TRANSPORT AND IMMOBILIZATION OF PHARMACEUTICALS IN HUMIC HYDROGEL

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Abstract

Pharmaceuticals can be found in soil and aquatic environments as a result of human activity. They are released into the environment where they undergo soil sorption, photodegradation, and chemical transformation. It is known that their migration ability and toxicity can be significantly affected by interactions with organic matter. The interactions can result in their immobilization in humic structure and decrease in their bioavailability. Humic substances as important constituents of natural organic matter are investigated from point of view of their interactions with different pollutants (including pharmaceuticals) mainly by means of traditional batch adsorption experiments. Our approach is different. Humic substances are used in the form of hydrogel which can be considered as a model system of soil with characteristic content of organic matter. The hydrogel form is advantageous for its defined parameters which can be reflected in initial and boundary conditions necessary for mathematical description of transport in the system. Ibuprofen (sodium salt) was studied from point of view of its transport and immobilization in humic hydrogel by the method of instantaneous planar source. It means that small defined amount of diffusing substance is placed on the circular surface of cylinder-shaped hydrogel (placed in glass tube to achieve one-dimensional diffusion). Diffusion study was complemented by adsorption experiments to compare traditional and our novel approaches. The mobility in humic systems as well as the immobilization ability and their effect on the bioavailability of pharmaceutical are discussed.

Keywords: Ibuprofen, humic acid, hydrogel, diffusion, immobilization

1. INTRODUCTION

The presence of pharmaceuticals in environment gains significant attention and raises health concern due to their adverse effect and environmental risks [1-3]. The fate of pharmaceuticals in the environment mainly depends on their adsorption behaviour in soils and sediments. The adsorption can affect their distribution between aqueous medium (such as soil solution) and solid surfaces, migration, and bioavailability [1,3]. The adsorption degree is influenced by pharmaceutical properties as well as environment characteristics such as temperature, pH, and content of minerals and organic matter [3,4]. Therefore, the adsorption studies are the most extensively used way of the investigation of interactions between pharmaceuticals and soils or sediments. Classical batch experiments [5-7], column experiments [8,9], and monitoring of pharmaceuticals in environment by means of the method of diffusive gradients in thin films (DGT) [10,11] are mostly performed to study of fate and immobilization possibilities of pharmaceuticals in environment.

Our study is focused on the transport of pharmaceuticals in systems containing humic acid as an active substance able to interact with diffusing drug particles. We believe that humic substances as main components of organic matter play important role in the immobilization of pollutants in nature. The significance of humic substances in interactions of pollutants with organic matter and the drug removal from environment and wastewater were reported in many studies, e.g. [1-4,12-15]. Our approach combines interaction (sorption) experiments and diffusion experiments and therefore allows the direct observation of the actual mobility of pollutants affected by their reactivity in the transport through model humic system. This approach was developed on the basis of general mathematical model for the diffusion accompanied by chemical reaction [16,17] and optimized for the application to the transport in humic hydrogels in our previous works, e.g.

[18-20]. In this study, the hydrogel based on the precipitation of humic acids resulting in the gel form prepared in defined shape and size which are necessary for the mathematical description and correctly defined initial and boundary conditions corresponding with our experimental arrangement. Ibuprofen, which was chosen for this study as an example of commonly used drug, is a clinically important pharmaceutical for the treatment of pain and inflammation [21,22]. Its high consumption can result in a contamination of aquatic environment including surface, drainage, and drinking waters [22,23] connected with toxic effects observed on non-target organisms even at low concentrations [24]. Our study is focused on the reactivity mapping of this pharmaceutical in systems containing humic substances, determination of its diffusivity, and immobilization potential of humic substances as active constituents of organic matter.

2. MATERIALS AND METHODS

Ibuprofen sodium salt (CAS 31121-93-4) was purchased from Sigma-Aldrich. Humic acids was extracted from lignite mined in the Czech Republic (Mikulčice in South Moravia) using a mixture of NaOH and $Na_4P_2O_7$ by the procedure described previously [18-20]; for more details of chemical structure and the isolation procedure see previous works [18-20,25].

Humic hydrogel was prepared by dissolving powdered humic acids in 0.5M NaOH and acidifying the solution with HCl to value of pH close to 1. After 24 hours, the gel phase was separated from the solution by repeated centrifugation followed by washing with deionized water [18-20]. The content of dry matter in prepared hydrogel was 15.9 ± 1.1% (wt.).

Diffusion experiments were performed with hydrogels pressed gently into glass tubes (length = 3 cm and diameter = 1 cm). A circular slice of filter paper (diameter = 1 cm) was sunk into a solution of ibuprofen with concentration equal to 232 mg /dm^3 (1 min) and then added to one side of the tube (filled with the hydrogel). The tube was packed with parafilm and aluminium foil to prevent the hydrogel drying. The durations of the diffusion experiments were 24, 48 and 72 h. Then, the hydrogel was sliced into thicknesses of 0.5 – 1 mm and each slice was extracted separately in 10 cm^3 of deionized water. Leachates were analysed using ultra-performance liquid chromatography (UHPLC Agilent 1290 Infinity LC) in tandem with triple quadruple (Bruker EVOQ LC-TQ) with electrospray ionisation (ESI). Chromatographically data resulted in concentration profiles of ibuprofen in humic hydrogels and determination of diffusion coefficient.

Adsorption and desorption experiments were performed in the ratio 1 g of powdered humic acids to 50 cm^3 of drug solution (adsorption) or deionized water (desorption). Initial concentrations of ibuprofen in solutions were in the range between 1 and 10 mg dm^{-3}. The solutions of were mixed with humic acids, stirred for 48 h, centrifuged, and analysed chromatographically as in diffusion experiments. Solid residues were mixed with deionized water, stirred for 48 h, centrifuged, and analysed as in the case of adsorption experiments. The equilibrium concentrations of solutions, adsorbed, and desorbed amounts were determined and used for the determination of adsorption capacity and the determination of free-mobile (leachable) and strongly bound (residual) fractions of ibuprofen in humic acids.

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

Diffusion experiment was based on the diffusion from instantaneous planar source. It means that a small defined amount of pharmaceutical was placed on the hydrogel surface, and it immediately diffuse into the hydrogel. A mathematical description was based on one-dimensional Fick's law

$$\frac{\partial c}{dt} = D_{\text{eff}} \frac{\partial^2 c}{\partial x^2} \quad (1)$$

Where *c* represents the concentration of the diffusing compound in the time *t* and the position *x* (the coordinate parallel to the direction of diffusion movement). Where D_{eff} is the so called "effective diffusion coefficient" in which two main effects (tortuous movement of the diffusing matter, chemical interactions in the system) are involved. While solving this partial differential equation, appropriate initial and boundary conditions must be applied according to the particular experimental settings [16-20]. If the diffusion is realised in inert medium, the effective diffusion coefficient includes only the tortuous movement of diffusing particles through the porous hydrogel structure.

A solution of the **Equation 1** for the diffusion from instantaneous planar source can be found in the form valid for the zero initial concentration of diffusing matter in hydrogel

$$c = \frac{n}{S\sqrt{\pi D_{\text{eff}} t}} exp\left(-\frac{x^2}{4D_{\text{eff}} t}\right) \quad (2)$$

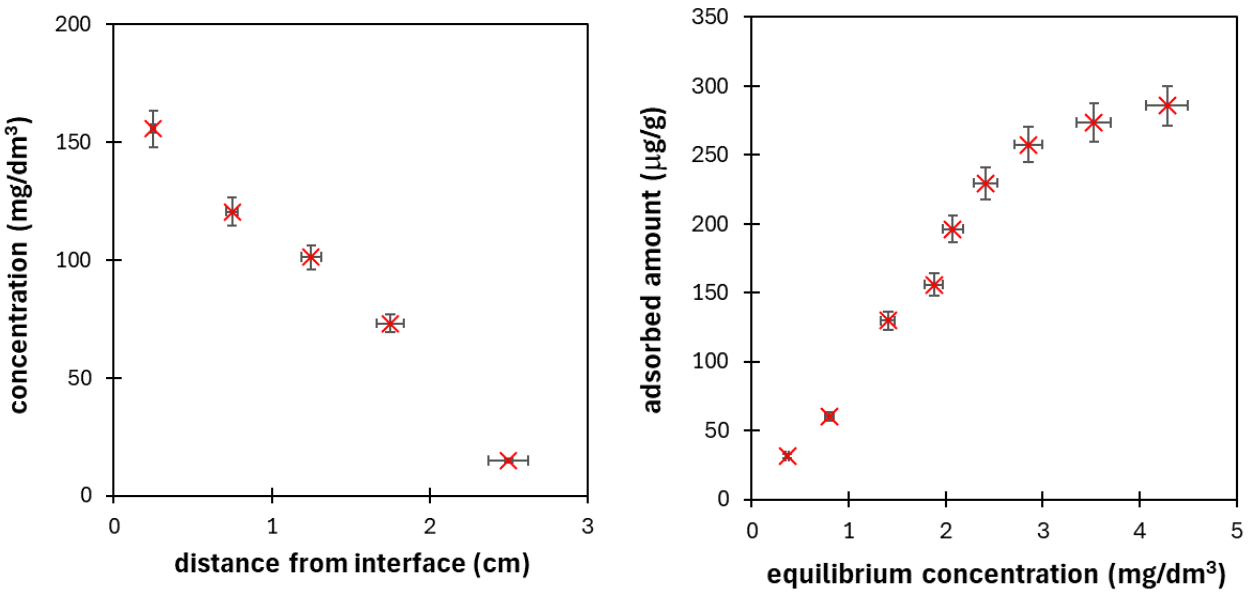
Where *n* stands for the total mass of diffusing compound applied in the form of a narrow pulse and *S* is the cross-section area available for the transport of the compound.

The **Equation 2** was linearized to calculate the effective diffusion coefficient directly from the experimental concentration profiles:

$$ln\, c = ln \frac{n}{S\sqrt{\pi D_{\text{eff}} t}} - \frac{x^2}{4D_{\text{eff}} t} \quad (3)$$

The example of measured concentration profile of ibuprofen in humic hydrogel is shown in **Figure 1a.** The concentration decreases gradually with the distance from the interface. According to **Equation 3**, the D_{eff} value can be determined from the slope of linear dependence of ln *c* on x^2 which is usual and commonly used calculation method [16-20]. The effective diffusion coefficient based on the slope of **Equation 3** was determined as $(3.42 \pm 0.04) \times 10^{-10}$ m^2/s. This value involves both the tortuous movement of the diffusing matter and chemical interactions of ibuprofen with humic acids, therefore the D_{eff} value is lower in comparison with its diffusion coefficient in water (5.0×10^{-10} m^2/s [26]). The question is if one of these effects predominates or if they contribute to the decrease in the diffusion coefficient to a similar degree. In consideration of the character of humic hydrogel, we are not able to compare experimental data with similar inert hydrogel. The humic hydrogel is composed of humic acids and water in ideal case, where majority component is water. Our experiences with diffusion of metal ions in humic hydrogel showed that their interactions with humic acids resulted in a noticeable decrease in the diffusion coefficient [16-20]. In previous works [19,27], we studied the effect of selective blocking of carboxylic groups of humic acids as active centres for the interactions on the diffusivity of copper(II) ions in the hydrogels. The decrease was stronger for hydrogels containing more active centres (less blocked functional groups) which corresponded with the hypothesis that the influence of hydrogel reactivity on the diffusion is considerable. Investigation of the diffusion in agarose hydrogel [28] showed that the diffusion coefficients for several drugs (including ibuprofen) were found to be essentially the same as in water. It means that the diffusion in the agarose hydrogel enriched by reactive humic acids should by caused mainly by the interactions of diffusing particles. Our experiences with the diffusion of pharmaceuticals in agarose hydrogels are different [29]. The diffusion coefficients obtained for sulphonamide antibiotics in agarose hydrogel were much lower in comparison with their values in water and an enrichment of hydrogel by humic acids resulted in further changes in their diffusivities.

In consideration to the discrepancy in our and published findings, we decided to investigate the interactions between ibuprofen and humic acids in details. The measured adsorption isotherm for ibuprofen and powdered humic acids is shown in **Figure 1b**. Adsorption experiments provided adsorption capacity of humic acids a_{max} and the ratio between adsorption and desorption rate constants *b* ($b = k_{ads}/k_{des}$) according to Langmuir isotherm [1,30] frequently used for chemical adsorption



(a) (b)

Figure 1 Concentration profile of ibuprofen in humic hydrogel after 72 h (a) and adsorption isotherm of ibuprofen on powdered humic acids (b).

$$a = a_{max}\ \frac{bc}{1+bc} \tag{4}$$

where *a* is adsorbed amount and *c* is equilibrium concentration. Parameters of adsorption isotherm (a_{max} and *b*) was determined on the basis of the linearized form of **Equation 4**:

$$\frac{1}{a} = \frac{1}{a_{max}b}\frac{1}{c} + \frac{1}{a_{max}}. \tag{5}$$

The adsorption capacity a_{max} was determined as 460 μg g^{-1} and the ratio *b* was equal to 0.39 dm^3 g^{-1}. The ratio between adsorption and desorption rate constants was lower than 1 which means that desorption should predominate, and more drug particles should be in mobile form. This was confirmed by the desorption experiments. Average leachability of adsorbed drug into water was 64 % and 36 % remained bound on humic acids. The effect of interactions between ibuprofen and humic acids thus is important but relatively big amount of ibuprofen can remain movable and bioavailable. As we can see in **Equation** 3, the calculation of effective diffusion coefficient can be based also on the intercept of the line where the total mass of diffusing drug applied on the interface is involved. The total mass is in the diffusion fractionated into movable mobile for and immobilized drug bound to humic acids. In reality, the diffusion coefficients based on the slope and intercept of the line cannot be the same as a result of the partial immobilization of diffusing particles and local equilibrium between movable and immobilized particles [16-20]. The difference between D_{eff} values based on the slope: (3.42 ± 0.04) × 10^{-10} m^2/s and the intercept: (4.82 ± 0.21) × 10^{-10} m^2/s can be considered as a degree of the immobilization and effect of the interactions between humic acids and diffusing pharmaceutical.

4. CONCLUSION

In this contribution, the diffusion of ibuprofen in humic hydrogel was studied. The hydrogel was used as a model medium of soil porous system containing reactive organic matter. The hydrogel should contain only humic acids (as a representative of organic matter) and water therefore can be considered as a model system for the investigation of transport of pharmaceutical through soil affected by their partial immobilization. It was found that the effective diffusion coefficient determined for the diffusion of ibuprofen in humic hydrogel is lower

in comparison with its diffusion in water which is caused by hydrogel porous structure and interactions in diffusion through reactive medium. Complementary adsorption/desorption experiments confirmed that ibuprofen can be partially immobilized. The average effectivity of adsorption was around 60%. The ratio between adsorption and desorption rate constants based on Langmuir isotherm as well as desorption experiments showed that the desorption predominated, and ibuprofen was leachable from humic acids in large measure (64 %) and a residue 36 % remained bound on humic acids.

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