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The Study of Sulfisoxazole Direct Photodegradation

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Abstract - In this paper, the photolytic behavior of the sulfonamide antibiotic sulfisoxazole in water was investigated in accordance with OECD Guideline 316 using a laboratory photoreactor approximating full-spectrum sunlight (SunTest CPS+). Direct photolysis of sulfisoxazole was relatively rapid, with a half-life of 3.1 h and 11.7 h, for the acid and neutral conditions, respectively. Direct photolysis rates was a pseudo-first order kinetics in neutral solution, but in acid solution after 2 h of photolysis the rate of SX rate increased. Identified photoproducts were sulfanilamide, and its OH addition products. The fragmentation of the isoxazole ring was also observed. These results suggest that photolytic processes could be a major removal mechanism of sulfisoxazole in aquatic systems.

***Keywords*:** Photodegradation, Sulfisoxazole, Kinetics, Intermediates of direct photodegradation.

1. Introduction

Sulfa pharmaceuticals are often used in aquaculture (Ternes, 1998), as veterinary pharmaceuticals, animal growth promoters (Hirsch et al., 1999; Battaglin et al., 2000), and human beings for the treatment of respiratory and urinary tract infections (Boreen et al., 2004). They share a common core chemical structure (p-aminobenzene sulfonamide), which inhibits multiplication of bacteria by acting as a competitive inhibitor of p-aminobenzoic acid in the folic acid synthesis cycle. This class of chemicals are excreted in urine and have been detected at concentrations as high as 7.9 µg/L in raw wastewater (Peng et al., 2006) with sulfisoxazole observed in effluents at range 19-34 ng/L (Spongberg & Witter, 2008). Sulfamonomethoxine, sulfadiazine, sulfisoxazole and sulfadimethoxine are permitted and sold as veterinary medicines for marine products (Chung, 2008).

In surface waters, the main removal processes of organic pollutants are biodegradation, sorption, hydrolysis (Bialk-Bielinska et al., 2012) and photodegradation. Photodegradation includes direct and indirect process. Direct photolysis requires an overlap of the pharmaceuticals' electronic absorption spectra and the irradiation wavelengths. If there is no overlap then the only photochemical process is indirect photolysis. The presence of photosensitizing species can significantly enhance the extent of indirect photolysis in environment. 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 (Boreen et al., 2004; Albinet et al. 2010; Manjun, 2009). Additionally, dissolved nitrates can mediate the formation of $^\bullet OH$ radicals in natural waters, and in some cases to a greater degree than DOM (Vione et al., 2006).

Sulfonamides absorb light in the environmentally relevant UV-B and UV-A ranges (280–400 nm) (Boreen et al., 2004). Thus, direct photolysis could be a significant mechanism for abiotic transformation in sunlit surface waters. Boreen et al (2004) reported that the rate of direct photolysis of five-membered heterocyclic sulfonamides is dependent upon the identity of the five-membered heterocyclic ring as well as the pH of the solution. The quantum yields calculated range from <0.005 for the neutral state of sulfamethizole to 0.7 ± 0.3 for the protonated state of sulfisoxazole, therefore the protonated state of five-

membered heterocyclic sulfonamides is the most photoreactive and varies among the sulfa drugs. Sulfanilic acid was identified as the main direct photodegradation product of sulfonamides (Boreen et al, 2004; Boreen et al 2005). Reaction of the sulfa drugs with hydroxyl radical was not modulated by the heterocyclic ring, and the rate constants were all near the bimolecular diffusion-controlled limit of 10^{10} M^{-1} s^{-1}.

The sulfa pharmaceuticals have been used for several decades, thus, it is very essential to study the transformation kinetics and mechanism involved of them in various environmental conditions. Moreover it is required by EU Directives on the monitoring of environmental quality. Regulatory demands on data quality for the environmental risk assessment of pharmaceuticals divides data into three categories where category I includes data which were obtained or generated according to internationally accepted test guidelines (e.g. OECD), or which were based on a specific testing guideline, or in which all the parameters described are closely related/comparable to a guideline method [Kuster et al, 2009; Klimish et al, 1997]. It is also stipulated that the environmental effects/fate of pharmaceuticals must be investigated using validated methods. Although more data on the photolysis of pharmaceuticals have become available [Aga, 2008; Reemtsma T.& Jekel, 2006; Gazpio et al., 2008], but they were obtained using different procedures. This makes comparison of results difficult, regardless of the uncertainty whether these data are representative of the actual behaviour of pharmaceutics in the environment. Such studies have determined phototransformation kinetics, products, and product pathways resulting either from direct or indirect (by photosensitising or reaction with oxidising intermediates) aqueous photolysis. The instructions in the European Medicines Agency guidelines require direct photolysis to be carried out using OECD Test Guideline 316 (OECD 2008).In the paper the data obtained by OECD 316 for sulfisoxazole have been presented.

2. Experimental

2.1 Photolysis Study

Photolysis studies were performed in accordance with OECD Guideline 316, including an initial screening by uptake of UV-Vis absorbance spectra and a full experimental study of any compound showing potential for degradation by natural sunlight. The full study employed an Atlas SunTest CPS+ instrument with a 1500 W arc xenon lamp and outdoor UV filters that restrict the transmission of light with wavelengths below 290 nm. To monitor the temperature, a black standard temperature sensor, was used. The device was refrigerated by an air cooled system. Solutions were irradiated in triplicate using 35 mL quartz tubes with an internal diameter of 1.8 cm. The samples were exposed until 90 % depletion of the analytes was observed, and the rate of photolytic degradation then determined. The exposure intensity was set to 550 W/m^2, which is equivalent to the maximal mid-summer solar irradiance at 40° latitude. The sulfisoxazole solution was added to sterilized buffer at pH=4,0 and pH=7.0 to give final concentrations of 37μM. Samples were collected at proper time intervals up to 8 hours. Control samples, wrapped in aluminium foil to exclude light, were also included to ensure no other concurrent degradation was occurring. The samples were analyzed by HPLC-UV and LC-(UV)MS. No reaction was observed for the pharmaceutical in the pH range 4.0–9.0 in blank control experiments performed in the dark.

The concentration of sulfisoxazole was determined by a HPLC with UV detector (Perkin Elmer, Series 200) equipped with a Phenomenex Gemini-NX C-18 column (150 × 4.6 mm, 5 μm). The injection volume of each sample was 50 μL and a flow rate was 0.7 mL min^{-1}. The UV/VIS detector set at 270 nm. Mobile phase A was 100 % acetonitryle and mobile phase B was H_2O with the addition of TFA at pH 3.5; both were selected in the isocratic program 45 % of mobile phase A and 55 % of mobile phase B

2.2. Identification of Photodegradation Products by LC-(UV)MS

Intermediates were identified by Agilent 1200 Series LC system (Agilent Technologies, Inc., Santa Clara, USA) coupled with HCT Ultra ion trap mass spectrometer (Brucker Daltonics, Bremen, Germany) with an electrospray ionization (ESI) source. Ions with m/z 35–400 were monitored in full scan mode, selected ions were monitored in SIM mode. Samples were separated on a Gemini C18-110A column (150

mm × 4.6 mm, 5 µm, Phenomenex Inc. Torrance, CA) (room temperature, wavelength 270 nm and 254 nm, injection volume 50 μL, flow rate 0.7 mL min^{-1}). Mobile phase A was 100 % of acetonitryle, and mobile phase B was 1 mM amonium acetete solution in mixtures ACN:H_2O (10:90, v:v); both were selected in the isocratic programme; 30 % mobile phase A and 70 % phase B. Chromatographic separation of sulfisoxazole and degradation products was achieved within less than 20 min.

3. Results and Discussion

Sulfisoxazole contains basic functional groups such as group of aniline ($H_3N(+)$-C_6H_4-) and heterocyclic base (-$C_4N_2H(CH_3)_2$). The acidic functional group is sulfonamide group, which is known to lose its proton relatively easily (pKa_3 = 5.0). Therefore the percentage of ionized and non-ionized species of the compound is depended on pH of solution. Agree to OECD 316 if the chemical is appreciably ionic anywhere within 4 - 9 pH range, the study should be conducted in one or more aqueous buffers at any of pH 4.0 ,7.0 and 9.0. At the initial pH 9.0 and 7.0 the main form of sulfisoxazole is anionic form (99%), while at pH=4.0 the neutral form (90%). The absorbance spectra of 37μM solution of SX and sulfisoxazole forms versus pH was presented in Fig. 1.

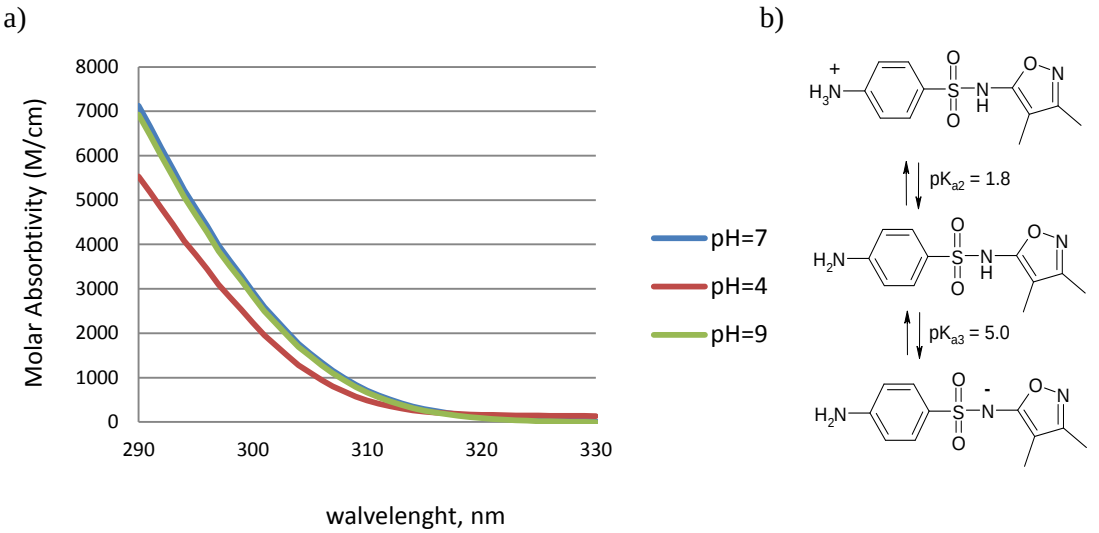


Fig. 1. The molar absorptivity of SX a) and structure of SX forms b) existed in solution versus pH.

The direct photodegradation rate constants measured for SX by Boreen et al (2004) in a variety of buffed solutions under simulated sunlight are listed in Table 1.

Table 1. Direct photolysis rate constant and half-life for SX.

pH	k (d^{-1})[a]	$t_{0,5}$ (h)[a]	k(d^{-1})[ba]
4.0	5.4	3.1	5.12 (pH=4.1)
7.0	1,4	11.7	2.16 (pH=6.8)

[a] – our results

[b-] data obtained by Boreen et al (2004) under natural sunlight on May 13, June 5, and June 9, 2003, in Minneapolis, MN (45° latitude), [SX]=100 μM, V=10mL.

The results are difficult to compare with the data obtained in other studies, because, the different experimental conditions such as sources of light (middle-pressure Hg lamp, sunlight with the cloudy or

sunny day) were utilized. The SX decay rate constants calculated by us are slightly different to these presented by Boreen et al (2004) (Table 1).

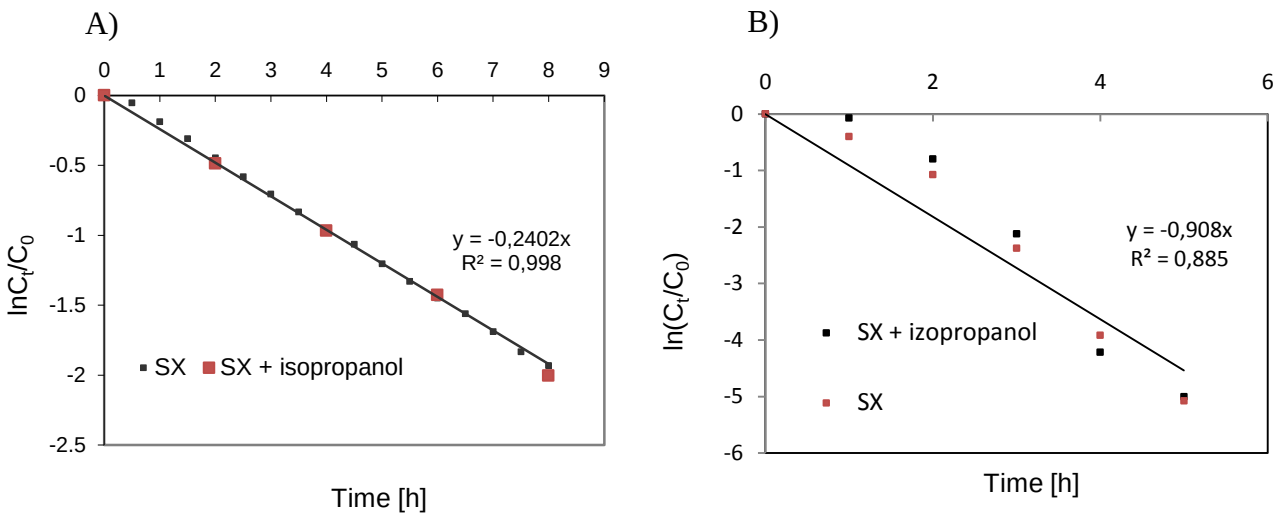


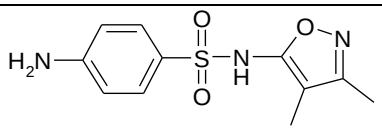
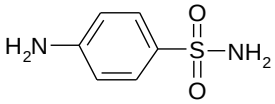
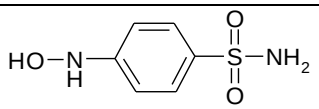
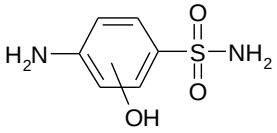
Fig. 2. Photodegradation of 37 μM SX in buffer at pH=7.0 A) and pH=4 B) in presence and absence of isopropanol.

In diluted solution the direct photolysis kinetics are theoretically first order. According to Fig. 2, at pH=7, there were the first order exponential decays of the normalized SX concentration (C_t/C_0) with time obtained with good linearity 0.998. In contrast to this, the photolysis of SX at pH=4 wasn't good fitted to the pseudo-first order kinetics. During 8 h of photolysis, the degradation showed two stages: slower for the first 2 h of irradiation, and more rapid after 2 hours of irradiation. Therefore the additional experiments with the scavenger of •OH radicals – entities, which take part in indirect photolysis, were carried out. The results showed, that at pH=4 the indirect photolysis didn't take part in the photodegradation of SX. The low fit of a linear correlation between $\ln C_t/C_0$ for SX at pH 4 could be due to presence of intermediates produced during the photolysis, which could act as promoter the decay of SX. Therefore after 2 hour of process the mechanism are complex.

Additionally the kinetic data for the photolysis demonstrated that SX is more stable at natural water pH in an anionic form. This observation is in good agreement with other studies (Boreen et al 2004), suggesting that photolysis rate is dependent on speciation in the given water sample.

To further elucidate the photolysis mechanism of SX, LC-(UV)-MS was applied to investigate the photolysis intermediates (Table 2). The photoproduct identified through MS analysis was sulfanilamide corresponding to $[M+H]^+ = 173$. Product $[M+H]^+ = 189$ was a hydroxyl derivative of sulfanilamide. Zhou and Moore (1994) have identified aniline as the main photolysis intermediate of other five-membered heterocyclic sulfonamide – sulfamethoxazole. However, aniline wasn't detected in this study. Next product $[M+H]^+ = 270$ was formed through the opening of the isoxazole ring of SX molecule. The unequivocal assignment of the structure for this compound is difficult because of the absence of specific fragments in the mass spectrum. However the fragmentation position of the isoxazole ring was different from previous reports (Trov´o et al., 2009). The process of SO_2 extrusion is well known in organic synthesis where UV-light is utilized (Givens et al., 1984) and the photodegradation of six-member heterocyclic ring-containing sulfonamides in the aquatic environment. (Guerard et al., 2009). In the study products $[(M-64)+H]^+$ was not observed.

Table 2. The proposed direct photolysis intermediates of SX

Compound	Retention time [min.]	$[M+H]^+$	Structure
Sulfisoxazole (SX)	12.2	268	
sulfanilamide	5.9	173	
hydroxyl derivative of sulfanilamid	8.2	189	 
product a	6.4	237	-
product b	6.0	289	-
product with opening of the isoxazole ring	12.7	270	-

4. Conclusion

The present study investigated the relevance of direct photochemical process on the persistence of the sulfonamide antibiotic drug, sulfisoxazole, in aquatic systems. Direct photolysis of SX varying with pH; at low pH, sulfisoxazole, predominantly in its neutral HS form, exhibited enhanced photoreactivity compared to its anionic S^- form. Although the absorptivity of sulfisoxazole increased as solutions became neutral. The direct photolysis of sulfisoxazole was rapid, with a half-life of 3.1 h and 11.7 h, for the acid and neutral conditions, respectively. However the data obtained from studies at pH=4 not good fitted to pseudo-first order kinetics, probably due to a complex mechanism. Therefore the further investigation explaining this behavior of SX should be carried out. In study, two photodegradation products of sulfisoxazole were identified: sulfanilamide and hydroxylated products of sulfanilamide. Overall, this investigation provides new overlook of the photochemical behavior of five-member heterocyclic ring containing sulfonamides in aquatic environments.

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