathways, leading to the introduction of a functional group, such as –OH, -SH, epoxide, -NH₂ or –COOH, with a usually modest increase in hydrophobicity. In Phase II, conjugations involve the attachment of a generally polar, readily available in vivo molecule to a susceptible functional group to form O- and N-glucuronides, sulfates and acetate esters, carboximides and gluthathionyl adducts, all with increased hydrophilicity relative to the unconjugated metabolite (Kalgutkar et al., 2002).

2.3. Occurrence and fate in the environment

With advances in medical technology and growing health-care spending, the consumption and uses of pharmaceuticals has been rising consistently. The consumption and application may vary considerably from country to country (Goossens et al., 2005; Ferech et al., 2006) and as a first approximation, the occurrence of a blockbuster drug that generates huge amount of annual sales could give an indication that relatively large quantities are entering the environment. They come principally from the manufacturers (even if emissions are assumed to be low in Europe and North America), from hospital wastes, as residues from private households (outdated medicines) which can end up on landfill sites where they can enter the landfill effluent, and form incomplete degradation in waste treatment plants (Kümmerer, 2009).

The input of pharmaceuticals, disinfectants, diagnostics and personal care products into the environment after use or outdated products, is a very typical and common situation and they are recognized as being an important part of the chemicals present in low concentrations in the environment. Medical substances have been detected in the effluent of medical care units, sewage and the effluent of sewage treatment plants, in surface water, ground water and even in drinking water, and from landfill sites (Heberer, 2002; Kolpin et al., 2002; Rabiet et al., 2006).

Pharmaceuticals are a large and varied class of compounds with diverse properties and applications. They are often grouped according to their therapeutic action: non-steroidal antiinflammatory drugs, antibiotics, beta-blockers, antiepileptics, blood lipid-lowering agents, antidepressants, hormones and antihistamines, which are classified according to various criteria including the volume of prescription, the toxicity and the evidence for presence in the environment (Khetan and Collins, 2007).



Figure 2.1. Fate of pharmaceuticals in the environment.

In 2006, there were more than 101 drugs that had sales exceeding 1 billion dollars per year, 35 of these had sales exceeding 2 billion dollars, and 16 surpassed the 3 billion mark (Khetan and Collins, 2007). Several health conditions such as cholesterol and triglyceride lowering stains, antiulcerants, antidepressants, and antihistamines (histamine H1 receptor antagonist), have been the target of multiple blockbusters. Another indicator of the great amount of pharmaceuticals production is the treatment of geriatric diseases like arthritis diabetes, high blood pressure, and elevated cholesterol, as the general population is getting older (Grimley, 2006).

The predominant fate processes for pharmaceuticals in the different environmental compartments are sorption (e.g. tetracyclines and quinolones) and (bio)degradation. Photodegradation and hydrolysis can also be significant in some cases. Substances reaching the environment may undergo different reactions, resulting in partial or complete transformation and/or degradation of the parent compound. Sometimes total degradation is stopped and hence

mineralization is not reached, generating intermediates that can be even more stable than the parent compounds. These generating intermediates can have higher toxicity then the parent drugs and present a higher potential for the environment. Bacteria and fungi are the groups of microorganisms that best degrade organic compounds. Hence, in sewage treatment plants (STPs), surface, ground and marine bacteria are assumed to be responsible of most biodegradation processes. The presence of pharmaceuticals in the aquatic environment evidences their incomplete degradation and elimination in sewage treatment (Kümmerer, 2009). Little is known about the occurrence, fate or activity of metabolites.

2.4. Toxicological effects

Pharmaceuticals are designed to target specific metabolic and molecular pathways in humans and animals, but they often have important side effects too. Their presence in the environment is impairing aquatic life forms, sometimes profoundly, and is producing changes that threaten the sustainability of the ecosphere on which our chemocentric civilization depends. They are often found to cause adverse ecological effects on aquatic and terrestrial organisms and they could present a risk to human health associated with consuming contaminated drinking water over a lifetime. Pharmaceuticals are present in the environment at trace levels, however, even in these infinitesimal concentrations, some of them have the potential to interfere detrimentally with normal development of aquatic life. Localized biogeochemical cycles are causing subtle modifications in plant growth, failure of larvae to molt or hatch, and anatomical deformities in a wide range of organism. Disturbances of the reproductive system and hormone system, immune depression and neurobehavioral changes can also been observed (Crane et al., 2006; Fent et al., 2006).

The amount of information available on the effects of active substances on organisms in the aquatic and terrestrial environment is low even though is increasing. There is a general lack of chronic toxicity data on pharmaceuticals. Many pharmaceuticals need more investigation about potential long-term ecotoxicological effects, particularly with respect to potential disturbances in hormonal homeostasis (endocrine disruption), immunological status, or gene activation and silencing during long-term exposure. Realistic concentration at measurable levels in the environment in chronic tests should be applied. The selection of species is also very important, there are presumably sensitive or insensitive species with respect to a certain endpoint and it has been found that standardized tests may underestimate effects (Fent et al., 2006; Kümmerer, 2009).

Some of the scant existing studies addressing the potential effects to human health from the presence of trace levels of pharmaceuticals in surface and drinking water claim that the concentration of many drugs and their metabolites are so low that they do not pose appreciable risk to human health. However, it was acknowledge that there are exceptions, such as antibiotics that have non-human target effect, estrogens that were developed for just one gender, genotoxic antineoplastics that have high potential fort allergic responses, or compounds that have very high bioaccumulation potential, which may be individually evaluated (Khetan and Collins, 2007).

All risk assessment is based on single compounds. Furthermore, pharmaceuticals do not occur as isolated pure substances in the environment, they are present as complex mixtures and it has been found that these mixtures might exhibit different effects than single compounds (Pomati et al., 2008). Some drugs act via the same or very similar modes of action and share the same receptor. Therefore, additive effects are to be expected. As for resistance against antibiotics, it is well known that cross resistance is quite common. Knowledge about the toxicity of mixtures is limited, making an accurate prediction of the chronic mixture toxicity indispensable for an environmental risk assessment.

2.5. Ecological risks of pharmaceutical metabolites in the environment

Generally, the efficiency of pharmaceuticals removal during water treatment is determined by the disappearance of the parent compounds and little attention has been given to the identification, let alone quantification, of the transformation products during the treatment. Persistent pharmaceutical metabolites require consideration for risk assessment because the effects resulting from exposure to a mixture of parent pharmaceutical and its metabolites may be quite different from what could be observed based only on toxicity of the single compound (Filby et al., 2007). Some of these formed metabolites show the potential to bind to proteins and other cellular constituents causing cellular function disruption, which may elicit a toxic effect and immune response (Thorpe et al., 2003; Sumpter and Johnson, 2005; Zhou et al., 2008). It is a challenge to assess individual and collective toxicities of metabolites and parent drugs because many metabolites have not been identified up to date. Mompelat et al. reported that approximately 160 pharmaceuticals and only 30 by-products (biotic and abiotic) have been in environmental investigations dealing with their occurrence, fata and ecotoxicology (Mompelat et

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al., 2009). Another important issue is the potential environmental effects of pharmaceutical mixtures from sewage treatment plant effluents. Several works have reported the increase of the toxicity of pharmaceutical mixtures, as well as the development of parasite-resistant strains and the change in the physiology of host organism, responding to the synergetic effect of pharmaceutical mixtures (Escher et al., 2005; Morley, 2009). Consequently, the identification and toxicity evaluation of transformation products formed during water treatment processes and biodegradation of pharmaceuticals has to be seriously taken into account. Consequently, it is critical to prioritize which metabolites are important for toxicity testing and risk assessment. This data are important starting points for the determination of the target compounds for monitoring aiming to reasonable predictions of potential ecotoxicities of metabolites. The lacking of this data on the chronic effects of pharmaceuticals' metabolites in the environment, make it difficult to do the necessary refinements to improve accuracy on existing models for risk assessment (Celiz et al., 2009).

2.6. The selected pharmaceuticals: Ranitidine and Furosemide

Furosemide (FRSM) is a sulfonamide derived from anthranilic acid. It is classified as loop diuretic and it is widely prescribed for the treatment of edematous states and hypertension (Pichette and Du Souich, 1996). This compound is not toxic in humans at therapeutic levels. However it has been associated with hypersensitivity and jaundice and has been found to show toxicity to some organisms (Peterson, 2012). Ranitidine (RNTD) is a H2-receptor antagonist, very widely prescribed for the treatment of peptic ulcer and gastroesophageal reflux disease. By 1988 it was the largest selling prescription drug (Khetan and Collins, 2007). It is oxidized in liver (30-70%) to *N*-oxide, *S*-oxide and *N*-demethylated metabolites (Martin et al., 1981). Both drugs have been unambiguously detected in sewage treatment plants, surface waters and sediments (Zuccato et al., 2000; Castiglioni et al., 2005; Fent et al., 2006; Gros et al., 2007; Rosal et al., 2010). Furthermore, FRSM and RNTD have been classified within the highest risk by a model dealing with the effects of contaminants on human Health (Besse and Garric, 2008).

2.7. Risk assessment

In 2006 the publication of the EU guideline on environmental risk assessment for human medicines was seen. The risk of adverse effects on humans through the ingestion of pharmaceuticals present in drinking water seems to be negligible, thus the risks posed to humans

from medicines seem to concern environmental hygiene rather than toxicology or pharmacology. This statement relies on some assumptions: 1) that effects and side effects during therapeutic use are the same in quality and quantity as for lifelong ingestion, 2) that the effects are the same for fetuses, babies, children, healthy adults and elderly people, and 3) that the risk posed by a single compound is comparable to the one posed by a mixture. Data enabling a realistic assessment for metabolites and transformation products are missing and we also have to consider that besides toxicity, persistence is of particular importance since persistent organic pollutants (POPs) increase the potential for long-term accumulation and hence varied effects, increasing the potential of multiple contamination of the ecosystem. In the United States as well as in most countries, water-quality standards do not regulate pharmaceuticals in reclaimed wastewater, drinking water or natural waters (Khetan and Collins 2007).

2.8. Risk management

Awareness of the presence of pharmaceuticals in the environment and evidence of the effects, make it clear that precautionary management action to reduce the release of pharmaceuticals into the environment should be considered. Combinations of management strategies will likely be most effective in mitigating the risks presented. The most effective management strategies in order to reduce the environmental impacts of pharmaceuticals are focused on: (i) advanced wastewater treatment technology, (ii) education of medical professionals to reduce over prescription, and (iii) pharmaceutical-return programs coupled with education and requirements for all municipalities to have at least a second cleansing step (Doerr-MacEwen and Haight, 2006). Alternatively and in accordance to the principle of green chemistry, the functionality of a chemical should not only include the properties of a chemical necessary for its application but also fast and easy degradability after its use (Anastas and J.C., 1998). Hence, improvement of sustainable synthesis and renewable feedstock are very prominent, making benign-by-design pharmacelogy a large, complex and fascinating subject that industry should address soon, if inherent problems can be faced, mainly economic issues.

2.9. Pharmaceutical removal in water treatment systems

2.9.1 The conventional methods

Conventional treatment plants, mainly based on the use of microorganisms, are inadequate to effectively destroy persistent organic compounds, due to their complex structure and low concentration in water, being the percentage of removal lower than 10%. Despite the lack of regulation concerning pharmaceuticals, the European Union Water Framework Directive, based on the precaution principle, produces an updated list of priority substances every four years (2000/60/EC) and has identified compounds from pharmaceuticals as potential pollutants (Jones et al., 2005).

In drinking water treatment systems, chlorine, chlorine dioxide and ozone are frequently used for disinfection. Chlorine is the most widespread conventional treatment for disinfecting drinking waters. Chlorination reaction is usually rapid with pharmaceutical products containing amines, giving rise to chlorinated compounds that can be toxic. The reaction rate can be strongly affected by the presence of different functional groups in the benzene ring. Chlorine dioxide is a more potent oxidant than chlorine and can oxidize numerous organic compounds. It is only effective for certain antibiotics and reacts selectively with functional groups with high electron density, such as tertiary amines and phenoxides. Ozone has more oxidation power tends to be the most reactive species against pharmaceuticals (Huber et al., 2003).

Generally, WWTPs comprise a primary system of physicochemical treatments and a secondary system that consists of a biological reactor formed by activated sludge. However, they have a limited capacity to remove pharmaceuticals since they are hardly metabolized by microorganisms; therefore they remain in effluents and contaminate surface and groundwater, representing the main source of drinking water. Tertiary water treatments include: biological systems (to remove nitrogen compounds such as ammoniac and ammonium ions), ion exchange, chemical precipitation for phosphorous, distillation for volatile organic compounds, adsorption on activated carbon, and AOPs to remove toxic biorefactory organic compounds, based on the generation of strong oxidants, mainly hydroxyl (*OH) radical, with a very high oxidation power. Many of these systems are under research and have yet to be applied on an industrial scale (Rivera-Utrilla et al., 2013).

2.9.2. Technologies based on AOPs

These processes are all based on the generation of free radicals (OH[•], O2^{•-}, HO2[•]), notably the hydroxyl radical ([•]OH), a very highly reactive species that can successfully attack most organic molecules with high reaction rate constants that range from 10⁷ to 10¹⁰ M⁻¹ s⁻¹. These radicals can be generated by different methods.

Key AOPs include heterogeneous and homogeneous photocatalysis based on near ultraviolet (UV) or solar visible radiation, electrolysis, ozonation, the Fenton's reagent, ultrasound and wet air oxidation, while less conventional but evolving processes include ionizing radiation, microwaves, pulsed plasma and the ferrate agent. Depending on the properties of the waste stream to be treated and the treatment itself, AOPs can be employed alone or coupled with other physicochemical or biological processes. For instance, they may be employed as a pre-treatment stage to convert initially biorecalcitrant compounds to more readily biodegradable intermediates followed by biological post-treatment. Regarding treatment efficiency, AOPs are generally capable of completely destroying the specific pharmaceutical in question but this is not necessarily accompanied by total mineralization. In several cases, degradation by-products are more biodegradable and less toxic than the original substrate, thus implying that a biological posttreatment may be feasible (Klavarioti et al., 2009).



Figure 2.2. Main technologies based on AOPs.

2.9.2.1. Ozonation

Ozone is a strong oxidant that either decomposes in water to form hydroxyl radicals which are stronger oxidizing agents than ozone itself, thus inducing the so-called indirect oxidation or
attacks selectively certain functional groups of organic molecules through an electrophilic mechanism. Moreover, treatment performance is enhanced if ozone is combined with light irradiation or hydrogen peroxide. Ozonation has been traditionally employed in drinking water treatment as well as (in some cases) for wastewater disinfection. Therefore, several studies have been carried out onsite in drinking water plants and WWTPs (Nakada et al., 2007). But special attention is given on WWTPs since pharmaceuticals usually exit secondary treatment unaffected (Klavarioti et al., 2009a; Rivera-Utrilla et al., 2013).

2.9.2.2. Fenton oxidation

The Fenton's reaction occurs in the presence of ferrous ions with hydrogen peroxide to generate hydroxyl radicals. It is considered to be a metal-catalyzed oxidation reaction in which ferrous iron acts as the catalyst (Tekin et al., 2006). The efficiency of the process is closely related to the solution pH, whose optimal values are 2.8-3.0 (however the process can occurs at pH between 2 and 4), concentration of reagents as well as their concentration ratio, $R = [H_2O_2]/[Fe^{2+}]$. Moreover, efficiency may be enhanced in the presence of UV irradiation as more radicals are produced in the so-called homogeneous photocatalysis or photo-Fenton process. Fenton oxidation is capable of mineralizing an important fraction of pollutants, decreasing toxicity and making the effluent more readily amenable to biological post-treatments. The major drawbacks of the Fenton process are: the narrow pH range of operation to avoid precipitation of iron oxyhydroxides, formation of process sludge and involvement of wasting reactions (reaction of *OH on H₂O₂ and Fe²⁺) decreasing treatment efficiency (Boufia-Chergui et al., 2010; Rivera-Utrilla et al., 2013)

2.9.2.3. Heterogeneous photocatalysis

The use of the semiconductor TiO_2 as a catalyst in a heterogeneous photocatalysis is a promising efficient technology. The illumination of an aqueous TiO_2 suspension with radiation with energy equal (or greater) than the band gap energy of the semiconductor to excite an electron from valence band to conduction band generates valence band holes and conduction band electrons. Valence band holes (h⁺) can react with water and hydroxyl ion to generate hydroxyl radicals, while conduction band electrons (e⁻) can react with adsorbed molecular oxygen reducing it to superoxide radical anion which can react with protons forming peroxide anions leading then to the formation of H₂O₂. The parameters affecting the performance of the process are the TiO₂ (catalyst) concentration, radiation wavelength and intensity, the solution pH and the addition (in

some cases) of H_2O_2 as an extra oxidant to promote reactions. Photocatalytic reactions usually obey to Langmuir-Hinshelwood model, which is reduced to pseudo-first or zero order kinetic depending on the operational conditions. Solar photocatalysis has gained considerable attention since it involves the use of a renewable source of energy (Doll and Frimmel, 2004, 2003).

2.9.2.4. UV radiation

Most pharmaceuticals are photoactive, absorbing luminous radiation. Various studies have demonstrated that numerous pharmacologically active compounds can be photodegradaded since they generally contain aromatic rings, heteroatoms and other functional groups allowing absorption. The effectiveness of direct photooxidation is governed by the contaminant absorption spectrum and the quantum yield of the process. However, when H_2O_2 is added during photooxidation, the predominant mechanism derives from the high reactivity of hydroxyl radicals formed by photolysis of H_2O_2 . In addition, irradiation with UV light is a well-established method for water disinfection and is increasingly used to treat pharmaceutical-polluted wastewater (Kang et al., 2004).

2.9.2.5. Gamma radiation

Radiolysis is based on the generation of radicals, highly reactive electrons, ions and neutral molecules through the exposure of water to high energy radiation. Reactive radicals are formed by a complex mechanism, including e_{aq} , H[•], and [•]OH, ions, and stable molecules (O₂, H₂ and H₂O₂), all of these chemical species are primary radiolytic products that subsequently modify and degrade the pollutant molecules. The proven efficiency of this technology to remove persistent pollutants has prompted research interest in its usefulness for the removal of pharmaceuticals (Song et al., 2008).

2.9.3. Electrochemical technologies

Among the AOPs, the application of electrochemical advanced oxidation processes (EAOPs) has increased in recent years. The use of electricity for water treatment was first suggested in 1889 (Chen, 2004) and since then many electrochemical technologies have been devised for the remediation of wastewaters. These processes can be classified in separation technologies, which isolate the xenobiotics from aqueous medium without altering their chemical structure (such as electrocoagulation), and degradation technologies, which involve chemical transformations. The

main advantage of the electrochemical technologies is their environmental compatibility because the main reagent is the electron, a clean reagent. They are versatile techniques showing high efficiency, amenability to automation, easy handing and safety because they operate under mild conditions. The main drawbacks include the cost related to electrical supply, the need of electrolytes and pH regulation, and the loss of activity and shortening of the lifetime of the electrodes by fouling due to the deposition of organic material on their surface (Chen, 2004; Brillas et al., 2009; Panizza and Cerisola, 2009; Sirés et al., 2014).

2.9.3.1 Electrocoagulation

This technique is based to use of a sacrificial (soluble) anode. A current is applied between two electrodes to dissolve Fe (or steel) or Al anodes immersed in a polluted water. The release of the corresponding metal ions yield to different Fe(II) (and/or Fe(III)) or Al(III) hydroxide species depending on the pH medium. These species act as coagulants or destabilizing agents that neutralize charges and separate colloids and ionic products from wastewater by sedimentation, accompanied by sludge production. The coagulated particles can also be separated by electroflotation when they are attached to the bubbles of H2 gas evolved at the cathode, being transported to the solution surface. The insoluble Fe(OH)₂ precipitates at pH>5.5, remaining in equilibrium with Fe²⁺ up to pH 9.5 or with monomeric species such as Fe(OH)⁺, Fe(OH)₂, and Fe(OH)₃⁻ at higher pH values. In the presence of O₂⁻², dissolved Fe²⁺ is oxidized to insoluble Fe(OH)₃. The insoluble Fe(OH)₂ and/or Fe(OH)₃ flocs retain colloids and ionic species and coagulate to form particles that are separated from the wastewater by sedimentation or electroflotation. Similar processes are involved with Al species (Chen, 2004).

 $Fe \rightarrow Fe^{2+} + 2e^{-}$ (1)

$$2H^{+} + 2e^{-} \rightarrow H_{2(g)}$$
⁽²⁾

$$2H_2O + 2e^- \rightarrow 2OH^- + H_{2(g)}$$
(3)

$$4Fe^{2^{+}} + 10H_{2}O + O_{2(g)} \rightarrow 4Fe(OH)_{3(s)} + 8H^{+}$$
(4)

Electrocoagulation has been successfully applied to the remediation of some synthetic and industrial pharmaceutical wastewaters and has also been envisaged as a pre-treatment of pharmaceutical industries wastewaters (Deshpande et al., 2010, 2005).

2.9.3.2. Electrochemical oxidation

The most popular electrochemical technique for wastewater remediation is the electrochemical oxidation, frequently called anodic oxidation (AO). This process involves the oxidation of pollutants in an electrolytic cell by direct electron transfer to the anode and by oxidation with heterogeneous M([•]OH) formed from water discharge at the anode or by mediated oxidation in the bulk with reactive oxygen species (ROS) formed by oxidation of some anions present in the solution such as Cl^{2} or SO_{4}^{2} . The physisorbed hydroxyl radical M([•]OH) is a powerful oxidant and able to involve the electrochemical combustion (transformation to CO₂) of organics present in the solution or oxidation of refractory organics into biodegradable products. High cell voltages are applied to achieve the simultaneous oxidation of pollutants and water, maintaining the anode activity, making this process very depending on the anode material, which have been classified into "active", such as Pt, IrO2 and RuO2, and "non-active", like PbO2, SnO2 and borondoped diamond (BDD), anodes. The process involves water oxidation leading to the formation of M([•]OH) by reaction (5). In the case of "active" anodes, the radicals' interaction with their surface is so strong that that it is transformed into chemisorbed "active oxygen", reaction (6) which mediates the electrochemical conversion of organic compounds (R) by reaction (7). On the other hand, the radicals' interaction with the surface of "non-active" ions is so weak that organics are directly oxidized by [•]OH until total mineralization(Comninellis, 1994).

$M + H_2O \rightarrow M(^{\bullet}OH) + H^+ + e^-$	(5)
--	-----

M(° OH)	\rightarrow MO +	H ⁺ + e ⁻	(6)

 $MO + R \rightarrow M + RO$

During AO process, different ROS apart from $^{\circ}$ OH, are produced, like H₂O₂ (reaction (8)) and O₃ from water discharge at the anode surface by reaction (9).

$2M(^{\bullet}OH) \rightarrow 2MO + H_2O_2$	(8)

$$3H_2O \rightarrow O_3 + 6H^{\dagger} + 6e^{-1}$$
 (9)

The BDD anode is the most potent "non-active" anode and it's considered the most suitable for the incineration of organics by AO (Panizza and Cerisola, 2009). When BDD is used, other weaker oxidizing species are formed from oxidation of the electrolyte ions present in the solution. Peroxodisulfate, peroxodicarbonate and /or peroxodiphosphate (Panizza and Cerisola, 2009). Additionally, when chloride ions are contained in wastewater, direct oxidation of this ion at the anode, yield soluble chlorine by reaction (10), which is rapidly hydrolyzed and then dissociated

(7)

(depending to solution pH) to hypochlorous acid and chloride ion (reaction (11)). Hypochlorous acid is weak acid with the equilibrium constant pKa=7.55.

$2Cl^{-} \rightarrow Cl_2(aq) + 2e^{-}$	(10)
$Cl_2(aq) + H_2O \leftrightarrow HCIO + Cl^- + H^+$	(11)
$HCIO \leftrightarrow CIO^- + H^+$	(12)

This species can attack the organics in competition with other ROS, and the formation and accumulation of toxic chloroderivates, such as trihalomethanes and chloramines is, a major drawback of the electro-oxidation in the presence of active chlorine (Bergmann et al., 2009; Martinez-Huitle and Brillas, 2009). The degradation rate and current efficiency of these processes is a function of experimental parameters such as pH, temperature, stirring rate, substrate concentration and current density, and their effects need to be studies to achieve the best operative conditions.

2.9.3.3. Electrochemical technologies based on the Fenton's reaction

This technology is based on the action of the heterogeneous [•]OH generated in the bulk solution, then minimizing the diffusional limitations in the AO systems. A general scheme of the chemistry of Fenton's reaction is presented in several review papers (Brillas et al., 2009; Pignatello et al., 2006), can be regarded in different stages, including initiation, catalysis, propagation and inhibition, according to reactions (13-28).

Initiation:

$$Fe^{2^+} + H_2O_2 + H^+ \rightarrow Fe^{3^+} + H_2O + {}^{\bullet}OH \qquad k_2 = 70 M^{-1} s^{-1}$$
 (13)

Catalysis

 $Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HO_2^{\bullet} + H^+$ $k = 3.1x10^{-3} M^{-1} s^{-1}$ (14)

$$Fe^{3+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^+ \qquad k_2 = 2x10^3 \text{ M}^{-1} \text{ s}^{-1} \qquad (15)$$

$$Fe^{3+} O_2^{\bullet-} \rightarrow Fe^{2+} + O_2 \qquad k_2 = 5x10^7 \text{ M}^{-1} \text{ s}^{-1} \qquad (16)$$

Propagation

$$HO_2^{\bullet} \leftrightarrow H^+ + O_2^{\bullet}$$
 $k_2 = 1.6 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ (18)

ArH +
$${}^{\bullet}OH \rightarrow ArHOH^{\bullet}$$
 k₂ = 10⁸-10¹⁰ M⁻¹ s⁻¹ (20)

Inhibition

$$Fe^{2^+} + {}^{\bullet}OH \rightarrow Fe^{3^+} + OH^ k_2 = 3.2 \times 10^8 M^{-1} s^{-1}$$
 (21)

$\mathrm{Fe}^{2^+} + \mathrm{HO_2}^{\bullet} \rightarrow \mathrm{Fe}^{3^+} + \mathrm{H_2O_2}$	$k_2 = 1.8 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$	(22)
$Fe^{2+} + O2^{\bullet-} + 2H^+ \rightarrow Fe^{3+} + H_2O$	$k_2 = 1.0 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$	(23)
$O2^{\bullet-} + HO2^{\bullet} + H^+ \rightarrow H_2O_2 + O_2$	$k_2 = 9.7 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$	(24)
$HO_2^{\bullet} + HO_2^{\bullet} \rightarrow H_2O_2 + O_2$	$k_2 = 8.3 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$	(25)
$HO_2^{\bullet} + {}^{\bullet}OH \rightarrow H_2O + O_2$	$k_2 = 7.1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$	(26)
$O_2^{\bullet} + {}^{\bullet}OH \rightarrow OH^{-} + O_2$	$k_2 = 1.9 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$	(27)
$^{\bullet}OH + ^{\bullet}OH \rightarrow OH^{-} + O_{2}$	$k_2 = 6.0 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$	(28)

2.9.3.3.1. Electro-Fenton Process

The oxidation of organics by Fenton reaction is a simply technology, but this process has a limited oxidation power because of a number of wasting reactions involved during treatment: The most important of these reactions are reactions (17) and (21) that consume both the reagents and formed [•]OHs. The necessity to use high quantity of chemicals and formation of process sludge are other inconvenient of this process. Electro-Fenton process is developed in order to avoid these inconvenient of the Fenton's reagent (Brillas et al., 2009; Oturan and Aaron 2014).

The accepted mechanism for the EF process involves the generation of [•]OH through the classical Fenton's reaction (13) using the Fenton's reagent (mixture of H_2O_2 and Fe^{2+}) in the bulk solution. In this case, this reagent is in situ electrochemically generated. The process occurs at an optimal pH range of 2.8-3.0 according to the catalytic behavior of the Fe^{3+}/Fe^{2+} couple. EF process allows the continuous electrogeneration and/or regeneration of the Fenton's reagent, thus increasing its efficacy and enhancing its environmental compatibility. In this context, H_2O_2 is continuously generated at a suitable cathode fed with pure O2 or air, reaction (29), along with the addition of a catalytic amount of ferrous iron yielding to the formation of heterogeneous [•]OH via Fenton's reaction (Oturan, 2000; Brillas et al., 2009; Sirés et al., 2014). The production of [•]OH is accelerated by the cathodic reduction of Fe^{3+} from reaction (30) that catalyzes the Fenton's reaction.

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$$
(29)
$$Fe^{3+} + e^- \rightarrow Fe^{2+}$$
(30)

The electro-Fenton technology can use two or three electrodes in divided or undivided cells; with cathode materials like graphite, carbon felt and gas diffusion electrodes (GDEs) (Sirés et

al., 2007a; Sirés et al., 2007b; Özcan et al., 2008; Panizza and Oturan, 2011; Dirany et al., 2012; Garcia-Segura et al., 2014) and anode materials including graphite, Pt, metal oxides, mixed metal oxides and BDD (Brillas et al., 2009). When an undivided cell is used, simultaneous destruction of pollutants takes place due to homogeneous or heterogeneous hydroxyl radicals and the generated ROS (non-active anodes) as a result of electrochemical oxidation. The efficiency of the process depends on the operational parameters solution pH, applied potential or current, catalyst nature and concentration, O₂ feeding, stirring rate electrolyte composition, and pollutant contents. This parameters are generally optimized for achieving the best current efficiency and lowest energy cost.

High flow rates of pure O₂ or air are normally used to maintain the solution saturated with O₂ for the greatest production of H2O2 according to reaction (29). High values of solution stirring rate or liquid flow rate is used for ensuring a fast homogenization and the enhancement of mass transfer of the reactants towards the electrode. Room temperature is generally employed as higher temperatures are detrimental because the considerable acceleration of H₂O₂ decomposition. The solution pH is a very important parameter to be taken into account. Several authors have reported maximum efficiencies at pH 3.0, which is close to pH 2.8, the optimal value where the maximum production of [•]OH is expected from Fenton' reaction. This optimum value is due mainly to effects on Fe(III) speciation (Pignatello et al., 2006). Other important operational parameters are the applied potential (Ecat) and current, which are related to the H₂O₂ production. Ecat depends on the used cathode material and it has been observed that a larger cathode area favors both H_2O_2 production and Fe^{2+} regeneration. It has been found that COD and TOC decays are significantly enhanced with increasing applied current up to a certain optimal value. The required catalyst concentration (Fe^{2+}/Fe^{3+}) is a function of the cathode utilized because of their different abilities for Fe²⁺ regeneration. On the other hand, increasing the initial pollutant concentration always represents a decrease in the kinetic and mineralization decays, which is due to the greater amount of matter that needs to be oxidized. In general, lower currents and greater organic contents lead to higher current efficiency and smaller energy consumption, however longer times are required for getting acceptable efficiencies (Brillas et al., 2009; Sirés et al., 2014).

Different woks have shown the efficiency of electro-Fenton process for the treatment of water polluted with pharmaceutical residues (Dirany et al., 2010; Sirés et al., 2010; Isarain-Chávez et al., 2011).



Figure 2.3. Generation of [•]OH during EF process using a BDD anode.

2.9.3.3.2 Photoelectrocatalysis

TiO₂ is the most spread catalyst used for photocatalysis. This process involves the irradiation of anatase TiO₂ (nano) particles in colloidal suspension by UV protons of λ <380 nm to promote an electron from the valence band to the conduction band (e⁻_{CB}) with a gap band of 3.2 eV, thus generating a positively charged vacancy or hole (h⁺_{VB}) from reaction (31). Organics are then oxidized from the photogerenated positive hole from reaction (32). In the same way, some other ROS can be produced from the photoinjected electron, reactions (33 to 36) (Boroski et al., 2009). However, the major loss in efficiency is due to the combination of the electrons promoted to the valence band, either by the unreacted holes by reaction (37) or by absorbed hydroxyl radical, reaction (38).

 $TiO_2 + h\nu \rightarrow e_{CB}^- + h_{VB}^+$ (31)

$$h^+_{VB} + H_2O \rightarrow {}^{\bullet}OH + H^+$$
 (32)

$$e_{CB}^{-} + O_2 \rightarrow O_2^{\bullet}$$
(33)

$$O_2^{\bullet-} + H^+ \rightarrow HO_2^{\bullet-}$$
(34)

$$2 \operatorname{HO}_2^{\bullet} \xrightarrow{} \operatorname{H}_2\operatorname{O}_2 + \operatorname{O}_2 \tag{35}$$

$$H_2O_2 + O_2^{\bullet} \rightarrow \bullet OH + OH^{\bullet} + O_2$$
(36)

 $e_{CB}^{-} + h_{VB}^{+} \rightarrow TiO_2 + heat$ (37)

$$e^{-}_{CB} + {}^{\bullet}OH \rightarrow OH^{-}$$
 (38)

The electrochemical application of photocatalysis, the photoelectrocatalysis (PEC), consists in the application of a constant anodic potential (E_{anode}) usually to a TiO₂-based thin film anode subjected to UV illumination. The photoinduced electrons are continuously extracted from the anode by an external electrical circuit to be injected into the cathode, thus producing a higher amount of holes from reaction (31) and [•]OH from reaction (5), inhibiting the reactions (33)-(38) and thus enhancing the efficiency of the process (Brillas et al., 2009). Some studies on the viability of PEC for pharmaceutical removal have been reported (Liu et al., 2009a, 2009b).

These electrochemical technologies have shown very good efficiencies in the destruction of pharmaceutical residues, being electro-Fenton with BDD anode the one showing the best results. In this contest, the synergetic action of BDD([•]OH) to mineralize organic pollutants and reduce the toxicity of the treated solutions, has also been confirmed (Dirany et al., 2011; Escher et al., 2006; Oturan et al., 2012; Randazzo et al., 2011). The main challenges for the upcoming application of these promising processes are the reduction of electrodes prices (particularly BDD) and the enhancement of their sustainability by the use of renewable energy sources.

2.9.4. The coupling of wastewater treatment technologies

The undeniable and well documented presence of persistent organic pollutants in the environment, including pharmaceutical residues, represents a major environmental problem that requires the development and application of effective technologies for their removal from water sources.

It is well known that conventional processes for wastewater treatment show a poor performance for the destruction of these persistent pollutants. Among the conventional processes, biological technologies are the most suitable owing to their low cost, good efficiencies and easy handling of operational conditions. Nonetheless they are not capable of degrading organics because in most cases they are highly recalcitrant and toxic to microorganisms, consequently, not been metabolized. Facing this problem, the application of more powerful chemical technologies, such as the AOPs was the only feasible option (except the nanofiltration or inverse osmosis) for the destruction of these contaminants. However, chemical oxidation for complete mineralization is generally expensive because of the generation of reaction intermediates, which are resistant to oxidation and thus, they consume more energy (radiation, ozone, electricity, etc.), chemical reagents (catalyst and oxidants) and treatment time (Muñoz and Guieysse, 2006). In this context, in the last years the combination between different wastewater

treatment technologies has become a center of interest within the scientific community. These combinations result in a considerable enhancement of the efficiency of pollutants degradation. Several works dealing with the coupling of different processes have been reported. They include numerous combinations between physicochemical technologies (Bes-Piá et al., 2002; Bhattacharya et al., 2013), AOPs (Prieto-Rodríguez et al., 2013; Sable et al., 2014; Quiñones et al., 2015), physico-chemical/AOPs (Kestioğlu et al., 2005; Zhou and He, 2007), Biological/AOPs (Cassano et al., 2011; Oller et al., 2011; Moreira et al., 2012; Bustillo-Lecompte et al., 2014), and among AOPs, coupling of EAOPs (Martínez-Huitle and Brillas, 2009; Sirés and Brillas, 2012; Feng et al., 2013; Sirés et al., 2014), and Biological/EAOPs (Mullot et al., 2009; (Fontmorin et al., 2014; Ganzenko et al., 2014). Among these coupled technologies, the most attractive and potential alternative is the application of a an AOP pre-treatment aiming to convert the initial persistent organic compounds into more biodegradable intermediates which could be then treated in a biological oxidation process with a significant low cost (Pulgarin et al., 1999; Sarria et al., 2003; Tabrizi and Mehrvar, 2004; Oller et al., 2011). When coupling pre-treatment AOPs/Biological systems, the percentage of mineralization should be minimal during the first stage in in order to avoid unnecessary expenditure of chemicals and energy.

Within the AOPs, electrochemical technologies possess various advantages as environmental compatible processes. They are versatile, high energy efficient, amiable to automation; they require simple equipment and operate under mild conditions (Anglada et al., 2009). They consequently are, as a pre-treatment followed by a biological process, very promising technologies for the degradation of persistent compounds.

2.10. Biotransformations

As it has been mentioned in the previous sections, the presence of pharmaceutical residues and their transformation products has been well documented. It has been established that they represent an environmental risk and toxicity for living organisms and the human being. They are generally found as complex mixtures that possess synergetic effects on toxicological risk. A better understanding on the chronic effects of pharmaceuticals metabolites is necessary for the improvement of risk assessment aiming to predict by-products and metabolites from pharmaceuticals transformations, and their ecotoxicities. Pharmaceutical transformation products can be formed from natural transformation of parent compounds in the environment, from the introduction of metabolized drugs inside the human body, or from the application of chemical (AOPs or EAOPs) or biological processes (biotic metabolites) during wastewater treatment. The identification and toxicity evaluation of these biotic and abiotic transformation products is thus necessary for risk assessment and accurate prediction of potential ecotoxicity.

Drugs and xenobiotic compounds going through the organism undergo metabolism by a series of enzymatic biotransformation changes. They suffer structural modification by enzymatic systems which lead to formation of relatively polar substances then easily excreted from the body. Drug metabolisms studies are traditionally conducted in vivo, using of model systems (usually whole animals systems) to produce the expected human metabolites of drugs. However, the use of microbial model has demonstrated a number of advantages, mainly the easiness and facility of working with microorganisms' cultures (Smith and Rosazza, 1975; Asha and Vidyavathi, 2009). The use of microbiganisms for simulating the mammalian metabolism of many molecules of pharmacological importance is well documented, and synthesis by using microbial models offers a few advantages compared to chemical synthesis, because it can be highly enantiomeric and region-selective under mild conditions (Azerad, 1999; Yoshida et al., 2001). The common way of metabolizing drugs involves the alteration of functional groups on the parent molecule via the cytochrome *P450* enzymes. These enzymes are most predominant in the liver but can also be found in the intestines, lungs and other organs. They require NADPH as a coenzyme and oxygen is used as a substrate (Vella, 1995).

Bioconversions are now considered by chemists as competitive and ecologically effective approach in organic synthesis since microorganisms are a major source of enzymes (Wohlgemuth, 2010). Enzymes present in many microbial strains allow access to a wide range of transformation products. This approach requires the selection of active microorganisms and is especially used in the preparation of mammalian metabolites (Azerad, 1999; Asha and Vidyavathi, 2009; Marvalin and Azerad, 2011).



Figure 2.4. Bioconversions schematization.

The hydrolytic and reductive capabilities of microorganisms especially fungi have been for longtime used in preparative reactions. Among fungi, *Cunninghamella* species have the ability to metabolize a wide variety of xenobiotic in region- and stereo-selective manners that are similar to those in mammalian enzyme systems (Jobling et al., 1998). *Cunninghamella* is a filamentous fungus found in soil and plant material, particularly at Mediterranean and subtropical zones. They possess cytochrome P450 monooxygenase systems analogous to those in mammals and phase II drug metabolism enzymes. This fungi species are able to metabolize a wide variety of xenobiotics using both, phase I (oxidative) and phase II (conjugative) biotransformation mechanisms, thus they have the ability to mimic mammalian metabolism and to perform novel biotransformations (Zhang et al., 1996; Sun et al., 2004; Asha and Vidyavathi, 2009).

Biotransformations can hence be a very useful tool for the preparation of drugs' transformation metabolites, aiming to strengthen and support predictive modeling and analysis on ecotoxicity and risk assessment.

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CHAPTER 3

Electrochemical Advanced Oxidation for Cold Incineration of the Pharmaceutical Ranitidine: Mineralization Pathway and Toxicity Evolution

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Chapter 3

Electrochemical Advanced Oxidation for Cold Incineration of the Pharmaceutical Ranitidine: Mineralization Pathway and Toxicity Evolution

Abstract

Ranitidine (RNTD) is a widely prescribed histamine H₂-receptor antagonist whose unambiguous presence in water sources appointed it as an emerging pollutant. Here, the degradation of 0.1 mM of this drug in aqueous medium was studied by electrochemical advanced oxidation processes (EAOPs) like anodic oxidation with electrogenerated H₂O₂ and electro-Fenton using Pt/carbon-felt, BDD/carbon-felt and DSA-Ti/RuO₂-IrO₂/carbon-felt cells. The higher oxidation power of the electro-Fenton process using a BDD anode was demonstrated. The oxidative degradation of RNTD by the electrochemically generated [•]OH radicals obeyed a pseudo-first order kinetics. The absolute rate constant for its hydroxylation reaction was 3.39×10^9 M⁻¹ s⁻¹ as determined by the competition kinetics method. Almost complete mineralization of the RNTN solution was reached by using a BDD anode in both anodic oxidation with electrogenerated H₂O₂ and electro-Fenton processes. Up to 11 cyclic intermediates with furan moiety were detected from the degradation of RNTD, which were afterwards oxidized to short-chain carboxylic acids before their mineralization to CO_2 and inorganic ions such as NH_4^+ , NO_3^- and SO_4^{2-} . Based on identified products, a plausible reaction pathway was proposed for RNTD mineralization. Toxicity assessment by the Microtox[®] method revealed that some cyclic intermediates are more toxic than the parent molecule. Toxicity was quickly removed following the almost total mineralization of the treated solution. Overall results confirm the effectiveness of EAOPs for the efficient removal of RNTD and its oxidation by-products from water.

Keywords: Anodic oxidation; BDD anode; Electro-Fenton, Hydroxyl radical; Ranitidine

3.1. Introduction

Over the last years, pharmaceuticals have been receiving increasing attention as potential bioactive chemicals in the environment (Kümmerer, 2009). They are considered as emerging pollutants because they still remain unregulated or are currently undergoing a regularization process (Esplugas et al., 2007). Their presence in aquatic systems is attributable to pharmaceutical industry wastes, hospital wastes, therapeutic drugs and personal care products. They enter into natural waters due to their incomplete elimination in wastewater treatment plants since they are recalcitrant to conventional processes like biodegradation, coagulation, sorption and photodegradation (Jones et al., 2005). The presence of drugs and their metabolites affect the quality of water and constitute a potential risk of toxicity for ecosystems and living beings (Klavarioti et al., 2009). Some drugs have been classified as endocrine disrupting compounds and it is usually accepted that some of them cause long-term, irreversible changes to micro-organisms genome even at low content, then having more resistance to them. (Fent et al., 2006). Moreover, these pollutants often occur as complex mixtures whose toxicity has been seldom predicted (Sirés and Brillas, 2012).

Ranitidine (RNTD) is a H2-receptor antagonist, very widely prescribed for the treatment of peptic ulcer and gastroesophageal reflux disease. By 1988 it was the largest selling prescription drug (Khetan and Collins, 2007). It is oxidized in liver (30-70%) to *N*-oxide, *S*-oxide and *N*-demethylated metabolites (Martin et al., 1981). It has been detected in European and US surface and wastewaters (Fent et al., 2006; Gros et al., 2007) and sediments (Zuccato et al., 2000). RNTD has been classified as one of the highest risk compounds by a model dealing with the effects of contaminants on human Health (Besse and Garric, 2008).

The development of clean and effective technologies for removing organic pollutants and particularly pharmaceuticals from water has been a major concern of researchers during the last decades. Among these technologies, advanced oxidation processes (AOPs) are known as effective treatment techniques for removing toxic and/or persistent organic pollutants from water (Pignatello et al., 2006; Oturan and Aaron, 2014). Several electrochemical AOPs (EAOPs) are being currently developed for water remediation because of their high oxidation/mineralization efficiency to remove organic pollutants (Brillas et al., 2009; Sirés and Brillas, 2012; Vasudevan and Oturan, 2014; Sirés et al., 2014). EAOPs are based on the in-situ electrochemical generation of hydroxyl radicals (*OH) which can non-selectively mineralize organics up to CO₂, water and inorganic ions. The characteristics of EAOPs like anodic oxidation (AO) and electro-Fenton (EF)

have been thoroughly reviewed (Brillas et al., 2009; Panizza and Cerisola, 2009; Feng et al., 2013). In AO, heterogeneous M([•]OH) radical is formed at the surface of a high O₂-overpotential anode by water oxidation from reaction (1) (Rodrigo et al., 2010; Brillas and Martínez-Huitle, 2011):

$$M + H_2O \rightarrow M(^{\bullet}OH) + H^+ + e^-$$

(1)

In the case of EF, [•]OH radical is produced in the bulk through Fenton's reaction (2) in which H_2O_2 is electrogenerated at a suitable cathode fed with O_2 or air by reaction (3) while a catalytic amount of Fe²⁺ ion (about 0.1-0.5 mM) is added. This ion can be cathodically regenerated from reaction (4) (Oturan et al., 1992; Oturan, 2000; Brillas et al., 2009; Özcan et al., 2009).

$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + ^OH$	(2)
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	(3)
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	(4)

The EF process has the advantage of producing oxidation reactions in the solution, whereas in AO the reactions are limited to the anode surface. However, AO with a boron-doped diamond (BDD) anode produces powerful heterogeneous BDD([•]OH). The use of a BDD anode in EF enhances strongly its oxidation/mineralization power owing to the formation of both, BDD([•]OH) and [•]OH at the anode surface by reaction (1) and in the bulk by Fenton's reaction (2), respectively (Oturan et al., 2012).

Several works have reported the destruction of different drugs by AO and EF using either a BDD or Pt anode with different carbonaceous cathodes (Sirés et al., 2007a, 2007b; Özcan et al., 2008; Panizza and Oturan, 2011; Dirany et al., 2012; Garcia-Segura et al., 2014). In contrast, very few studies have utilized dimensionally stable anodes (DSA) (Ihos et al., 2013; Oturan et al., 2013) because although these electrodes possess high surface area and excellent mechanical and chemical resistance at high current and in strongly acidic media, they have low ability for M([•]OH) generation (Martínez-Huitle and Brillas, 2009).

The degradation of RNTD in aqueous media has been described by direct photolysis (Latch et al., 2003), heterogeneous photocatalysis (Addamo et al., 2005) and ozonation (Rivas et al., 2009). Recently, Radjenović et al. (2010) reported its complete disappearance by photo-Fenton and solar photocatalysis, but only with 55% of total organic carbon (TOC) removal. However, these works have not reported any mechanistic study because the oxidation products of RNTD from [•]OH attack have not been yet identified.

This work presents a detailed study on the degradation of RNTD by AO with electrogenerated H_2O_2 (AO- H_2O_2) and EF processes using different anodes like Pt, DSA and BDD.

The effect of current on drug removal, mineralization rate and mineralization current efficiency (MCE) induced by [•]OH attack, as well as a toxicity assessment, was comparatively examined. Cyclic intermediates were identified by ESI-TOF-MS, aliphatic acids were quantified by HPLC and released inorganic ions were followed by ion chromatography. Based on detected products, a plausible mineralization pathway for RNTD is proposed.

3.2. Experimental

3.2.1. Chemicals

Ranitidine hydrochloride (purity > 98%) was of reagent grade from Fluka. Anhydride sodium sulfate used as background electrolyte and heptahydrated iron (II) sulfate used as catalyst in EF were of analytical grade from Acros Organics. All solutions were prepared with ultrapure water from a Millipore Milli–Q system with resistivity >18 M Ω cm. Reagent grade sulfuric acid from Merck was used to adjust their initial pH to 3.0. All the other chemicals used were either of HPLC or analytical grade from Prolabo, Fluka and Acros Organics.

3.2.2. Electrochemical treatments

All electrolysis were carried out in an undivided cylindrical cell containing 230 mL solutions under vigorous stirring with a magnetic bar. The anode was a cylindrical Pt mesh of 4.5 cm height and 3 cm internal diameter (EF-Pt process), a 25 cm² thin-film BDD onto a Nb substrate from Condias Gmbh (EF-BDD process) or a 25 cm² DSA-Ti/RuO₂-IrO₂ (EF-DSA process). The cathode was a carbon felt of 15 cm × 4 cm × 0.5 cm in dimension from Carbon-Lorraine. In all cases, the anode was centered in the cell and was surrounded by the cathode, which covered the inner wall of the cell. The solution was continuously saturated by O₂ at atmospheric pressure by bubbling compressed air at 1 L min⁻¹ before 10 min of starting the electrolysis. The EF treatments of 0.1 mM RNTD solutions were assessed in 0.05 M Na₂SO₄ with 0.1 mM Fe²⁺ as catalyst at pH 3.0, room temperature and constant current between 100 and 500 mA provided by a Hameg HM8040 triple power supply. Comparative AO trials were made using a BDD/carbon-felt cell with H₂O₂ generation (AO-H₂O₂-BDD process) but without Fe²⁺ addition.

3.2.3. Instruments and analytical procedures

The solution pH was measured with a CyberScan pH 1500 pH-meter. The mineralization of RNTD solutions was assessed from their TOC decay determined on a Shimadzu VCSH TOC analyzer. The time-course of RNTD concentration was followed by reversed-phase HPLC using a Merck Lachrom LC fitted with a RP-18 (5 μ m, 250 mm × 4.6 mm) column at 40 °C and coupled with a L-7455 UV-Vis detector selected at λ = 280 nm. These analyses were carried out with a 28:72 (v/v) methanol/water (10 mM ammonium acetate) mixture at 0.7 mL min⁻¹ as mobile phase. Generated aliphatic acids were followed by ion-exclusion HPLC using an Altech LC fitted with a Supelco, Supelcogel H (9 μ m, 25 cm × 4.6 mm) column at room temperature and coupled with a Dionex AD20 UV detector set at λ = 210 nm, using 1% H₂SO₄ at 0.2 mL min⁻¹ as mobile phase. The released inorganic ions were detected by ion chromatography using a Dionex ICS-1000 Basic Ion Chromatography System coupled with a Dionex DS6 conductimetric detector containing a cell at 35 °C. The NH₄⁺ content was determined with a Dionex CS12A (25 cm × 4 mm) cationic column and a mobile phase of 9 mM H₂SO₄ at 1.0 mL min⁻¹. The NO₃⁻ and SO₄²⁻ contents were obtained with a Dionex AS4A-SC (25 cm × 4 mm) anion column using a 1.8 mM Na₂CO₃ + 1.7 mM NaHCO₃ solution at 2.0 mL min⁻¹ as mobile phase.

Cyclic organic products formed during the EF-BDD were identified by electrospray time of flight mass spectrometer (ESI-TOF-MS) using an Applied Biosystems QSTAR Pulsar I operating in positive mode.

Toxicity measurements were performed with the Microtox[®] method based on the inhibition of bioluminescence of the marine bacteria *Vibrio fischeri*. A luminometer Berthold Autolumat Plus LB 953 was used following the international procedure OIN 11348-3. The bacteria and LCK 487 LUMISTOX as activation reagent were from Hach Lange France SAS. The electrolytic trials were made at 500 and 1000 mA and the bioluminescence intensity was measured after 5 min of exposition to the samples at 15 °C.

3.3. Results and Discussion

3.3.1. Effect of current on the decay kinetics of RNTD

The applied current is the most important parameter in EAOPs since it controls the formation of hydroxyl radicals through reactions (1)-(4). To clarify its effect on RNTD decay, 230 mL of 0.1 mM drug solutions with 0.05 M Na₂SO₄ were treated by EF-Pt, EF-BDD and EF-DSA with

0.1 mM Fe²⁺ and by AO-H₂O₂-BDD operating between 100 and 500 mA. Fig. 3.1 shows that RNTD was completely removed in all cases and at given current, it disappeared completely at increasing times in the sequence: EF-BDD \leq EF-Pt < EF-DSA << AO-H₂O₂-BDD. In the latter process, RNTD was only destroyed by M(•OH) generated at the anode surface from reaction (1), whereas in EF, additional homogeneous [•]OH was simultaneously produced in the solution bulk from Fenton's reaction (2) between electrogenerated H₂O₂ and electrochemically regenerated Fe²⁺, strongly accelerating the oxidation kinetics of RNTD. Therefore, the EF-BDD treatment is much powerful than AO-H₂O₂-BDD because of the greater production of hydroxyl radicals, leading to a fast drug oxidation by the simultaneous action of these radicals at the BDD surface and primordially in the bulk. Concerning the anodes used in EF, Fig. 3.1 clearly demonstrates that BDD exhibited better performance than Pt and DSA, as expected by its higher oxidation ability (Martínez-Huitle and Brillas, 2009; Oturan et al., 2012). This behavior can be attributed to the higher O₂-overpotential of BDD that promotes greater amounts of reactive BDD([•]OH) because of its weak physisorption on the anode surface.



Figure 3.1. Effect of current on the time-course of ranitidine concentration during different treatments of 230 mL of 0.1 mM drug solutions in 0.05 M Na₂SO₄ at pH 3.0 and room temperature using: (a) Pt/carbon-felt, (b, d) BDD/carbon-felt and (c) DSA-Ti/RuO₂-IrO₂/carbon-felt cells. In cells (a)-(c), electro-Fenton (EF) process with 0.1 mM Fe²⁺. In cell (d), anodic oxidation with electrogenerated H₂O₂ (AO-H₂O₂-BDD), without Fe²⁺ addition. Applied current: (\blacklozenge) 100 mA, (\blacksquare) 200 mA, (\blacktriangle) 300 mA, (\blacklozenge) 400 mA and (\bigstar) 500 mA.

Fig. 3.1 also shows that shorter time was needed for the total disappearance of RNTD at higher current in all treatments (see Table 1), as expected from the increasing rate of reactions (1)-(4) producing greater quantities of M([•]OH) and [•]OH. However, similar concentration-time plots were obtained for high currents in all cases, indicating a little positive effect on RNTD kinetics with rising applied current. This phenomenon can be explained by the progressive enhancement of parasitic reactions like H_2O_2 reduction and H_2 evolution at the cathode, along with self-destruction of M([•]OH) at the anode (Oturan et al., 2011; Sirés et al., 2014), evidencing that current oversupply gives a current waste, lowering the treatment efficiency. This behavior can also be deduced from the relative small rise in apparent rate constant (k_{app}) with increasing current from 100 to 500 mA, collected in Table 3.1 for the oxidation of RNTD by hydroxyl radicals assuming a pseudo-first-order reaction kinetics. Note that [•]OH is a very reactive species, which cannot be accumulated in the medium because of its high destruction rate and very short lifetime, and the quasi-stationary state approximation can then be applied to its concentration. The excellent linear correlations thus obtained, as shown in the inset panels of Figs. 3.1a and 3.1b, confirm that a constant [•]OH concentration reacted with RNTD at each current.

Table 3.1. Apparent rate constant and degradation time for the complete disappearance of 0.1 mM RNTD by the applied EAOPs at different currents. A pseudo-first order kinetics for the drug oxidation by hydroxyl radicals was assumed.

	k _{app} (min ⁻¹) (Degradation time (min))				
Process	100 mA	200 mA	300 mA	400 mA	500 mA
EF-Pt	0.57(10)	0.66 (10)	0.72 (7)	0.84 (7)	0.98 (5)
EF-BDD	0.54(10)	0.60 (10)	0.71 (7)	0.91(5)	1.00 (5)
EF-DSA	0.37(12)	0.45 (12)	0.58 (10)	0.81 (7)	0.82 (7)
AO-H ₂ O ₂ -BDD	0.07(60)	0.11 (45)	0.38 (20)	0.52 (15)	0.72 (10)

The above kinetic model allowed determining the absolute (second order) rate constant (k_{RNTD}) of the reaction between RNTD and [•]OH, not previously reported in the literature. To do this, the competition kinetic method was applied selecting *p*-hydroxybenzonic acid (*p*-HBA) as standard competitor with absolute rate constant $k_{p-HBA} = 2.19 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (Hanna et al., 2005) and
using an equal 0.1 mM concentration for both, RNTD and p-HBA under the operating conditions of Fig. 1a at 100 mA. The absolute rate constant was calculated from Eq. (5):

$$k_{RNTD} = k_{p-HBA} \frac{[k_{app,RNTD}]}{[k_{app,p-HBA}]}$$
(5)

and $k_{\text{RNTD}} = 3.39 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ was found. This value is of the same magnitude order of the absolute rate constant reported for the analogous oxidation of other pharmaceuticals (Brillas et al., 2009; Dirany et al., 2012).

3.3.2. Effect of current on the mineralization process

Figs. 3.2a and 3.2b highlight a rapid TOC decay of the 0.1 mM RNTD solution by EF-BDD and AO-H₂O₂-BDD, respectively, attaining an almost total mineralization in 8 h. The mineralization rate was enhanced with increasing current, confirming the production of oxidant hydroxyl radicals during long time without BDD fouling. This behavior can then be explained by the greater amount of BDD([•]OH) formed from reaction (1) and/or [•]OH generated from reaction (2), which oxidize more quickly both, RNTD and its oxidation intermediates.

Fig. 3.2c shows the comparative percentage of TOC removal after 4 h of electrolysis of 230 mL of 0.1 mM RNTD solutions in 0.05 M Na₂SO₄ at pH 3.0 and room temperature by the different treatments at 500 mA. The mineralization of the drug solution reached ca. 94% TOC abatement for EF-BDD and AO-H₂O₂-BDD, whereas TOC was reduced by 89.47% for EF-Pt and only 80.38% for EF-DSA. The very slow change in TOC after this time suggests the formation of very refractory intermediates, mainly carboxylic acids. This behavior can be observed on the inset panels of Figs. 3.2a and 3.2b from the dramatic drop in MCE with prolonging electrolysis time at each current. This parameter was calculated as follows (Brillas et al., 2009):

$$MCE(\%) = \frac{n F V_s \,\Delta(TOC)_{exp}}{4.32 x 10^7 \, m \, l \, t} \, 100 \tag{6}$$

where *F* is the Faraday constant (96,487 C mol⁻¹), *V*_s is the solution volume (L), $\Delta(TOC)_{exp}$ is the experimental TOC decay (mg L⁻¹), 4.32 x 10⁷ is a conversion factor (3,600 s h⁻¹ x 12,000 mg mol⁻¹), *m* is the number of carbon atoms in RNTD molecule (13), *I* is the applied current (A) and *t* is the time (h). The number *n* of electrons consumed per RNTD molecule during mineralization was taken as 58 from reaction (7), assuming the release of NH₄⁺ and SO₄²⁻ as major ions, as will be discussed below.

$$C_{13}H_{22}N_4O_3S + 25H_2O \rightarrow 13CO_2 + 4NH_4^+ + SO_4^{2-} + 60H^+ + 62e^-$$
(7)



Figure 3.2. Influence of current on the percentage of normalized TOC removal vs. electrolysis time for the treatment of 230 mL of 0.1 mM RNTD solutions in 0.05 M Na₂SO₄ at pH 3.0 and room temperature by (a) EF-BDD with 0.1 mM Fe²⁺ and (b) AO-H₂O₂-BDD using a BDD/carbon-felt cell at: (\blacklozenge) 100 mA, (\blacksquare) 200 mA, (\blacktriangle) 300 mA, (\blacklozenge) 400 mA and (\bigstar) 500 mA. The inset panels show the corresponding mineralization current efficiency (c) Percentage of TOC removal for different EAOPs after 4 h of electrolysis.

The inset panels of Figs. 3.2a and 3.2b show a drop in efficiency with rising current, an opposite trend to that of TOC removal. This decay can be related to a gradual loss in the relative quantity of BDD($^{\circ}$ OH) and $^{\circ}$ OH by parallel non-oxidizing reactions, including the oxidation of BDD($^{\circ}$ OH) to O₂ by reaction (8), the dimerization of $^{\circ}$ OH by reaction (9) and its reaction with Fe²⁺ and H₂O₂ by reactions (10) and (11), respectively (Brillas et al., 2009). The quicker generation of other weaker oxidants at the BDD anode, like peroxodisulfate (S₂O₈²⁻) ion from reaction (12) and

ozone from reaction (13) (Panizza and Cerisola, 2009), also contributes to the fall of generated BDD([•]OH) and hence, of TOC removal.

$2BDD(^{\bullet}OH) \rightarrow 2BDD + O_2 + 2H^+ + 2e^-$	(8)
$2 ^{\circ}\text{OH} \rightarrow \text{H}_2\text{O}_2$	(9)
$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^{-}$	(10)
$H_2O_2 + {}^{\bullet}OH \rightarrow HO_2 {}^{\bullet} + H_2O$	(11)
$2 \operatorname{SO_4}^{2-} \rightarrow \operatorname{S_2O_8}^{2-} + 2e^-$	(12)
$3 \text{ H}_2\text{O} \rightarrow \text{O}_3 + 6 \text{ H}^+ + 6\text{e}^-$	(13)

Results of Fig. 3.2 highlight that the use of a BDD anode accelerates remarkably TOC removal and mineralization current efficiency as a result of its greater ability to produce active BDD([•]OH) radicals that can oxidize effectively all by-products (Oturan et al., 2012), even those refractory to the EF-Pt and EF-DSA processes. This confirms that the nature of the anode used in EAOPs plays a significant role in the oxidation/mineralization of organic pollutants. Consequently, EF-BDD and AO-H₂O₂-BDD are very efficient for the treatment of RNTD solutions.

3.3.3. Mineralization pathway

The RNTD degradation during AO-H₂O₂ and EF processes leads to the formation of primary oxidation products which are subsequently destroyed with hydroxyl radicals up to mineralization. To clarify its reaction sequence, the treated solutions were analyzed to determine not only cyclic organic products but also short-chain carboxylic acids as end-products before conversion to CO₂ and inorganic ions released to solution. From these results, a scheme including various oxidation pathways for RNTD mineralization is proposed in Fig. 3.3.

Cyclic organic intermediates were detected by ESI-TOF-MS analysis of solutions treated by the most potent EF-BDD process. From this technique, 12 intermediates, 11 of which with furan moiety (compounds **A-M** in Fig. 3), were identified. This suggested an attack of [•]OH on different sites of RNTD according to pathways **I**, **II** and **III**. Route **I** consists of the oxidative cleavage of the C-S bond of RNTD to form **A** and **B** from the attack of [•]OH onto S atom along with elimination of a sulfate ion. Route **II** involves the formation of the sulfoxyde **C** from simultaneous oxidation of S atom and [•]OH addition onto its alpha position, followed by its hydroxylation to yield **D**. Compound **E** is a *N*,*N*-demethylated product from **C**. The mineralization of **E** with release of NH_4^+ and $NO_3^$ ions leads to **F**, which can be oxidized to the sulfonated **G**. Product **B** along **H** can also be obtained from the oxidative breaking of **E** and/or **D**. Route **III** starts by the attack of [•]OH onto the *N*,*N*- dimethylamino group generating the demethylated product I, followed by its deamination to J. The latter compound yields either K by partial mineralization of the side chain or M from its hydroxylation at the level of the carbon adjacent to S atom. Finally, compound L obtained from K hydroxylation gives the sulfonated G via deamination and oxidation of S atom.



Figure 3.3. Proposed reaction pathway for RNTD mineralization by the EF-BDD process with a carbon-felt cathode involving all identified cyclic organic and aliphatic intermediates, as well as released inorganic ions.

Successive hydroxylation of the above cyclic intermediates are expected to promote their ring cleavage yielding short-chain carboxylic acids, along with release of NH_4^+ , NO_3^- and $SO_4^{2^-}$ ions (Brillas et al., 2009; El-Ghenymy et al., 2014; Oturan and Aaron, 2014). The evolution of final carboxylic acids found for EF-BDD and EF-DSA at 300 mA is depicted in Figs. 3.4a and 3.4b, respectively. Acetic, pyruvic, malic, oxamic, formic and oxalic acids were identified. As shown in Fig. 3.3, oxidation of the three former acids leads to oxalic and formic acids (Brillas et al., 2009), whereas oxamic acid can be formed from precedent *N*-derivatives. Finally, oxamic, oxalic and formic acids are directly oxidized to CO_2 (Garcia-Segura and Brillas, 2011). All these acids form Fe(II) complexes (Sirés et al., 2007a) that require long destruction times due to their lower reactivity with heterogeneous M(*OH) constituting residual TOC on longer times. For example, only 0.027 mM formic acid (2.25% of initial TOC) was found after 8 h of EF-BDD (see Fig. 3.4a). Comparison of Figs. 3.4a and 3.4b reveals a much more efficient removal of carboxylic acids in EF-BDD, in agreement with the greater oxidation ability of BDD(*OH). This confirms the superiority of BDD anode giving faster TOC removal and higher mineralization degree (see Fig. 2c).



Figure 3.4. Time-course of the concentration of the main short-chain carboxylic acids detected during the EF treatment of 230 mL of a 0.1 mM RNTD aqueous solution in 0.05 M Na₂SO₄ with 0.1 mM Fe²⁺ at pH 3.0 using (a) BDD/carbon-felt and (b) DSA/carbon-felt cells at 300 mA and room temperature. Acids: (\blacklozenge) oxalic, (\blacksquare) oxamic, (\blacktriangle) formic, (\blacklozenge) pyruvic, (\bigstar) malic and (\Box) acetic.

Fig. 3.5 depicts the evolution of NH_4^+ , NO_3^- and SO_4^{2-} ions formed from the initial N and S of RNTD for EF-BDD at 300 mA. NH_4^+ was the most largely accumulated ion up to ca. 0.15 mM, as expected from deamination of cyclic organic products. However, NO_3^- ion was accumulated in lesser extent and after reaching 0.08 mM at 1 h, its content dropped to ca. 0.01 mM at 8 h. This phenomenon can be related to: (i) its oxidation on BDD yielding volatile compounds like N_xO_y . and (ii) its reduction on the carbon-felt cathode to give N_2 , ammonia and hydroxylamine from reactions (14)-(17) (Lévy-Clément et al., 2003; Mook al., 2012):

$$NO_{3}^{-} + 3H_{2}O + 5e^{-} \rightarrow 1/2N_{2} + 6 OH^{-}$$
(14)

$$NO_3^{-} + 6H_2O + 8e^{-} \rightarrow NH_3 + 9 OH^{-}$$
 (15)

$$NO_3^- + H_2O + 2e^- \rightarrow NO_2^- + 2 OH^-$$
 (16)

$$NO_2^- + 4H_2O + 4e^- \rightarrow NH_4OH + 5OH^-$$
(17)



Figure 3.5. Time-course of the concentration of (\blacklozenge) NH₄⁺, (\blacktriangle) NO₃⁻ and (\blacksquare) SO₄²⁻ ions released during the EF-BDD treatment of 230 mL of 0.1 mM of RNTD in 0.05 M Na₂SO₄ and 0.1 mM Fe²⁺ at pH 3.0 using a BDD/carbon-felt cell at 300 mA and room temperature.

At the end of EF-BDD, however, the total N concentration as NH_4^+ and NO_3^- only attained about 40% of the initial N in solution. This negative mass balance highlights the loss of a large proportion of N in electrochemical oxidation/reduction reactions. In contrast to this complex behavior, Fig. 3.5 shows a rapid release of SO_4^{2-} ion from the beginning of the electrolysis, reaching 0.97 mM that is practically equal to the initial S (0.1 mM) in the RNTD solution, evidencing solution mineralization along with TOC abatement.

3.3.4. Evolution of toxicity of RNTD solutions during mineralization

The change in toxicity of the 0.1 mM RNTD solution under AO-H₂O₂ treatment was monitored from the bioluminescence inhibition of *V. fischeri* using BDD/carbon-felt and DSA/carbon-felt cells at 500 and 1000 mA. These trials represented an approximate assessment of the potential risks of this drug and its degradation products in the environment, taking into account the lack of data about their ecotoxicity (Laurencé et al., 2014).



Figure 3.6. Evolution of the toxicity of 230 mL of a 0.1 mM RNTD solution with electrolysis time during the AO-H₂O₂ process with (a) BDD/carbon-felt and (b) DSA/carbon-felt cells in terms of the percentage of inhibition of the bioluminescence of *V. fischeri* bacteria after 5 min of exposure. The solution contained 0.05 M Na₂SO₄ at pH 3.0 and the experiments were made at (\blacklozenge) 500 mA and (\blacksquare) 1000 mA and room temperature.

Figs. 3.6a and 3.6b show that in all cases the bioluminescence inhibition increases at the early treatment stages reaching values as high as 99%, which can be related to the formation of cyclic organics more toxic than RNTD. The continuous generation and destruction of such products is responsible of the pronounced fluctuations in toxicity during the first hour of electrolysis. At longer time, the rapid drop in bioluminescence inhibition indicates a drastic fall in toxicity, as expected by the disappearance of cyclic compounds (responsible of toxicity) as reflected in TOC decay (see Fig. 1d). Figs. 3.6a and 3.6b also show a more effective detoxification using a BDD

anode at 1000 mA due to the quickest TOC removal under these conditions. The low bioluminescence inhibition rate at longer electrolysis times demonstrates the effectiveness of the electrochemical treatment. The remaining residual toxicity can then be related to the presence of carboxylic acids that are readily biodegradable, as reported elsewhere (Dantas et al., 2008; Oturan et al., 2008; Dirany et al., 2011).

3.4. Conclusions

It has been demonstrated that different EAOPs like EF-Pt, EF-BDD, EF-DSA and AO-H₂O₂ are very effective for the complete removal and almost total mineralization of the drug RNTD in aqueous medium. The use of a 3D carbon-felt cathode ensured the continuous electrogeneration of H_2O_2 and Fe^{2+} to produce homogeneous [•]OH in the bulk solution. The BDD anode gave large amounts of active heterogeneous BDD([•]OH), making EF-BDD and AO-H₂O₂-BDD the most efficient processes for RNTD mineralization. Both EAOPs yielded about 94% TOC abatement after 4 h of electrolysis at 500 mA, whereas TOC removal dropped to 89.47% for EF-Pt and 80.38% for EF-DSA. The RNTD decay always obeyed a pseudo-first-order kinetics and an absolute rate constant of 3.39×10⁹ M⁻¹ s⁻¹ was determined for its hydroxylation by the competition kinetics method. Analysis of treated solutions allowed the identification of 11 cyclic organic intermediates with furan moiety, 6 short-chain carboxylic acids and inorganic ions like NH_4^+ , NO_3^- and SO_4^{2-} . From these products, a plausible reaction pathway for RNTD mineralization is proposed. The toxicity assessment showed the formation of intermediates that are more toxic than RNTD itself, although total detoxification was attained at the end of AO-H₂O₂ treatments, regardless of the anode used, thereby demonstrating the effectiveness of the EAOPs tested. These results highlight that AO-H₂O₂ and EF are viable environmentally friendly technologies for the remediation of wastewaters containing pharmaceutical residues like RNTD and their oxidation products.

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A Pre-Pilot Flow Plant Scale for the Electro-Fenton and Solar Photoelectro-Fenton Treatments of Acidic Solutions of the Pharmaceutical Ranitidine

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Chapter 4

A pre-pilot flow plant scale for the electro-Fenton and solar photoelectro-Fenton treatments of acidic solutions of the pharmaceutical ranitidine

Abstract

A 2.5 L capacity pre-pilot plant equipped with a Pt/air-diffusion cell coupled with a solar photoreactor was used for a comparative study on the mineralization of the pharmaceutical Ranitidine by the electro-Fenton process. The compound is oxidized by the hydroxyl radical (*OH) generated from the reaction between H_2O_2 generated at the cathode and the added Fe^{2+} and/or under the action of sunlight. SPEF process was found to be more efficient, reaching up to 70% TOC removal, while EF yielded much poorer degradation. The effect of current density and both Fe^{2+} and drug concentrations on the degradation rate and mineralization efficiency of EF and SPEF, was examined. It was found that RNTD decay followed a pseudo first order kinetics, with a greater rate in SPEF due to the additional generation of .OH induced by sunlight on Fe(III) species. The electrochemical degradation of RNTD yields to the formation of aromatic by-products which are afterwards oxidized to aliphatic carboxylic acids before their conversion to CO_2 and inorganic ions (NH₄⁺, NO₃⁻, CI⁻ and SO₄²⁻). Short-chain carboxylic acids (mainly oxalic acid) formed complexes with Fe(III) present in the solution in EF, which are hardly destroyed by *OH. Nonetheless, sunlight can quickly photolize Fe(III)-oxalate complexes thus explaining the higher oxidation ability of SPEF.

Keywords: Hydroxyl radical; Electro-Fenton; Oxidation products; Ranitidine; Solar photoelectro-Fenton; Water treatment

4.1. Introduction

During the last decade, the development of effective and clean technologies for the treatment of toxic and/or biorefractory organic compounds from waters has been a major concern. Among these technologies, electrochemical advanced oxidation processes (EAOPs) have shown to be very effective for removing organic pollutants from waters due to their great oxidation/mineralization ability (Brillas et al., 2009; Sirés and Brillas, 2012b; Sirés et al., 2014a; Vasudevan and Oturan, 2014). EAOPs are based on the in-situ electrochemical generation of hydroxyl radical ($^{\circ}OH$), which is the second strongest oxidant known because it has so high standard reduction potential (E° ($^{\circ}OH/H_2O$) = 2.80 V/SHE) that can non-selectively react with most organics up to their mineralization to CO₂, water and inorganic ions (Özcan et al., 2009; Rosales et al., 2009; Feng et al., 2013).

Electro-Fenton (EF) is the most common EAOP based on Fenton's reaction chemistry used for the decontamination of acidic waters (Brillas et al., 2009; Özcan et al., 2009; Panizza and Oturan, 2011; Feng et al., 2013; Sirés et al., 2014; Vasudevan and Oturan, 2014). In EF, H_2O_2 is continuously electrogenerated at a carbonaceous cathode from reaction (1) and a catalytic amount of Fe²⁺ ion is added to the contaminated solution to react with it to yield Fe³⁺ ion and homogeneous [•]OH in the bulk from the well-known Fenton's reaction (2) with optimum pH = 2.8. An advantage of EF over the classical Fenton's reagent treatment is that Fe²⁺ ion can be cathodically regenerated from Fe³⁺ via reaction (3), thereby accelerating Fenton's reaction (2) and enhancing the mineralization process (Brillas et al., 2009; Garcia-Segura et al., 2011).

$O_2 + 2 H^+ + 2 e^- \rightarrow H_2O_2$	(1)
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$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-$$
(2)

$$Fe^{3+} + e^{-} \rightarrow Fe^{2+}$$
(3)

The oxidation power of the EF process can be strongly enhanced if the solution is simultaneously irradiated with UVA light, giving rise to the photoelectro-Fenton (PEF) process (Sirés et al., 2007; Wang et al., 2008; Anotai et al., 2011; Khataee et al., 2013). The main drawback of using artificial UVA lamps is their high energy cost for practical application. To solve this problem, we have proposed the direct use of sunlight as renewable and inexpensive energy source in the so-called solar PEF (SPEF) process (Guinea et al., 2010; Almeida et al., 2011; Isarain-Chávez et al., 2011; Ruiz et al., 2011; Salazar et al., 2012; El-Ghenymy et al., 2013) . The very positive action of UV irradiation in these photo-assisted EAOPs is due to: (i) the photolysis of Fe(OH)²⁺, the preferential Fe³⁺ species at pH near 3, regenerating more Fe²⁺ and producing more **°**OH from

photo-Fenton reaction (4) and (ii) the photodecarboxylation of some generated Fe(III)-carboxylate complexes according to the general reaction (5).

$$Fe(OH)^{2+} + hv \rightarrow Fe^{2+} + {}^{\bullet}OH$$

$$Fe(OOCR)^{2+} + hv \rightarrow Fe^{2+} + CO_2 + R^{\bullet}$$
(5)

The use of an undivided cell in EF and SPEF with a high O₂-overpotential anode (M) also promotes the parallel attack of organic pollutants by heterogeneous M([•]OH), produced as intermediate from water reduction from reaction (6) (Martínez-Huitle and Ferro, 2006; Panizza and Cerisola, 2009):

$$M + H_2O \rightarrow M(^{\bullet}OH) + H^+ + e^-$$
(6)

The preferred anodes for EAOPs are boron-doped diamond (BDD) thin-film electrodes because of their higher ability to produce more amounts of reactive BDD([•]OH) than other common anodes like Pt and PbO₂, allowing to mineralize aromatic and aliphatic organic pollutants in much larger extent (Ciríaco et al., 2009; Flox et al., 2009; Panizza and Cerisola, 2009; Brillas et al., 2010; Rodrigo et al., 2010; Tsantaki et al., 2012; Cavalcanti et al., 2013). However, it has been found that using a SPEF system, organic pollutants are mainly destroyed in the bulk by the great generation of [•]OH from reaction (2) along with the photolytic action of sunlight via reactions (4) and (5) (Sirés and Brillas, 2012; Sirés et al., 2014). For this reason, less powerful anodes such as Pt, yielding less energy consumption, can also be useful for organics destruction by SPEF (Isarain-Chávez et al., 2011; El-Ghenymy et al., 2013; Moreira et al., 2013).

Pharmaceuticals have been receiving increasing attention as potential bioactive chemicals in the environment (Kümmerer, 2009). They are accumulated into natural waters because of their incomplete removal in wastewater treatment plants since they are recalcitrant to conventional physicochemical methods (Jones et al., 2005; Homem and Santos, 2011). Pharmaceuticals and their metabolites represent a potential risk of toxicity for ecosystems and living beings (Klavarioti et al., 2009). It has been documented that some drugs cause long-term, irreversible changes to micro-organisms genome even at low content in water, then having more resistance to them (Crane et al., 2006; Fent et al., 2006). The development of potent oxidation processes to destroy synthetic drugs from waters is then necessary for avoiding their potential hazardous effects in the environment over living beings.

Several works have reported the degradation of some pharmaceuticals by EF using a Pt or BDD anode different carbonaceous cathodes like carbon-felt or gas-diffusion electrodes (Ignasi Sirés et al., 2007a, 2007b, 2007c; Dirany et al., 2010; Guinea et al., 2010; Isarain-Chávez et al., 2011; Dirany et al., 2012; Garcia-Segura et al., 2012; El-Ghenymy et al., 2013). However, a reduced number of papers have been devoted to investigate the removal of drugs using SPEF (Guinea et al., 2010; Isarain-Chávez et al., 2011; El-Ghenymy et al., 2013). More research efforts are then required to know the characteristics of this process to assess its viability to destroy wastewaters polluted with pharmaceuticals at industrial level. Among these compounds, ranitidine (RNTD, see chemical structure in Fig. 1) is a H2-receptor antagonist, very widely prescribed for the treatment of peptic ulcer and gastroesophageal reflux disease (Khetan and Collins, 2007), which has been classified as one of the highest risk drugs on human health (Besse and Garric, 2008). It has been found in European and US surface and wastewaters (Fent et al., 2006; Gros et al., 2007) and sediments (Zuccato et al., 2000).

This paper presents a comparative study on the degradation of acidic RNTD solutions by EF and SPEF using a 2.5 L pre-pilot plant equipped with a Pt/air diffusion cell and a flat solar photoreactor for the latter method. Our aim was to clarify the action of oxidizing agents and UV radiation supplied by sunlight over the performance of the SPEF process of such drug. The effect of current density (*j*) and Fe²⁺ and substrate concentration on total organic carbon (TOC), mineralization current efficiency (MCE) and energy consumption was examined. The kinetics for RNTD decay and the evolution of generated carboxylic acids and released inorganic ions were followed by chromatographic techniques.

4.2. Materials and methods

4.2.1. Chemicals

Ranitidine hydrochloride (purity > 98%) was of reagent grade supplied by Fluka. Anhydride sodium sulfate used as background electrolyte and heptahydrated iron (II) sulfate used as catalyst were of analytical grade purchased from Acros Organics. All solutions were prepared with high-purity water from a Millipore Milli–Q system with resistivity >18 M Ω cm. Reagent grade sulfuric acid supplied by Merck was used to adjust their initial pH to 3.0. All the other chemicals used were either of HPLC or analytical grade purchased from Prolabo, Fluka and Acros Organics.

4.2.2. Batch recirculation flow plant

Fig. 4.1 shows a scheme of the 2.5 L pre-pilot plant used to carry out the EF and SPEF assays in batch recirculation mode under galvanostatic conditions. The RNTD solution was

introduced in the reservoir and recirculated through the plant by a peristaltic pump at a flow rate of 200 L h^{-1} , being its temperature maintained at 35 °C by two heat exchangers.



Fig. 4.1. Batch circulation pre-pilot flow plant with an undivided filter-press cell with a 20 cm² Pt anode and a 20 cm² air-diffusion cathode used for the solar photoelectro-Fenton (SPEF) degradation of 2.5 L of RNTD solutions in 0.05 M Na₂SO₄ at pH 3.0 and 35 °C.

The solution then passed through a one-compartment filter-press reactor with 20 cm² electrodes separated 1.2 cm, and further circulated through a flat solar photoreactor. The electrochemical cell contained a Pt sheet anode of 99.99% purity from SEMPSA and a carbon-PTFE air-diffusion cathode from E-TEK, which was fed with air pumped at an overpressure of 8.6 kPa for continuous H₂O₂ generation by reaction (1). A constant *j* was provided to the cell by an Agilent 6552A DC power supply, directly measuring the applied potential difference. The solar photoreactor consisted of a 24 cm x 24 cm x 2.5 cm polycarbonate box (600 mL of irradiation volume) with a mirror at the bottom and tilted 41° to best collect the incident sun rays. The SPEF experiments were conducted starting from noon in sunny and clear days during the summer of 2014 within the facilities of the University of Barcelona, Spain (latitude 41°21'N, longitude 2°10'E). The UV irradiation intensity (300-400 nm) was in the range of 30-32 W m⁻², as measured with a Kipp&Zonen CUV 5 radiometer. Comparative EF trials were carried out by covering the pre-pilot plant with an opaque plastic to avoid UV irradiation on the system. The air-diffusion cathode was previously activated by electrolyzing 2.5 L of 0.05 M Na₂SO₄ at pH 3.0 and 150 mA cm⁻² for 240 min.

4.2.3. Apparatus and analytical procedures

The pH of the solution was determined on a Crison GLP 22 pH-meter. Samples withdrawn from the treated solutions were alkalinized to stop the degradation process and microfiltered with 0.45 μ m PTFE filters purchased from Whatman before analysis. The mineralization of RNTD solutions was monitored from their TOC abatement, determined with a Shimadzu VCSN TOC analyzer. Reproducible TOC values with an accuracy of ±1% were found by injecting 50 μ L aliquots to the analyzer. From these data, the mineralization current efficiency for each trial at current *I* (in A) and time *t* (in h) was then estimated from Eq. (7) (Ruiz et al., 2011):

MCE (%) =
$$\frac{nFV_s \Delta (TOC)_{exp}}{4.32 \times 10^7 m I t} 100$$
 (7)

where *F* is the Faraday constant (96487 C mol⁻¹), *V*_s is the solution volume (in L), Δ (TOC)_{exp} is the experimental TOC decay (in mg L⁻¹), 4.32×10^7 is a conversion factor to homogenize units (= 3600 s h⁻¹ × 12000 mg carbon mol⁻¹) and *m* is the number of carbon atoms of RNTD (13 atoms). The number of electrons (*n*) consumed per each drug molecule was taken as 62 considering its total mineralization to CO₂ and sulfate and ammonium as pre-eminent ions, as will be discussed below:

$$C_{13}H_{22}N_4O_3S + 27 H_2O \rightarrow 13 CO_2 + SO_4^{2-} + 4 NH_4^+ + 60 H^+ + 62 e^-$$
(8)

The specific energy consumption per unit TOC mass (EC_{TOC}) was calculated as follows (Ruiz et al., 2011):

$$EC_{TOC}(kWh g^{-1} TOC = \frac{E_{cell} I t}{V_s \Delta (TOC)_{exp}}$$
(9)

where E_{cell} is the average potential difference of the cell (in V).

The time-course of RNTD concentration was followed by reversed-phase HPLC using a Waters 600 LC fitted with a Thermo Electron Corporation Hypersil ODS 5 μ m, 150 mm x 3 mm (i.d.), column at room temperature and coupled with a Waters 996 photodiode array detector set at λ = 280 nm. These analyses were carried out with a 28:72 (v/v) methanol/water (10 mM ammonium acetate) mixture at 0.7 mL min⁻¹ as mobile phase. Generated aliphatic acids were quantified by ion-exclusion HPLC using the above LC fitted with a Bio-Rad Aminex HPX 87H, 300 mm x 7.8 mm

(i.d.), column at 35 °C and the array detector selected at $\lambda = 210$ nm, and circulating 4 mM H₂SO₄ at 0.6 mL min⁻¹ as mobile phase. The released inorganic ions were detected by ion chromatography using a Shimadzu 10 Avp LC coupled with a Shimadzu CDD 10 Avp conductivity detector. The NH₄⁺ content was determined with a Shodex IC YK-421, 125 mm x 4.6 mm (i.d.), cation column at 40 °C under circulation of 5.0 mM tartaric acid, 24.2 mM boric acid, 2.0 mM dipicolinic acid and 15.0 mM crown esther solution at 1.0 mL min⁻¹. The NO₃⁻ and SO₄²⁻ concentrations were obtained with a Shim-Pack IC-A1S, 100 mm x 4.6 mm (i.d.), anion column at 40 °C using a 2.5 mM phthalic acid and 2.4 mM tris(hydroxymethyl)aminomethane solution at 1.5 mL min⁻¹ as mobile phase.

4.3. Results and discussion

4.3.1. Electro-Fenton degradation of ranitidine solutions

A key parameter to regulate the amount of [•]OH produced in the bulk from Fenton's reaction (2) is the Fe²⁺ concentration acting as catalyst in EAOPs based on Fenton's reaction chemistry. To find the optimum content of this species using the pre-pilot plant, a series of EF trials was made by electrolyzing 2.5 L of 33.8 mg L⁻¹ RNTD solutions in 0.05 M Na₂SO₄ with Fe²⁺ concentration ranging from 0.20 to 2.0 mM at pH 3.0, 35° C and 100 mA cm⁻² for 360 min. This pH was set because it has been found optimal for the destruction of many aromatics under similar EF conditions (Guinea et al., 2010; Isarain-Chávez et al., 2011; Ruiz et al., 2011). In all these experiments, the solution pH was not regulated because it remained practically constant, only dropping to a final value of 2.7-2.8 probably due to the formation of acidic products like short-linear carboxylic acids (Sirés and Brillas, 2012), as will be discussed below.

For the above trials, Fig. 4.2 highlights a gradual TOC decay with electrolysis time in all cases, although at times longer than 2 h electrolysis, the mineralization process was strongly inhibited. This behavior is indicative of the generation of very recalcitrant compounds such as Fe(III)-carboxylate compounds that are hardly destroyed by homogeneous [•]OH and heterogeneous Pt([•]OH) formed from reactions (2) and (6), respectively (Brillas et al., 2009; Garcia-Segura et al., 2011; Sirés and Brillas, 2012). Fig. 4.2 also shows a faster TOC abatement when Fe²⁺ content rose from 0.20 to 0.50 mM, whereupon this parameter underwent a gradual fall up to 2.0 mM Fe²⁺. After 360 min of electrolysis, TOC was reduced by 61% as maximal using 0.50 mM Fe²⁺. The enhancement in mineralization from 0.20 to 0.50 mM Fe²⁺ can be accounted for by a higher

production of **•**OH from Fenton's reaction (2) by the presence of more catalyst, thereby accelerating the destruction of the drug and its products. In contrast, the progressive inhibition in mineralization when Fe²⁺ concentration increased from 0.50 to 2.0 mM can be related to the gradual quicker attack of this ion on generated **•**OH in the bulk from the parasitic reaction (10) (Ruiz et al., 2011; Salazar et al., 2012):

$$Fe^{2^+} + {}^{\bullet}OH \rightarrow Fe^{3^+} + OH^-$$
(10)

The above results allowed concluding that 0.50 mM Fe²⁺ was an optimum catalyst content for the Fenton's reaction (2) taking place under the experimental conditions tested and hence, it was chosen for all the subsequent RNTD treatments made by EF and SPEF.



Figure 4.2. Effect of catalyst concentration on TOC decay with electrolysis time for the electro-Fenton (EF) treatment of 2.5 L of a 33.8 mg L⁻¹ RNTD solution in 0.05 M Na₂SO₄ at pH 3.0 and 35 °C in the pre-pilot plant with a Pt/air-diffusion reactor of 20 cm² electrodes at 100 mA cm⁻² and liquid flow rate of 200 L h⁻¹. Fe²⁺ concentration: (*****) 0.20 mM, (**△**) 0.50 mM, (**●**) 1.0 mM and (**♦**) 2.0 mM.

The current density is another key variable in EAOPs since it regulates the generation of oxidizing species for removing organic pollutants (Brillas et al., 2009; Sirés and Brillas, 2012). To assess the effect of *j* on the EF process in the pre-pilot plant, a solution with 112.6 mg L⁻¹ RNTD in 0.05 M Na₂SO₄ with optimum 0.50 mM Fe²⁺ and pH 3.0 was degraded between 25 and 100 mA cm⁻². As can be seen in Fig. 4.3a, an increase in *j* promoted TOC removal as a result of a greater production of Pt([•]OH) from reaction (6) and of H₂O₂ from reaction (1) (Flox et al., 2007) allowing the generation of more amounts of [•]OH from Fenton's reaction (2) and enhancing the mineralization of organics. After 360 min of electrolysis, increasing TOC removal of only 23%, 37% and 41% was found for 25, 50 and 100 mA cm⁻². In contrast, Fig. 4.3b shows an opposite trend for

MCE which decays as rising *j*. The best efficiency was then obtained for 25 mA cm⁻² where it rose up to a maximal of 24% at the starting of the process, then dramatically dropping to a final value of about 10% owing to the formation of hardly oxidizable products with hydroxyl radicals like short-chain carboxylic acids. This decay in MCE was even more pronounced at higher *j* values (see Fig. 4.3b). The existence of lower efficiency at higher *j* can be associated with a progressive loss in the relative production of hydroxyl radicals as a result of the increase in rate of their non-oxidizing reactions yielding fewer attacks on organics. These parasitic reactions involve primordially the oxidation of Pt(°OH) to O₂ at the anode by reaction (11), as well as the dimerization of °OH in the bulk by reaction (12) and its reaction with H₂O₂ to form the weaker oxidant hydroperoxyl radical (HO₂[•]) via reaction (13) and with Fe²⁺ by reaction (10) (Brillas et al., 2009; Panizza and Cerisola, 2009):

$2 \text{ Pt}(^{\bullet}\text{OH}) \rightarrow 2 \text{ BDD} + \text{O}_2 + 2 \text{ H}^+ + 2 \text{ e}^-$	(11)

$$2 ^{\bullet}OH \rightarrow H_2O_2 \tag{12}$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow HO_2 {}^{\bullet} + H_2O$$
(13)

The change of EC_{TOC} with electrolysis time for the above trials is depicted in Fig. 4.3c. As expected, the energy consumption dropped with rising *j* by the concomitant increase in E_{cell} . Nevertheless, at given *j* EC_{TOC} underwent a slight increase with prolonging the EF treatment and thus, final values of about 1.0, 1.6 and 2.4 kWh g⁻¹ TOC for 25, 50 and 100 mA cm⁻², respectively, were found. The above findings indicate that the application of lower *j* favors the efficiency and energy consumption of the EF degradation of RNTD, although poorer mineralization is achieved.

The influence of RNTD concentration between 33.8 and 112.6 mg L⁻¹ under optimum EF conditions at 100 mA cm⁻² was also examined. Fig. 4.4a depicts that the normalized TOC abatement decreased as drug concentration increased, a behavior that can be simply related to the fact that a smaller proportion of organics can be mineralized under the action of a similar concentration of hydroxyl radicals produced at the same *j*. Nevertheless, the MCE values calculated for these experiments from Eq. (7) and presented in Fig. 4.4b reveal that they became greater as the starting RNTD content grew, then being superior for the highest value tested of 112.6 mg L⁻¹. This tendency suggests that in the presence of higher amounts of organic matter, the parasitic reactions (10)-(13) of generated Pt(°OH) and °OH are progressively decelerated because more quantities of both hydroxyl radicals are able to react with greater quantities of organic pollutants enhancing the efficiency of the mineralization process. This behavior is also reflected in the concomitant decay in EC_{TOC} when the solution contained more initial RNTD, as shown in Fig.



4.4c. The lowest energy consumptions of near 1.75 kWh g^{-1} TOC was thus obtained for the greatest content of 112.6 mg L⁻¹.

Figure 4.3. Effect of current density on (a) TOC removal, (b) mineralization current efficiency and (c) energy consumption per unit TOC mass vs. electrolysis time during the electro-Fenton (EF) treatment of 2.5 L of a 112.6 mg L⁻¹ RNTD solution in 0.05 M Na₂SO₄ with 0.50 mM Fe²⁺ at pH 3.0 and 35 °C in the pre-pilot plant at liquid flow rate of 200 L h⁻¹. Applied current density: (\blacklozenge) 25 mA cm⁻², (\blacklozenge) 50 mA cm⁻² and (\blacktriangle) 100 mA cm⁻².

The aforementioned results evidence a poor degradation of acidic RNTD solutions by EF. The mineralization process is favored operating at lower j values and higher drug content, conditions under which the reactivity of Pt([•]OH) and [•]OH is enhanced yielding more efficiency and less energy consumption. Once clarified the role of oxidizing agents in this EAOP, the SPEF process was further studied under comparable conditions in order to assess its oxidation power and the influence of UV radiation from sunlight over the mineralization of drug solutions, as will be described in subsection below.



Figure 4.4. Influence of drug concentration on (a) normalized TOC abatement, (b) mineralization current efficiency and (c) energy consumption per unit TOC mass vs. electrolysis time during the EF treatment of 2.5 L of RNTD solutions in 0.05 M Na₂SO₄ with 0.50 mM Fe²⁺ at pH 3.0 and 35 °C in the pre-pilot plant at 100 mA cm⁻² and liquid flow rate of 200 L h⁻¹. RNTD concentration: (\blacklozenge) 33.8 mg L⁻¹, (\blacklozenge) 67.6 mg L⁻¹ and (\blacktriangle) 112.6 mg L⁻¹.

4.3.2. Solar photo-Electron treatment of ranitidine solutions

The effect of applied *j* on the SPEF process was firstly examined for 2.5 L of a 112.6 mg L⁻¹ drug solution with 0.05 M Na₂SO₄ and 0.50 mM Fe²⁺ at pH 3.0 operating between 25 and 100 mA cm⁻².



Figure 4.5. Influence of current density on the change of (a) TOC, (b) mineralization current efficiency and (c) energy consumption per unit TOC mass with electrolysis time during the solar photoelectro-Fenton (SPEF) treatment of 2.5 L of a 112.6 mg L⁻¹ RNTD solution in 0.05 M Na₂SO₄ with 0.50 mM Fe²⁺ at pH 3.0 and 35 °C in the pre-pilot plant with a Pt/air-diffusion reactor coupled to a flat solar photoreactor of 600 mL irradiation volume at liquid flow rate of 200 L h⁻¹. Applied current density: (\blacklozenge) 25 mA cm⁻², (\blacklozenge) 50 mA cm⁻² and (\blacktriangle) 100 mA cm⁻².

Fig. 4.5a depicts a high increase in TOC removal with increasing *j*, attaining a final mineralization of 44%, 50% and 67% after 360 min of electrolysis at 25, 50 and 100 mA cm⁻², respectively. Compared with EF, the SPEF process is much more potent, allowing destroying about 1.4-1.9 times more of TOC under comparable conditions. The superiority of SPEF can be explained by the photolytic power of sunlight on several intermediates, like Fe(III)-carboxylate complexes, causing faster mineralization of the treated solution. It is noteworthy that this process cannot completely mineralize the ranitidine solutions, suggesting the formation of very recalcitrant products that cannot be destroyed by the combined action of Pt([•]OH), [•]OH and UV irradiation. The higher oxidizing ability of SPEF than EF can also be deduced by comparing Figs. 4.3b and 4.5b, where the comparative MCE-time plots for the 112.6 mg L^{-1} drug solution at the same j values are given. Results of Fig. 4.5b show a gradual decay in efficiency as *j* rose, as expected if the mineralization of organics is controlled by the relative amounts of generated hydroxyl radicals that decrease at higher *j*, as stated above. The positive effect of sunlight accelerating the destruction of some intermediates is confirmed from the higher MCE values achieved in SPEF compared to EF. Decreasing maximum efficiencies of 62%, 43% and 30% were determined at the beginning of the electrolysis at growing *j* values of 25, 50 and 100 mA cm⁻², which further dropped to 20%, 13% and 8.7%, respectively, at 360 min of SPEF. The strong decay in MCE with prolonging the treatment can be ascribed to the accumulation of hardly recalcitrant products as well as the presence of less organic matter in solution (Panizza and Cerisola, 2009). The fact that the SPEF process was much more efficient than EF was also corroborated by the lower energy consumptions spent in the comparable experiments. Fig. 4.5c highlights that EC_{TOC} of 0.24 kWh g⁻¹ TOC for 25 mA cm⁻², which rose to 0.95 kWh g⁻¹ TOC for 100 mA cm⁻², were finally obtained for SPEF, values much lower than those found for the comparative EF treatments (see Fig. 4c). The UV radiation from sunlight in SPEF then causes a more efficient and less expensive degradation of RNTD solutions.

The influence of drug content in the range 33.8-112.6 mg L⁻¹ on the SPEF process was further considered operating with 0.50 mM Fe²⁺ at pH 3.0 and 100 mA cm⁻². As can be seen in Fig. 4.6a, the normalized TOC decayed more slowly as more organic matter was initially added to the solution, being finally reduced by 83%, 73% and 67% for 33.8, 67.6 and 112.6 mg L⁻¹, respectively. This TOC abatement by SPEF was much higher than those obtained by EF under comparable conditions (see Fig. 4.4a), thereby confirming the superiority of the former process under all the experimental conditions tested. Fig. 4.6b highlights that the MCE values found for the SPEF treatment rose rapidly with increasing the starting RNTD concentration, as expected by the

gradual reaction of more quantities of Pt(*OH) and *OH with organics coming from the inhibition of their parasitic reactions (10)-(13), then leading to higher amounts of organics that can be more quickly photolyzed by sunlight. This behavior was also verified from the concomitant decay in EC_{TOC} , which varied, for example, from 3.88 to 0.92 kWh g⁻¹ TOC at the end of the degradations performed from 33.8 to 112.6 mg L⁻¹ of the drug, as shown in Fig. 4.6c.



Figure 4.6. Effect of drug content on the variation of (a) normalized TOC, (b) mineralization current efficiency and (c) energy consumption per unit TOC mass with electrolysis time during the SPEF degradation of 2.5 L of RNTD solutions in 0.05 M Na₂SO₄ with 0.50 mM Fe²⁺ at pH 3.0 and 35 °C in the pre-pilot plant at 100 mA cm⁻² and liquid flow rate of 200 L h⁻¹. RNTD content: (\blacklozenge) 33.8 mg L⁻¹, (\blacklozenge) 67.6 mg L⁻¹ and (\blacktriangle) 112.6 mg L⁻¹.

The aforementioned findings evidence that the combined action of Pt(*OH), *OH and UV radiation from sunlight in SPEF leads to a more potent oxidation process to decontaminate acidic RNTD solutions than EF and hence, it is more viable for a possible industrial application. However, the SPEF treatment of these solutions only allows a partial mineralization of the drug because of the generation of very stable products than cannot be destroyed by generated hydroxyl radicals and/or photolyzed. To better clarify this behavior, the kinetics for RNTD decay and the evolution of generated carboxylic acids and released inorganic ions were followed by chromatographic techniques, as detailed below.

4.3.3. Kinetic analysis of ranitidine decay by electro-Fenton and solar photoelectron-Fenton

The reaction or the drug with generated hydroxyl radicals (Pt($^{\circ}OH$) and $^{\circ}OH$) in the EF and SPEF treatments of 2.5 L of a 112.6 mg L⁻¹ RNTD with 0.50 mM Fe²⁺ at pH 3.0 and 35° C in the prepilot plant, was followed by reversed-phase HPLC, where it displayed a well-defined peak at retention time (t_r) of 5.1 min. Prior to these measurements, it was corroborated that the drug concentration of the above solution remained constant under circulation in the solar plant, indicating that RNTD was not directly photolyzed by sunlight.



Figure 4.7. Drug abatement vs. electrolysis time for the $(\blacklozenge, \blacktriangle)$ EF and $(\diamondsuit, \bigtriangleup)$ SPEF processes of 2.5 L of a 112.6 mg L⁻¹ RNTD solution in 0.05 M Na₂SO₄ with 0.50 mM Fe²⁺ at pH 3.0 and 35 °C in the pre-pilot plant at liquid flow rate of 200 L h⁻¹. Applied current density: $(\diamondsuit, \diamondsuit)$ 50 mA cm⁻² and $(\blacktriangle, \bigtriangleup)$ 100 mA cm⁻².

Fig. 4.7 depicts the comparative concentration decay of RNTD in both EAOPs by applying j values of 50 and 100 mA cm⁻². The drug was rapidly removed to disappear in less than 50 min in

most cases, indicating that the slow degradation detected for the solution was due to the destruction of products that react more difficultly with hydroxyl radicals than the parent compound. A quicker RNTD decay can be observed in Fig. 4.7 for SPEF compared to EF at given *j*, as expected by the higher production of [•]OH in the bulk induced by the photolytic reaction (4) taking place in the former process. Moreover, the removal of the drug was always enhanced when *j* rose from 50 to 100 mA cm⁻² due to the greater generation of Pt([•]OH) and [•]OH, in agreement with the faster TOC removal found under these experimental conditions (see Figs. 4.3a and 4.5a).

The above concentration decays were then analyzed from kinetic equations related to simple reaction orders. Excellent linear straights were obtained considering a pseudo-first-order reaction, as presented in the inset panel of Fig. 4.7. From this kinetic analysis, an apparent rate constant (k_1) of 0.068 min⁻¹ ($R^2 = 0.998$) at 50 mA cm⁻² and 0.153 min⁻¹ ($R^2 = 0.997$) at 100 mA cm⁻² for EF, which upgraded to 0.095 min⁻¹ ($R^2 = 0.997$) at 50 mA cm⁻² and 0.251 min⁻¹ ($R^2 = 0.998$) at 100 mA cm⁻² for SPEF, was obtained. This behavior can be explained because hydroxyl radicals cannot be accumulated in the medium due to its high destruction rate and very short lifetime, and thus, the quasi-stationary state approximation can be applied to their concentration giving rise to a pseudo-first-order reaction for RNTD, as experimentally found.

4.3.4. Time-course of generated carboxylic acids and released inorganic ions

The quick oxidation of RNTD by Pt(°OH) and °OH yields cyclic intermediates which are then expected to be transformed into short-linear aliphatic carboxylic acids (Brillas et al., 2009; Sirés and Brillas, 2012). This was confirmed by analyzing a 112.6 mg L⁻¹ (0.358 mM) drug solution treated by EF and SPEF with optimum 0.50 mM Fe²⁺ at pH 3.0 and 100 mA cm⁻² for 360 min by means of ion-exclusion HPLC. These chromatograms exhibited well-defined peaks for oxalic (t_r = 6.4 min), malic (t_r = 9.5 min), pyruvic (t_r = 9.8 min), oxamic (t_r = 10.7 min), formic (t_r = 13.4 min) and acetic (t_r = 16.0 min) acids. Malic, pyruvic and acetic acids can be formed from the destruction of cyclic intermediates and are oxidized to oxalic and formic acids (Brillas et al., 2009; Sirés et al., 2014), whereas oxamic acid can be produced from the oxidation of *N*-intermediates. Oxalic, oxamic and formic acids are known to be the ultimate carboxylic acids that are directly converted into CO₂ (Garcia-Segura and Brillas, 2011; Sirés and Brillas, 2012). All these acids are largely present in the medium in the form of Fe(III) complexes (Garcia-Segura and Brillas, 2011; Sirés and Brillas, 2012).



Figure 4.8. Time-course of the concentration of the main short-chain carboxylic acids detected during the (a) EF and (b) SPEF degradations of 2.5 L of a 112.6 mg L⁻¹ RNTD solution with 0.05 M Na₂SO₄ and 0.50 mM Fe²⁺ at pH 3.0 and 35 °C using the pre-pilot plant at 100 mA cm⁻², 35 °C and liquid flow rate of 200 L h⁻¹. Acids: (\blacklozenge) oxalic, (\blacksquare) malic, (\blacktriangle) pyruvic, (\blacklozenge) acetic, (\bigstar) formic and (\bigstar) oxamic.

Fig. 4.8a shows that in EF, malic, pyruvic and acetic acids were poorly accumulated and completely removed, indicating that Fe(III)-malate, Fe(III)-pyruvate and Fe(III)-acetate complexes are destroyed by generated Pt(°OH) and/or °OH. In contrast, oxalic, oxamic and formic acids were rapidly accumulated to 0.47, 0.39 and 0.37 mM, respectively, which decayed to 0.28, 0.22 and 0.34 mM at the end of the treatment. This evidences the poor efficiency of generated hydroxyl radicals to mineralize Fe(III)-oxalate, Fe(III)-oxamate and Fe(III)-formate species. A simple mass balance reveals that all these acids contribute in 16.1 mg L⁻¹ TOC, corresponding to about 55% of the final TOC remaining in the final solution (see Fig. 4.3a), and hence, they are the main products present in it. For SPEF, however, a different behavior can be observed in Fig. 4.8b. The effective photolysis of Fe(III)-oxalate and Fe(III)-oxamate complexes yielded the total disappearance of oxalic and oxamic acids from the medium in 360 min. In contrast, the Fe(III)-formate species were

much more slowly photolyzed and 0.027 mM formic acid still remained in the medium at the end of SPEF. This ultimate acid corresponds to 0.32 mg L⁻¹ TOC, only representing about 2% of the TOC of the final treated solution (see Fig. 4.6a). It can then be inferred that the greater oxidation ability of SPEF is due to the efficient photolysis of final Fe(III)-carboxylate complexes, which are very stable predominant products in EF. These results also confirm the large production of very recalcitrant products, related to about 31% of final TOC in SPEF, since they are not removed by generated oxidizing species and/or UV radiation from sunlight.



Figure 4.9. Evolution of the concentration of (\blacklozenge) SO₄²⁻, (\blacklozenge) NH₄⁺ and (\blacktriangle) NO₃⁻ ions released during the experiment shown in Fig. 8.

The released inorganic ions like NH_4^+ , NO_3^- and SO_4^{2-} coming from the N and S atoms present in ranitidine (see Fig. 4.9) during the above experiments were quantified by ion chromatography and the results obtained are presented in Figs. 4.9a and 4.9b for EF and SPEF, respectively. As can be seen, NH_4^+ ion was accumulated in a larger extent than NO_3^- ion in both EAOPs, as proposed in reaction (8). After 360 min of electrolysis, 0.38 mM NH_4^+ (29% of initial N) and 0.15 mM NO_3^- (12% of initial N) were determined for EF, while 0.61 mM NH_4^+ (48% of initial N) and 0.36 mM NO_3^- (28% of initial N) were obtained for SPEF. Consequently, only 41% and 76% of the initial N content was lost as ions in EF and SPEF, respectively. This indicates that part of *N*-derivatives remaining in the medium in EF can be degraded under UV irradiation in SPEF yielding more amounts of inorganic ions, although the formation of volatile compounds like N₂ and N_xO_y is also feasible (Sirés et al., 2014). On the other hand, Figs. 4.9a and 4.9b show that SO₄²⁻ ion was gradually released up to 0.30 mM (93% of initial S) for EF and 0.31 mM (96% of initial S) for SPEF. This indicates that most S content of RNTD is always released as SO₄²⁻ ion, as stated in reaction (8).



Figure 4.10. Chemical structure of ranitidine (RNTD).

4.4. Conclusions

It has been demonstrated that SPEF is an efficient and viable process for the treatment of RNTD solutions of pH 3.0 at a pre-pilot plant equipped with a Pt/air-diffusion cell coupled to a flat solar photoreactor. About 80% mineralization was achieved for this procedure using 0.50 mM Fe²⁺ as optimum catalyst, showing a much better performance that EF under comparable conditions as a result of the combined action of Pt([•]OH), [•]OH and UV radiation from sunlight to destroy organics. In all cases, the increase of *j* enhanced drug mineralization, but decreased the MCE values due to the acceleration of parasitic reactions consuming the hydroxyl radicals. The use of lower *j* and higher organic load gave lower energy consumptions. The RNTD concentration decay always obeyed pseudo-first order kinetics, with greater apparent rate constant for SPEF because of the additional production of [•]OH in the bulk induced from reaction (4). The oxidation of the molecule and its cyclic intermediates in both EAOPs yielded final carboxylic acids like malic, pyruvic, acetic, oxalic, oxamic, and formic. The three latter acids were the main products accumulated in the final solution treated by EF due to the large stability of their Fe(III)-carboxylate complexes in front the attack of hydroxyl radicals. The quick and total photolysis of final Fe(III)-oxalate and Fe(III)oxamate species, as well as the slower photodecomposition of Fe(III)-formate complexes, explained the greater oxidation power of SPEF. Initial S of the drug was almost completely released as SO_4^{2-} ion, whereas its initial N was mainly lost as NH_4^+ ion along with a smaller proportion of NO_3^- ion. All these results highlight the potentiality of the SPEF process as a sustainable and environmentally friendly technology for the degradation of RNTD, since it utilizes the electron as clean reagent and sunlight as renewable and inexpensive energy source, suggesting that this method could be useful at industrial scale to treat waters contaminated with pharmaceuticals.

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Electro-Oxidation of the Pharmaceutical Furosemide: Kinetics, Mechanism and by-products

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Chapter 5

Electro-Oxidation of the Pharmaceutical Furosemide: Kinetics, Mechanism and By-products.

Graphical abstract



Abstract:

Furosemide is a widely prescribed diuretic whose unambiguous presence in water sources appointed it as an emerging pollutant. This work focuses on the electrochemical degradation of this drug in aqueous solutions by the Electrochemical Advanced Oxidation Processes (EAOPs) electro-Fenton and anodic oxidation, using Pt/carbon-felt and BDD/carbon-felt cells with H_2O_2 electrogeneneration. The higher oxidation power of the electro-Fenton process using a BDD anode was demonstrated. The oxidative degradation of furosemide by the electrochemically generated [•]OH radicals follows a pseudo-first order kinetics. The absolute rate constant of the oxidation reaction of furosemide by [•]OH n was determined using competition kinetics method and found to be 3.4 x 10^9 M⁻¹ s⁻¹. The evolution of the TOC removal during treatment as mineralization efficiency parameter was investigated. It was found that the electrochemical degradation of the furosemide yields to the formation of aromatic by-products which are afterwards oxidized to aliphatic carboxylic acids before their conversion to CO₂ and inorganic ions (NH₄⁺, NO₃⁻, Cl⁻ and

SO₄²⁻). Toxicity assessment by the Microtox[®] method revealed that oxidation reaction intermediates more toxic than the parent molecule are formed. Nevertheless, the overall results confirm the high effectiveness of anodic oxidation and electro-Fenton processes for the removal of the furosemide and its by-products from aqueous media.

Keywords: Furosemide, Emerging pollutants, Anodic oxidation, BDD, Electro-Fenton.

5.1. Introduction

Over the last years, pharmaceuticals have been receiving increasing attention as potential bioactive chemicals in the environment (Kümmerer, 2009). These substances are considered as emerging pollutants because they still remain unregulated or are currently undergoing a regularization process (Esplugas et al., 2007). Pharmaceuticals and their bioactive metabolites are continuously introduced in the aquatic environment. Their presence in natural water is attributable to personal hygiene products, pharmaceutical industry waste, hospital waste and therapeutically drugs. Pharmaceuticals and their metabolites or degradation products accumulate in the environment mainly because of their incomplete elimination in wastewater sewage plants due to their relative recalcitrance to the process such as biodegradation, deconjugation, sorption and photodegradation (Jones et al., 2005). The presence and occurrence of these compounds affect the water quality and may constitute a potential risk of toxicity for the ecosystems and the human and animal well-being in the long term (Klavarioti et al., 2009). It is usually accepted that some of them may cause long-term, irreversible changes to micro-organisms genome, even at low content, increasing their resistance to them. Furthermore, some drugs have been classified as endocrine disrupting compounds. In addition, these pollutants often occur as complex mixtures whose toxicity has been seldom predicted (Sirés and Brillas, 2012).

Furosemide (FRSM) is a sulfonamide derived from antranilic acid. It is classified as loop diuretic and it is widely prescribed for the treatment of oedematous states and hypertension (Pichette and Du Souich, 1996). It has been unambiguously detected both in sewage treatment plants and rivers (Castiglioni et al., 2005; Rosal et al., 2010). Furthermore, furosemide has been classified as one of the highest risk compounds by a model dealing with the effects of contaminants on human health (Besse and Garric, 2008). This compound is not toxic in humans at therapeutic levels. However it has been associated with hypersensitivity and jaundice and has been found to show toxicity to some organisms (Peterson, 2012).

Advanced oxidation processes are known as effective treatment techniques for removing toxic and/or persistent organic pollutants from water (Pignatello et al., 2006; Oturan and Aaron, 2014). Along these processes, several electrochemical advanced oxidation processes (EAOPs) are being currently developed for water remediation, these technologies have shown high oxidation/mineralization efficiency in the removal of organic pollutants from wastewater (Oturan et al., 1992; Brillas et al., 2009; Sirés et al., 2014). These processes are based on the in-situ electrochemical generation of hydroxyl radicals ('OH) which can non-selectively oxidize any

organic pollutant leading to its almost complete mineralization to CO₂, water and inorganic ions when starting molecules contain heteroatoms. Their characteristics have been thoroughly reviewed with emphasis on electro Fenton (EF) and anodic oxidation (AO) processes (Brillas et al., 2009; Panizza and Cerisola, 2009; Rodrigo et al., 2010; Feng et al., 2013; Vasudevan and Oturan, 2014). Both of these powerful EAOPs use heterogeneous M(°OH) and homogeneous °OH formed at the anode and in the bulk solution respectively, as oxidizing agents. In the AO process, heterogeneous M(°OH) are formed on the anode surface according to Eq. (1) by oxidation of water (Bensalah et al., 2005; Cañizares et al., 2005; Panizza and Cerisola, 2005) on a high O₂-evolution overpotential anode (M) such as boron doped diamond (BDD) electrode :

 $BDD + H_2O \rightarrow BDD(^{\bullet}OH) + H^+ + e^-$

(1)

In the case of EF process, these radicals are homogeneously generated in the bulk solution through Fenton's reaction (Eq. (4)) via in situ electrogenerated Fenton's reagent (Eqs. (2) and (3)) using a suitable cathode fed with O_2 or air, along with the external addition of a catalytic amount (about 10^{-4} M) of a soluble iron(II) salt as source of Fe²⁺ ions (catalyst) (Oturan, 2000; Lahkimi et al., 2007; Brillas et al., 2009; Özcan et al., 2009). The electrochemical regeneration of Fe²⁺ (Eq. (3)) allows the catalysis of Fenton's reaction and continuous production of 'OH (Oturan et al., 2008a).

$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	(2)
$Fe^{3+} + e^{-} \rightarrow Fe^{2+}$	(3)
$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH^-$	(4)

EF process has advantage of producing oxidation reaction in the mass of the solution in contrast of AO in which the reaction is limited to anode surface. Several works have been reported during the last decade on destruction of different antibiotics and other drugs by these EAOPs using either a BDD or a Pt anode with different carbonaceous materials as cathodes (Sirés et al., 2007a, 2007b; Özcan et al., 2008; Dirany et al., 2011; Garcia-Sefura et al., 2011; Panizza and Oturan, 2011; Dirany et al., 2012; Garcia-Segura et al., 2011; El-Ghenymy et al., 2013; Ali). Recently Oturan et al. (Oturan et al., 2012) have shown that the performance of EF process can be significantly enhanced by its coupling with AO by using a BDD anode. In this case hydroxyl radicals are simultaneously produced both on the anode surface by water oxidation (Eq. (1)) and in the bulk of solution by Fenton's reaction (Eq. (4)).

Some studies have been already conducted on the treatment of FRSM in aqueous media (Pérez et al., 2010; Urtiaga et al., 2013) achieved good removal rates of FRSM and other drugs by combining AO process with others treatment technologies such as ultrafiltration and reverse osmoses. The heterogeneous photocatalytic degradation of FRSM and other pharmaceuticals has also been reported(Molinari et al., 2006). Nevertheless, there are no thorough reports concerning oxidative degradation of FRSM and mechanistic studies by EAOPs and furthermore its oxidation products formed from [•]OH attack haven not been yet identified, highlighting the environmental problematic due to the presence of medicinal pollutants and their environmental transformation products in the aquatic bodies.

In this study we applied EAOPs to remove FRSM, chosen as model pollutant, from water and through this example to highlight that EAOPs constitute very attractive technologies for the treatment of wastewaters contaminated by pharmaceutical pollutants. Therefore the present paper aims to investigate the mineralization of the diuretic FRSM by AO, EF and their coupling in order to clarify the roll of electrochemically generated hydroxyl radicals in the degradative oxidation processes utilizing different anode materials such as BDD and Pt electrodes, the effect of experimental parameters on FRSM decay and mineralization rate, and finally the identification of formed by-products to propose a general reaction mechanism for Furosemide mineralization.

5.2. Materials and Methods

5.2.1. Chemicals

Furosemide (> 99.0%) was purchased from TCI Europe N.V and used as received. Analytical grade anhydride sodium sulfate used as background electrolyte and heptahydrated iron (II) sulfate as catalyst (Fe²⁺) source, were purchased from Acros Organics. Reagent grade sulfuric acid supplied by Merck was used to adjust their initial pH to 3.0. All the other chemicals used were either of HPLC or analytical grade purchased from Prolabo, Fluka and Acros Organics. All solutions were prepared with high-purity water from a Millipore Milli–Q system with resistivity >18 M Ω cm.

5.2.2. Instruments and analytical procedures

Electrolyses were performed with a Hameg HM8040 triple power supply at constant current. Solution pH was measured with a CyberScan pH 1500 pH-meter. Mineralization of the FRSM aqueous solutions was assessed from the decay of dissolved organic carbon determined by a Shimadzu VCSH TOC analyser. The time course of the concentration of FRSM was followed by reversed-phase HPLC using a Merck Lachrom liquid chromatograph equipped with a L-7100 pump, fitted with a RP-18 (5µm, 4,6 mm x 250 mm) column at 40 °C and coupled with a L-7455 UV-Vis

detector selected at 240 nm (optimal wavelength for FRSM absorption). All the analyses were carried out isocratically with a mobile phase consisting in a 45:55 (v/v) methanol/H₃PO₄ mixture at pH 3 at 0.7 mL min⁻¹ flow rate. The generated aliphatic acids were identified and quantified by ion-exclusion HPLC technique using an Altech liquid chromatograph equipped with a Model 426 pump, fitted with a Supelco, Supelcogel H 9 mm, 25 cm x 4.6 mm column at room temperature and coupled with a Dionex AD20 UV detector selected at 210 nm. A 1% H₂SO₄ solution at a flow rate of 0.2 mL min⁻¹ was used as the mobile phase.

The aromatic oxidation products formed med during electrochemical treatment were identified mass spectrometry analysis using an electrospray time of flight mass spectrometer (ESI-TOF-MS) operating in positive mode (QSTAR Pulsar I from Applied Biosystems).

The inorganic ions released in the treated solutions were determined by ion chromatography using a Dionex ICS-1000 Basic Ion Chromatography System. The analysis of anions was monitored using an IonPac AS4A-SC, 25 cm x 4 mm, anion exchange column linked to an IonPacAG4A-SC, 5 cm x 4 mm column guard. For the determination of the cations, an IonPac CS12A, 25 cm x 4 mm, cation exchange column linked to an IonPac CG12A, 5 cm x 4 mm column guard, was used. The system was equipped with a DS6 conductivity detector containing a cell heated at 35° C.

5.2.3. Electrochemical treatments

Electrolyses were carried out in a single-compartment mixed cell of 250 mL capacity. Electro-Fenton treatments were performed using either a cylindrical Pt mesh of 4.5 cm of height and 3 cm of internal diameter (EF-Pt) or a thin-film BDD electrode (25 cm²) (EF-BDD) as anode, and a large surface area carbon-felt piece (Carbone-Lorraine) as cathode. In all cases the anode was centred in the electrolytic cell and was surrounded by the cathode, which covered the inner wall of the cell. Continuous saturation of solutions by oxygen at atmospheric pressure was ensured by bubbling compressed air into the system.

The electrolyses of aqueous solutions containing up to 0.1 mM FRSM with 0.1 mM Fe²⁺ as catalyst were assessed in 0.05 M Na₂SO₄ at pH 3.0 and room temperature under the application of a constant current in the range of 50-500 mA. All the experiments were performed with solutions of 230 mL volume with a vigorous stirring by a magnetic bar.

5.2.4. Toxicity measurements

Toxicity measurements were performed using the Microtox[®] method, based on the determination of the inhibition of bioluminescence of the marine bacteria *V. fischeri*. A luminometer Berthold Autolumat Plus LB 953 was employed, according to the international procedure (OIN 11348-3). The bacteria and the activation reagent, LCK 487 LUMISTOX, were provided by Hach Lange France SAS. The measurements were performed on samples collected from 0.1 mM FRSM solutions having undergone the electro-oxidation treatment for regular time periods. Constant currents of 500 and 1000 mA were applied. In all cases the bacteria's bioluminescence intensity was measured after 5 min of exposition to the samples at 15 °C.

5.3. Results and Discussion

5.3.1. Analysis of the oxidation kinetics

The applied current is the most important parameter in the EAOPs since the formation of hydroxyl radicals is controlled by this parameter through Eqs. (1) – (4). To clarify the effect of this parameter, the oxidative degradation of 230 mL FRSM solutions with 0.05 M Na₂SO₄ in presence of 0.1 mM Fe²⁺ was carried out by applying currents in the range of 100 to 500 mA. Results depicted in Fig. 5.1 show the effect of the applied current on the decay kinetics of 0.1 mM FRSM solutions by EF with Pt/carbon-felt (EF-Pt) and BDD/carbon-felt (EF-BDD) cells, and AO, without Fe²⁺ addition, using a BDD/carbon-felt cell including H₂O₂ electrogeneration (AO-H₂O₂).

It can be seen that a complete degradation of the drug was achieved in all cases, however with a slower rate by AO-H₂O₂ process. The total disappearance of FRSM needs 5, 7 and 60 min for EF-BDD, EF-Pt and AO-H₂O₂ cells respectively. In the anodic oxidation, heterogeneous BDD(•OH) are generated on the anode surface by oxidation of water in addition of weaker oxidant H₂O₂ formed at carbon-felt cathode, whereas in EF these hydroxyl radicals are produced both at the anode surface (Eq. (1) as well as in homogeneous medium from Fenton reaction (Eq. (4)) between electrogenerated H₂O₂ and electrochemically regenerated Fe²⁺ (catalyst), making its performance superior because of the greater generation of these radicals. Therefore, the oxidation power of this EF-BDD cell is better since pharmaceutical molecules are oxidized both at the surface of the anode and in the bulk solution. It is important to notice that mass transport limitations inherent to an electrode process can be as well minimized when applying this process. Concerning the application of a different anode material during EF process (BDD/carbon-felt and Pt/carbon-felt systems), the former cell exhibits better performance due to the higher oxidation power of the BDD anode compared to the Pt one (Oturan et al. 2012). This can be attributed to the high $O_{2^{-}}$ evolution overpotential and the adsorption mode (physisorption) of hydroxyl radicals in the case of the former.



Figure 5.1. Time course of FRSM concentration during different electrochemical treatments of 230 mL, 0.1 mM of FRSM in 0.05 M Na₂SO₄ at pH 3.0 and room temperature. (a) Pt/carbon-felt (EF-Pt) cell with $[Fe^{2+}] = 0.1$ mM (b) BDD/carbon-felt (EF-BDD) cell with $[Fe^{2+}] = 0.1$ mM and (c) BDD/carbon-felt (AO-H₂O₂) without Fe²⁺ addition. (\diamondsuit): 100 mA, (\blacksquare): 200 mA, (\bigtriangleup): 300 mA, (\bigcirc): 400 mA, (*) 500: mA. The inset panel shows the corresponding kinetic analysis assuming a pseudo-first-order reaction.

As can be seen from Fig. 5.1, a shorter time was needed for the total disappearance of FRSM at higher currents as expected from the higher [•]OH and BDD([•]OH) production rate from Eqs. (1)-(4). However, the application of a higher current did not accelerate the oxidation process after

reaching an optimum value, which can be explained by the progressive enhancement of the parasitic reactions, mainly the H₂ evolution at the cathode and the rapid oxidation of [•]OH to O₂ at the anode, evidencing that a current oversupply leads to a current waste and decrease the efficiency of the treatment. In Tab. 5.1, the apparent rate constants for the oxidative degradation of FRSM are summarized. The decay of the drug concentration with time is in good agreement with the pseudo first-order reaction kinetics with excellent linear correlations (as can be shown in the inset panel depicted in Fig. 5.1), suggesting that a constant [•]OH/BDD([•]OH) concentration reacts with FRSM at a given current. This assumption can be made considering "quasi-stationary state" hypothesis for the concentration of hydroxyl radicals as they are very reactive (no accumulation in the medium) and thus have a very short life span of a few nanoseconds.

Table 5.1. Apparent rate constants and degradation times for complete disappearance of FRSM by the applied EAOPs, assuming a pseudo-first order kinetics for FRSM's oxidation by [•]OH.

	Degradation time (min)		k _{app} (min ⁻¹)	
Process / I (mA)	300	500	300	500
EF (anode Pt)	10	5	0.91	1.09
EF (anode BDD)	7	5	0.99	1.19
A0-H ₂ O ₂	60	45	0.09	0.15

The absolute rate constant (k_{FRSM}) of the oxidation of FRSM by hydroxyl radicals was also determined, conducting competition kinetic experiments, using equal concentrations of both, FRSM and a standard competition substrate, the p-hydroxybenzoic acid (p-HBA) with a well-defined absolute rate constant ($k_{\text{p-HBA}} = 2.19 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$) (Beltrán et al., 2009). The absolute rate constant was then calculated from Eq. (5):

$$k_{FRSM} = k_{p-HBA} \frac{[k_{app,FRSM}]}{[k_{app,p-HBA}]}$$
(5)

And a value of $k_{\text{FRSM}} = 3.39 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, was obtained. This value is of the same order of rate constants reported for the oxidation of other pharmaceuticals (Brillas et al., 2009; Dirany et al., 2012; Sirés and Brillas, 2012) but differs significantly from the one reported by Wols et al. 2013

who estimated a kinetic constant of $1.1 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$ from the UV/H₂O₂ photocatalytic degradation of furosemide.

5.3.2. Effect of current intensity in the mineralization efficiency

The mineralization kinetics (in terms of TOC removal) of 0.1 mM FRSM aqueous solution vs. electrolysis time as function of the applied current is given in Fig. 5.2. As can be seen, the mineralization rate was higher as increasing the current, confirming the trend observed for oxidation kinetics, according to the greater production rate of 'OH/BDD('OH) at higher current values for hours without observing the fouling of the electrodes. This increase in the mineralization rate in function of current rise can be explained in terms of the greater amount of BDD('OH) produced at the surface of the anode (AO-H₂O₂) and supplementary 'OH generated in the bulk (EF-BDD) which can oxidize more quickly both, the FRSM and its oxidation intermediates. The mineralization of the drug solution is almost complete (more than 95% TOC removal) after 4 h treatment, when applying the EF-BDD and AO-H₂O₂ processes. The very slow change after this time, suggests that the residual TOC is formed by some refractory intermediates, mainly carboxylic acids. This behavior can be clearly observed on the Fig. 5.2b showing the dramatic drop in mineralization efficiency (MCE) with increasing currents values determined according to Eq. (6) (Brillas et al. 2009):

$$MCE(\%) = \frac{nFV_{s}\Delta(TOC)_{exp}}{4.32x10^{7}mlt} 100$$
(6)

where $\Delta(TOC)_{exp}$ is the experimental TOC decay (mg L⁻¹), t is the treatment time (h), F is the Faraday constant (96487 C mol⁻¹), V_s is the volume of treated solution (L), 4.32 x 10⁷ is a conversion factor to homogenize units (3600 s h⁻¹ x 12,000 mg mol⁻¹), *m* is the number of carbon atoms (12) in FRSM molecule and *I* is the applied current (A). The number *n* of electrons consumed per FRSM molecule was taken as 48 according to reaction (7) and considering transformation of N atoms of FRSM to NH₄⁺.

$$C_{12}H_{11}N_{2}CIO_{5}S + 23H_{2}O \rightarrow 12CO_{2} + 2NH_{4}^{+} + CI^{-} + SO_{4}^{2-} + 49H^{+} + 48e^{-}$$
(7)

The calculated MCE values are depicted in the inset panel of Fig. 5.2b. An opposite tendency can be observed while the efficiency drops as current increases. It can be related to a

gradual loss in the relative quantity of generated BDD([•]OH) and [•]OH in the wasting (parallel nonoxidizing) reactions for which reaction rate enhanced by rising applied current value. These parasitic reactions primordially include the oxidation of BDD([•]OH) via reaction (8), the dimerization of [•]OH from reaction (9), and reaction of [•]OH with Fenton's reagent (reactions (10) and (11)) (Brillas et al., 2009; Panizza and Oturan, 2011). The quicker generation of some other oxidants at the BDD anode, like the peroxodisulfate ($S_2O_8^{2-}$) ion and ozone by reactions (12) and (13), respectively, can also contribute to the fall of the amount of generated BDD([•]OH).



Figure 5.2. TOC removal vs. electrolysis time for the mineralization of 230 mL of 0.1 mM FRSM aqueous solution in 0.05 M Na₂SO₄ at pH 3.0 and room temperature: (a) Pt/carbon-felt (EF-Pt) cell with $[Fe^{2+}] = 0.1$ mM, (b) BDD/carbon-felt (EF-BDD) with $[Fe^{2+}] = 0.1$ mM, and (c) (BDD/carbon-felt (AO-H₂O₂ without Fe²⁺ addition. (\diamondsuit): 100 mA, (\blacksquare): 200 mA, (\bigtriangleup): 300 mA, (\blacklozenge): 400 mA, (\bigstar): 500 mA, (\Box): 1000 mA.

$2 \text{ BDD}(^{\bullet}\text{OH}) \rightarrow 2 \text{ BDD} + \text{O}_2 + 2\text{H}^+ + 2 \text{ e}^-$	(8)
$2 ^{\circ}\text{OH} \rightarrow \text{H}_2\text{O}_2$	(9)
$Fe^{2+} + OH \rightarrow Fe^{3+} + OH^{-}$	(10)
$H_2O_2 + {}^{\bullet}OH \rightarrow HO_2{}^{\bullet} + H_2O$	(11)
$2 \text{ SO}_4^{2-} \rightarrow \text{S}_2 \text{O}_8^{2-} + 2 \text{ e}^{-}$	(12)
$3 \text{ H}_2\text{O} \rightarrow \text{O}_3 + 6 \text{ H}^+ + 6 \text{ e}^-$	(13)

Fig. 2 highlights that the use of the BDD anode in AO and EF processes produce a remarkable acceleration of the TOC removal. This is due to its much greater ability to produce active radicals, making this kind of electrode able to completely degrade all the by-products, even those refractory to EF-Pt (Oturan et al., 2012). This fact confirms that the nature of the anode plays a significant role in the oxidation of organic compounds by EAOPs, specially observed at low current values because at higher current values the oxidation in the bulk solution became the predominant mechanism. Consequently the EF-BDD cell seems to be very efficient for the treatment of FRSM solutions, being even better than anodic oxidation alone, because of the mass transport limitation inherent to this process and limitation of the oxidation process to anode surface.

5.3.3. Mineralization pathway: Analysis of oxidation intermediates and end-products

5.3.3.1. Identification of aromatic by-products

Oxidation of FRSM leads to the formation of a number of aromatic oxidation products which are subjected to further oxidation reactions with hydroxyl radicals generated in the EAOPs applied. To clarify the mineralization pathway, the aromatic intermediates were identified by ESI-TOF-MS analysis. Based on the identified aromatic intermediates, aliphatic end-products and released inorganic ions as well as TOC removal results, a plausible mineralization reaction pathway is proposed in Fig. 5.3.

According to the structure of FRSM we can clearly see that there are two sites strongly active to oxidation: the furan ring and the nitrogen atom of the amino group. The identification of aromatic intermediates is in accordance with this observation since products resulting from the attack of **°OH/BDD(°OH)** to the furan ring, which can afterwards suffer intramolecular rearrangements, especially those from the nucleophilic attack to the carbonyl group, were found (structures from A to C), as well as the intermediates coming from the breakage of FRSM following

the oxidative attack to the amine group (structures F1, F2 and HF). The identified intermediates as aniline (H), the γ -ketoenal (B) and the pyridinium derivate (C) have also been identified as the resulting products from FRSM by metabolic oxidation processes (Hezari and Davis, 1992; Antoine et al., 2007; Chen and Burka, 2007). Furthermore, aniline was as well obtained by electrochemical oxidation of FRSM (Laurencé et al., 2011). Additionally, it is well known that the addition of [•]OH on unsaturated bond (hydroxylation) is one of the main mechanisms of the reaction between [•]OH and organic compounds having unsaturated bonds (Pignatello et al., 2006; Brillas et al., 2009), in well agreement with the formation of the observed hydroxylated by-products: structure A from FRSM, E from hydroxylation of C and I from hydroxylation of G (with oxidation of amine group) and/or from H with desulfonation/deamination). Structure J can be formed by electrochemical dechlorination at the cathode.



Figure 5.3. Suggested mineralization pathway for the mineralization of FRSM by hydroxyl radicals following identified aromatic/aliphatic intermediates, inorganic ions and TOC removal value.

5.3.3.2. Formation and evolution of short-chain carboxylic acids and inorganic ions.

The cleavage of the benzenic ring of aromatic products and the oxidation of their lateral groups are expected to form short-chain carboxylic acids (Brillas et al., 2009; Garcia-Segura et al., 2012; El-Ghenymy et al., 2013; Oturan, 2014) whose formation and evolution were followed by ion-exclusion HPLC and results were depicted in Fig. 4. Acetic (K), pyruvic (L), maleic (M), oxamic (N), formic (O) and oxalic (P) acids were identified (see Fig. 5.4). Oxidation of pyruvic and maleic acids can conduct to the formation of oxalic and formic acids (M. A. Oturan et al., 2008b; Sirés and Brillas, 2012), while oxamic acid can be formed from precedent amino compounds. Oxamic, oxalic and formic acids are afterwards directly oxidized to CO₂ (Brillas et al. 2009). These compounds require longer destruction times due to their lower reactivity with hydroxyl radicals constituting residual TOC on longer treatment times. It is also important to note that carboxylic acids were more efficiently degraded in the BDD/carbon-felt (EF-BDD) cell, in agreement with the greater mineralization power of BDD([•]OH) added to [•]OH formed in the bulk and therefore the faster TOC removal efficiency with this cell . A simple mass balance at 60 min of EF-BDD and AO-H₂O₂ at 300 mA indicated that the carboxylic acids represented 66% and 33% of TOC content, respectively, of the treated solution, this contribution being more than 90% at 6 h treatment in both cases. The release and evolution of inorganic ions (NH_4^+ , NO_3^- , Cl^- and SO_4^{-2-}) from the heteroatoms N, Cl and S present initially in the structure of FRSM was assessed by ion chromatography. Fig. 5.5 shows that NH_4^+ , NO_3^- , Cl⁻ and SO_4^{2-} ions were accumulated in the solution progressively from the beginning of the electrolysis. It can be seen that NH4⁺ is the most largely accumulated ion in agreement with the presence of amine and the aminosulfonyl (H₂N-SO₂-) groups which are highly susceptible to oxidation, leading to the generation of this ion. NO₃ is formed in a less extent and not accumulated in the medium because of its oxidation to N₂ mainly by BDD([•]OH). Another explanation to non-accumulation of NO3⁻ can be related to its electrochemical reduction on the carbon-felt cathode to nitrogen gas, ammonia and hydroxylamine according to Eqs. (14) - (17) as reported in the literature (Lévy-Clément et al., 2003; Mook et al., 2012).

$$NO_3^{-} + 3H_2O + 5e^{-} \rightarrow 1/2N_2 + 9OH^{-}$$
 (14)

$$NO_3 + 6H_2O + 8e^- \rightarrow NH_3 + 9OH^-$$
(15)

 $NO_3^{-} + H_2O + 2e^{-} \rightarrow NO_2^{-} + 2OH^{-}$ (16)

$$NO_2^{-} + 4H_2O + 4e^{-} \rightarrow NH_4OH + 5OH^{-}$$
(17)



Figure 5.4. Time-course of the concentration of the main short-chain carboxylic acids accumulated in solution during the mineralization of 230 mL of 0.1 mM FRSM aqueous solution in 0.05 M Na₂SO₄ at pH 3.0 and room temperature: (a) BDD/carbon-felt (EF-BDD) at 300 mA with $[Fe^{2+}] = 0.1$ mM, and (b) BDD/carbon-felt (AO-H₂O₂) at 300 mA without Fe²⁺. (\diamondsuit): oxalic acid, (\blacksquare): oxamic acid, (\bigtriangleup): formic acid, (\bullet): pyruvic acid, (\star): maleic acid, (\Box): acetic acid.

Thus, the total nitrogen concentration $(NH_4^+ \text{ and } NO_3^-)$ attained almost 90% of the initial nitrogen amount at the end of the treatment for both with EF-BDD and AO-H₂O₂ cells. On the other hand, as shown in Fig. 5.5, Cl⁻ does not reach the total initial content of 0.1 mM. During AO it is slowly generated from the BDD([•]OH) attack on chloroorganics and oxidized to chorine at the BDD anode (Martinez-Huitle and Brillas, 2008). This phenomenon can be better observed for EF-BDD process where Cl⁻ was more rapidly released due to the greater oxidation ability of BDD([•]OH)/[•]OH and then its quick oxidation to Cl₂.Concerning SO₄²⁻ ions, they are rapidly released from the beginning of the electrolysis, accounting the total amount of the initial S in FRSM molecule at the end of the treatment. These results bring additional information on the mineralization of FRSM solution in complement of TOC abatement measurement.



Figure 5.5. Time-course of the formation and evolution of inorganic ions released during the electrolysis of 230 mL 0.1 mM of FRSM in 0.05 M Na₂SO₄ at 300 mA, pH 3.0 and room temperature: (a) BDD/carbon-felt cell (EF-BDD) with $[Fe^{2+}] = 0.1$ mM and (b) BDD/carbon-felt (AO-H₂O₂) cell without Fe²⁺. (\diamondsuit): ammonium, (\blacksquare): nitrate, (\bigtriangleup): sulfate, (\blacklozenge): chloride.

5.3.4. Evolution of toxicity during mineralization treatment of FRSM aqueous solution.

The evolution of solution toxicity in term of the percentage of inhibition of luminescence of *V. fischeri* marine bacteria as a function of electrolysis time at 500 and 1000 mA with EF-BDD and AO-H₂O₂ cells after 15 min of exposure time, was studied (Fig. 5.6), aiming to conduct an approximate assessment of the potential risks derived from the presence of this drug (and also others) in the environment, taking into account the lack of data dealing with the ecotoxicity of pharmaceuticals and their metabolites or other environmental transformation products (Laurencé et al., 2014). It can be seen from Fig. 5.6 that in all cases the rate of inhibition of bacterial luminescence increases from early stages quickly reaching high values up to 99%, which can be explained by formation of aromatic intermediates more toxic than FRSM in early treatment times. The continuous generation and destruction of the different oxidation intermediates resulting from the attacks of hydroxyl radicals is responsible of the observed fluctuations in the toxicity of treated solutions. The rapid decrease in bacteria luminescence inhibition, after having reached a maximum value, indicates a drop in toxicity that is completely in agreement with the

disappearance of cyclic compounds reflected in the TOC decrease. Thereby, the variation in the solution toxicity during the first two h of electrolysis is due to the presence of various degradation by-products with different toxicities. After this time, the diminution of the toxicity is consistent with the decrease in the TOC value, indicating the disappearance of aromatic/cyclic compounds which are responsible of the toxicity. The low luminescence inhibition rate in the later treatment stages demonstrates the effectiveness of the electro-oxidation with EF-BDD and AO-H₂O₂ processes using BDD anode. The residual toxicity is due to the presence of carboxylic acids that are readily biodegradable. This behavior has also been reported in previous works (Dantas et al., 2008; Dirany et al., 2011).



Figure 5.6. Evolution of solution toxicity (in term of inhibition of the luminescence of *Vribrio fischeri* bacteria, after 5 min of exposure) during the mineralization of 0.1 mM FRSM in 0.05 M Na₂SO₄ at pH 3, using: a) BDD/carbon-felt cell (EF-BDD) with $[Fe^{2+}] = 0.1$ mM and (b) BDD/carbon-felt cell (AO-H₂O₂) without Fe²⁺. (\diamondsuit): 500 mA and (\blacksquare): 1000 mA.

5.4. Conclusion

The EAOPs: EF-Pt (Pt/carbon-felt), EF-BDD (BDD/carbon-felt) and AO-H₂O₂ (BDD/Carbon-felt without Fe²⁺) studied in this work were found to be very effective methods for the complete degradation and almost total mineralization of the diuretic FRSM. The use of the 3D carbon-felt cathode ensured the continuous electrogeneration of H₂O₂ and regeneration of Fe²⁺, whereas the BDD anode produced large amounts of active BDD([•]OH) radicals, making EF-BDD and AO-H₂O₂ the

most efficient processes for efficient removal of FRSM from water, achieving complete destruction of the drug and 95% TOC abatement at the end of 8 h treatment while the mineralization rate was only 85% in the case of EF-Pt cell.

Short-chain aliphatic carboxylic acids were identified as aliphatic by-products and the almost overall mineralization of the pharmaceutical was confirmed by the quasi quantitative release of inorganic ions $SO_4^{2^-}$, NO_3^- , NH_4^+ and CI^- .

The toxicity assessment showed the formation of intermediates that are more toxic than FRSM itself, although total detoxification was attained at the end of the electrochemical treatment, thereby demonstrating the effectiveness of electro-oxidation with BDD anode in EAOPs.

All these results highlight that the EAOPs are viable environmentally friendly technologies for the remediation of wastewaters containing pharmaceutical residues and their degradation metabolites.

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CHAPTER 6

A Combined Electro-Fenton Pre-Treatment and a Biological Process for the Mineralization of the Pharmaceuticals Furosemide and Ranitidine

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Chapter 6

A Combined Electro-Fenton Pre-Treatment and a Biological Process for the Mineralization of the Pharmaceuticals Furosemide and Ranitidine

Abstract:

A coupled electro-Fenton – biological treatment has been employed to enhance the biodegradability and complete the mineralization of the pharmaceuticals Furosemide (FRSM) and Ranitidine (RNTD). In the first part, the electrochemical degradation of the drugs in aqueous solution was assessed by the electro-Fenton process (EF), using a BDD/carbon-felt cell with H_2O_2 electrogenenration. The evolution of the TOC removal during electrolysis as mineralization efficiency parameter was investigated. It was found that the electrochemical degradation of the drugs yields to the formation of cyclic/aromatic by-products which are afterwards oxidized to aliphatic carboxylic acids, which can be more easily biodegraded, before their conversion to CO_2 and inorganic ions (NH_4^+ , NO_3^- , CI^- and $SO_4^{2^-}$). Toxicity evaluation by the Microtox® method revealed that oxidation intermediates are more toxic than the parent molecules, but solution toxicity is also considerably decreased during electro-Fenton pre-treatment. Thereafter, the application of an aerobic treatment was conducted according to the enhancement of the biodegradability of the FRSM and RNTD solutions (BOD₅/COD ratios of 0.41 and 0.37, respectively) and the decrease of the toxicity after electrolysis. A significant part of the by-products resulting from the electrolysis were satisfactorily removed by means of the aerobic treatment.

Keywords: Furosemide, Ranitidine, Anodic oxidation, BDD, Electro-Fenton, Biodegradability.

6.1. Introduction

The presence of pharmaceutical residues in the environment is now considered as an important environmental problem owing to the health and environmental hazards associated with these bioactive chemicals. There is not concise legislation concerning these so-called emerging pollutants and their regularization procedures are in process (Espulgas et al., 2007; Kümmerer, 2009). The main sources introducing pharmaceuticals and their transformation products to the environment are household residues, pharmaceutical and veterinarian industry, hospital waste, and the effluents from wastewater treatments plants, as they are recalcitrant to the used conventional processes for wastewater treatment (Klavariotu et al. 2009).

At current environmental concentration (normally found at trace levels), pharmaceuticals have been associated with adverse developmental effects in aquatic organisms as well as on human health. Some of them are classified as endocrine disrupting compounds. In addition, health effects arise from the synergistic action of mixtures of drugs and micropollutants form other sources (Khetan and Collins, 2007; Sirés et al., 2012).

The very widely prescribed drugs Furosemide and Ranitidine, have been detected in sewage treatment plants effluents and natural aquatic bodies (Kolpin et al., 2002; Castiglioni et al., 2005; Castiglioni et al., 2006; Gros et al., 2007; Conley et al., 2008; Rosal et al., 2010). FRSM is a loop diuretic prescribed for the treatment of edematous states and hypertension (Pichette et al., 1996), while RNTD is a H2-receptor antagonist employed for the treatment of peptic ulcer and gastroesophageal reflux disease (Khetan and Collins, 2007). FRSM has shown toxic effects to some organisms and it has been associated with hypersensitivity and jaundice (Peterson, 2012). Both drugs have been classified as one of the highest risk compounds by a model dealing with the effects of contaminants on human health (Besse and Garric, 2008).

Biological processes, the most utilized technologies for wastewater treatment, do not provide satisfactory results for the degradation of these persistent contaminants, since many of them are toxic or resistant to the applied microorganisms (Pulgarin and Kiwi, 1996; Muñoz and Guieysse, 2006). Advanced oxidation processes are known as effective treatment techniques for removing toxic and/or persistent organic pollutants from water (Pignatello et al., 2006; Oturan and Aaron, 2014). Among these oxidative technologies, several electrochemical advanced oxidation processes (EAOPs) are being currently developed for water remediation, showing high oxidation/mineralization efficiency in the removal of organic pollutants (Oturan et al., 1992; Brillas et al., 2009; Sirés and Brillas, 2012; Sirés et al., 2014). These processes are based on the in-situ electrochemical generation of hydroxyl radicals ('OH) which non-selectively oxidize any organic pollutant leading to its almost complete mineralization to CO₂, water and inorganic ions when starting molecules contain heteroatoms. Their characteristics have been thoroughly reviewed with emphasis on electro Fenton (EF) and anodic oxidation (AO) processes (Brillas et al., 2009; Panizza and Cerisola, 2009; Rodrigo et al., 2010; Feng et al., 2013; Vasudevan and Oturan, 2014). Both of these powerful EAOPs use heterogeneous M('OH) and homogeneous 'OH formed at the anode and in the bulk solution respectively, as oxidizing agents. In the AO process, heterogeneous M('OH) are formed on the anode surface according to Eq. (1) by oxidation of water (Panizza and Cerisola, 2005; Bensalah et al., 2005; Cañizares et al., 2005) on a high O₂-evolution overpotential anode (M) such as boron doped diamond (BDD) electrode :)

 $BDD + H_2O \rightarrow BDD(^{\bullet}OH) + H^+ + e^-$

(1)

(3)

In the case of EF process, hydroxyl radicals are homogeneously generated in the bulk solution through Fenton's reaction (Eq. (4)) via in situ electrogenerated Fenton's reagent (Eqs. (2) and (3)) using a suitable cathode fed with O_2 or air, along with the external addition of a catalytic amount (about 10^{-4} M) of a soluble iron(II) salt as source of Fe²⁺ ions (catalyst) (Oturan, 2000; Lahkimi et al., 2007; Brillas et al., 2009; Özcan et al., 2009). The electrochemical regeneration of Fe²⁺ (Eq. (3)) allows the catalysis of Fenton's reaction and continuous production of 'OH (Oturan et al., 2011).

$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	(2)
	· · ·

 $Fe^{3+} + e^- \rightarrow Fe^{2+}$

 $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH$ (4)

EF process has advantage of producing oxidation reaction in the mass of the solution in contrast of AO in which the reaction is limited to anode surface. Different carbonaceous materials have been used as cathodes for the electrochemical degradation of several pharmaceuticals (Özcan et al., 2007; Sirés et al., 2007a, 2007b; Dirany et al., 2011, 2012; Panizza and Oturan, 2011; El-Ghenymy et al., 2013; Garcia-Segura et al., 2014). Recently Oturan et al. (2012) have shown that the performance of EF process can be significantly enhanced by its coupling with AO by using a BDD anode. In this case hydroxyl radicals are simultaneously produced both on the anode surface by water oxidation (Eq. (1)) and in the bulk of solution by Fenton's reaction (Eq. (4)).

In general, complete mineralization of persistent organics is generally expensive when applying AOPs because the oxidation intermediates formed during the treatment tend to be more and more resistant to oxidation and hence they necessitate increasing treatment times and consequently consume more energy. As an attractive and very promising alternative, the application of these AOPs in a pre-treatment has been studied by different authors, aiming to transform the initially persistent organic compounds into more biodegradable intermediates that can then be biologically treated at considerably lower costs. In this manner, the AOP pre-treatment improves biodegradability, permitting the application of a biological treatment (Jeworski and Heinzle, 2000; Tabrizi and Mehrvar, 2004; Oller et al., 2011; Ganzanko et al., 2014).

The present work deals with the potentiality of the combination of an electro-Fenton pretreatment and a biological process as a promising and feasible technology for the removal of pharmaceutical residues in water, highlighting the great ability of the EF process for the increasing of biodegradability and the detoxification of the treated solutions. On our behalf, few studies, including the recent works by Foucade's group (Fontmorin et al., 2014; Mansour et al., 2014; Ferrag-Siagh et al. 2013), have been reported concerning these EF-biological coupled systems for the degradation of pharmaceutical pollutants (Ganzenko et al., 2014), which is imperative for a better understanding and further optimization of the process for upcoming applications.

6.2. Materials and Methods

6.2.1. Chemicals

Ranitidine hydrochloride (purity > 98%) was of reagent grade from Fluka and furosemide (> 99.0%) was purchased from TCI Europe N.V. Anhydride sodium sulfate used as background electrolyte and heptahydrated iron (II) sulfate used as catalyst in EF were of analytical grade from Acros Organics. All solutions were prepared with ultrapure water from a Millipore Milli–Q system with resistivity >18 M Ω cm. Reagent grade sulfuric acid from Merck was used to adjust their initial pH to 3.0. All the other chemicals used were either of HPLC or analytical grade from Prolabo, Fluka and Acros Organics.

6.2.2. Instruments and analytical procedures

Electrolysis were performed with a Hameg HM8040 triple power supply at constant current. Solution pH was measured with a CyberScan pH 1500 pH-metter. Mineralization of the pharmaceuticals aqueous solutions was assessed from the decay of dissolved organic carbon determined by a Shimadzu VCSH TOC analyzer. The time course of the concentration of FRSM and RNTD was followed by reversed-phase HPLC using a Merck Lachrom liquid chromatograph equipped with a L-7100 pump, fitted with a RP-18 (5 μ m, 4,6 mm x 250 mm) column at 40 °C and coupled with a L-7455 UV-Vis detector selected at 240 nm for FRSM and 280 nm for RNTD . All the analyses were carried out isocratically with a mobile phase consisting in a 45:55 (v/v) methanol/H₃PO₄ mixture at pH 3 at 0.7 mL min⁻¹ flow rate for FRSM and 28:72 (v/v) methanol/Amonium acetate 10 mM mixture, at 0.7 mL min⁻¹ for RNTD. The generated aliphatic acids were identified and quantified by ion-exclusion HPLC technique using an Altech liquid chromatograph equipped with a Model 426 pump, fitted with a Supelco, Supelcogel H 9 mm, 25 cm x 4.6 mm column at room temperature and coupled with a Dionex AD20 UV detector selected at 210 nm. A 1% H₂SO₄ solution at a flow rate of 0.2 mL min⁻¹ was used as the mobile phase.

6.2.3. Electrochemical treatments

Electrolysis were carried out in a single-compartment mixed cell of 230 mL capacity. Electro-Fenton treatments were performed using a thin-film BDD electrode (25 cm²) (EF-BDD) as anode, and a large surface area carbon-felt piece (Carbone-Lorraine) as cathode. In all cases the anode was centered in the electrolytic cell and was surrounded by the cathode, which covered the inner wall of the cell. Continuous saturation of solutions by oxygen at atmospheric pressure was ensured by bubbling compressed air into the system.

The electrolysis of aqueous solutions containing up to 0.1 mM FRSM or RNTD with 0.1 mM Fe^{2+} as catalyst were assessed in 0.05 M Na_2SO_4 at pH 3.0 and room temperature under the application of a constant current of 500 mA. All the experiments were performed with solutions of 230 mL volume with a vigorous stirring by a magnetic bar.

6.2.4. Toxicity measurements

Toxicity measurements were performed using the Microtox[®] method, based on the determination of the inhibition of bioluminescence of the marine bacteria *V. fischeri*. A luminometer Berthold Autolumat Plus LB 953 was employed, according to the international procedure (OIN 11348-3). The bacteria and the activation reagent, LCK 487 LUMISTOX, were provided by Hach Lange France SAS. The measurements were performed on samples collected from 0.1 mM FRSM or RNTD solutions having undergone the electro-oxidation treatment for regular time periods. A constant current of 500 mA were applied. In all cases the bacteria's bioluminescence intensity was measured after 5 min of exposition to the samples at 15 °C.

6.2.5. Biodegradability tests

A respirometric method was used to determine the Biochemical Oxygen Demand at 5 days (BOD₅) using the OxiTop[®] control system (WTW) (Reuschenbach et al., 2003). Bacteria extracted with KCl at 9 g L-1 (30 mL with 5 g of dried soil) and a IKA-MS1 mini-shaker (1800 rpm during 1 min) from a sample of uncontaminated soil, were added, just before adding the samples, to an aqueous solution saturated in oxygen (9.1 mg $O_2 L^{-1}$ at 20 °C) containing a phosphate buffer solution and a saline solution (Rodier et al., 2009). All the samples were adjusted to circum-natural pH. All the bottles containing the solutions were equipped with a rubber sleeve in which pure NaOH pellets were added to trap the CO₂ formed during biodegradation. The samples were incubated at 20 °C during 5 days in dark conditions. D(+)-Glucose. H₂O was used as a reference. A blank, representing the endogenous respiration, prepared with UPW and the seed solution was taken into account for calculation. The BOD₅ measured in each blank was insignificant compared to the BOD_5 of the samples and so causes no interference. Chemical oxygen demand (COD) analyses were carried out by a photometric method using a Spectroquant[®] NOVA 60 (merck) instrument. The samples were prepared adding 2 mL of each one into COD Cells test (15-300 mg O₂ L⁻¹ range) (Merck) and heating at 148 °C during 2 h in a Spectroquant[®] TR 420 (merck). The tubes were cooled to room temperature before analysis.

6.2.6. Aerobic treatment

12 pure cultures were selected from the collection of the laboratory of Molécules de Comunication et Adaptation des Micro-organismes of the Muséum National d'Histoire Naturelle. Cultures were maintained on agar slants (Streptomyces bacteria: ISP medium 2; other bacteria and filamentous fungi: bactopeptones 5 g/L, yeast extract 5 g/L, malt extract 5g/L, glucose 20 g/L and agar 20 g/L) and stored at 4° C. Liquid culture media containing (g/L) glucose 16, yeast extract 4, malt extract 10 and soybean peptones 5 (YMS medium) were sterilized without glucose at 120° C for 20 min. Flasks (100 mL) containing 50 mL of YMS culture medium were inoculated with glycerol suspension of microorganism and incubated at 30° C and 200 rpm for 60 h.

Biomasses were harvested by centrifugation (bacteria) or filtration (fungi) and directly suspended in 200 mL of the previously 2 h-electrolyzed solutions of FRSM and RNTD in the BDD-carbon felt cell. NaOH was added to adjust pH to 7.0. The experiments were performed at 30° C and 200 rpm for 7 days and monitored by ion-exclusion HPLC in order to follow the evolution of the carboxylic acids that had already been formed during the electrochemical treatment.

6.3. Results and discussion

6.3.1. Electrochemical oxidation assessment

In our previous works we studied the electrochemical degradation of RNTD and FRSM by the EF and the AO processes using a Pt or a BDD anode, determining the apparent rate constants of the oxidation of the drugs by the 'OH/BDD('OH), as well as the optimal conditions of the electrochemical treatment in means of the applied current and the catalyst concentration(Olvera-Vargas et al., 2014a; Olvera-Vargas et al., 2014b). According to the obtained results, EF and AO processes with a BDD anode and a carbon felt anode at 500 mA of applied current and 0.1 mM of Fe²⁺ as catalyst were chosen for the electrochemical pre-treatment of both drugs. Complete degradation of both drugs was achieved before 10 minutes of electrolysis.



Figure 6.1. TOC removal vs. electrolysis time for the mineralization of 230 mL of 0.1 mM of FRSM (\blacksquare) and RNTD (\blacksquare) aqueous solutions in 0.05 M Na₂SO₄ at pH 3.0, room temperature and 500 mA of current, using a BDD/carbon-felt (EF-BDD) cell with [Fe²⁺] = 0.1 mM.

The mineralization kinetics was measured in terms of TOC removal. Figure 6.1 shows the TOC decay of 0.1 mM FRSM and RNTD aqueous solution vs. electrolysis. It can be observed that the mineralization of the drugs' solutions is almost complete after 4 h treatment, reaching a 95% of TOC removal. The very slow change after this time suggests that the residual TOC is due to some refractory intermediates, mainly short-chain carboxylic acids. These results highlight the great ability of the BDD anode to quickly oxidize organic molecules and their cyclic/aromatic

intermediates by the large production of active radicals, both at the surface of the anode and the supplementary 'OH formed in the bulk solution (Oturan et al., 2012).

It is important to notice that a considerable TOC abatement is achieved during the first 2 hours of electrolysis; however, the remaining organic matter can (50% of the initial TOC after 1h-electrolysis and 30% after 2h) can be a source of carbon for the microorganisms during the biological phase.

The EF-BDD cell showed to be a very efficient technology for the treatment of both FRSM and RNTD solutions, achieving very good mineralization rates.

6.3.2. Mineralization pathway: Analysis of oxidation intermediates and end-products

Oxidation of FRSM and RNTD leads to the formation of a number of cyclic/aromatic oxidation products that undergo further oxidation reactions with hydroxyl radicals generated during the electrochemical treatment. The successive hydroxylation onto different positions of the different cyclic intermediates weakens them and promotes their final oxidative ring cleavage to yield several aliphatic compounds that are afterwards oxidized to short-chain carboxylic acids, along with the release of inorganic ions Cl⁻, $SO_4^{2^-}$, NH_4^+ and NO_3^- (Brillas et al., 2009; El-Ghenymy al. 2014, Oturan and Aaron 2014).

Figure 6.2 depicts a reaction pathway for the complete mineralization of FRSM and RNTD by [•]OH, adapted from Olvera-Vargas et al. It can be seen that Oxidation of FRSM and RNTD leads to the formation of a number of cyclic/aromatic oxidation products that are further oxidized until almost complete mineralization. The successive hydroxylation onto different positions of the cyclic intermediates weakens them and promotes their final oxidative ring cleavage to yield several aliphatic compounds that are afterwards oxidized to short-chain carboxylic acids, along with the release of inorganic ions Cl⁻, SO₄²⁻, NH₄⁺ and NO₃⁻, according to the heteroatoms present in the molecule (Oturan et al., 2008a; Brillas et al., 2009; El-Ghenymy al. 2014, Oturan and Aaron 2014).

The carboxylic acids resulting from the oxidation of cyclic compounds (acetic (I), pyruvic (II), maleic (III), oxamic (IV), formic (V) and oxalic (VI) in the case of FRSM; and acetic (I), pyruvic (II), oxamic (IV) formic (V) and oxalic (VI) and malic (VII) for RNTD) require longer destruction times due to their lower reactivity with the [•]OH, thus representing longer treatment times. Nevertheless, these compounds can be more easily assimilated by microorganisms, as they are simpler structures that can be incorporated into their metabolic routes as a source of energy.

After one hour of electrochemical treatment, more than 60% of the TOC content has already been achieved in all cases. Moreover, a simple mass balance at this stage of the EF-BDD process, at = 500 mA, indicated that the carboxylic acids represented 65.49% and 55.13% of the TOC content of the FRSM and RNTD treated solution, respectively.



Figure 6.2. Suggested mineralization pathway for the oxidation of a) FRSM and b) RNTD by hydroxyl radicals ([•]OH), according to the identified cyclic/aromatic and organic acids by-products.

6.3.3. Evolution of toxicity during mineralization treatment of the pharmaceuticals aqueous solution.

The evolution of the toxicity of the drugs solutions in term of the percentage of inhibition of luminescence of *V. fischeri* marine bacteria as a function of electrolysis time at 500 mA, with the EF-BDD cell after 5 min of exposure time, was studied (Figure 3) aiming to approximately assess the potential risks derived from the presence of these kind of compounds in the environment, taking into account the lack of data dealing with the ecotoxicity of pharmaceuticals and their transformation products (Laurencé et al., 2014). It can be seen from Figure 3 that in all cases the rate of inhibition of bacterial luminescence rapidly increases from the early stages of electrolysis, reaching high values up to 99%, evidencing the formation of aromatic/cyclic intermediates more toxic than the parent molecules. Consequently, the continuous generation and destruction of the different oxidation intermediates resulting from the attacks of hydroxyl radicals is responsible of the observed fluctuations in the toxicity of the treated solutions.



Figure 6.3. Evolution of solution toxicity (in terms of inhibition of the luminescence of Vribrio fischeri bacteria, after 5 min of exposure) during the mineralization of 0.1 mM FRSM (\blacklozenge) and RNTD (\blacksquare) solutions in 0.05 M Na₂SO₄ at pH 3 using: the BDD/carbon-felt cell (EF-BDD) at 500 mA with [Fe²⁺] = 0.1 mM: a) FRSM electrolysis and b) RNTD electrolysis.

The rapid decrease in bacteria luminescence inhibition, after having reached a maximum value, indicates a drop in toxicity that is completely in agreement with the disappearance of cyclic compounds, also reflected in the TOC decrease. The diminution of the toxicity after 2h-electrolysis is consistent with the decrease in the TOC value, indicating the disappearance of aromatic/cyclic
compounds which are responsible of the increase in the luminescence inhibition. The residual toxicity is due to the presence of carboxylic acids that are readily biodegradable. This behavior has also been reported in previous works (Dirany et al., 2011; Dantas et al., 2008; Otruan et al., 2008b).

6.3.4. Biodegradability tests

Biodegradability tests were performed to the samples taken at 1 and 2 h of electrolysis of the drugs solutions. The results are summarized in table 6.1. It can be seen that the BOD₅ increased after 1 h treatment and decreased after two hours, but keeping a higher value that the initial one, corresponding to the non-treated compounds. The increase of the BOD₅/COD ratio showed an improvement of the biodegradability after the electro-Fenton pre-treatment, reaching values near to the biodegradability threshold of 0.4 (Pulgarin et al., 1999), rendering the solution suitable for the application of a biological treatment.

Table 6.1. Results of the evolution of solutions biodegradability (in term of the BOD5/COD ratio) during the electrolysis of 0.1 mM FRSM and RNTD aqueous solutions in 0.05 M Na₂SO₄ at pH 3 using: the BDD/carbon-felt cell (EF-BDD) at 500 mA with $[Fe^{2+}] = 0.1$ mM.

	COD (mg L ⁻¹)		BOD ₅ (mg L ⁻¹)		BOD ₅ /COD	
Time (h)	FRSM	RNTD	FRSM	RNTD	FRSM	RNTD
0	13.8	16	0.483	0.448	0.035	0.028
1	3.312	4.8	0.994	1.344	0.30	0.28
2	1.656	2.176	0.679	0.805	0.41	0.37

Considering the enhancement of the solution's biodegradability after the electro-Fenton treatment due to generation of biocompatible compounds such as short-chain carboxylic acids, which constitute an important part of the residual TOC, along with the considerable abatement of the solution's toxicity after 2 h of electrochemical pre-treatment; the application of a biological treatment was assessed aiming to reduce energy costs, inasmuch as these formed refractory compounds requiring longer treatment times and consequently a greater energy consumption.

6.3.5. Biological process

The increase in the biodegradability of the drugs solutions after electrolysis demonstrates that during the electrochemical treatment, pharmaceuticals are oxidized into biodegradable compounds, mainly short-chain carboxylic acids, which could be assimilated by microorganisms. In this context, the RNTD and FRSM solutions after 2h of electrolysis were subjected to an aerobic process, aiming to biodegrade the remaining products. The evolution of the previously formed carboxylic acids was followed, and results are depicted in Figures 6.4a and b.

As can be seen, in both cases the initial concentration of acetic, oxalic, oxamic and formic acids increased reaching a maximum value, then decreasing until complete disappearance after 7 days treatment. It is well known that microorganisms metabolize substrates by a series of enzymatic biotransformation changes. This is how some species are able to metabolize a wide variety of xenobiotics using both phase I (oxidative) and phase II (conjugative) biotransformation mechanisms (Zhang et al., 1996; Levsen et al., 2005). The involved degradation pathways are complicated processes implicating many enzymes and different catabolic routes as well as production and consumption of several intermediates (Kagle et al., 2009; Haidibarata and Kristanti, 2012). In this way, it was observed that the metabolism of the used cultures without substrate addition, led to the generation of different compounds that were accumulated in the medium. Within these compounds, carboxylic acids that were identified after electrolysis (oxamic, oxalic, acetic and formic acids) were also found. The results of the blank experiment effectuated without substrate addition are shown in Figure 6.4c. It can be noted that these organic acids are also consumed as part of the biomass' metabolism, being almost totally removed after 7 days. The above mentioned explains the increase in the concentration of the carboxylic acids during the biological treatment, along with the assimilation of the remaining cyclic and aliphatic compounds coming from the partial electrochemical oxidation of the drugs, which are progressively and continuously consumed by the microorganisms.

The quicker accumulation of the acetic acid at the first stage of the aerobic process, suggests that it could be the first formed metabolite, being probably transformed afterwards into formic acid, which is also consumed until complete disappearance. The other carboxylic acids are more slowly formed and assimilated.



Figure 6.4 Time-course of the concentration of the main short-chain carboxylic acids during the aerobic treatment of 230 mL of the electrolyzed FRSM (b) and RNTD (c) aqueous solutions using the BDD/carbon-felt cell at 500 mA with $[Fe^{2+}] = 0.1 \text{ mM}$, a) representing the blank. (\blacklozenge) oxalic acid, (\blacksquare) oxamic acid, (\blacktriangle) formic acid, (\blacklozenge) pyruvic acid, (\bigstar) acetic acid, (\Box) malic acid.

The relevance of the application of an electro-Fenton pre-treatment was verified since the biodegradability of the drugs solutions increased after electrolysis, being as a significant part of the generated by-products was readily biodegradable, which was confirmed by their successful removal by the aerobic treatment applied afterwards reaching undetectable concentrations after

7 days of exposure.. These results highlight the potentiality of the coupled EF-biosystem for the efficient and feasible treatment of wastewaters containing pharmaceutical residues.

6.4. Conclusion

The efficiency of the electro-Fenton process for the pre-treatment of aqueous solutions of FRSM and RNTD was demonstrated, being that the biodegradability of the solutions after 2-h electrolysis was improved, achieving BOD₅/COD ratio values of 0.41 and 0.37 for FRSM and RNTD respectively, which are in the limit of biodegradability. Additionally, detoxification of the solutions was also attained. The produced biocompatible effluent was afterwards efficiently treated by an aerobic system, completely removing the by-products formed during electrolysis.

The beneficial effects of this two-steps treatment have therefore been confirmed. Electro-Fenton pre-treatment was able to remove the biorecalcitrant compounds and toxicity, and to produce biocompatible intermediates required for further biological treatment. The results indicate that a combined electro-Fenton – bioprocess is an effective approach for the treatment of pharmaceuticals in water.

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CHAPTER 7

Preparation and Identification of Furosemide's TPs by Electro-Fenton Process and Microbial Bioconversion. A Contribution to the Fate of Pharmaceutical Residues in the Environment

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Chapter 7

Preparation and Identification of Furosemide's TPs by Electro-Fenton Process and Microbial Bioconversion. A Contribution to the Fate of Pharmaceutical Residues in the Environment

Abstract :

The presence of pharmaceutical residues and their transformation products (TPs) has been well documented. It has been established that they represent an environmental risk and toxicity for living organisms and the human being. Pharmaceuticals transformation products can be formed from natural transformation of parent compounds in the environment (coming from industrial effluents), from the introduction of metabolized drugs inside the human body, or from the application of chemical (AOPs or EAOPs) or biological processes (biotic metabolites) during wastewater treatment. The identification and toxicity evaluation of these biotic and abiotic transformation products is thus necessary for risk assessment and accurate prediction of potential ecotoxicity.

In the present study, the identification of different FRSM TPs transformation products from both, biotic (bioconversion) and abiotic (chemical/electrochemical advanced oxidation), was achieved. Among them, saluamine and pyridinium were formed chemically and biologically, suggesting the high possibility of their presence in environmental compartments. The toxicity tests during the treatment by electro-Fenton (EF) treatment of the drug suggest that the identified TPs can represent a toxicological risk for some living organisms. The identification of a new TP from FRSM bioconversion evidences the great diversity of degradation compounds that can be generated. Consequently, this study is presented as a very useful tool for the preparation of drugs' transformation by-products, aiming to strengthen and support predictive modeling and analysis on ecotoxicity and risk assessment. Additionally, dealing with the difficulty to degrade these kinds of organic persistent contaminants, the application of EF process is highlighted as a very promising technology for the efficient mineralization and detoxification of solutions containing pharmaceutical residues since 94% mineralization was achieved after 6 h treatment by this process.

7.1. Introduction

Pharmaceuticals are classified as emergent pollutants that represent an environmental and toxicological risk, and whose impact on ecosystems still remains unclear (Khetan and Collins, 2007). They have been unequivocally detected in effluents of sewage treatment plants (STPs), surface water, ground water, and even in drinking water (Heberer, 2002). Despite their presence in low concentrations, their continuous release into the environment, mainly after excretion and from incomplete degradation during wastewater treatment, makes them pseudo-persistent and thence a focal point of toxicity risk.

Once in the environment, pharmaceutical products are subjected to conversion processes that can induce a great variety of transformation products (TPs). They can be metabolized by different microorganisms (biotic transformations) and/or undergo photolysis, hydrolysis or chemical oxidation (abiotic transformations). In like manner, similar processes can occur during sewage and drinking water treatment, resulting in the generation of different biologically and chemically transformed products (Zwiener and Frimmel, 2003). These products may preserve the mode of action of the parent compound or even be biologically more active (Rosal et al., 2010). Determining the toxicity and ecotoxicity of these TPs formed during biological and photolytic natural processes, and during water and wastewater treatment, is fundamental and a prerequisite for a complete risk assessment study aiming a comprehensive protection of the environment. In this context, studies dealing with this relevant issue are increasing. Nevertheless, neither conclusions nor general remarks can be extracted due to the inconsistencies concerning the followed methodology, experimental conditions, the use of a variety of bioassays, species and endpoints, and the different aqueous matrices analyzed (Fatta-Kassinos et al., 2011).

The great variety of applicable treatments and experimental conditions, along with the numerous pharmaceutical compounds existing, is a clear evidence of the many possible TPs that can be formed during water and wastewater treatment. Therefore, the big challenge facing this problem is the selection of an efficient technology (or a combination of them) for the ideal fast and full mineralization of the target compounds and their complex mixtures.

During the last years, many advanced oxidation processes (AOPs) have been developed and applied for the degradation of compounds recalcitrant to biological degradation. The main concern relates to the formation of divers products from the non-selective attack of in situ generated strong oxidants, mainly hydroxyl radicals (*OH), the main oxidant that may trigger complex reaction pathways up to complete mineralization (Oturan, 2000). Among these technologies, electrochemical advanced oxidation processes (EAOPs), which are based on the insitu generation of the oxidizing species (mainly [•]OH) have shown very good efficiencies for the degradation and mineralization of organic persistent compounds (Martínez-Huitle and Brillas, 2009; Feng et al., 2013; Oturan, 2014; Sirés et al., 2014). Electro-Fenton (EF) process is the most common used and applied EAOP, in which [•]OH radicals are produced in the bulk through the Fenton's reaction (1), in which H₂O₂ electrogenerated at a suitable cathode, reaction (2), reacts with the catalytic amount of Fe⁺² ions externally added to the solution and electrocatalytically regenerated in the process reaction (3) (Oturan et al., 1999; Brillas et al., 2009; Özcan et al., 2009).

$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$	(1)
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	(2)
$Fe^{3+} + e^- \rightarrow Fe^{2+}$	(3)

With the aim of accurately studying TPs by biological ways, we point out bioconversions as competitive and ecologically effective approach now considered in organic synthesis, since microorganisms are a major source of enzymes (Wohlgemuth, 2010) that able to convert organic molecules. Enzymes present in many microbial strains allow access to a wide range of transformation products. This approach requires the selection of active microorganisms and is especially used in the preparation of mammalian metabolites (Azerad, 1999; Asha and Vidyavathi, 2009; Marvalin and Azerad, 2011).

The hydrolytic and reductive capabilities of microorganisms, especially fungi, have been for longtime used in preparative reactions. Among fungi, *Cunninghamella* species have the ability to metabolize a wide variety of xenobiotic in region- and stereo-selective manners that are similar to those in mammalian enzyme systems (Davis, 1988). *Cunninghamella* is a filamentous fungus found in soil and plant material, particularly at Mediterranean and subtropical zones. They possess cytochrome P450 monooxygenase systems analogous to those in mammals and phase II drug metabolism enzymes. This fungi species are able to metabolize a wide variety of xenobiotics using both, phase I (oxidative) and phase II (conjugative) biotransformation mechanisms, thus they have the ability to mimic mammalian metabolism and to perform novel biotransformations (Zhang et al., 1996; Sun et al., 2004; Asha and Vidyavathi, 2009). Biotransformations can hence be a very useful tool for the preparation of drugs' transformation metabolites, aiming to strengthen and support predictive modeling and analysis on ecotoxicity and risk assessment.

The present work faces the main challenges of the existing environmental risk due to the presence of pharmaceuticals and their TPs in water sources. It highlights the efficiency of the EF

process as a very promising technology for the mineralization of pharmaceutical substances from water, as it is capable to destroy both, the parent compound and the transformation products formed during water treatment. It deals as well with the identification of both, abiotic and biotic transformation by-products, suggesting that common intermediates to chemical oxidation and biological metabolism are highly likely to be found in natural aquatic bodies, presenting a toxicity risk for certain microorganisms. This study aims to contribute for the development and enrichment of risk assessment and modeling for accurate (eco)toxicological predictions on the fate of pharmaceutical residues in the environment.

7.2. Materials and methods

7.2.1. Chemicals

Furosemide (purity > 98%) was of reagent grade from Fluka. Anhydride sodium sulfate used as background electrolyte and heptahydrated iron (II) sulfate used as catalyst in EF were of analytical grade from Acros Organics. All solutions were prepared with ultrapure water from a Millipore Milli–Q system with resistivity >18 M Ω cm. Reagent grade sulfuric acid from Merck was used to adjust their initial pH to 3.0. All the other chemicals used were either of HPLC or analytical grade from Prolabo, Fluka and Acros Organics.

7.2.2.Instruments and analytical procedures

7.2.1.1. Electro-Fenton

Electrolyses were performed with a Hameg HM8040 triple power supply at constant current. Solution pH was measured with a CyberScan pH 1500 pH-meter. Mineralization of the FRSM aqueous solutions was assessed from the decay of dissolved organic carbon determined by a Shimadzu VCSH TOC analyser. The time-course of the concentration of FRSM was followed by reversed-phase HPLC using a Merck Lachrom liquid chromatograph equipped with a L-7100 pump, fitted with a RP-18 (5 μ m, 4,6 mm x 250 mm) column at 40 °C and coupled with a L-7455 UV-Vis detector selected at 240 nm (maximum adsorption wavelength of FRSM). All the analyses during EF were carried out isocratically with a mobile phase consisting in a 45:55 (v/v) methanol/H₃PO₄ mixture at pH 3 and 0.7 mL min⁻¹ flow rate. The generated aliphatic acids were identified and quantified by ion-exclusion HPLC technique using an Altech liquid chromatograph equipped with a Model 426 pump, fitted with a Supelco, Supelcogel H 9 mm, 25 cm x 4.6 mm column at room

temperature and coupled with a Dionex AD20 UV detector selected at 210 nm. A 1% H₂SO₄ solution at a flow rate of 0.2 mL min⁻¹ was used as the mobile phase. The inorganic ions released in the treated solutions were determined by ion chromatography using a Dionex ICS-1000 Basic Ion Chromatography System. The analysis of anions was monitored using an IonPac AS4A-SC, 25 cm x 4 mm, anion exchange column linked to an IonPacAG4A-SC, 5 cm x 4 mm column guard. For the determination of the cations, an IonPac CS12A, 25 cm x 4 mm, cation exchange column linked to an IonPac CG12A, 5 cm x 4 mm column guard, was used. The system was equipped with a DS6 conductivity detector containing a cell heated at 35° C. An electrospray time of flight mass spectrometer (ESI-TOF-MS) operating in positive mode (QSTAR Pulsar I from Applied Biosystems), was used for the identification of the aromatic by-products formed during the EF treatment.

7.2.1.2. Bioconversion

Biotransformations were performed under an air atmosphere at 27° C and 200 rmp and monitored by HPLC and MS. HPLC analysis were performed on Gilson system (pump 305, pump 306, gradient dynamic mixer 811B and auto-injector 234) with an Agilent C18, 5 μ m (250 mm × 4.6 mm) column. The system was controlled and the results were analyzed by Unipoint Gilson software. The HPLC solvents were: water with 0.1% TFA (A) and acetonitrile/water with 0.1% TFA (B). Column was eluted with an appropriate gradient program: 90% A/10% B to 50% A/50% B in 15 min, then to 30% A/70% B in 10 min, held for 10 min before being carried to 50%A/50% B in 3 min, to a final composition of 90% A/10% B, achieved in 7 min. The detection was at UV 240 nm.

Mass spectra data were recorded using an electrospray time of flight mass spectrometer (ESI-TOF-MS) operating in the negative mode (QSTAR Pulsar I of Applied Biosystems).

All NMR experiments were recorded on Bruker Avance III HD 400 MHz spectrometer (Wissembourg, France)

7.2.3. Electrochemical treatment

Electrolysis were carried out in a single-compartment mixed cell of 230 mL capacity. Electro-Fenton treatments were performed using either a cylindrical Pt mesh of 4.5 cm of height and 3 cm of internal diameter (EF-Pt) or a thin-film BDD electrode (25 cm²) (EF-BDD) as anode, and a large surface area carbon-felt piece (Carbone-Lorraine) as cathode. In all cases the anode was centered in the electrolytic cell and was surrounded by the cathode, which covered the inner wall of the cell. Continuous saturation of solutions by oxygen at atmospheric pressure was ensured by bubbling compressed air into the system. The electrolysis of aqueous solutions containing 0.1 mM FRSM with 0.1 mM Fe^{2+} as catalyst was performed in 0.05 M Na_2SO_4 at pH 3.0 and room temperature under the application of a constant current of 500 mA. All the experiments were performed with solutions of 230 mL volume with a vigorous stirring by a magnetic bar.

7.2.4. Bioconversion test

The *Cunninghanella echinulate* var. *elegans* ATCC 9245 culture was maintained on agar slants (bactopeptones 5 g/L, yeast extract 5 g/L, malt extract 5g/L, glucose 20 g/L and agar 20 g/L) and stored at 4° C. 500 mL of liquid culture media containing (g/L) glucose 16, yeast extract 4, malt extract 10 and soybean peptones 5 (YMS medium) were sterilized without glucose at 120° C for 20 min and inoculated with a glycerol suspension of microorganisms. Culture medium was incubated at 27° C in rotatory shaker (200 rpm) for 60 h.

Biomass was harvested by filtration and suspended in 500 mL of 0.1 M sodium citrate where 200 mg of FRSM were added after been dissolved in 600 μ L N,N-dimethylformamide, the final FRSM concentration in the solution was 0.4 g L⁻¹. Biotransformations were performed under an air atmosphere at 27° C and 200 rmp and monitored by HPLC and MS. The samples were diluted with methanol and centrifuged at 13000 g for 5 min. The bioconversion of the isolated keto-alcohol TP was carried out under the same conditions.

7.2.5. Toxicity measurements

Toxicity measurements were performed using the Microtox[®] method, based on the determination of the inhibition of bioluminescence of the marine bacteria *V. fischeri*. A luminometer Berthold Autolumat Plus LB 953 was employed, according to the international procedure (OIN 11348-3). The bacteria and the activation reagent, LCK 487 LUMISTOX, were provided by Hach Lange France SAS. The measurements were performed on samples collected during the EF treatment of a 0.1 FRSM solution at 500 mA constant current. In all cases the bioluminescence intensity of bacteria was measured after 5 min of exposition to the samples at 15 °C.

7.3. Results and discussion

7.3.1. EF treatment

Based on the results obtained in our previous works dealing with the application of EAOPs for the mineralization of organic persistent pollutants, the degradation of FRSM by EF process using Pt/carbon-felt (EF-Pt) and BDD/carbon-felt (EF-BDD) in undivided cells under the obtained optimal operating conditions, was studied. Figure 7.1 depicts the decay kinetics of the oxidative degradation of 230 mL of a 0.1 mM FRSM solution in 0.05 M Na2SO4 with 0.50 mM Fe²⁺ at 500 mA constant current and, pH 3.0 and room temperature by EF-Pt and EF-BDD. Total degradation of the drug was achived in very short times, 10 min, when using both electrochemical cells. The determined pseudo-first order apparent rate constants of the oxidation reaction of FRSM by °OH (k_{app} = 0.99 min⁻¹ for EF-BDD and k_{app} = 0.92 min⁻¹ for EF-Pt), which is depicted in the inset panel of Fig. 7.1, demonstrates the great ability of both, heterogeneous M(°OH) formed at the anode surface, and homogeneous °OH formed in the bulk solution. Nonetheless, the small difference between both apparent rate constants suggests that FRSM is mainly oxidized by °OH formed in the bulk solution.



Figure 7.1. Time course of FRSM concentration during electrolysis by EF-Pt (*) and EF-BDD (\bullet) cells, at 500 mA of 230 mL of a 0.1 mM FRSM solution, in 0.05 M Na₂SO₄, in presence of 0.1 mM Fe²⁺ as catalyst, at pH 3.0 and room temperature.

Figure 7.2 depicts the evolution of mineralization of 0.1 mM FRSM aqueous solution in terms of TOC changes, as mineralization efficiency parameter, during the electrolysis of 230 mL solution containing 0.05 M Na₂SO₄ and 0.1 mM Fe²⁺ at 500 mA, pH 3.0 and room temperature by EF-BDD and EF-Pt. The EF-BDD cell exhibits a better performance due to the high O₂-evolution

overpotential of BDD anode and its greater ability to produce active radicals which can destroy refractory compounds to the EF-Pt cell (Oturan et al., 2012). More than 90% of TOC removal was attained after 4 h EF-BDD treatment, reaching up to 94% at 8 h. The slow change of TOC decay after 4 h suggests that residual TOC is composed of refractory intermediates (mainly short-chain carboxylic acids) which are hardly oxidized by [•]OH, The enhancement of the rate of wasting reactions (4) and (5) that become predominant because of relatively low concentration of organic matter in solution constitutes another reason to this lower TOC removal rate.

$$Fe^{2+} + {}^{\bullet}OH \to Fe^{3+} + OH^{-} \qquad k = 3.2 \times 10^{8} \text{ M}^{-1} \text{ s}^{-1}$$
(4)
$$H_{2}O_{2} + {}^{\bullet}OH \to HO_{2}^{\bullet} + H_{2}O \qquad k_{2} = 3.3 \times 10^{7} \text{ M}^{-1} \text{ s}^{-1}$$
(5)

As can be seen in Fig. 7.2, the overall TOC evolution during oxidation of FRSM by EF shows up how FRSM is quickly destroyed, resulting in the formation of aromatic and aliphatic by-products. Aromatic products, being more reactive towards [•]OH than the aliphatic ones, FRSM and formed aromatic intermediates are rapidly degraded by [•]OH, leading to the formation of aliphatic compounds, mainly carboxlylic acids, which are more largely accumulated and remain longer time in the solution as they are hardly oxidized, therefore, constituting most of the TOC content. Acetic, pyruvic, maleic, oxalic, formic and oxalic acids were identified, being oxamic, oxalic and formic acids the most largely accumulated. During the FRSM electrochemical oxidation, heteroatoms present in FRSM structure, are released as NH_4^+ , NO_3^- , CI^- and SO_4^{2-} ions. Their formation and determination were monitored by ion chromatography. SO_4^{2-} attains almost the total initial amount of the corresponding sulphur atom present in the molecule. Concerning N, NH_4^+ is the most largely accumulated ion, whereas NO_3^- reaches a maximum concentration, then decreasing due to either its oxidation in the anode or its reduction in the cathode (Lévy-Clément et al., 2003).



Figure 7.2. a) TOC removal vs. electrolysis time during the mineralization of 230 mL of 0.1 mM FRSM aqueous solution in 0.05 M Na₂SO₄ at 500 mA, pH 3.0 and room temperature by EF-BDD. (---) total TOC (EF-BDD), (\blacksquare) total TOC (EF-Pt), (--): TOC by FRSM, (**X**): TOC by carboxylic acids, (\bullet) TOC by cyclic by-products. b) evoluction of carboxylic acids (\blacklozenge): SO₄²⁻, (*****): NH₄⁻, (\blacktriangle): NO₃⁻.

7.3.2. Identification of oxidation by-products formed during electrolysis

Mass spectrometry analysis of the treated solution during the EF degradation of FRSM allowed the identification of several oxidation by-products, permitting the proposal of a general pathway for the mineralization of the drug by [•]OH (Fig. 7.3). Accordingly with [•]OH typical oxidation mechanisms, mineralization of the molecule goes through a series of reactions, mainly hydroxylation, hydrogen atom abstraction, desulfonation, deamination and oxidative ring breaking of hydroxylated aromatics rigs, furthermore yielding to the formation of simpler aliphatic products, mainly short-chain carboxylic acids, which are the ultimate end-products before complete mineralization to CO_2 , H_2O and inorganic ions (Pignatello et al., 2006; Brillas et al., 2009). Attack of [•]OH to the very reactive furan ring of FRSM, leads to the formation of a γ -ketoenal intermediate (B), which is afterwards oxidize to pydinium derivate (C). Both products, (A) and (B),

had been identified as transformation products of FRSM metabolism (Antoine et al., 2007). The radical attack to the amino group of the molecule results in the formation of structure E, saluamine, which is furthermore oxidized to compound F. This compound has similarly been found as TP of FRSM bioconversion (Hezari and Davis, 1992) and electrochemical oxidation (Laurencé et al., 2011, 2014). These cyclic intermediates are furthermore oxidized to aliphatic compounds before their almost complete mineralization.



Figure 7.3. Suggested mineralization pathway for the mineralization of FRSM by hydroxyl radicals following identified aromatic/aliphatic intermediates.

7.3.3. Toxicity tests during EF treatment

Toxicity in term of inhibition luminescence percentage of marine bacteria *V. Fischeri* after 5 min of exposure time, was assessed during the electrolysis of a 0.1 mM FRSM solution at 500 mA and 1000 mA using the EF-BDD/carbon-felt cell (Fig. 7.4). The formation of some toxic intermediates from the early stages of electrolysis can be deduced from the great increase of the inhibition rate of bacteria luminescence, with values as high as 99%. The fluctuations in toxicity during the firs hour of electrolysis can be attibuated to the variety of the aromatic/cyclic by-products continiously formed and distroyed by the oxidative action of *OH. Nevertheless, solution toxicity is diminished while electrolysis proceeds, owing to the disappearance of these aromatic/cyclic compounds. The remained residual toxicity observed after 2 h treatment is due to the readily biodegradable carboxylic acids present in the solution. These results demonstrate the effectiveness of EF also for the solution detoxification. Similar behaviors have as well been reported in previous studies (Dantas et al., 2008; Oturan et al., 2008; Dirany et al., 2011).



Figure 7.4. Evolution of solution toxicity (in term of inhibition of the luminescence of *Vribrio fischeri* bacteria, after 5 min of exposure) during the mineralization of 0.1 mM FRSM in 0.05 M Na_2SO_4 , 0.1 mM Fe²⁺ at pH 3, using the EF-BDD cell with carbon-felt cathode at (\blacksquare): 500 mA and (\bigcirc): 1000 mA applied current.

The presence of toxic by-products in the first stages of FRSM oxidation, along with the identification of some of those cyclic/aromatic compounds, suggests that saluamine, pyridinium and the γ -keto-carboxylic acid product, which have also been identified as TPs of bioconversion processes, are very likely to be toxic to *Vibrio fischeri* bacteria.

7.3.4. FRSM bioconversion

The bioconversion of FRSM by *C. echinulate* was monitored by reverse-phase HPLC and MS. The chromatograms showed the presence of 2 peaks apart from that of FRSM at Rt=12.8 min (I) and Rt=29.5 min (II). The obtained products were purified by preparative chromatography. The MS spectrum of the purified product (I) in negative mode showed a molecular ion m/z 249, corresponding to the $[M-H^+]^-$ ion. Along with the ¹H NMR analysis, where protons form the furan ring were not observed, the formation of saluamine (I) was confirmed. This compound resulting from the N-dealkylation of FRSM, is generated by hydroxylation in the α -position of the amino group involving monooxygenase like cytochrome P450 enzyme, followed by hydrolysis of the hemiaminal intermediate (Hezari and Davis, 1992; Dinnocenzo et al., 1993; Shaffer et al., 2002; Williams et al., 2007).



Figure 7.5. Proton NMR spectrum obtained for the keto-alcohol metabolite.

The MS spectrum of the second purified compound (II), solubilized in DMSO, showed a molecular ion ($[M-H^+]^-$ at m/z 349) in negative mode, corresponding to an increase of 20 u compared to furosemide, which resulted from the addition of one oxygen and four hydrogen atoms. The presence of the chlorine atom in this metabolite is confirmed by the fact that the MS spectrum shows three peaks at m/z 349, 351 and 353 with relative intensity of 100, 30 and 10

respectively. Additionally, the interpretation of the ¹H NMR, ¹³C NMR, along with the two dimensional COSY, HSQS and HMBC spectrums, allowed the unambiguous identification of a ketoalcohol product with a molecular mass of 350.77 g mol⁻¹. In the ¹H NMR spectrum showed in Fig. 7.5, the triplet at 3.5 ppm demonstrates the presence of the primary alcohol present in the molecule, while the multiplet and triplet at 1.7 and 2.5 ppm, respectively, correspond to the protons of the carbons in the aliphatic chain. The chemical shift of the signal at 4.3 ppm, evidences the presence of the amino and the ketone groups next these protons. The ¹³C spectrum depicted in Fig. 7.6 confirms the presence of the four -CH2- carbons in the aliphatic chain, appearing in the rage of 20 to 60 ppm. The signals with the greater chemical shift (60.5 and 52.4 ppm) in this range of 20 to 60 ppm correspond to the carbon atoms bonded to the OH- and R-NH- groups, respectively.



Figure 7.6. ¹³C NMR spectrum obtained for the keto-alcohol metabolite.

This keto-alcohol compound results from the oxidation of the furan ring, whose mechanism is thought to proceed through one of two general paths: the direct formation of an epoxide, or the addition of the high valent iron (IV)-oxospecies to the π -system of the furan ring to produce a tetrahedral intermediate or cationic σ complex that can be rearrange to yield either an epoxide or a zwitterionic intermediate. Both intermediates can rearrange to form a cis-enedione(Guengerich, 2003; Peterson, 2012). The double bond and the aldehyde group of the resulting cis-enedione intermediate can be further reduced to form the keto-alcohol product (II).



Figure 7.7. Mechanistic pathway of the formation of the keto-alcohol product through an epoxide intermediate.

Fig. 7.7 depicts the mechanism of the generation of the keto-alcohol product through the epoxide route. As mentioned in former sections, Antoine et al. (Antoine et al., 2007) detected a similar intermediate, a γ -keto-carboxylic acid product, as one on the major FRSM biliary metabolites in rats. It has been reported that aldehydes can be either reduced to the corresponding alcohol in microorganisms or oxidized to the corresponding carboxylic acid in animals (Lacroix, 1997). This carboxylic acid compound was also found during the applied EF treatment for the degradation of the drug.

Interestingly, the MS spectrum in negative mode of the keto-alcohol compound solubilized in methanol, revealed a protonated molecular ion m/z 380, which correspond to the ketal adduct resulting from the methanol addition.

The presence molecular ion ($[M-H^+]^-$, m/z 329), suggested the presence of the anteriorly identified pyridinium (III) during electrochemical oxidation. This compound can be formed from initial intramolecular condensation of the amino group with the aldehyde of the *cis*-enedione intermediate(Chen and Burka, 2007). According to the HPLC and MS analysis, the bioconversion of the isolated keto-alcohol resulted in the formation of sulamine (I) and very likely of pyridinium (III). These results confirm the great ability of this culture of microorganisms to oxidize the α -position of the amino group and the furan ring. Fig. 7.8 depicts structures of the bio-TPs of FRMS.



Figure 7.8. Identified metabolites resulting from the bioconversion of FRSM by fungus *Cunninghanella echinulate* var. *elegans* ATCC 9245.

It is of major importance to notice that the TPs saluamine and pyridinium have been detected in both electrochemical oxidation and biological transformation, which suggests that these compounds are likely to be present in the environment, as represented in Fig. 7.9.



Figure 7.9. Main transformation products of FRSM formed by both, biological and electrochemical routes.

7.4. Conclusions

The great efficiency of the EF process for the mineralization of FRSM, has been demonstrated, reaching up to 94% of TOC abatement after 6 h of electrolysis. The [•]OH and M([•]OH) generated during the process are capable of efficiently mineralize FRSM and its cyclic/aromatic by-products, and they have as well shown to be capable of diminishing the solution toxicity accompanied by increase of biodegradaility as a result of aromatics conversion into more biodegradable short-chain compounds.

The obtained results highlight the environmental problem presented by the presence of pharmaceuticals and their biotic and abiotic TPs in the environment. It has been demonstrated that FRSM can be converted by different techniques, leading to the same TPs, which result to be the more alike degradation compounds under different oxidation conditions, therefore highly likely to be found in environmental bodies. Among the different biotic and abiotic prepared and identified TPs, saluamine and pyridinium were generated at both, electrochemical and biological transformations. The high toxicity levels found during EF treatment, evidences the presence of toxic by-products resulting from the chemical degradation of FRSN, suggesting that biotic and abiotic TPS saluamine and pyridinium, as well as the new metabolite (α keto-alcohol derivate) identified from the FRSM bioconversion, represent a toxicological risk for certain organisms. Nevertheless, further toxicity tests are required.

In this context, this approach seems to be a good implement and source of information for assessing the fate and impact of pharmaceutical residues in the environment. Obtained results evidenced that FRSM's TPs can be generated by different methods, provide useful data about the more likely transformation products of FRSM that can be found in natural water sources as a result of chemical and biological conversions.

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Concluding Remarks and Future Perspectives

Chapter 8

Concluding Remarks and Future Perspectives

8.1. Electrochemical Advanced Oxidation Processes for the degradation of pharmaceutical residues.

Facing the environmental risk presented by the well documented presence of pharmaceutical residues in the environment, the interest in developing and applying electrochemical technologies have considerably increased in recent years, owing to the high efficiency they have shown for mineralization of persistent organic contaminants and their degradation by-products.

In the first stage, the present study deals with the application of the EAOPs EF, AO and SPEF with different anode materials, for the degradation of the pharmaceutical products FRSM and RNTD, aiming to point out the optimal experimental conditions and thoroughly assess the kinetic and mechanistic aspects of these processes. It has been demonstrated that the different applied EAOPs like EF-Pt, EF-BDD, EF-DSA and AO-H₂O₂ are very effective for the effective removal and almost total mineralization of both drugs RNTD and FRSM in aqueous medium. The use of a 3D carbon-felt cathode ensured the continuous electrogeneration of H₂O₂ and Fe²⁺ to produce homogeneous [•]OH in the bulk solution. The BDD anode produces large amounts of active heterogeneous BDD([•]OH) radicals, making the EF-BDD and AO-H₂O₂ (with BDD anode) processes the most efficient in the mineralization of both drugs. In all cases, EF-BDD and AO-H₂O₂ yielded about 94% TOC abatement after 4 h of electrolysis at 500 mA, whereas TOC removal exceeded 85% in the case of EF-Pt and 80% for EF-DSA when applied for RNTD incineration. The degradative oxidation of the drugs always obeyed a pseudo-first-order kinetics, and the absolute rate constant of their oxidation by [•]OH were determined by the competition kinetics method, being 3.4×10^9 M⁻¹ s⁻¹ for RNTD and 3.39×10^9 M⁻¹ s⁻¹ for FRSM

The chromatographic and MS analysis of both RNTD and FRSM treated solutions allowed the identification of several cyclic/aromatic organic intermediates, short-chain carboxylic acids and inorganic ions (NH_4^+ , NO_3^- and SO_4^{2-}). A plausible reaction pathway for both pharmaceuticals mineralization by [•]OH, was therefore proposed. In the case of both drugs, FRSM and RNTD, the toxicity assessment showed the formation of intermediates whose toxicity is higher than that of the parent compounds. However, total detoxification was attained at the end of the EF-BDD and AO- H_2O_2 treatments, thereby demonstrating the effectiveness of the applied EAOPs. These results highlight the potentiality of AO- H_2O_2 and EF as environmentally friendly efficient technologies for the remediation of wastewaters containing pharmaceutical residues and their oxidation by-products.

8.2. Scaling up the electrochemical technologies: EF and SPEF in a pre-pilot flow plant

Numerous studies have been conducted reporting the best operational conditions for the application of EAOPs in laboratory scale, evidencing their great ability to mineralize persistent organic contaminants. However, when scaling up these kinds of technologies, aiming to demonstrate and implement their industrial feasibility, diverse difficulties emerge, owing to the multiple factors intervening and affecting the whole process. Responding to this challenge, several authors have reported the application of different EAOPs at pilot and industrial scale. Nevertheless, much work is still left to be done, envisaging the operating industrial application of these promising electrochemical technologies. For which, it is necessary to take into account the main facts representing this challenge: operational costs and the utilization of renewable energy sources for sustainability enhancement.

In response to the presented issue, the electrochemical degradation by EF and SPEF processes with a Pt anode in a pre-pilot flow plant was assessed. It has been demonstrated that SPEF at a pilot-plant scale is an efficient and sustainable viable process for the degradation of RNTD in aqueous solutions, achieving total disappearance of the drug and more than 80% of mineralization after 6h electrolysis, showing a much better performance that EF. It was observed that the increase of *i* enhanced drug mineralization but decreases MCE due to the acceleration of parasitic reactions consuming the main oxidant [•]OH. On the other hand, the lower EC_{TOC} values were obtained at lower values of applied current and higher drug concentrations. Accordingly to what has been found for RNTD degradation using 250 mL capacity electrochemical cells, concentration decay follows a pseudo-first order kinetics, with greater rate constants when applying SPEF, because of the additional photoassisted production of [•]OH promoted by solar irradiation. The Fe(III) complexes formed with the generated carboxylic acids after oxidation of the cyclic/aromatic intermediates were photolized during SPEF process. These results highlight the potentiality of the SPEF process at greater scale as a sustainable and environmentally friendly

technology for the degradation of pharmaceuticals from water, inasmuch as it utilizes a clean reagent: the electron, and sunlight as a renewable source of energy

8.3. The potentiality of EF as a pre-treatment followed by a biological process

It has been demonstrated that the main backgrounds of the electrochemical technologies based on AOPs lies in the increasing energy consumption, which represents additional costs, due to the longer treatment times required as a consequence of the refractory compounds formed during oxidation of the organic contaminants. As these generated refractory compounds, mainly short-chain carboxylic acids, are usually highly biodegradable, the application of an electrochemical pre-treatment aiming to increase the biodegradability of the solution, followed by a conventional biological process, has emerged as a promising coupled-technology for the efficient treatment or persistent organic compounds at relatively moderate costs. In this context, the evaluation of the EF as a pre-treatment stage followed by a biological degradation, was conducted. The efficiency of the combined process for the degradation of RNTD and FRSM solutions was confirmed. EF pre-treatment was able to increase the biodegradability of the RNTD and FRSM solutions, removing biorecalcitrant compounds. The formed biodegradable products after EF, mainly short-chain carboxylic acids, were afterwards completely removed by the microorganisms applied. Additionally, detoxification of the solution was also attained after 2 H of electrochemical oxidation, therefore evidencing the beneficial effects of this two-steps technology for the treatment of water containing pharmaceutical products. However, more research needs to be conducted in order to optimize operational conditions.

8.4. Preparation and identification of the most plausible TPs of FRSM

The development of more powerful analytical techniques has evidenced the high environmental risk presented by the presence of pharmaceutical residues and their transformation products in natural sources of water. The studies reporting the toxicological risk of these substances along with the lacking of information concerning the identities and (eco)toxicity of their TPs, demonstrates that arduous work is required in order to evaluate the fate and impact of these environmental contaminants. The identification of the oxidation by-products of the studied pharmaceuticals by EF and AO processes, as well as the toxicity tests assessed during the electrochemical treatments, permitted to confirm that the oxidation of pharmaceuticals, mainly by the strong oxidant, in which AOPs are based, leads to the generation of different reactive and toxic intermediates. As pharmaceuticals can also been exposed to biological transformations during wastewater treatment plants and directly in the environment, bioconversions on FRSM were conducted aiming to verify the generation of TPs. The results show that both, biological and electrochemical methodologies, can convert the molecule into the same TPs (saluamine and pyridinium), suggesting that these compounds are the most likely TPs of FRSM formed under different oxidation conditions, and therefore the most probably degradation compounds to be found in environmental compartments. The obtained results highlight the useful information contributed by the present study for the assessment of the fate of pharmaceutical residues in the environment.

8.5. A general overview

Responding to the environmental problem caused by pharmaceutical residues present in the environment, the present work deals with the main challenges related to the management of this increasingly worrying issue.

The first challenge lies on the necessity of developing and applying efficient technologies for the destruction of these organic contaminants. Facing to this problem, the obtained results highlight the potentiality of the electrochemical technologies EF and AO as very promising processes for the mineralization of pharmaceuticals in aqueous medium. Additionally, it was demonstrated that the coupling of an EF pre-treatment and a biological treatment rises as a very efficient alternative, inasmuch as the beneficial combination of both technologies reduce the inconvenient presented by EF, owing to the increased energy consumption and longer treatment times needed due to the formation of refractory by-products.

The second challenge is presented by the increasing exigencies concerning sustainability. The use of sustainable technologies and renewable sources of energy is increasingly becoming mandatory. In this context, the utilization of solar radiation was confirmed to highly improve the efficiency of the EF process during the SPEF treatment of the pharmaceutical RNTD in a pre-pilot flow plant, hence demonstrating the potentiality of this technology for the treatment of wastewater containing pharmaceutical compounds, as well as its potential applicability in pilot and industrial scale as a very environmentally friendly process.

The last challenge concerns the environmental impact of the transformation products of pharmaceutical residues. Several studies have demonstrated that these compounds can be even more active and toxic that their parent substances. The reported assessment of the biotic and
abiotic transformation products of the studied drugs, demonstrated the effectiveness of this approach for the preparation of several FRSM transformation products presenting a toxicological risk, from which, those found as TPs of both, biotic and abiotic transformations, are highly likely to be found in the environment. Consequently, this approach can be seen as an important tool for risk assessment and accurate toxicological modeling for prediction of the fate and impact of these environmental contaminants.

8.6. Future perspectives

8.6.1. Identification and toxicity evaluation of pharmaceuticals TPs

Most of the studies out in the literature dealing with toxicological tests are focused on parent compounds and very little information is available concerning their TPs. Some studies, including the present work, have put in evidence the generation of transformation by-products showing higher toxicity levels that the departing drug (Dantas et al., 2008; Oturan et al., 2008; Dirany et al., 2011). Hence, the evaluation of the toxicity, after their respective identification, of the formed TPs, is imperative for risk assessment. In the same line, it is mandatory to carry on analysis on real water samples aiming to detect the present TPs for accurate prediction of ecotoxicological risk. These tasks also involve the development of adequate analytical methods dealing with the complex matrices and the very low concentrations.

8.6.2. Toxicity tests to solutions containing mixtures of pharmaceuticals and their TPs

Degradation of pharmaceuticals residues as well toxicity assessments, are generally reported for single compounds solutions. However, these substances are present in the environment as complex mixtures, whose synergistic effects have been reported by several authors(Pomati et al., 2008). This is thus necessary to work with these kinds of medicines cocktails with the objective of closer imitate real samples and better understand the effects of this multiple interactions on toxicity towards living organisms.

8.6.3. Bench-scale studies and the coupling of treatment technologies

The selection of a suitable wastewater treatment depends on the quality standards to be met and the most effective option with the lowest reasonable costs. Therefore, multiple factors need to be considered such as the quality of the original wastewater, conventional treatment options, facility of handling, economical issues, sustainability, potentiality for using the treated water, and so on. The possibilities and compatibilities of the available conventional methods are widely known. Nonetheless, information concerning the efficiency and the optimal conditions of the new technologies: AOPs, which are in general, designed certain specific contaminants, is primordial(Oller et al., 2011). For this reason, bench-scale and pilot-plant studies are required for the development of these innovative technologies and the generation of information on new industrial processes. Such studies become even more decisive when combining several technologies. The coupling of these coupling processes appears to be a very promising option for wastewater treatment, as the benefits of the single methods can be exploited, and the drawbacks avoided, as a result of the combination.

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Annex 1 Valorization of the PhD research

Publications

- [1] Olvera-Vargas, H., Oturan, N., Brillas, E., Buisson, D., Esposito, G., & Oturan, M. A. (2014).
 Electrochemical advanced oxidation for cold incineration of the pharmaceutical ranitidine: Mineralization pathway and toxicity evolution. Chemosphere, 117, 644–651.
- [2] Olvera-Vargas, H., Oturan, N., Buisson, D., van Hullebusch, E. D., & Oturan, M. A. (2014). Electro-oxidation of the pharmaceutical Furosemide: Kinetics, mechanism and by-products. CLEAN - Soil, Air, Water (in press), doi:10.1002/clen.2014DD656
- [3] Olvera-Vargas, H., Oturan, N., Oturan, M. A., & Brillas, E. (2014). A pre-pilot flow plant scale for the electro-Fenton and solar photoelectro-Fenton treatments of acidic solutions of the pharmaceutical ranitidine. Separation and Purification Technology. (Submitted)
- [4] Olvera-Vargas, H., Oturan, N., Buisson, D., & Oturan, M.A. (2014). A Combined Electro-Fenton Pre-Treatment and a Biological Process for the Mineralization of the Pharmaceuticals Furosemide and Ranitidine. (In preparation).
- [5] Olvera-Vargas, H., Buisson, D., Léroy, S., Martins, T., & Oturan, M.A. (2014). Preparation and Identification of Furosemide's TPs by Electro-Fenton Process and Microbial Bioconversion. A Contribution to the Fate of Pharmaceutical Residues in the Environment. (In preparation).

Proceeding:

Olvera-Vargas, H., Oturan, N., & Oturan, M. A. (2012). Degradation of Dye Azure B by Electrochemical Advanced Oxidation Processes. In S. Eslamian (Ed.), 3rd International Conference on Development, Energy, Environment, Economics (pp. 99–104). Paris, France: WSEAS Press.

Communications

- Olvera-Vargas, H., Oturan, N., Oturan, M.A. (2013). Etude de la Dégradation du Furosémide par les Procédés Electrochimiques d'Oxidation Avancée. *Journées d'électrochimie2013* (oral presentation). Paris, France.
- Olvera-Vargas, H., Oturan, N., Oturan, M.A. (2013). Electro-oxidation of Ranitidine by Electrochemical Advanced Oxidation Processes. *International Conference on Envrionmental Science and Technology* (oral presentation). Nevsehir, Turkey.
- Olvera-Vargas, H., Oturan, N., Oturan, M.A. (2012). Degradation of Dye Azure B by Electrochemical Advanced Oxidation Processes. 3rd International Conference on Development, Energy, Environment and Economics (DEEE'12) (oral presentation). Paris, France.

Summer school presentations

- Olvera-Vargas, H., Oturan, N., Buisson, D., van Hullebusch, E., Oturan, M.A. (2014). Studies on the Fate of Pharmaceuticals in Aqueous Media: Electrochemical Degradation and Biotransformation. *Summer School: Biological Treatment of Solid Waste*. Cassino, Italy.
- Olvera-Vargas, H., Oturan, van Hullebusch, E., Oturan, M.A. (2012). Studies on the Fate of Pharmaceuticals in Aqueous Media: Synthesis, Characterization and Detection of Abiotic transformation Products using Advanced Oxidation Processes. *Summer School on Contaminated Soils: from Characterization to Remediation*. Paris, France.

Annexes

Annex 2 Curriculum Vitae



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Publications

- [1] Olvera-Vargas, H., Oturan, N., Buisson, D., van Hullebusch, E. D., Oturan, M. A. (2014).
 Electro-oxidation of the pharmaceutical Furosemide: Kinetics, mechanism and by-products.
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