MOBILITY OF SULFONAMIDE ANTIBIOTIC SULFAPYRIDINE IN DIFFERENT TYPES OF SOIL

[1]Petra ZÁVODSKÁ, [1]Denisa JANEBOVÁ, [1]Jan FUČÍK, [1]Martina KLUČÁKOVÁ, [1]Miloslav PEKAŘ,
[2]Victory JAQUES, [2]Tomáš ZIKMUND, [2]Jozef KAISER

[1]*Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic, EU, Petra.Zavodska@vut.cz*
[2]*CEITEC, Brno University of Technology, Brno, Czech Republic, EU*

Abstract

This contribution focuses on monitoring the mobility of sulfapyridine in different soil types. The main difference in the used types of soil was the content of soil organic matter. Simple sorption and desorption experiments in buffer solutions with different pH values were made. Adsorbed and desorbed amount of sulfapyridine was measured using UV-VIS and liquid chromatography. The water solution of sulfapyridine was used for experiments in real conditions when a plastic tube was put into the soil and watered with an aqueous antibiotic solution. For the following days, tubes were taken out of soils, separated and adsorbed amount was measured with UV-VIS. In addition, computed tomography was used to make models for molecular diffusivity. From these measurements, the diffusion coefficient was determined along with adsorbed and desorbed amount of sulfapyridine in different types of soils and in different values of pH. It was confirmed that the pH value and type of soil has an influence on the sorption of used antibiotic.

Keywords: Soil, sorption, desorption, sulfapyridine, tomography

1. INTRODUCTION

Pharmaceuticals are used widely in the world to treat human and animal diseases. But they also have an impact on the Environment. Due to incomplete absorption and metabolism, an unutilized part of drugs is discarded into the Environment through urine or faeces [1]. Pharmaceuticals have been found in the Environment in low concentrations (from nanogram to microgram per litre). Not only can they get into the environment by urine but also by improper disposal or use. Sometimes, the drugs are discarded via the toilet, or the drain or are thrown into waste. If the drugs are not eliminated or adsorbed by soils, they may get into the water and all the aquatic environment [2,3,4].

Sulfapyridine is a sulfonamide antibiotic. This term is generic for derivates of paraaminobenzenesulfonamide structure is shown on the **Figure 1**). Sulfapyridine was firstly found in the thirties of 20th century. Sulfonamides in general act as competitive inhibitors to para-aminobenzoic acid (PABA). Not only do they act as inhibitors to PABA but also have a wide range of antimicrobial activity against Gram-positive and Gram-negative bacteria. In the drug therapy, they had only a bacteriostatic effect, which means they only prevented the growth of bacteria. Therefore, they had a very limited role as single agents [5,6].

The main motivation for this work was the fact that sulfapyridine and many other sulfonamides antibiotics can get through wastewater treatment in unchanged form. As was mentioned, many antibiotics are excreted in large quantities into the environment as active compounds. A significant amount of antibiotics gets into the water, that is used for
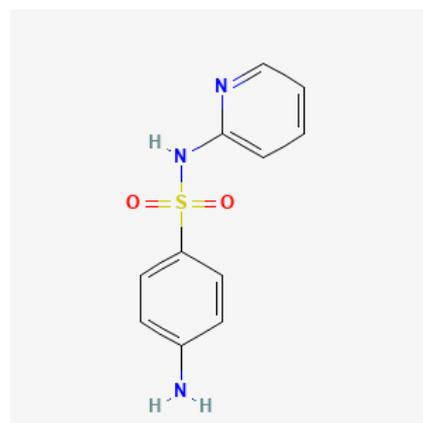

Figure 1 Chemical structure of Sulfapyridine [7]

watering or fertilizing agricultural soil. In soil, most of the antibiotics undergo a biological and physicochemical transformation, but they still have their antimicrobial activity and thus have a potentially toxic effect on the bacteria in soils. They can have an impact on soil enzyme activity as well. This enzyme activity is involved in the bioavailability of nutrients necessary for plants [8].

2. MATHERIAL AND METHODS

2.1. Sorption experiments

Firstly, simple sorption and desorption experiments were performed. Three types of soil, differed by the content of soil organic matter (SOM), were used (**Table 1**). Approximately 0.5 g of each soil was put into a centrifuge tube and filled with 25 ml of drug solution with a concentration 10 mg/l. As a drug solution Britton-Robinson buffer (BRB), with pH 3, 5, 7, and 10, was used.

Table 1 Amount of organic matter in soil samples

Sample	A	B	C
SOM (w.%)	5.75	1.17	6.86

For precisely 48 hours, samples were mixed on a magnetic stirrer. After the period, samples were centrifuged. After 20 minutes, samples were taken out. The supernatant was filtered using 0.45 μm syringe filters and measured with UV/VIS. Samples in BRB with pH 7 and 10 needed to be diluted three times. Part of the supernatant was filtered using 0.22 μm syringe filters and ten times diluted for liquid chromatography measurements (LC).

Sediment from centrifuge tube was transferred to Petri dish and dried. Dry sediment was put back into the clean centrifuge tube along with 25 ml of distilled water. For another 48 hours, samples were mixed on the magnetic stirrer. After the period, for another 20 minutes, they were centrifuged. The supernatant was filtered using 0.45 μm syringe filters for UV/VIS measurements and 0.22 μm for LC measurements. Once again, samples in pH 7 and 10 were three times diluted for UV/VIS. Samples for LC were ten times diluted.

2.2. Sorption experiments in real conditions

Further experiments were performed to simulate real conditions. Plastic syringes (volume of 150 ml) were put into the soil and watered with the drug solution (concentration 1 mmol/l). Syringes were covered with parafilm and for the next days left in the soil. 11 syringes in total were put into the soil. At different times the syringes with the soil were taken out and the soil was divided into 6 parts. Approximately 5 g from each part was put into 25 ml of tap and distilled water and into an $MgCl_2$ solution (concentration 1 mol/l). The leachates were filtered using the 0.45 um syringe filters and measured with UV/VIS. From these measurements, diffusion coefficients were obtained.

2.3. CT measurements

In addition, diffusion coefficients were used in computed tomography (CT). Samples were taken from permanent grassland near the Brno University of Technology, Faculty of Chemistry in Brno, in an intact condition using a transparent plastic tube (**Figure 2**). Samples were transferred to the CT lab and measured by GE phoenix v|tome|x L240. CT measurements were used for creating a model of molecular diffusivity.

Figure 2 Transparent plastic tube used for sampling a CT measuring

3. RESULTS AND DISCUSSION

3.1. Sorption experiments

Sorption experiments were made as described in section 2.1. Using four pH values (3, 5, 7, and 10±0,05) and three types of soil with different amounts of SOM (**Table 1**) showed differences in adsorbed and desorbed amounts of sulfapyridine between soil types and even between values of pH.

As shown in the **Figure 3 (A)** adsorbed amount reaches up to 260 µg/g. There is no evident trend while comparing pH values. All samples have a maximum at pH 3 (sample A 259 µg/g, sample B 198 µg/g, and sample C 194 µg/g). Except for sample C, they have their minimum at pH 7, which is close to the pKa of sulfapyridine (8.43). Sample A has its minimum equal to 154 µg/g, while sample B is equal to 132 µg/g. As mentioned before, sample C has its minimum at pH 10 equal to 57 µg/g. The **Figure 3 (B)** shows the adsorption efficiency of soil samples. As mentioned before, all samples reached their maximum at pH 3. The sample A even reaches 100 % of adsorption efficiency, while the efficiency of the sample B is 67.6 % and the efficiency of the sample C is only 63.3 % The minimum value of efficiency of the sample A is equal to 47.8 %, while the sample B equals 36 % at pH 7 and the minimum for the sample C equal to 15.2 % at pH 10. Therefore, it's apparent, that the best pH value for sulfapyridine adsorption equals 3.
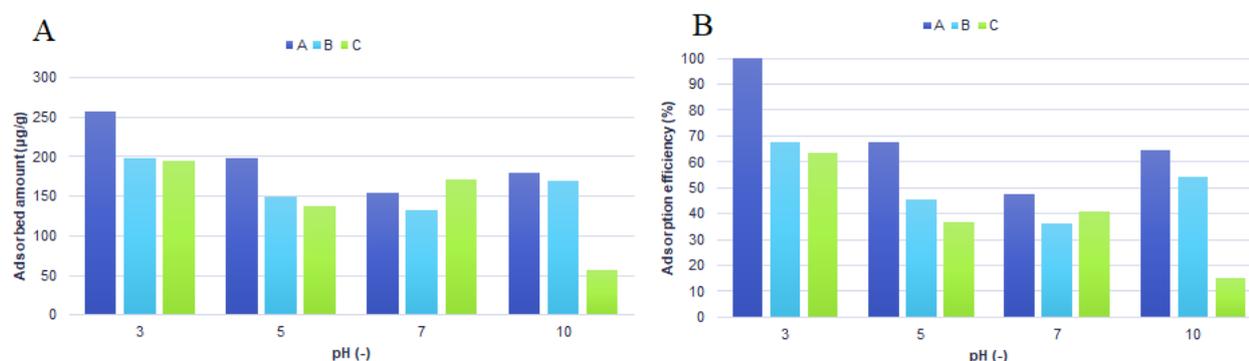

Figure 3 Adsorbed (A) amount of sulfapyridine in three types of soil in different values of pH and adsorption efficiency (B)

Comparing the efficiency of the soil within each sample it's evident that the sample A has the largest adsorbed amounts and the highest adsorption efficiency while the sample C, which has the highest content of SOM, has the lowest efficiency along with adsorbed amounts. Therefore, adsorption does not depend only on the content of SOM, but on the other soil properties (one of them may be the content of calcium).
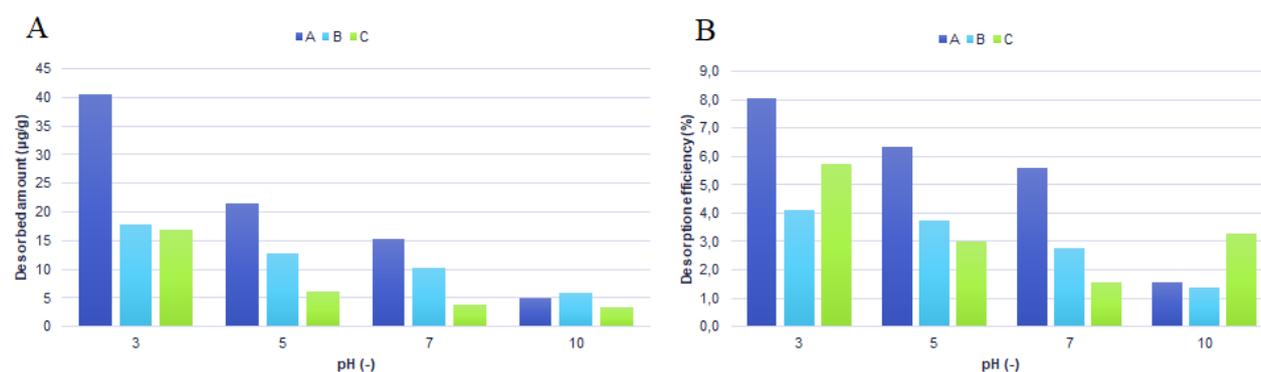

Figure 4 Desorbed amount (A) of sulfapyridine and desorption efficiency (B)

Desorbed amounts are shown in the **Figure 4** (A). There is an evident trend when all the samples have their maximum at pH 3 (the sample A 40.6 ug/g, sample B 17.8 ug/g, and the sample C 16.9 ug/g) and the amounts are decreasing to pH 10, where all samples have their minimum (the sample A 4.9 ug/g, the sample B 5.9 ug/g, and the sample C 3.4 ug/g). It's apparent that the sample A has the highest desorbed amounts, while the sample C has the lowest amounts. Therefore, increasing the pH reduces the desorption of sulfapyridine. The **Figure 4** (B) compares desorption efficiencies. As in the case of adsorption, the sample A has the highest desorbed amounts (at pH 3 up to 8.1 %). The sample A then is the best adsorbent within the samples but also the best desorbent. Contrary to the sample A, sample C is considered the worst adsorbent and also the worst desorbent, which means that the sample C was able to adsorb sulfapyridine molecules and retain them.

3.2. Sorption experiments in real conditions

Sorption experiments were made as described in the section 2.2. Using second Fick's law for nonstationary diffusion, three diffusion coefficients (distilled water, tap water, $MgCl_2$) were obtained (**Table 2**). $MgCl_2$ was used for its ability to adsorb instead of sulfapyridine. Comparing the coefficient obtained from our measurement with results from [9], where the measurements were carried out in diffusion cells using a gel as a membrane, it's apparent that the coefficients are quite similar. In the $MgCl_2$ solution, the diffusion coefficient has the largest value, which supports the assumption of adsorbing instead of sulfapyridine. In tap water the diffusion coefficient is higher than in distilled water, because in tap water there are some minerals (such as magnesium, calcium or chlorine), that can affect adsorption of sulfapyridine.

Table 2 Diffusion coefficients for distilled water, tap water and $MgCl_2$

	D (m^2/s)
Distilled water	$4.23 \cdot 10^{-10}$
Tap water	$4.39 \cdot 10^{-10}$
$MgCl_2$	$5.59 \cdot 10^{-10}$

3.3. CT measurements

For CT measurements the uppermost part of the soil was taken and measured with CT. Using VG studio and a scan of soil the model of molecular diffusivity was made. The **Figure 5** (B) shows the model of molecular diffusivity of soil sample of sulfapyridine in water. The **Figure 5** (A) shows the cut-out part of the model and it's apparent, that molar concentration decreases from top to bottom. The **Figure 5** (C) shows streamlines, as possible pathways of solution through the soil.
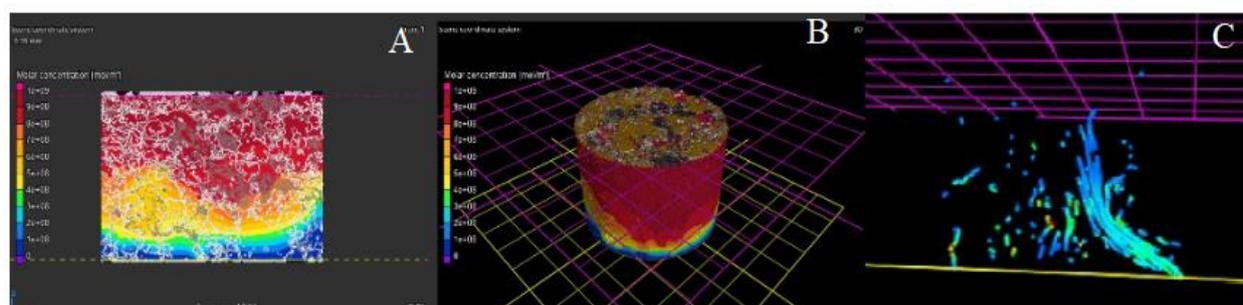

Figure 5 Model of molecular diffusivity (A, B) with streamlines (C)

4. CONCLUSION

Sorption experiments in the lab and real conditions were made along with CT measurements. The first experiments show the ability of soil to adsorb sulfapyridine with high efficiency and its ability to hold it within

its structure. The soil A with 5.75 wt. % of organic matter turned out to be the best for the adsorption of sulfapyridine when at pH 3 adsorption reached 100 % of efficiency. Contrary to this, the soil C with 6.86 wt.% of organic matter turned out to be the worst adsorbent. The efficiency of the soil C reached only 63.4 %. Comparing soils shows that not only the content of organic matter is responsible for adsorption, but also other parameters which might be the content of calcium or magnesium. Desorption experiments showed that the best adsorbent (the soil A) is also the best desorbent, thus it releases much more molecules of sulfapyridine than the other two samples (desorption efficiency of the soil A reaches 8.1 %). On the contrary the sample B, that turned out to be worse adsorbent than the soil A, was the worst desorbent, thus it adsorbs sulfapyridine and holds it in its structure (desorption efficiency reaches only 4.1 %).

Experiments in real conditions were made to compare diffusion coefficients obtained from measurements in diffusion cells. Using diffusion cells in publication [9] writers got a diffusion coefficient equal to $4.75 \cdot 10^{-10}$ m^2/s. In our experiments in the soil column, diffusion coefficients were equal to $4.23 \cdot 10^{-10}$ m^2/s in distilled water, $4.39 \cdot 10^{-10}$ m^2/s in tap water and $5.59 \cdot 10^{-10}$ m^2/s. Results showed the possibility of using this method for measuring the diffusion coefficients.

In addition, the CT measurements were made. Using the VG studio to create a model of molecular diffusivity showed possible ways of diffusion of sulfapyridine solution. Both CT and real condition experiments are only preliminary results and will be develop in next years, but it's apparent that the usage is possible.

Experiments in real conditions in soil columns are new and for now, have some advantages (diffusion in real soil instead of gel) but also some disadvantages (the need for intact and large space in the field). But it's important to keep monitoring the drugs in soils and their behaviour.

ACKNOWLEDGEMENTS

REFERENCES

[1]   DRILLIA, Panagiota, Katerina STAMATELATOU and Gerasimos LYBERATOS. Fate and mobility of pharmaceuticals in solid matrices. *Chemosphere*. 2005, vol. 60, no. 8, pp. 1034-1044.

[2]   KÜMMERER, Klaus. 2010. Pharmaceuticals in the Environment. *Annual Review of Environment and Resources*. 2010, vol. 35, no. 1, pp. 57-75.

[3]   AUS DER BEEK, Tim, Frank-Andreas WEBER, Axel BERGMANN, Silke HICKMANN, Ina EBERT, Arne HEIN and Anette KÜSTER. 2016. Pharmaceuticals in the environment-Global occurrences and perspectives. *Environmental Toxicology and Chemistry*. 2016, vol. 35, no. 4, pp. 823-835.

[4]   CHRISTENSEN, F.M. Pharmaceuticals in the Environment—A Human Risk?. *Regulatory Toxicology and Pharmacology*. 1998, vol. 28, no. 3, pp. 212-221.

[5]   Sulfonamides. *Meyler's Side Effects of Drugs*. 16th. Elsevier, 2016, pp. 555-569.

[6]   CHRISTENSEN, S.B. Drugs That Changed Society: History and Current Status of the Early Antibiotics: Salvarsan, Sulfonamides, and β-Lactams. *Molecules.* 2021, vol. 26, 6057.

[7]   PubChem. *Sulfapyridine*. [online]. 2005. [viewed: 2022-09-15]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/Sulfapyridine

[8]   MOLAEI, Ali, Amir LAKZIAN, Rahul DATTA, Gholamhosain HAGHNIA, Alireza ASTARAEI, MirHassan RASOULI-SADAGHIANI and Maria T. CECCHERINI. Impact of chlortetracycline and sulfapyridine antibiotics on soil enzyme activities. *International Agrophysics*. 2017, vol. 31, no. 4, pp. 499-505.

[9]   CHEN, Chang-Er, Hao ZHANG, Guang-Guo YING and Kevin C. JONES. 2013. Evidence and Recommendations to Support the Use of a Novel Passive Water Sampler to Quantify Antibiotics in Wastewaters. *Environmental science & technology*. 2013, vol. 47, no. 23, pp. 13587-13593.