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Photocatalytic degradation of 4-amino-6-chlorobenzene-1,3-disulfonamide stable hydrolysis product of hydrochlorothiazide: Detection of intermediates and their toxicity☆

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ABSTRACT

In this work we have investigated in details the process of degradation of the 4-amino-6-chlorobenzene-1,3-disulfonamide (ABSA), stable hydrolysis product of frequently used pharmaceutical hydrochlorothiazide (HCTZ), as one of the most ubiquitous contaminants in the sewage water. The study encompassed investigation of degradation by hydrolysis, photolysis, and photocatalysis employing commercially available TiO_2 Degussa P25 catalyst. The process of direct photolysis and photocatalytic degradation were investigated under different type of lights. Detailed insights into the reactive properties of HCTZ and ABSA have been obtained by density functional theory calculations and molecular dynamics simulations. Specifically, preference of HCTZ towards hydrolysis was confirmed experimentally and explained using computational study. Results obtained in this study indicate very limited efficiency of hydrolytic and photolytic degradation in the case of ABSA, while photocatalytic degradation demonstrated great potential. Namely, after 240 min of photocatalytic degradation, 65% of ABSA was mineralizated in water/TiO_2 suspension under SSI, while the nitrogen was predominantly present as NH_4^+. Reaction intermediates were studied and a number of them were detected using LC-ESI-MS/MS. This study also involves toxicity assessment of HCTZ, ABSA, and their mixtures formed during the degradation processes towards mammalian cell lines (rat hepatoma, H-4-II-E, human colon adenocarcinoma, HT-29, and human fetal lung, MRC-5). Toxicity assessments showed that intermediates formed during the process of photocatalysis exerted only mild cell growth effects in selected cell lines, while direct photolysis did not affect cell growth.

1. Introduction

Diuretic, 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide (hydrochlorothiazide, HCTZ) is frequently used for the treatment of hypertension, congestive heart insufficiency, renal tubular acidosis, prevention of rock formation in kidneys, sometimes even for treatment of hypercalciuria (Brigante et al., 2005; Mahajan et al., 2012). O'Grady et al. (1999) have reported that HCTZ does not metabolize completely and at least 62.6% of the oral dose is eliminated by the kidney unchanged within 24 h. Thieme et al. (2001) had reported that hydrolysis process already starts in urine.

HCTZ is one the most ubiquitous contaminants in the sewage (Radjenović et al., 2009; Estrada-Arriaga et al., 2016) and river waters (López-Serna et al., 2013). Petrović et al. (2014) were detected HCTZ in Novi Sad (Serbia) in drinking water, the Danube River, canal water and municipal waste water in concentration from 24 to 1070 ng/L.

A common property observed for thiazides is hydrolysis in aqueous media. In the case of HCTZ hydrolysis only one of degradation product was identified – 4-amino-6-chlorobenzene-1,3-

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disulfonamide, ABSA (Deventer et al., 2009). Brigante et al. (2005) have studied photolytic degradation of HCTZ in pure water and sewage treatment plant water with simulated and direct sunlight. They have identified three photoproducts: ABSA, 6-hydroxy-3,4-dihydro-2*H*-1,2,4-benzothiadazine-7-sulfonamido-1,1-dioxide and 4-amino-6-hydroxy-1,3-benzenedisulfonamide. Oxidation degradation of HCTZ under mild conditions (3% H_2O_2, 80 °C, during 1 h) leads to the formation of 6-chloro-2-oxy-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, while harder oxidation conditions (6% H_2O_2, 80 °C, during 2 h) lead to the formation of ABSA, beside aforementioned compound (Mahajan et al., 2012). Studies using photoproducts or metabolites of pharmaceuticals showed that they can be less, equally, or more phototoxic (Selvaag and Thune, 1996). Ozone-treated HCTZ samples indicate that parent compounds and transformation products are not harmful towards bacteria bioluminescence tests (Borowska et al., 2016). Han et al. (2000) demonstrated that the photodegradation products of HCTZ with chloride play important roles in their phototoxicity and could induce photohaemolysis of erythrocytes. The increase in haemolysis value is directly correlated to the UV irradiation time of HCTZ. However, the photodegradation products of HCTZ demonstrated no phototoxicity, as measured in the *Candida albicans* test.

Highly efficient way for mineralization of organic compounds is based on the application of semiconductors as catalysts (Hoffmann et al., 1995; Abramović et al., 2011, 2015). Although it is available in different crystal forms, commercial TiO_2 Degussa P25 exhibits outstanding activity and supremacy over other available TiO_2 (Klavarioti et al., 2009).

Computational simulations provide an insight into the structure of target compounds and enable easier interpretation of experimentally obtained results (Sarazen et al., 2016). After conducted geometrical optimization, important information for the understanding of reactivity and stability of organic molecules can be obtained (Armaković et al., 2016).

Multi endpoint bioassays that are based on whole-cell response of mammalian cell lines are powerful indicators of metabolic, biochemical, and genetic alterations that arise under the influence of evaluated compounds (Četojević-Simin et al., 2012), and to our knowledge there are no results for influence of HCTZ or ABSA to mammalian cell lines.

According to the available literature, investigation of stability and toxicity of ABSA and its intermediates has not been performed, although a number of authors suggested (Brigante et al., 2005; Deventer et al., 2009) that aforementioned compound is formed as a main product of HCTZ degradation. Within this study detailed experimental and computational analysis of hydrolysis process of HCTZ has been performed. Density functional theory (DFT) and molecular dynamics (MD) simulations have been performed for the investigation of fundamental and reactive properties of investigated molecules. Also, the main part of the present study was investigation of photolytic and photocatalytic degradation of ABSA under different type of lights. Reaction intermediates were studied and a number of them were detected. The toxicity of the mixtures formed by degradation were studied by employing mammalian cell lines (rat hepatoma, H-4-II-E, human colon adenocarcinoma, HT-29, and human fetal lung, MRC-5).

2. Experimental and computational details

2.1. Chemicals and solutions

All chemicals were of reagent grade and were used without further purification. The solutions were made using double distilled water (DDW). (±)-Hydrochlortiazide (≥99% purity) and ABSA (≥98% purity) were purchased from Sigma–Aldrich. The TiO_2 Degussa P25 (hereafter TiO_2, 75% anatase and 25% rutile, surface area 50 ± 1.0 m^2/g, crystallite size about 20 nm, according to the producer's specification, specific surface area of 53.2 m^2/g and total pore volume 0.134 mL/g (Tomić et al., 2015)) was used as photocatalyst. Other applied chemicals are given in the Supplementary Material.

2.2. Degradation procedures

Hydrolysis of 0.05 mmoL/L HCTZ solution was carried out in dark in a closed volumetric flask (Duran®), while direct photolysis was investigated at daylight during the whole day from February 19, 2014 to October 15, 2014. All experiments under influence of sunlight have been performed at ambient temperature (25 ± 2 °C).

Direct photolysis and photocatalytic degradation, under simulated lights (UVA and simulated sunlight irradiation, SSI), was described in detail in Supplementary Material. Briefly, experiments were performed using 20 mL of 0.05 mmoL/L ABSA containing 2.0 mg/mL of TiO_2, except for the study of direct photolysis, effect of catalyst loading and initial concentration of ABSA. All experiments were performed at the natural pH, without any adjusted, which declines during the photodegradation, from pH 5 to 4.

2.3. Analytical procedures

Kinetics of HCTZ and ABSA were monitored with Ultra Fast Liquid Chromatography with Diode Array Detection (UFLC–DAD, Shimadzu) (Armaković et al., 2015). Detail description of UFLC–PDA analysis was given in Supplementary Material. The UV/vis PDA detector was set at 223 nm (wavelength of maximum absorption of HCTZ and ABSA).

Concerning TOC analysis, 10 mL aliquots of the reaction mixture were taken at regular time intervals, diluted to 25 mL and analyzed after filtration on an Elementar Liqui TOC II analyzer, according to Standard US 120 EPA Method 9060A. For each measuring of mineralization degree a new suspension was prepared.

For ion chromatographic determinations, aliquots of 3 mL of the reaction mixture were taken at regular time intervals, filtered through membrane filters and analyzed on an ion chromatograph Dionex ICS 3000 Reagent Free IC system with conductometric detector. Detail description of ion chromatographic analysis was given in Supplementary Material.

For the liquid chromatography, with electrospray ionization-tandem mass spectrometry (LC–ESI–MS/MS) evaluation of intermediates, 10 μL samples were analyzed on an Agilent Technologies 1200 series HPLC with Agilent Technologies 6410A series electrospray ionization triple-quadrupole MS/MS, using Agilent Technologies Zorbax XDB-C18 column (50 mm × 4.6 mm i.d., particle size 1.8 μm, 25 °C) (Finčur et al., 2017). Detailed information about LC–ESI–MS/MS analysis can be found in the Supplementary Material.

Cell growth activity was carried out under the experimental conditions described in Supplementary Material. The growth effects were assessed using the cell lines H-4-II-E (ATCC CRL-1548), HT-29 (ECACC 91072201) and MRC-5 (ECACC 84101801).

2.4. Computational details

All DFT simulations were performed using Jaguar 9.4 program (Bochevarov et al., 2013), while MD simulations have been performed with Desmond program (Bowers et al., 2006; Guo et al., 2010; Shivakumar et al., 2010), as implemented in Schrödinger Materials Science Suite 2016-4 (Schrödinger Inc, 2016). Detailed computational information can be found in the Supplementary Material.

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