TRANSPORT AND INTERACTIONS OF SULFAPYRIDINE IN AGAROSE HYDROGELS ENRICHED BY HUMIC ACIDS

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Abstract

Knowledge of behaviour of pharmaceuticals as contaminants in nature is essential for their mobility and toxicity in the soils and surface- and ground-water environments. Their migration ability and toxicity can be affected by their interactions with organic matter. In this contribution, sulfapyridine (a sulfanilamide drug used for antibacterial medication) is studied from the point of view of its transport in hydrogels containing humic substances. Agarose hydrogel was used as a model medium for the transport experiments. Standard Elliot soil humic acid (4S102H) purchased from International Humic Substances Society was incorporated into the hydrogel as an active nanomaterial for interactions with drug and potential suppression of their migration and toxicity in soil systems. The method of diffusion couple was applied on the diffusion experiments. Donor part was saturated by sulfapyridine and acceptor part was free of drug. After connecting of both parts, the time development of concentration profiles in the couple was monitored. The effective diffusion coefficients for the transport of sulfapyridine in hydrogel enriched by humic acids and pure agarose hydrogel were determined and compared. It was found that the transport of drug is influenced by interactions with humic acids which can immobilize partially sulfapyridine in humic structure. Simultaneously, sorption/desorption experiments were realized. Their results confirmed high degree of drug immobilization (60 - 90 % in the dependence on drug concentration).

Keywords: Sulfapyridine, humic acids, agarose hydrogel, diffusion, interaction

1. INTRODUCTION

The use of many different pharmaceuticals in human and veterinary medicine and their persistence can result in potential dangerous behaviour in soil and aqueous environments [1-5]. Drugs are not completely eliminated, therefore their residues leave the body and pass into water, sediments and soils [6-12]. The degradation of drugs are very different in the dependence on their chemical character and physicochemical circumstances. An important role is attributed to soil properties and quality of organic matter. The migration ability and toxicity of pharmaceuticals in nature can be significantly affected by their interactions with soil components.

Humic substances as important constituent of soil organic matter can form complexes with drugs of different strengths therefore strongly bond, ion exchangeable and extractable fractions of complexes co-exist in nature [5,13,14]. The binding character varies between electrostatic interactions, hydrogen bonds and hydrophobic interactions [5,13-18]. Sulfonamides are one of the most commonly used antibiotic in human and veterinary medicine. Their overuse and misuse led to their widespread occurrence in nature [19-21], e.g. their representative - sulfapyridine - is one of the most frequently antibiotics detected in wastewater effluents and surface waters [22-24]. They reach agricultural soils via contaminated sewage sludge, wastewater, and manure used for fertilization [25-27]. The occurrence, fate, ecotoxicity and uptake of antibiotics by edible crops were described in detail in thorough reviews [28-30]. Studies on interactions of, soil, organic matter and humic substances with antibiotics are focused mainly on adsorption processes [31-35]. Chen et al. [31] studied the adsorption of sulfamethoxazole and sulfapyridine on peat soil and composted manure. They resulted that

content of organic carbon greatly enhanced their adsorption on the soil and suggested the hydrophobic partition as the major mechanism. The pyridine group was mainly responsible for the adsorption at high pH values (in the case of sulfapyridine). Haham et al. [32] investigated sorption-desorption behavior of sulfapyridine with three different soils. The showed important roles of soil mineral matrices and dissolved organic matter. Similarly, the important effect of dissolved organic matter on the adsorption of sulfonamides on graphene oxides was observed by Liu et al. [33]. Thiele-Bruhn et al [34] studied sorption of sulfonamides on whole soils and their particle-size fractions. According to their results, the adsorption increased with aromaticity and electronegativity of functional groups attached to the sulfonyl-phenyl-amine core. Modeling and molecular mechanics calculations showed preferred site-specific sorption via hydrogen bonds and van der Waals interactions. Thiele [35] investigated the adsorption of sulfapyridine on Chernozem. They observed that the adsorption was substantially higher in moist soil in comparison with dried sample. They concluded also that the N-heterocycle significantly contributed to the adsorption of sulfapyridine.

Zhi et al. [36] stated that traditional adsorption models (Langmuir, Freundlich) cannot exhibit their behavior under different influencing conditions. Thus, they are not able to reflect the precise adsorption mechanism of antibiotics in soil. Schwarz et al. [25] concluded that the mechanism of immobilization of sulfapyridine varied from reversible sorption to the formation of non-extractable bound residues. The character of interactions, immobilization degree and mobility of antibiotics in nature thus are significantly influences by soil properties and character of organic matter [31,34,37].

Our approach is different. The contribution is focused on the diffusivity of sulfapyridine in model hydrogel matrix and the effect of humic substances incorporated in hydrogel on the migration of the antibiotics. The study is based on our previous experiences with diffusion experiments of different pollutants in hydrogel matrices containing humic substances [38,39] and the effect of the interactions on the mobility [40-43]. The method of diffusion couple [38] was chosen for the investigation of humic-drug interactions effect on the mobility of sulfapyridine in model hydrogel based on agarose enriched by humic acids [39,41].

2. MATERIALS AND METHODS

Sulfapyridine (as sulfonamide antibotics) and agarose (routine use class) were purchased from Sigma-Aldrich. Elliot soil humic acids (4S102H) were purchased from the International Humic Substances Society. The main characteristics of humic acids (e.g. elemental composition or the contents and properties of acidic functional groups) can be found on the website of the International Humic Substances Society.

The preparation of hydrogels was based on the thermo-reversible gelation of agarose solution. It was dissolved in deionized water (1 % wt.), heated at 80 °C and stirred, and finally sonicated to remove gasses. Afterwards, the solution was slowly poured into the PMMA spectrophotometric cuvette. Its orifice was immediately covered with pre-heated plate of glass to prevent drying and shrinking of gel. Flat surface of the boundary of resulting hydrogels was provided by wiping an excess solution away. Gentle cooling of cuvettes at the laboratory temperature led to the gradual gelation of the mixture. Hydrogels enriched by humic acids were prepared from 1 % wt. agarose solution containing 0.01 % wt. of humic acids. Donor hydrogels were prepared by means of agarose (1 % wt.) dissolved in 1 mM sulfapyridine solution.

The diffusion couple was assembled from two parts: donor and acceptor hydrogels. Donor hydrogel was formed from agarose containing sulfapyridine as diffusing drug particles. Acceptor hydrogels were based on agarose or agarose enriched by humic acids without antibiotics (their initial concentrations in acceptor hydrogels were equal to zero).

The distributions of sulfapyridine in donor and acceptor hydrogels were determined in selected time intervals. The cuvettes were disconnected and the UV-VIS spectra were measured at various distances from the orifice by means of Varian Cary 50 UV-VIS spectrophotometer equipped with the special accessory providing controlled fine vertical movement of the cuvette in the spectrophotometer. Using the collected UV-VIS spectra,

the concentrations of drug were determined at different positions in hydrogels. The obtained data were used to compute the concentration profiles of drug in the donor and acceptor parts of diffusion couple. The diffusion fluxes in given times were determined as the total contents of sulfapyridine in acceptor hydrogel (normalized on the area of interface). The content of drug in acceptor hydrogel at given time should be the same as the decrease in drug content in the donor part of diffusion couple.

Sorption experiments were realized with sulfapyridine solutions with concentration range 1-10 mg.dm^{-3}. Solid humic hamic (in form of powder) were mixed with the drug solution (1 g / 50 cm^3) and stirred. After equilibration (48 h), the powder was centrifuged and mixed with deionized water in order to determine the amount of extractable (mobile) phase of sulfapyridine. The decrease in concentration of drug solutions as well as the concentration of leachates were determined by means of UV/VIS spectrometry (see above).

All experiments were triplicated and performed at laboratory temperature (25 ± 1 °C). Data are presented as average values with standard deviation bars.

3. RESULTS AND DISCUSSION

In this work, the effect of humic-drug interaction on the diffusivity of sulfapyridine in agarose hydrogels were studied. In Figure 1 the example of experimental data obtained after 4 days are shown. We can see that the crossing of drug particles from donor hydrogel into acceptor hydrogels with and without of humic acids are different. As assumed, the presence of humic acids as active substance can cause interactions between their binding sites and sulfapyridine which can result in a partial immobilization of drug particles. The effect of humic-drug interactions should be more noticeable for longer times of diffusion. The mathematical description of realized experiments were based on second Fick's law valid for non-stationary diffusion [38,40-43]. In the case of diffusion couple, the boundary conditions of semi-infinite mediums and equality between the decrease in drug content in donor hydrogel and the increase in drug content in acceptor hydrogel in given time were applied. The concentration at interface between both hydrogels was equal to the half of initial donor concentration. The initial boundary condition was the homogeneous initial concentration of drug in donor hydrogel (c_0) and zero initial concentrations of sulfapyridine in acceptor hydrogels.

The mathematical solution of the second Fick's law is

$$c(x,t) = \frac{1}{2} c_0 erfc \frac{x}{\sqrt{4D_{ef}t}} \quad (1)$$

and the equation for the total diffusion flux m_t which goes through the interface between donor and acceptor hydrogels (x = 0) in time t can be expressed as

$$m_t = c_0 \sqrt{\frac{D_{ef}t}{\pi}} \quad (2).$$

The effective diffusion coefficient D_{ef} is the main parameter characterizing rate of the diffusion process. It allows for two main factors: the tortuous movement of the diffusing particles in the porous structure of hydrogel and interactions of diffusing particles with active components incorporated in hydrogels. If we assume a local equilibrium between free mobile drug and immobilized particles, its equilibrium constant K can be expressed as the radio between concentrations of immobilized and free sulfapyridine and involved into the value of D_{ef}:

$$D_{ef} = \frac{D_0}{1+K} \quad (3).$$

Diffusion coefficients for of drug for pure agarose hydrogel and hydrogel enriched by humic acids were determined by means of equation 2. In this contribution, D_0 thus represents the diffusivity of drug particles in non-reactive agarose hydrogel (without humic acids) and D_{ef} involves the influence of interactions. Their values

were calculated as D_0 = 1.01×10^{-10} m^2.s^{-1} and D_{ef} = 9.85×10^{-11} m^2.s^{-1}. The equilibrium constant K is then (according to equation 3) equal to 0.37.

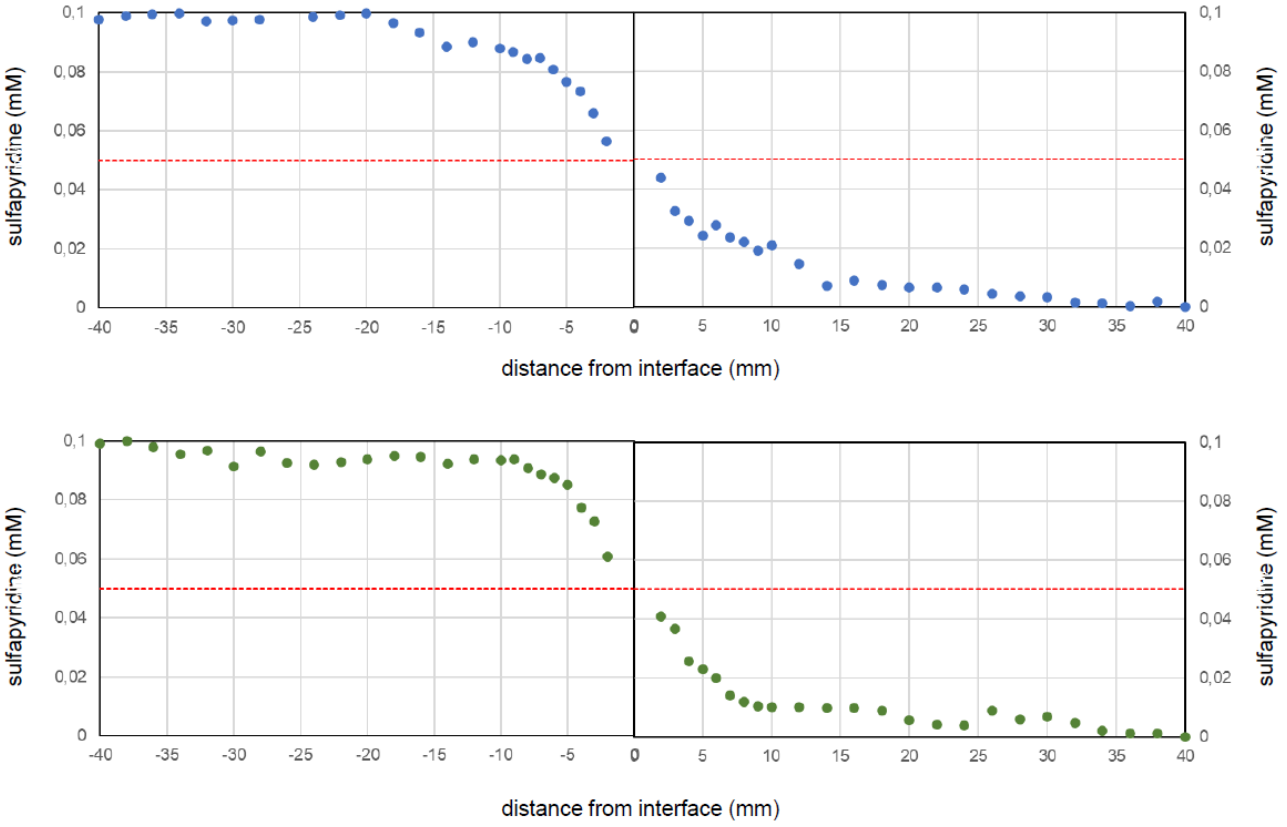

Figure 1 The concentration profiles of sulfapyridine in the diffusion couple formed from donor hydrogel and acceptor hydrogels based on pure agarose (blue) and agarose enriched by humic acids (green) after 4 days. Dashed lines (red) represent theoretical homogeneous concentration of sulfapyridine after attainment of equilibrium.

Adsorption/desorption experiments showed that humic acids are able to immobilize sulfapyridine with adsorption capacity 0.28 mg.g^{-1} (based on Langmuir adsorption isotherm [44]). The desorption experiments showed that the content of mobile fraction increased from 16.4 % (for initial concentration of 1 mg.dm^{-3}) up to 38.5 % (for 10 mg.dm^{-3}). It means that humic acids were able to strongly bind a majority of drug and its percentage decreased as the capacity of humic acids were gradually saturated.

4. CONCLUSION

In this work, the diffusivity of sulfapyridine in model hydrogels was studied. The diffusion couple was formed by donor agarose hydrogel with incorporated drug particles and acceptor hydrogel with initially zero drug concentration. Two types of acceptor hydrogel (with and without humic acids) were used in order to investigate the influence of humic-drug interactions on the mobility of sulfapyridine. It was found that drug particles were partially immobilized by the interactions which resulted in lower value of effective diffusion coefficient. The percentage of non-leachable drug fraction achieved 60-80 % in the dependence on the total drug content.

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