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Aquatic photochemistry of the sulfonamide antibiotic sulfapyridine

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ABSTRACT

The photolytic behavior of the sulfonamide antibiotic sulfapyridine in water was investigated using a laboratory photoreactor approximating full-spectrum sunlight. Direct photolysis of sulfapyridine was rapid, with a half-life of 2.6 h and 31 min, and an observed quantum yield of 0.0013 ± 0.0002 and 0.013 ± 0.001, for the neutral species and fully deprotonated species, respectively. Direct photolysis rates increased dramatically with degree of deprotonation, with measured pKa_1 and pKa_2 values of 2.22 ± 0.03 and 8.58 ± 0.02, respectively. Indirect photolysis was assessed using water from constructed wetland mesocosms. A four-fold increase in the dissipation rate of sulfapyridine was observed due to the influence of high levels of dissolved organic carbon, after accounting for light screening by such materials. Nitrates had no observable effect on indirect photolysis rates. Major photoproducts identified were SO_2 extrusion and OH addition products. These results show that photolytic processes are a major removal mechanism of sulfonamide drugs in aquatic systems.

1. Introduction

Human and veterinary-use pharmaceuticals are a significant class of contaminants that can enter aquatic environments. These chemicals find their way into surface waters primarily via wastewater discharge or agricultural runoff, and are normally not completely removed in sewage treatment processes. Although acute effects from such chemicals are generally unlikely at the low concentrations prevalent in the environment, little is known of the potential effects of sublethal chronic exposure concentrations on non-target organisms, particularly antibiotics [1]. These drugs are among the most prominent therapeutic pharmaceuticals detected in the environment [1], and are of particular concern due to the potential for development of microbes bearing antibiotic resistance genes [2–5].

Sulfonamides are a major class of broad-spectrum synthetic antibiotics, widely used to treat respiratory, skin, and urinary tract infections, and as animal growth promoters. This class of chemicals are excreted in urine and have been detected at concentrations as high as 7.9 μg/L in raw wastewater [6], with sulfapyridine observed in effluent at 63–135 ng/L [7]. The strong bacteriostatic properties of sulfonamides can have significant effects on the ecological functioning of microorganisms. For example, sulfapyridine was shown to significantly reduce microbial activity in soils, with EC_{10} values ranging from 0.14 to 160 ng/g based on the Fe(III) reduction test, with an incubation time of 7 d [8]. This, along with the promotion of antibiotic resistance [2,3], strongly suggest that the environmental presence of sulfonamides may pose a hazard to ecosystem health.

Sulfonamides absorb light in the environmentally relevant UV-B and UV-A ranges (280–400 nm) [9]. Thus, direct photolysis could be a significant mechanism for abiotic transformation in sunlit surface waters, as observed for other five- and six-member ringed sulfonamides for which protonation state has a major influence on degradation rate [9,10]. Furthermore, the presence of photosensitizing species can significantly enhance the extent of indirect photolysis. Dissolved organic matter (DOM) present in natural waters can sensitize chemical photodegradation either by direct transfer of energy, or through the formation of reactive intermediates such as singlet oxygen ($^1O_2$), superoxide anion ($O_2^{-\bullet}$) and hydroxyl ($^\bullet$OH), hydroperoxy ($HO_2^\bullet$), alkylperoxy ($RO_2^\bullet$), and carbonate ($CO_3^{-\bullet}$) radicals [9,11–15]. Additionally, dissolved nitrates can mediate the formation of $^\bullet$OH radicals in natural waters, and in some cases to a greater degree than DOM [16].

While a number of studies have examined the photochemical fate of sulfonamides, few have investigated sulfapyridine, despite its common occurrence [7]. Photodegradation rates of sulfapyridine differed in wastewater treatment plant effluent compared to in pure HPLC-grade water [17]. Degradation rates were 5-fold faster in the effluent ($t_{1/2} = 2$ h) versus in the pure water in that study ($t_{1/2} = 10$ h), indicating that indirect photolysis plays a significant

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role in limiting the persistence of sulfapyridine. Sulfamethazine, on the other hand, showed little evidence of indirect photolysis in the effluent matrix [17]. Quantum yields were not reported for either chemical. Despite the structural similarities that exist within the class of sulfonamide drugs, photochemical behavior differs significantly from compound to compound [9,10], suggesting that the study of the photochemical fate of individual sulfonamides on a case-by-case basis is necessary.

The objective of the present study was to characterize the photochemical behavior of the sulfonamide antibiotic drug sulfapyridine. Specifically, direct photochemical degradation rates and quantum yields of sulfapyridine were examined in water with a laboratory photoreactor. The effect of speciation on direct photolysis was examined by conducting irradiations at acidic, neutral and basic pH. Indirect photolysis was assessed by irradiating sulfapyridine in water from field mesocosms designed to replicate constructed wetlands, that can help eliminate such chemicals [18]. Two photodegradation products were identified. These results will help define the fate of sulfapyridine in the aquatic environment. Furthermore, this study will further our understanding of the removal mechanisms responsible for eliminating pharmaceuticals from natural and engineered waters, and characterize exposure routes, therefore enhancing risk assessment of this chemical in aquatic ecosystems.

2. Materials and methods

2.1. Chemicals and reagents

Sulfapyridine (≥99%), *p*-nitroanisole (PNA, 97%), and pyridine (PYR, ≥99.9%) were purchased from Sigma–Aldrich (St. Louis, MO). HPLC grade methanol was purchased from Fisher Scientific (Fair Lawn, NJ). Solutions for direct photolysis were prepared with nanopure water (>17 MΩ-cm, Milli-Q RG, Millipore Corp., Ann Arbor, MI), and buffered using di- and tri-basic potassium phosphate (K_2HPO_4, K_3PO_4, ≥98%, Sigma–Aldrich) titrated with hydrochloric acid (HCl, Fisher Scientific) to the desired pH. Ultra-grade potassium nitrate (KNO_3, ≥99.5%, Sigma–Aldrich) was used for indirect photolysis experiments. Liquid chromatographic solvents were prepared with nanopure water and HPLC grade acetonitrile (ACN, Fisher Scientific) or methanol (MeOH) buffered with formic acid (95%, Sigma–Aldrich).

2.2. Instrumentation and equipment

2.2.1. Laboratory photoreactor

All irradiations were performed using a Rayonet Merry-Go-Round Photochemical Reactor (model RPR-100, The Southern New England Ultraviolet Company, Branford, CT). The photoreactor had 16 medium-pressure mercury lamps with spectral emission ranging from 250 to 400 nm, centered at 300 nm. Irradiation vessels used were 50 mL cylindrical Pyrex tubes which filtered wavelengths <290 nm.

2.2.2. Chemical analyses

A Shimadzu (Columbia, MD) UV-2501PC spectrophotometer was used to record UV–vis absorption spectra in the determination of molar absorptivities of sulfapyridine and pK_a values via spectrophotometric titration. Analyses of irradiated solutions were done with an Agilent Technologies (Mississauga, ON) 1200 high performance liquid chromatograph (HPLC) equipped with a UV diode array detector. Sulfapyridine was separated on a Waters (Milford, MA) Symmetry C_{18}, 4.6 mm × 150 mm, 3.5 μm analytical column with a Phenomenex (Torrance, CA) SecurityGuard C_{18} Guard Cartridge (4 mm × 3.0 mm ID). PNA/PYR were separated using an Agilent Zorbax HILIC plus column (2.1 mm × 100 mm, 3.5 μm). Mobile phases were water and ACN buffered with 0.05% formic acid (10 mM) to pH 3. Sulfapyridine was separated isocratically with 70:30 H_2O:ACN, at 1 mL/min, while PNA/PYR was separated with 80:20 H_2O:ACN at 0.5 mL/min. Quantitation was monitored at 260 and 320 nm for sulfapyridine and 320 nm for PNA/PYR.

Identification of photoproducts was done via ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC/MS/MS) using an Agilent 1200 binary UHPLC pump coupled to a 6410 triple quadrupole MS/MS (Agilent Technologies). Separation was achieved with a Zorbax C_{18} column (2.1 mm × 100 mm, 1.8 μm) with a Phenomenex SecurityGuard C_{18} Guard Cartridge (4 mm × 3.0 mm ID) using gradient elution at 0.5 mL/min commencing with 95:5 H_2O:MeOH (buffered with 10 mM formic acid), ramping linearly to 100% MeOH over 5 min, followed by a 3 min re-equilibration. Injection volume was 5 μL. Analytes were ionized using an electrospray interface operating in both positive and negative ionization modes with the following conditions: capillary voltage, 4000 V; nebulizer pressure, 15–55 psi; drying gas flow, 10–11 L/min; drying gas temperature, 300 °C. The source fragmentor voltage ranged from −51 V in negative mode to +129 V in positive mode. Nitrogen was the collision gas, with collision energies between 11 and 20 V. The cell accelerator voltage was maintained at 7 V for all analyses.

2.3. pK_a determination

Spectra of sulfapyridine were recorded at 0.1–0.2 pH intervals between pH 12 and 1. Aqueous solutions were buffered with K_3PO_4 (50 mM) titrated to the desired pH with concentrated HCl. At each pH a 10 mL aliquot of the buffer was removed and spiked with 100 μL of sulfapyridine providing a 5 mg/L solution. Differences in absorbance at single wavelengths were plotted as a function of pH and the resultant data were fit to a non-linear sigmoidal regression (Prism v. 5.01, GraphPad Software, La Jolla, CA) to determine pK_a. Additional details are available in Supplemental Information.

2.4. Photolytic studies

2.4.1. Chemical actinometry

The PNA/PYR actinometer established by Dulin and Mill [19] was used to monitor photon flux, which was then used to estimate the photoreaction quantum yield of sulfapyridine. Photoreactor experiments used 4.6×10^{-5} M PNA and 0.01 M PYR. Irradiation and dark experiments for the actinometer system were carried out in parallel.

2.4.2. Direct photolysis

Stock solutions of sulfapyridine were prepared at 4.0×10^{-4} M (100 mg/L) in nanopure water. Triplicate laboratory irradiations were conducted with 40 mL solutions of sulfapyridine, each at 5 mg/L, in Pyrex tubes. Dark experiments were carried out in an oven that matched the temperature and time of irradiation in the photoreactor, which reached a maximum temperature of 45 °C at irradiation times >2 h. All experiments were performed in triplicate over five time points. Photolyses were conducted in 50 mM of the appropriate potassium phosphate buffer at pH 5.2, 7.2 and 11.1.

2.4.3. Indirect photolysis

Laboratory photolysis of sulfapyridine was also investigated in simulated natural water from established field mesocosms. Absorbance spectra of the mesocosm water was obtained using a UV–vis spectrophotometer, and used to determine screening factors of the water. These simulated constructed wetland mesocosms contained sediment, water (originally sourced from City of Winnipeg tapwater), macrophytes, invertebrates, and microbial