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Analysis and degradation of pharmaceuticals and fluorinated surfactants

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INTRODUCTION

Focus in this study was on pharmaceuticals and perfluorinated compounds since they play an important role in our daily activities and in increasing the living standard, but on the other hand they are not so friendly to the environment.

1.1. PERFLUORINATED COMPOUNDS

Perfluorinated compounds (PFCs) are organofluorine compounds where all the C–H bonds have been replaced by C–F bonds. They contain a hydrophobic fluorinated carbon tail and a polar, hydrophilic head attached to one end of the carbon chain. PFCs have surface-active properties and are excellent surfactants and surface protectors due to their unique properties of repelling both water and oil. They are also used in a variety of products such as textile protectors, coatings, plastics, fire fighting foams, shampoos, stain repellents for furniture, carpets and pesticides. PFCs are released to the environment after production, use or disposal and persist although some may undergo chemical degradation or biodegradation [1].
The widespread detection of environmentally persistent perfluorinated acids (PFCAs) such as perfluorooctanoic acid (PFOA) and its longer chained homologues (C9>C15) in biota has instigated a need to identify potential sources. It has recently been suggested that fluorinated telomer alcohols (FTOHs) are probable precursor compounds that may undergo transformation reactions in the environment leading to the formation of these potentially toxic and bioaccumulative PFCAs [2].

1.1.1. Materials and methods

Perfluorinated carboxylic acids (PFBA, PFPeA, PFHxA, PFOA, PFNA, PFDA, PFDUnA, PFDoA, PFTrA, PFTeA) and flurotelomer alcohols (10:2-, 6:2-, 8:2-FTOH) were purchased from Neochema (Mainz, Germany). ^{13}C labelled standards were M-8:2 FTOH, MPFBA, MPFHxA, MPFOA, MPFNA, MPFDA, MPFUnA, MPFDoA all purchased from Wellington laboratories (Ontario, Canada). All solvents used were of the highest purity purchased from Merck (Darmstadt, Germany).

The investigated perfluorinated compounds were extracted from the washing water samples on Oasis WAX catridges (60 mg, 3 cm^3) using an apparatus for solid phase extraction. Before water application, cartridges were conditioned in three steps. First they were conditioned with 2 mL (MeOH+0.1% NH_3), then with 2x2 mL MeOH and in the end with 3x3 mL H_2O. A sample volume of 200 mL was applied to the cartridges. After washing step with 3 mL H_2O/MeOH (80/20) cartridges were dried for 10 min with nitrogen. Retained compounds were eluted in two steps. The first step was for neutral and basic compounds and was done with 2x1.5 mL MeOH+0.05% HOAc, and the second step was for acidic compounds and was done with 2x2 mL MeOH+0.1% NH_3. Elution one was vortexed and filled into HPLC vials, while elution two was evaporated to drynes at 50 ^0C, reconstituted in 500 µL H_20/MeOH 1/1 and filtered through membrane filter.

The instrumental chromatographic setup consisted of two Series 200 Micro Pumps, a Series 200 vacuum degasser, and a Series 200 autosampler (Perkin Elmer, Norwalk, CT, USA). Separations were carried out on a HALO C18 column (Advanced Materials Technology, Wilmington, DE, USA), 50 x 2.1 mm, 2.7 µm particle size. The chromatograph was coupled to a hybrid triple quadrupole linear ion trap tandem mass spectrometer Q Trap 3200 (Applied Biosystems, Foster City, CA, USA).

1.1.2. Results and discussion

Aim of this study was to investigate how much PFCs are released from the different jackets during washing process into washing water, which end up in the environment. An earlier study showed presence of PFCs in the jackets used for this study.

Preliminary experiments showed that washing water with detergent is too complex matrix for PFCs detection because of high concentration of surfactants.

In order to biodegrade surfactants present in detergent, and on that way try to simplify the matrix degradation experiments were done using fixed-bed bioreactors (FBBRs). FBBRs consisted of a tank or storage bottle connected to a fixed bed, consisting of a glass column filled with glass beads. The mixture of washing water and effluent wastewater (1:4) was circulated on a closed loop through this column by means of a vacuum pump, allowing the microorganisms of the water matrix to accumulate on the surface of the glass beads. A second membrane pump aerated the water in the storage bottle. Samples were taken through a three-way valve at the top of the fixed bed. Shematic setup of FBBR can be seen on the Figure 1.

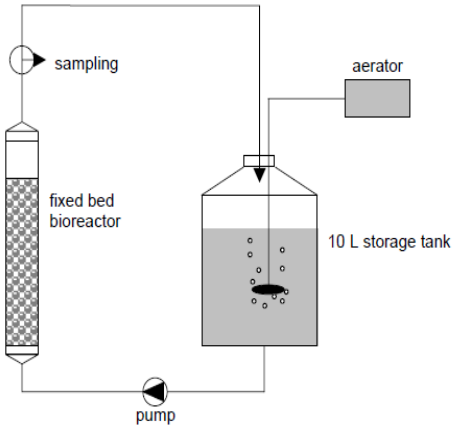

Figure 1. Shematic setup of the FBBR

After two weeks of experiment running at room temperature, water was taken, filtrated, spiked with PFCs and enriched. Generally, the results were better compared with those obtained by only washing water spiked with PFCs. Although compounds were detected, they could not be quantified because of the high background. But at least it was obvious that detection of these compounds, especially acidic was much better after biodegradation of surfactants.

In the next set of experiments microorganisms accumulated on the surface of the glass beads were taken and put together with washing water on biodegradation. Washing water was spiked only with native compounds while labelled compounds were added after biodegradation, prior SPE procedure. Biodegradation was done at room temperature, shaking bottles with washing water and microorganisms. Aerobic conditions were maintained by aerating the bottles regularly with an aquarium pump. Biodegradation could lead to degradation not only surfactants but also fluorinated telomer alcohols since it was demonstrated that FTOHs are potential sources of PFCAs as a consequence of degradation [2].
After four weeks of biodegradation, water was taken and analyzed. PFCs could not be quantified because of the background. So, it can be concluded that washing water with detergent is too complex matrix for detection PFCs.

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1.2. PHARMACEUTICALS

Among all classes of pharmaceuticals, the presence of antibiotics is likely to be of most concern because it could lead to the development of bacteria with antibiotic resistance genes and to the development of allergenic responses. They are extensively used in both human and veterinary medicine against microbial infections; in addition a certain fraction of antibiotics is used to increase the rate of growth in animal farms and fisheries [1]. Once in the body of man or animal pharmaceuticals undergo a series of biochemical reactions. Metabolic changes include two types of biochemical reactions known as biotransformation reactions of the first and second phase [2, 3]. In most instances, human drugs are metabolized in the body to more polar compounds that are more likely to pass through the waste water treatment plants (WWTPs) and end up in the environment.

Photodegradation might be an important elimination process for pharmaceuticals if they are light sensitive. It takes place mainly in clear surface water and may not occur when compounds are present in turbid water, soil and sewage since they have low light exposure. The effectiveness of photodecomposition depends on light intensity and frequency, and also it varies with season, latitude, pH and hardness of the water, the type of matrix and location [4].

Sulfonamides (SAs) represent one of the most commonly used families of antibiotics in veterinary medicine. As they are weak acids and both fairly water-soluble and polar compounds, SAs are retained weakly in soil systems, having a high potential for leaching or running off to ground waters and surface waters respectively after their release into the environment [5, 6]. One of possible way to limit SAs concentration in the environment is photolysis.

1.2.1. Materials and methods

Trimethoprim (TMP), sulfadiazine (SDIAZ), sulfamethazine (SMETH) were obtained from Veterina Animal Health Ltd. (Kalinovica, Croatia), while sulfamethoxazole (SMETOX) was supplied by Sigma-Aldrich (St. Louis, MO). N^4-acetylsulfadiazine (Na-SDIAZ), N^4-acetylsulfamethazine (Na-SMETH), N^4-acetylsulfamethoxazole (Na-SMETOX) were purchased from Toronto Research Chemicals (North York, ON, Canada). All solvents used were of the highest purity available and were from Merck (Darmstadt, Germany).

Suntest CPS+ simulator (Atlas, Germany) equipped with a Xenon lamp and temperature sensor was used as the source of artificial sunlight in the wavelength range of 300-800 nm. Pharmaceutical solutions were exposed to simulated sunlight for a continuous duration of 24 h. During the experiments the radiation intensity was maintained at 500 W m^{-2} and the reaction temperature was kept at (25 ± 2) °C. In order to establish that the degradation of the investigated pharmaceuticals was affected only by the light, control samples were also analyzed. They had the same composition as the investigated samples and were performed under the same conditions but protected from the effects of light (vessels with a control sample were wrapped in aluminum foil).

To investigate the appearance of photodegradation products, the samples were analyzed by LC-MS/MS, which was performed with an Applied Biosystems triplequadropole mass spectrometer

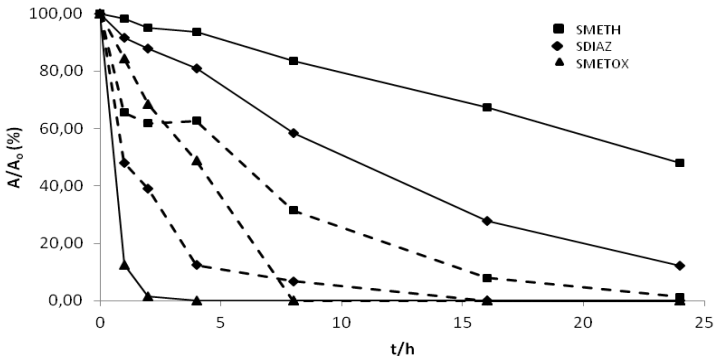
(Applied Biosystems, API 2000, USA) coupled with a HPLC system (Agilent Series 1100). Separations were carried out on Aqua Perfect C18 column (MZ Analysentechnik GmgH, Mainz, Germany), 50 x 2.1 mm, 5 µm particle size.

1.2.2. Results and discussion

During the irradiation a decrease in the concentration of all SAs and their metabolites was observed, no degradation was determined under light exclusion.

As can be seen form Figure 1., SAs and their metabolites showed susceptibility to light, but it is possible to notice their different behavior. Considering the fact that, at least, 88% of degradation for almost all compounds (except for SMETH) was achieved, further irradiation was not extended for a longer period.

A)



B)

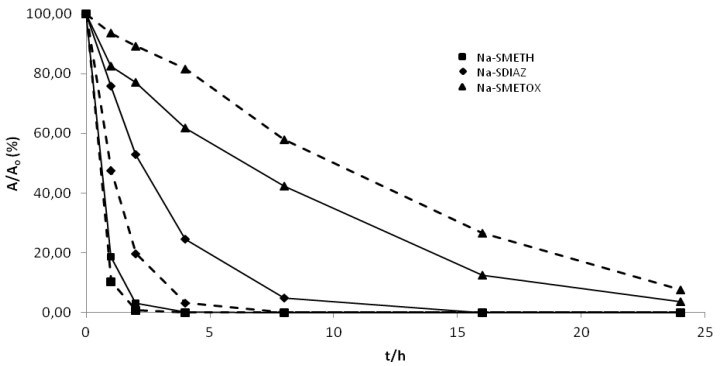


Figure 1. Degradation profiles of investigated sulfonamides (A) and their N^4-acetylated metabolites (B) at pH 7 (dashed line) and pH 4 (solid line).

The results of photodegradation experiments of investigated pharmaceuticals were plotted as a function of irradiation time, and the data were assuming pseudo-first order kinetics. The pseudo-first-order model is represented with following equation:

$A = A_0 \times \exp(-kt)$ (1)

where, A_o is the initial pharmaceuticals concentration (in our case integrated area of the chromatographic peak), A is the residual pharmaceuticals concentration at sampling time t *(h)* and k *(h^{-1})* is the rate constant. Half-lives were calculated following equation (2):

$t_{1/2} = \ln(2)/k$ (2)

Fitting results showed that photolysis of all SAs and their metabolites followed pseudo first order kinetics at both pH levels with correlation coefficient (R^2) usually higher than 0.99. The rate constants together with other kinetic parameters are listed in Table 1.

Table 1. Pseudo-first order kinetic parameters (k, rate constant; R^2, correlation coefficient; $t_{1/2}$, half-life).

Analyte	Water pH	k(h^{-1})	R^2	$t_{1/2}$ (h)
SDIAZ	4	0.0871	0.9909	7.96
	7	0.3650	0.9775	1.90
SMETH	4	0.0300	0.9826	23.10
	7	0.1710	0.9818	4.05
SMETOX	4	2.1009	0.9999	0.33
	7	0.1807	0.9983	3.84
Na-SDIAZ	4	0.3809	0.9979	1.82
	7	0.8726	0.9981	0.79
Na-SMETH	4	1.8354	0.9991	0.38
	7	2.4955	0.9973	0.28
Na-SMETOX	4	0.1358	0.9936	5.00
	7	0.1040	0.9736	6.66

SAs are dissociating compounds which exist as cationic, neutral or anionic species as a function of their two dissociation constants (pK_a) and the surrounding pH [7]. As a consequence of this, the SAs reactivity is pH dependent which plays an important role for assessing their environmental behaviour. In our case, photolysis of all investigated compounds was carried out at two pH levels (4 and 7).

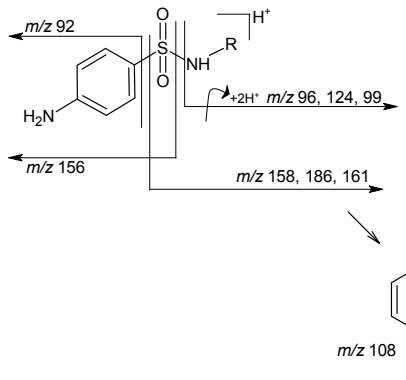
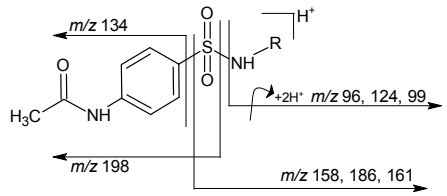
With the increase of pH from 4 to 7, the photolysis rate constants increased for almost all SAs, except in the case of SMETOX and its metabolite which behaviour was totally different. Their photolysis rate constants decreased with pH increase. So, it is clear that pH highly influence the pharmaceuticals direct photolysis rate.

Typically, the photolysis of organic pollutants could lead to the structural decomposition and eventually to the mineralization to CO_2. For most antiobiotics, complete mineralization is usually difficult because of the structural stability.

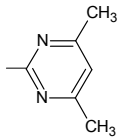
The fragmentation process of SAs yielded in group-specific ions such as *m/z* 156, 108 and 92 which have been already known from the literature [8, 9]. Fragment ion at *m/z* 156 was result of cleavage of the S-N bond yielding the stable sulfonamide moiety $(M\text{-}RNH_2)^+$. Loss of SO_2 leaded to the ion at *m/z* 92 $(M\text{-}RNH_2\text{-}SO_2)^+$, while a rearrangement leading to loss of SO occured in ion at *m/z* 108 $(M\text{-}RNH_2\text{-}SO)^+$ (Table 2.). Except these characteristic fragment ions containing the sulfonamide moiety, also fragment ions typical for each compound, containing the aminopyrimidine ring were detected. All three fragments *m/z* 96 (SDIAZ), *m/z* 124 (SMETH) and *m/z* 99 (SMETOX) were created in the same way as can be seen from the Table 2.

Similarly, acetylated SAs showed also the group-specific product ions such as *m/z* 198, 134 and 108. Higher *m/z* values for the first two fragment ions were result of the presence of acetyl group. Result of cleavage of the sulphonamide bond was fragment ion at *m/z* 198, while further loss of SO_2 results in the formation of the ion at *m/z* 134. Fragment ion at *m/z* 108 and ions containing the aminopyrimidine ring for each compound were the same as in the case of their unmetabolized compounds (Table 2.). Fragmentation of these three compounds produced ions such as *m/z* 158, 186 and 161 which correspond to $(RNH_2+SO_2)^+$. In the mass spectrum of each compound there was also ion at *m/z* 93 which was the result of loss of acetyl group and $(RNH_2+SO_2)^+$. Fragment ions *m/z* 96, *m/z* 124 and *m/z* 99 containing the aminopyrimidine ring were also detected as in the case of their unmetabolized compounds.

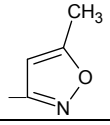
Table 2. Structures and substances properties, and their proposed fragmentation pathways.

R	Sulfa- (SA)	N^4-acetylsulfa- (Na-SA)
		

	pKa_1	pKa_2	*m/z*	pKa^a	*m/z*
-diazine 	2	6.4	251	6.3±0.3	293
-methazine	2.4	7.4	279	7.2±0.5	321



-metoxazole



1.8	6.0	254	5.6±0.5	296	

[a] Calculated values using Advanced Chemistry Development (ACD/Labs) Software Solaris V4.67 [10]

Photolysis of SAs and their metabolites resulted in total six different photodegradation products. Four of them were desulfonated products, two were result of sulfonamide bond cleavage and there were also some isomers in the case of SDIAZ, SMETH and SMETOX.

TMP is very often prescribed with SAs, especially with SMETOX due to their synergistic activity. Therefore, photolysis of TMP was also studied under the same conditions as in the case of sulfonamides. As can be seen from the Figure 2., no significant difference was observed at two different pH values. Therefore, it could be concluded that influence of pH on degradation rate of TMP is insignificant.

Degradation of TMP did not follow pseudo first-order kinetics. During the first 4 h, TMP intensity was almost the same, starting dropping rapidly in the moment of formation of its photodegradation products. This faster mechanism could be the result of autocatalytic effect [11]. Irradiation of TMP with the initial concentration 10 mg L^{-1} resulted in total degradation after 16 hours of irradiation, at both pH values.
Photodegradation of TMP resulted in two degradation products, *m/z* 305 and *m/z* 307.

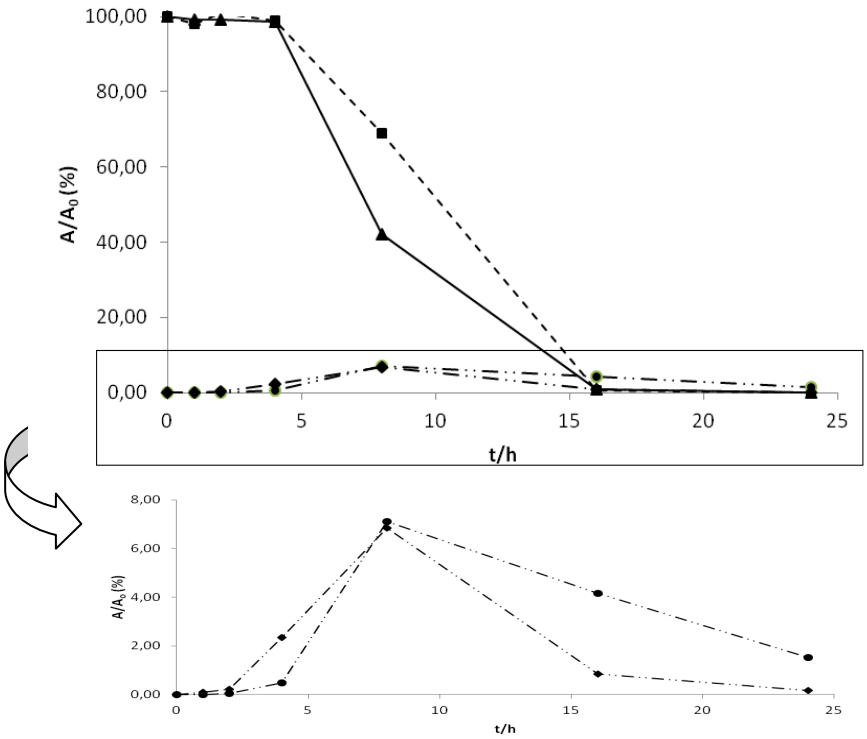


Figure 2. Degradation profiles of trimethoprim at pH 4 (solid line) and pH 8 (dashed line); and evoluation of its photodegradation products, *m/z* 305 (●) and *m/z* 307 (◆).

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