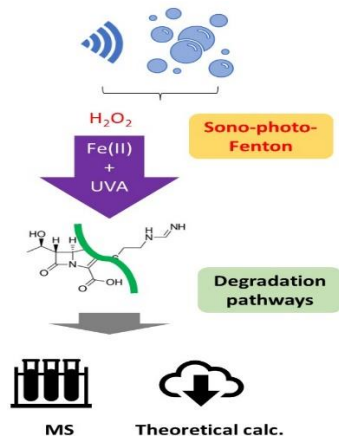


Degradation pathways elucidation of the antibiotic imipenem by hybrid sono-photo-Fenton process

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The presence of emerging contaminants such as antibiotics in water, is a growing concern due to the negative effects that can be induced on the ecosystem and public health. Thus, alternative processes to efficiently degrade the antibiotics before their entrance into municipal sewage systems are needed. In this context, ultrasound-based processes have an opportunity. Herein, the combination of sonochemistry with photo-Fenton, taking advantage of the sono-generated H₂O₂ is presented for the degradation of imipenem (considered the most reliable last-resort treatment for infections caused by multidrug-resistant bacteria). To understand the action of the combined process on imipenem (IMI) degradation, theoretical calculations and experimental analysis were done using atomic charge analysis and mass spectrometry (MS), respectively. It was identified hydroxyl radical as the main responsible for the antibiotic degradation, the theoretical results indicated the imine, β -lactam, alcohol, and thioether moieties in IMI are more susceptible to be transformed by $\cdot\text{OH}$, which was confirmed experimentally by MS.

Introduction

The presence of emerging contaminants (ECs) in water, especially antibiotics, is a growing concern due to the negative effects that can be induced on the ecosystem and public health, e.g. the generation and proliferation of antibiotic-resistant genes and bacteria. Carbapenem antibiotics (such as imipenem) are considered the most reliable last-resort treatment for infections caused by multidrug-resistant bacteria. However, the rapid spread of resistance to carbapenems, mainly Gram-negative bacteria, appears to rapidly reduce this option, generating a global public healthcare problem [1]. For instance, the presence of carbapenem-resistant genes in enterobacteria was reported in hospitals from 2013 to 2017 in Peru [2]. Several works have pointed to wastewater treatment plants (WWTP) as the sites where bacteria can develop resistance to antibiotics and spread into the environment. The presence of antibiotics in the WWTP effluents is because conventional treatment methods (e.g., biological processes) are not able to eliminate them. Thus, alternative processes to efficiently degrade the antibiotics before their entrance into the WWTP are needed. Moreover, alternative processes should be applied in the primary sources of pollution by antibiotics such as hospitals or factories of pharmaceuticals. In this context, Advanced Oxidation Processes (AOPs) have an opportunity. AOPs have emerged as an efficient alternative to eliminate CEs, being high-frequency ultrasound (US) one of the most promising non-photochemical AOPs. In the US, pollutant degradation can occur via pyrolysis or by attacks of sono-generated hydroxyl radicals ($\cdot\text{OH}$) coming from the water molecules cleavage. US process is also able to generate hydrogen peroxide as a by-product of radical combination. Thus, iron ions and UV light can be added to the ultrasound, producing a hybrid sono-(photo)-Fenton process. This

hybrid process may enhance the degradation [3].

On the other hand, from a fundamental point of view, if a hybrid process is applied, it is relevant to consider the elucidation of transformation products (intermediates), especially when total mineralization is not achieved (as frequently observed for US-based processes). Therefore, this work aims to elucidate the primary intermediates formed during the imipenem (a very representative carbapenem antibiotic) degradation by a hybrid sono-photo-Fenton process. Experimental and theoretical analyses were performed by Mass Spectrometry and atomic charge calculations, respectively. Thus, herein, we present the following items: 1) the identification of the degradation routes involved in the system for the antibiotic study, 2) the reactivity of the organic pollutant toward the oxidizing species, and 3) the elucidation of the possible chemical structures of the primary intermediates.

Material and Methods

Imipenem (IMI) (> 95% purity) was purchased from Matrix Scientific. Acetonitrile (grade HPLC) was obtained by Supelco. Iron sulfate heptahydrate (FeSO₄·7H₂O) and catalase (2000-5000 units/mg) were acquired from Sigma-Aldrich. Quantitative analysis of imipenem (IMI) was measured at 300 nm by HPLC Agilent 1100. Degradation of IMI was performed in a batch glass reactor (Meinhardt Ultrasounds) at 578 kHz, 24 W, and 5 mg L⁻¹ of ultrasonic frequency, power, and Fe(II) dosage, respectively, containing 300 mL of 20 mg L⁻¹ of meropenem solution at pH 6.4 ± 0.2. The temperature of the ultrasonic reactor was controlled at 19 ± 2 °C in a cooled water bath and the UVA light experiment was performed with a 4W Philips cylindrical black lamp (maximum emission at 365 nm). Theoretical analysis of the atomic charge for IMI was performed using a free online atomic charge calculator

(versión 1.0.17.1.26). The products of primary transformations during degradation were measured using UPLC-Qtof-MS, Waters. Chromatographic conditions were as mobile phase, acetonitrile: water (20:80), operated positive, with collision energies for fragmentation of 10 to 20 eV. All experiments were conducted at least in duplicate with good reproducibility.

Results and Discussion

IMI was initially subjected to the sonochemical treatment (sonolysis). This system degraded ~47% after 120 min. As imipenem is a non-volatile compound, it is not degraded by pyrolysis. Indeed, it was noted that the H_2O_2 accumulation rate in the imipenem presence is lower than the BK (sonication of distilled water in the antibiotic absence), supporting the reaction of the pharmaceutical with the sonogenerated $\bullet OH$. As mentioned above, hydrogen peroxide is an intrinsic by-product of the sonochemical treatment (during IMI treatment, H_2O_2 was accumulated at a rate of $0.76 \mu M \text{ min}^{-1}$). Then, as a strategy to use this sonochemical by-product, ferrous ions and UVA, were added to the sonochemical system, forming the sono-photo-Fenton process. Under this last system, ~90% of IMI was degraded in 2 h. As Fe or UVA alone led to removals of IMI lower than 15%, the degradation improvement involved the extra $\bullet OH$ through photo-Fenton reactions [4].

After determining the radical routes involved in the degradation of IMI, the theoretical calculations of atomic charges for the antibiotic were employed to identify the most likely sites for the attacks by the $\bullet OH$ generated in the sono-photo-Fenton system. Figure 1 shows the distribution of atomic charges on the IMI structure. This analysis indicated that red-colored atoms belong to the electron-rich regions, suggesting that the imine, β -lactam, alcohol, and thioether moieties in IMI are more susceptible to be transformed by $\bullet OH$.

Conclusions

The addition of ferrous ions and UVA improved the IMI sonodegradation thanks to the extra production of hydroxyl radicals via photo-Fenton reactions. The pathways of hydroxylation and oxidation of thioether plus the plausible opening of the β -lactam ring were linked to the IMI transformation. Such pathways were consistent with the predictions based on the atomic charge analyses for the antibiotic which pointed out the imine, β -lactam, alcohol, and thioether groups as electron-rich moieties susceptible to radical attacks.

Acknowledgments

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To corroborate the theoretical results, the structure of the primary intermediates was established experimentally. Thus, by-product formation was evaluated at ~50% of IMI degradation. The detection of some primary stable transformation products is illustrated in Figure 2.

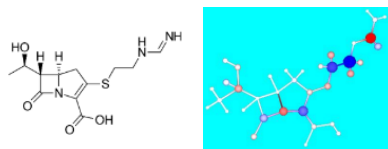


Figure 1. Distribution of atomic charges on IMI structure according to the theoretical calculations.

The experimental results showed that the by-products formed during treatment come from the hydroxylation and oxidation of thioether. Also, the opening of the β -lactam ring is plausible. These findings indicated that there was a good fitting between the prediction by the atomic charge analysis and the intermediates obtained by Mass Spectrometry.

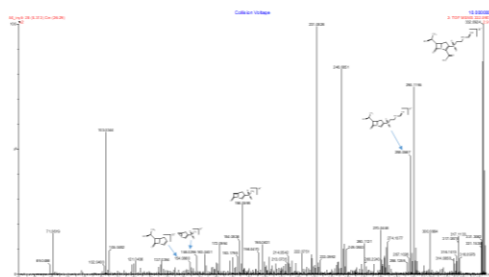


Figure 2. Transformations of IMI under the action of $\bullet OH$ generated in the sono-photo-Fenton system.