

Photocatalytic degradation of macrolide antibiotic azithromycin in aqueous sample

Čizmić M.¹, Vrbat K.¹, Ljubas D.², Čurković L.^{3,*} And Babić S.¹

¹Department of Analytical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 19, 10000 Zagreb, Croatia

²Department of Energy, Power Engineering and Environment, Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb, Ivana Lučića 5, 10000 Zagreb, Croatia

³Department of Materials, Faculty of Mechanical Engineering and Naval Architecture, University of Zagreb, Ivana Lučića 5, 10000 Zagreb, Croatia

*corresponding author:

e-mail: lidija.curkovic@fsb.hr

Abstract

Azithromycin (AZI) is widely used macrolide antibiotic and its presence in waste water treatment plant (WWTP) influent is unequivocal. Many studies have shown that AZI is not being removed from the waste waters during the conventional waste water treatment. Therefore it can reach the environment and have possible adverse effect on the living organisms. It is of great importance to develop methods for waste water treatment that could completely remove pollutants such as AZI. Photocatalysis, one of advanced oxidation processes (AOP), has been presented as effective in removing organic pollutants such as pharmaceuticals in numerous studies. In this work aqueous samples of AZI have been subjected to photocatalytic degradation. Nanostructured sol-gel TiO₂ was used as a catalyst but in the form of a film which makes the possible future implementation more applicable. Photocatalytic degradation was optimized in order to find the best experimental conditions for complete removal of AZI. Sample analysis was conducted on HPLC-MS/MS which was also used for monitoring of AZI degradation and determination of possible degradation products.

Keywords: photocatalysis, sol-gel TiO₂ film, azithromycin, HPLC-MS/MS

1. Introduction

Antibiotics are widely used pharmaceuticals and so they have been detected in different water samples such as waste water treatment plant influent and effluent, surface waters, drinking water and groundwater (Bhandari *et al.*, 2008, Grujić *et al.*, 2009 and Loganathan *et al.*, 2009). Macrolides, as one of the most commonly used families of antibiotics, have been widely used in human and veterinary medicine to treat several diseases and infections. AZI is the first macrolide antibiotic belonging to the azalide group which is derived from erythromycin by adding a nitrogen atom into the lactone ring of erythromycin. AZI is released in water body and the observation of AZI in surface water was reported as high as 3 ng L⁻¹, 69 ng L⁻¹ in effluents and

257 ng L⁻¹ in groundwater (Voigt and Jaeger, 2017). Over the past decade, various strategies have been employed in order to solve the problem of antibiotic waste water because the conventional waste water treatments are often ineffective (Petrovic *et al.*, 2011). Recently, AOPs (UV254, UV/H₂O₂, Fenton and the photo-Fenton oxidation, and the heterogeneous photocatalysis) have been proposed as alternative methods for the degradation of pharmaceuticals (Chong *et al.*, 2010; Cruz *et al.*, 2012, Čizmić *et al.*, 2017, Li and Shi, 2016, Moriera *et al.*, 2015). Among AOPs, heterogeneous photocatalysis using TiO₂ based photocatalyst has shown a great potential as a low-cost and eco-friendly technique to degrade and mineralize the antibiotics in water. Generally, TiO₂ has been used in two forms: highly dispersed fine particles or suspended particles in liquid medium and thin films on supported materials. The main disadvantages of photocatalyst application in form of suspensions are (i) difficult separation of TiO₂ after the treatment, and (ii) the low quantum efficiency of these processes. However, this problem could be avoided by immobilization of TiO₂ in the form of a film on different substrates. A number of methods have been employed to fabricate TiO₂ films, including sputtering, chemical vapour deposition, and sol-gel process (Čurković *et al.*, 2014; Čizmić *et al.*, 2017). However, the sol-gel process is one of the most appropriate technologies to prepare thin oxide coating. The interest in application of sol-gel method is due to several advantages including: good homogeneity, ease of composition control, low processing temperature, large area coatings, low equipment cost, and good photocatalytic properties (Šegota *et al.*, 2011; Čurković *et al.*, 2014).

Tong *et al.* (2011) reported occurrence of AZI in environmental waters and described the photolysis of azithromycin under simulated solar radiation. They were observed seven photoproducts. To the best of our knowledge, there is no available literature data regarding to kinetics of photocatalytic degradation of AZI catalyzed by sol-gel TiO₂ thin films and elucidation of degradation pathways of AZI.

Therefore, the goal of this study is investigation of application of sol-gel TiO₂ film for photocatalytic degradation of AZI.

2. Experimental part

2.1. Materials

Analytical standard of azithromycin was obtained from Pliva (Zagreb, Croatia). 0.1 mol/L HCl and 0.1 mol/L NaOH (Kemika, Zagreb, Croatia) were used for pH adjustment. For chromatographic analysis HPLC grade acetonitrile (J. T. Baker, Deventer, Netherlands) and formic acid (Merck, Darmstadt, Germany) were used. Ultra pure water was used in all experiments and was prepared by a Millipore Simplicity UV system (Millipore Corporation, Billerica, MA, USA). Solutions of azithromycin used for photolytic and photocatalytic experiments (1 and 10 mg L⁻¹) were freshly prepared before every experiment by accurate weighing of powder standard and dissolving in water to achieve the certain concentration. All solutions were kept in the dark under 4 °C.

2.1. Preparation of sol-gel TiO₂ films

For the preparation of colloidal solution (TiO₂ sol), the following components were used: (i) titanium (IV) isopropoxide (Ti(C₃H₇O₂)₄)-TIP as a precursor, (ii) isopropanol (C₃H₇OH)-PrOH as a solvent, (iii) acetylacetonate (C₅H₈O₂)-AcAc as a chelating agent, (iv) nitric acid (HNO₃)-HN, 0.5 M as a catalyst, (v) 2 g polyethylene glycol, PEG (H(OCH₂CH₂)_nOH), $M_r = 5000-7000$, as an organic/polymer additive. All chemicals were analytical grade reagents. The molar ratio of these reactants was: TIP:PrOH:AcAc:HN = 1:35:0.63:0.015. The TiO₂ nanostructured film was deposited on the inner walls of a borosilicate glass cylinder. The procedure of deposition, synthesis and characterization of the sol-gel TiO₂ film was described in details elsewhere (Ćurković *et al.*, 2014).

2.2. Photolytic and photocatalytic experiments

All experiments were carried out in the 0.11 L borosilicate glass cylinder reactor (with 200 mm in height and 30 mm in diameter) thermostated at temperature of 25±0.2 °C with continuous purging with air. Two types of experiments were conducted using this reactor: (i) photolysis (with irradiation of 185/254 nm and 365 nm) and (ii) photocatalysis where TiO₂ was catalyst in a form of a nanostructured film and irradiation sources were the same as in photolysis. Scheme and description of photoreactor can be found in previously published manuscript (Ćurković *et al.*, 2014).

Two different UV-radiation lamps were used: model Pen-Ray 90-0019-04, with $\lambda_{max} = 365$ nm and incident photon flux $N_p = 4.295 \cdot 10^{-6}$ einstein s⁻¹ (UV-A lamp) and model Pen-Ray 90-0004-07 with $\lambda_{max} = 254/185$ nm (UV-C lamp) and incident photon flux $N_p = 1.033 \cdot 10^{-6}$ einstein s⁻¹ (UVP, Upland, CA, USA). Incident photon flux was determined by actinometric experiments following the procedure described in (Kuhn, Braslavsky *et al.*, 2004). The lamps

were placed in the center of reactor and the UV radiation reaches the inner wall of the reactor through the solution, causing the photolytic/photocatalytic oxidation process in reactor. During the experiments samples for chromatographic analysis were taken from the reactor at particular time intervals and stored in dark under 4 °C until analysis.

2.3. HPLC-ESI-MS/MS analysis

Samples taken from the reactor during photodegradation experiments were analyzed on an Agilent Series 1200 HPLC system (Santa Clara, CA, USA) connected to a triple quadrupole mass spectrometer Agilent 6410 with an ESI interface. The column used for chromatographic separation of the degradation products was Synergi Polar (100 mm × 2.0 mm, particle size 2.5 μm) supplied by Phenomenex (Torrance, CA, USA). The mobile phase was MilliQ water acidified with 0.1% formic acid (A) and acetonitrile acidified with also 0.1% formic acid (B) in gradient elution. The analyses were performed in the positive ionisation mode. The conditions of the ion source of the mass spectrometer were: drying gas temperature 350 °C, capillary voltage 4 kV, drying gas flow 11 L min⁻¹ and nebulizer pressure 35 psi. Injection volume was 5 μL. For acquisition and data processing Agilent MassHunter software version B.01.03 was used.

3. Results and discussion

3.1. Photolysis

Photolysis was done using two different irradiation sources: 185/254 nm (UV-C) and 365 nm (UV-A) at different concentrations of AZI to investigate the influence of starting concentration of target substance. Firstly, experiment with no irradiation was performed. Solution of AZI was placed in the reactor with TiO₂ nano film and samples were taken at defined times (Figure 1, A). The results after HPLC-MS/MS analysis showed no decrease in AZI concentration during the investigated time (Figure 1A) confirming that decreasing of AZI concentration in photolytic and photocatalytic experiments is only due to effects of light and catalyst. Low concentration of AZI was very slowly degraded and high concentration of AZI was not degraded during the photolytic experiment with irradiation of 365 nm (Figure 1B). On the other hand it was degraded under the influence of 185/254 nm at both of investigated concentrations. In the solution where the starting concentration was 1 mg L⁻¹ AZI was completely removed after 10 minutes of irradiation whereas where the starting concentration was 10 mg L⁻¹ the complete removal took longer. The complete degradation occurred after 20 min. Although AZI shows low absorption at 254 nm it is still successfully degraded. It should be noted that 254 nm irradiation is a high energy radiation which is able to degrade complex molecules such as AZI. Irradiation at 254 nm has successfully been used for disinfection of drinking waters because of its efficiency (Klavarioti *et al.*, 2015). On the other hand AZI does not absorb at 365 nm and the energy of this radiation is low which results in almost no degradation.

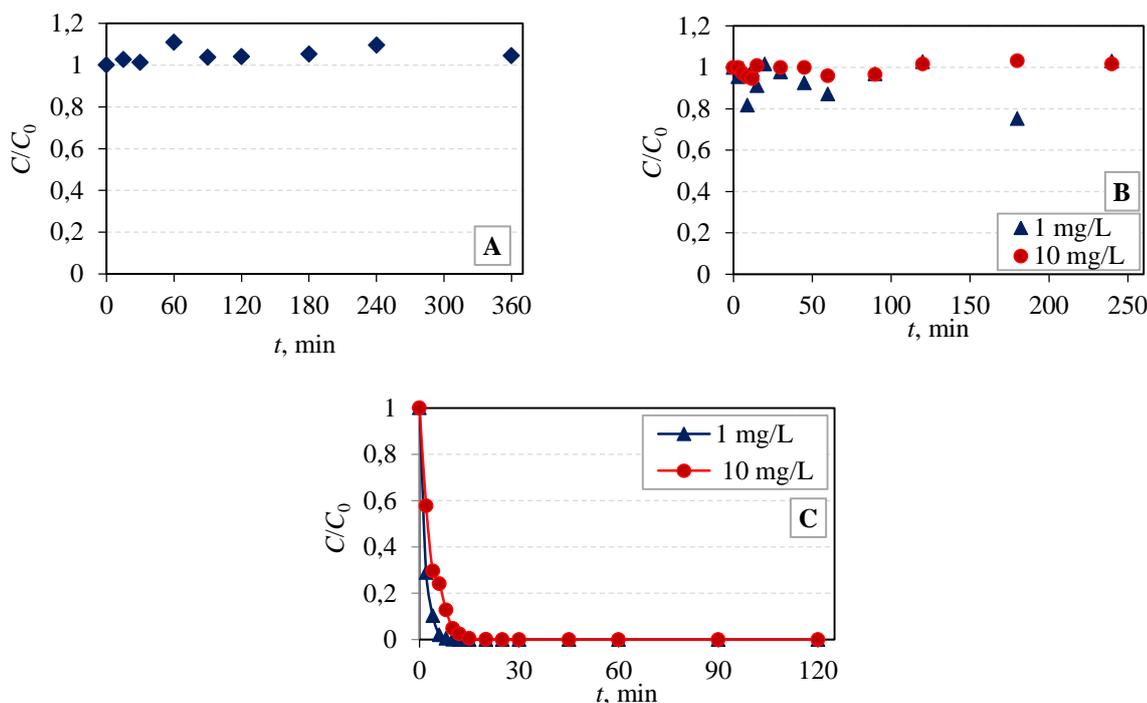


Figure 1. Photolytic degradation using different light sources and different concentrations of AZI: (A) no irradiation; (B) 365 nm; (C) 185/254 nm

3.2. Photocatalysis

Aqueous solutions of AZI were subjected to photocatalytic degradation using TiO₂ nanostructured film as catalyst. The influence of initial concentration of AZI and the influence of pH on efficiency of photocatalytic degradation was monitored at 254 nm and 365 nm. In all experiments where the concentration of AZI was 10 mg L⁻¹ the

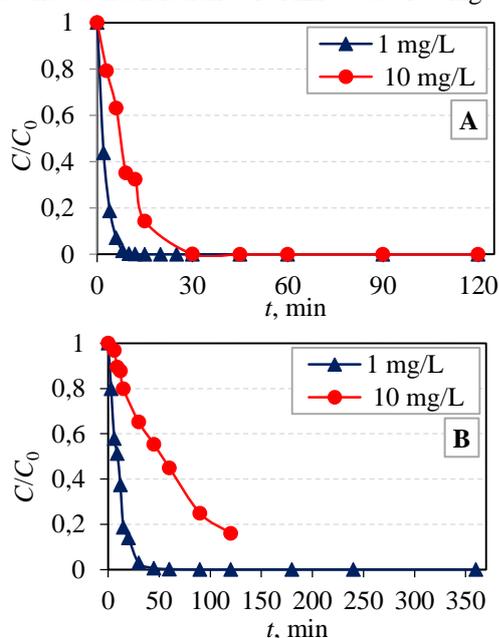


Figure 2. Photocatalytic degradation at different starting concentrations of AZI: (A) 185/254 nm + TiO₂; (B) 365 nm + TiO₂

Photocatalytic experiments with different pH values were conducted in order to determine the effect of pH on the degradation rate of AZI. The pH was adjusted initially at 3,

efficiency of degradation was lower than for the experiments where the concentration of AZI was 1 mg L⁻¹ (Figure 2). In the experiment with 254 nm irradiation (Figure 2A) AZI was completely removed regardless of concentration while in experiment with 365 nm (Figure 2B) it was completely removed in the investigated time only when initial concentration was 1 mg L⁻¹.

7 and 10 with HCl or NaOH. Based on the obtained results (Figure 3) it can be seen that the degradation rates under pH 3 and 7 were almost the same. But, when the starting pH of the solution was 10, the degradation was enhanced. During the photocatalysis many reactions take place but considering that the degradation rate at pH 10 was faster than in lower pH it is possible that the main mechanism responsible for degradation is the reaction of valence bandholes with hydroxide ions and formation of hydroxyl radical which is a powerful oxidant (Klavarioti *et al.*, 2009).

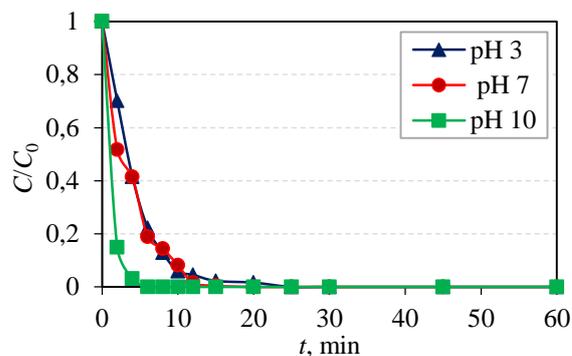


Figure 3. Photocatalytic degradation of AZI at different pH

3.3. Degradation products

Degradation products were investigated in solutions where the initial concentration of AZI was 10 mg L⁻¹ in order to track their formation easier and more precisely.

Five degradation products were detected cumulatively with their *m/z* as follows: 290, 368, 434, 591 and 606. Further analysis of degradation products and their tentative structural formulae is in progress. To our present knowledge there are no reported degradation products of AZI after photocatalysis.

4. Conclusions

Azithromycin is widely used macrolide antibiotic and its presence in the environment is unequivocal. Its influence on the environment and living organisms is still underinvestigated. Therefore the means of its removal from the waste waters is necessary. AOPs proved to be efficient in removal of different pollutants and so in this work it was investigated the efficiency of removal of AZI via photocatalytic degradation using TiO₂ nano film.

AZI is susceptible to photocatalytic degradation and it was completely removed from the solution using 254 nm and 365 nm as irradiation sources. The influence of initial concentration of AZI and the initial pH of solution was investigated considering degradation rate. Optimal degradation was achieved when the initial concentration of AZI was 1 mg L⁻¹ with initial pH of 10 in photocatalytic degradation with 254 nm.

During the experiments degradation products were formed. Five different degradation products were identified. Additional research is in progress to propose tentative structural formulae and degradation pathways.

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