Chemical Engineering Journal 331 (2018) 785–793

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

New insights into the chlorination of sulfonamide: Smiles-type rearrangement, desulfation, and product toxicity

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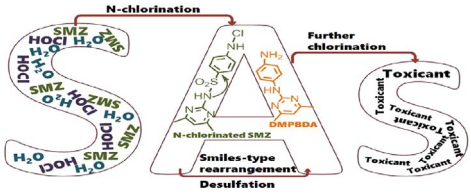
GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Sulfonamide
Chlorination
Smiles-type rearrangement
Pathway
Toxicity

ABSTRACT

Sulfamethazine (SMZ) is a typical sulfonamide antibiotic that has been frequently detected in municipal sewage, surface waters and drinking water. In this study, the oxidation of SMZ by free chlorine ($HOCl/OCl^-$) was investigated in detail, including the reaction kinetics, transformation products (TPs), reaction pathway, and toxicity change. Free available chlorine (FAC) was effective to eliminate SMZ, with a half-life of 0.98 min in the presence of 3 mg/L FAC and 10 μg/L SMZ at pH 7. The removal favoured neutral pH ranging from 6.08 to 7.98. Twelve oxidation products were identified using HPLC-QTOF-MS and HPLC-MS/MS. The Smiles-type rearrangement and the following desulfation process were speculated as the major pathway, via which approximate 40% of SMZ were transformed. Quantum chemistry calculation indicated that the substitution of chlorine to the amino group increased the electrophilicity of the aniline carbon, and thus facilitated the rearrangement. The rearrangement and desulfation product, N-(4,6-dimethylpyrimidin-2-yl)benzene-1,4-diamine (DMPBDA), was identified using the corresponding standard substance. Most of the other TPs were originated from DMPBDA through further chlorination and coupling reaction. Toxicity assays employing the photobacterium *V. fischeri* indicate the generation of more toxic TPs compared to SMZ. As the reaction progressed, toxicity continued to increase, and the percentage inhibition reached 97% and 99% on the 7th day and 14th day, respectively. This study presented a new possible reaction pathway for the chlorination of sulfonamide antibiotics and provided useful information for better evaluation of the effectiveness and safety of chlorination.

1. Introduction

Sulfonamide antibiotics (SAs) refer to a class of synthetic antimicrobial agents that contain the sulfonamide group [1]. They are widely used for human medicine and livestock husbandry due to their low cost and broad spectrum antibacterial property. Nowadays, SAs are also known as a kind of micropollutant that receives a lot of attention due to their continually occurrence in the aquatic environment. They are frequently detected in surface waters and ground waters at concentrations ranging from 1 ng/L to 1.9 μg/L [2–4]. Higher concentrations up to 2 μg/L have been reported in sewage treatment plant (STPs) effluent [3,5]. They are also detected in drinking water samples with

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http://dx.doi.org/10.1016/j.cej.2017.09.024
Received 30 April 2017; Received in revised form 27 July 2017; Accepted 2 September 2017
Available online 07 September 2017
1385-8947/

concentrations ranging from 0.1 to 60 ng/L [6]. STP effluent is a major source for SAs introduced into the aquatic environment, since they cannot be removed completely in STPs [7]. Other major routes of SAs are the discharge of wastewater of livestock husbandry and the land application of STPs sludge and contaminated manure [8,9]. The main concern about SAs is that they have the potential to exert selective pressure on microflorae and stimulate dissemination of antibiotic resistance genes among microorganisms and even to pathogens [10].

Chlorination (by HOCl/OCl^-) is the most common disinfection process used all over the world. As a kind of frequently-detected micropollutant, the chlorination behaviors of SAs are of great environmental significance, because the mineralization of SAs by chlorine can hardly be achieved, thus generating numerous transformation products (TPs), of which the toxicity remain unknown [11]. However, few researches have been launched on the chlorination of SAs. So far, only the chlorination pathway of a five-membered heterocyclic SA named sulfamethoxazole was studied in detail. Dodd et al. and Gao et al. suggested that the main TPs resulted from the direct attack of chlorine on the sulfamethoxazole aniline nitrogen, generating N-chlorinated sulfamethoxazole, N,N-dichlorinated sulfamethoxazole and azosulfamethoxazole by coupling reaction. N,N-dichlorinated sulfamethoxazole was further transformed to N-chloro-p-benzoquinoneimine through the cleavage of S-N bond [12,13]. Nevertheless, small modification of structure might affect the reaction behavior dramatically. Therefore, detailed studies on other SAs are necessary. Gaffney et al. once investigated the main chlorination products of sulfamethazine, sulfamerazine, sulfamethoxazole, sulfapyridine, sulfathiazole and sulfadiazine [14]. However, in that study, only few major products were speculated without the generation pathway and further identification. Wang et al. also studied the chlorination products of three SAs (sulfamethoxazole, sulfathiazole, and sulfadimethoxine). Several types of products generated through the chlorine substitution, S-C cleavage, S-N hydrolysis, desulfonation, oxidation, hydroxylation and conjugation reactions were detected. However, their study focused on the screening of disinfection byproducts without the details of the reaction mechanisms [15].

The chlorination products of SAs are rarely studied, not to mention their toxicity. To our knowledge, the available toxicity evaluations only involved the photodegradation, ozonation, and photo-Fenton TPs of some SAs [16–19]. For example, Dirany et al. investigated the toxicity of sulfachloropyridazine and its TPs generated after electro-Fenton treatment using *V. fischeri*. A sharp increase of toxicity was observed at the first 10 min due to the generation of cyclic compounds, which were decomposed to aliphatic carboxylic acids and resulted in a decrease of toxicity after 120 min [16]. González et al. observed a decrease of toxicity during the sulfamethoxazole removal by photo-Fenton [17]. Unlike the oxidation treatments that employ strong oxidizing hydroxyl radicals, after chlorination, some harmful halogenated products may form [18,19]. Hence, the toxicity of chlorination products of SAs should receive adequate attention.

Considering the above-mentioned research status, chlorination behavior of a frequently-detected SAs named sulfamethazine (SMZ) was investigated in detail in this study. The aims of this study are: (1) to investigate the reaction kinetics of free available chlorine (FAC) with SMZ under different pH and FAC dosages; (2) to identify the major chlorination products of SMZ and speculate their reaction pathways; (3) to evaluate the toxicity of SMZ chlorination products.

2. Materials and methods

2.1. Reagents and materials

SMZ (99%), sodium hypochlorite (4.4%), and formic acid (HPLC grade) were purchased from Sigma-Aldrich (USA). Sodium thiosulfate ($Na_2S_2O_3$), sodium hydroxide, monosodium phosphate, and disodium phosphate were purchased from Fuchen (China). Acetonitrile (HPLC grade) was obtained from Merck (Germany). Ultrapure water was prepared using a Milli-Q Integral 5 system (Millipore, USA). N-(4,6-dimethylpyrimidin-2-yl)benzene-1,4-diamine (DMPBDA) was purchased from J & K Scientific (China). The freeze-dried photobacteria *V. fischeri* were obtained from Hamamatsu Photonics (China).

2.2. Reaction kinetics

Reactions were conducted in 200 mL beakers at 25 °C (± 1 °C in air-conditioned room). 100 mL solutions containing 10 μg/L SMZ and 10 mmol/L phosphate buffer saline (PBS) that prepared using ultrapure water were constantly mixed by Teflon-coated stir bar on a magnetic stirrer at 300 rpm [20–23]. Reactions were initiated by adding FAC stock solution. Samples were collected at 5, 10, 20, 30 s and 1, 2, 5, 8, 10, 20, 30 min. $Na_2S_2O_3$ was spiked into each sample to quench the residual FAC (molar ratio 1:1) [24]. Different FAC dosages of 1 and 5 mg/L were also tested at pH 7. FAC concentration was measured by HACH DR3900 spectrophotometer (HACH, USA) following the Hach DPD method (EPA approved HACH 8021). pH were measured before initiating reactions and at 1, 5, 10, 20, 30 min, which were found almost constant (± 0.01).

Detection of SMZ was fulfilled by an Applied Biosystems/SCIEX API 3200 triple-quadrupole mass spectrometer (MS/MS) (AB Sciex, USA) equipped with a Shimadzu 20A high-performance liquid chromatography (HPLC) system (Shimadzu, Japan) performed in electrospray positive ionization (ESI+) and multiple reaction monitoring (MRM) mode. The MS/MS analysis parameters were listed in Table S1. Chromatographic separation was accomplished using a C18 column (Agilent, Poroshell 120, EC-C18, 2.1 × 100 mm, 2.7 μm). The flow rate was 0.2 mL/min. Mobile phase A was ultrapure water and mobile phase B was acetonitrile, both with 0.1% formic acid as mobile phase additive. The following gradient was used: 0–5 min, 5% B to 100% B; 5–6 min, 100% B; 6–7 min, 100% B to 10% B; 7–10 min, equilibrate with 5% B.

2.3. Product identification

To analyse the TPs and reaction pathway, high concentration of SMZ (0.36 mmol/L) was chlorinated with 3.6 mmol/L FAC at pH 7 in 75 mmol/L PBS. Mixtures were sampled respectively at 8 min, 5 h, 12 h, 1 d, 3 d, 5 d, 7 d, 14 d and preliminarily analysed (without quenching agent) by HPLC-MS/MS system with the same chromatographic column, flow rate and mobile phases mentioned in "2.2 Reaction kinetics". The following gradient was used: 0–13 min, 10% B to 100% B; 13-15 min, 100% B; 15–16 min, 100% B to 10% B; 16–20 min, equilibrate with 10% B. The injection volume was 10 μL. The MS analysis was performed in ESI+ and Q1 scan mode. Major TPs were further analysed by an Applied Biosystems/SCIEX Q-Star time-of-flight mass spectrometer (QTOF-MS) (AB Sciex, USA) equipped with a Agilent 1200 HPLC system (Agilent, USA) to provide more accurate molecular weights. The same HPLC column, mobile phases and gradient were used and HPLC-QTOF-MS analysis was performed in ESI+ and product ion scan mode. HPLC-MS/MS analysis parameters of all TPs in MRM mode, including declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP), were optimized and listed in Table S1.

2.4. Computational methods

The density functional theory (DFT) calculation was performed applying the Gaussian 09 software package [25]. Fully optimizations of geometry and the following vibrational frequency calculation were fulfilled at the B3LYP level with the 6-311G(d,p) basis set. Atomic dipole moment corrected Hirshfeld (ADCH) charges were obtained by the Multiwfn software (version 3.3.9) [26].