DIFFUSION OF DICLOFENAC IN REACTIVE HUMIC HYDROGEL

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Abstract

Transport and reactivity mapping of pharmaceuticals in soil and aquatic environments is vitally important for environmental safety. Pharmaceuticals can interact with organic matter which can result in their partial immobilization and suppression of their migration, bioavailability, and toxicity. Humic substances as important constituents of natural organic matter play important role in this self-healing ability of soils. In this work, they were used for the preparation of hydrogel medium for the monitoring of drug transport accompanied by the interactions between humic substances and drug. Humic hydrogel can be considered as a model system of soil with characteristic content of organic matter. Diclofenac was chosen for the investigation of their transport in hydrogels as a representative of widely used non-steroidal anti-inflammatory drugs. The method of instantaneous planar source was used for the transport and reactivity mapping of diclofenac in humic hydrogel. The method is based on a small defined amount of diffusing substance placed on the circular surface of cylinder-shaped hydrogel (placed in glass tube to achieve one-dimensional diffusion). The method can provide diffusion parameters as effective diffusion coefficients characterizing drug transport affected by interactions with active sites of humic substances and apparent equilibrium constant between immobilized and free movable drug particles. The difference between values based on slope and intercept of linearized mathematical model indicated relatively strong interactions between humic substances as active constituents of hydrogel and diclofenac. The interactions were confirmed by results of adsorption/desorption experiments when around 50 % of diclofenac was not leachable and remained in immobilized form.

Keywords: Diffusion, immobilization, humic substances, hydrogel, diclofenac, adsorption, desorption

1. INTRODUCTION

Pharmaceuticals have been extensively used in the last decades in large quantities. Since, they are incompletely absorbed and metabolised by the human organism, soils, sediments, surface and ground water are contaminated which results in a decline in the quality of the environment, an increase in the exposition of organisms to Pollution and human health hazard [1-4]. One of the widely used over-the-counter drugs is diclofenac (sodium salt of 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetic acid) [5-7]. It is a common non-steroidal anti-inflammatory drug associated with pain-relieving action with pronounced antirheumatic, anti-inflammatory, analgesic, and antipyretic properties [8-11]. Diclofenac is often recognized as the 'world's most popular pain killer' and is also the most commonly used anti-inflammatory drug [1,6]. Diclofenac is relatively persistent in the aquatic environment due to its hydrophilicity and stability [10,12]. Generally, diclofenac has an estimated degradation range from 20 to 40% [13]. A negligible degradation was observed mainly under anaerobic conditions [8,14]. Being that diclofenac is recalcitrant to degradation, its removal efficiency from wastewater in conventional sewage treatment plants is relatively low [5,8]. Therefore, it is often detected in environment as well as in in tap/drinking waters in concentrations which can result in adverse effects on aquatic life and potential risk to human health [3,6,15-17].

In soils, diclofenac can be partially immobilized by interactions with soil organic matter which has an important influence on its mobility, bioavailability, and ultimately its fate in the environment [1,2,7,8,12,13]. Other factors affecting the behaviour of diclofenac in soils are mineral content and composition, pH, temperature, cation exchange capacity [2,7,12]. From point of view of the soil heterogeneity and complicated structure and composition, the interactions are the result of different mechanisms including surface complexation, H-bonding, cation bridging, and ion-exchange [18-22]. There are many studies dealing with adsorption/desorption processes in traditional batch arrangement [2,7,11-14,17,23-25]. Some of them are focused on column experiments which can better capture the migration ability of drug in soil [8,25-29]. Our approach is different. In previous work [30], an agarose hydrogel enriched by humic acids was used as a model system for the reactivity mapping of pharmaceuticals (including diclofenac) in transport through hydrogel. The method of diffusion cells was used, and the effective diffusion coefficients were determined on the basis of time lag and the slope of time dependence of concentration in acceptor part of diffusion cells for inert (2.34 and 1.86 $\times$ 10^{-10} m^2/s) and humic reactive hydrogels (1.15 and 1.46 $\times$ 10^{-10} m^2/s) [30]. In this work, humic hydrogel prepared as a precipitate of sodium humate by acidifying with HCl [32,33]. Therefore, humic hydrogel contains no carrier medium (such as e.g. agarose) and it is comprised only by humic acid in aqueous environment. The advantage of this arrangement is that all observed effects are caused by humic acids and its hydrogel network and no other substance can affect the transport in this type of hydrogel.

2. MATERIALS AND METHODS

Diclofenac (CAS 15307-79-6) 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 $Na_4P_2O_7$. More details can be found in our previous works [31,32]. The main characteristics of humic acids are listed in **Table 1**.

Table 1 Characterization of humic acids (elemental composition normalized on dry ash-free sample)

C (% at.)	H (% at.)	N (% at.)	S (% at.)	O (% at.)	acidity (mmol/g)	COOH (mmol/g)
42.69	41.80	0.90	0.27	14.34	7,24	5.23

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. Final mass content of humic acids hydrogel was equal to 15.9 % 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 saturated solution of diclofenac (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 tubes with hydrogel were disconnected and each tube was extracted separately in 10 cm^3 of deionized water. Leachates were analysed spectrophotometrically (Hitachi U-3900H). UV/VIS spectra resulted in concentration profiles of diclofenac in humic hydrogels and determination of diffusion coefficient [31].

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 diclofenac in solutions were in the range between 0.5 and 2.5 mg dm^{-3}. The solutions of were mixed with humic acids, stirred for 48 h, centrifuged, and analysed spectrophotometrically 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 described in detail in our previous work [31]. In beginning (t = 0), the small defined amount of diffusion substance (n) is placed on the boundary (x = 0) and then this substance diffused into the hydrogel and its concentration (c) in the dependence of the position (x) and time (t) is monitored. The total amount of diffusion substance (n) does not change and is the sum of its amount in different positions ($\sum n_i$). No diffusing particle can achieve "the end" of hydrogel ($x \to \infty$) and the concentration there remains equal to zero). A mathematical description was based on one-dimensional Fick's law [30-32] and initial and boundary conditions (**Table 2**).

Table 2 Initial and boundary conditions for the diffusion from instantaneous planar source

$t = 0$	$x \in \langle 0; \infty \rangle$	$c = 0$
$t > 0$	$x \to \infty$	$c = 0$
$t > 0$	$x \in \langle 0; \infty \rangle$	$\sum n_i = n$

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 final solution for the diffusion from instantaneous planar source can be expressed as

$$c = \frac{n}{S\sqrt{\pi D_{\text{eff}} t}} exp\left(-\frac{x^2}{4D_{\text{eff}} t}\right) \tag{1}$$

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 and S is the cross-section area available for the diffusion.

For the calculation of the effective diffusion coefficient, the **Equation 1** was used in its linearized form

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

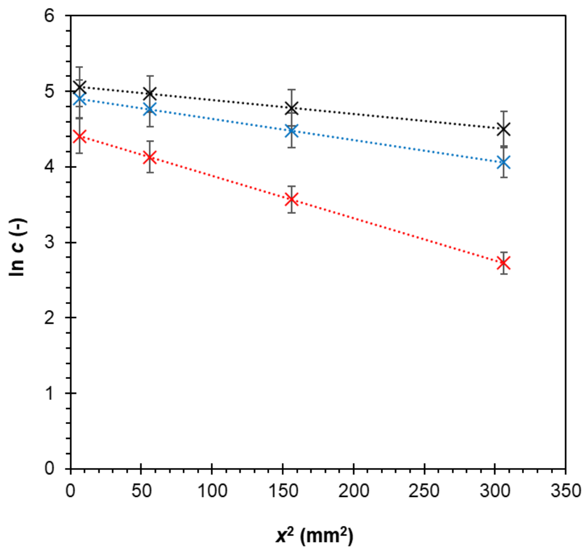
As can be seen, the effective diffusion coefficient can be determined on the basis of the slope as well as the intercept of linear dependence (**Equation 2**). If diffusing substance cannot interact with hydrogel and remains free and movable, D_{eff} based on slope and intercept should be the same. In contrast, the difference between both D_{eff} values indicates that a part of the substance is immobilized. The problem is that the total amount n comprises both free and immobilized diclofenac which was applied on the interface in the beginning of experiment, while spectrometer detects only free movable diclofenac (concentration c). Thus, this discrepancy can result in different values of diffusion coefficients which indicates that the diffusing substance can interact with humic acids and be partially immobilized.

In **Figure 1a**, the experimental concentration profiles fitted by **Equation 2** are shown. We can see that obtained dependencies are strictly linear, which means that our experiments complies with initial and boundary conditions (**Table 2**). The effective diffusion coefficient based on the slope (**Equation 2)** was determined as (5.19 ± 0.15) × 10^{-10} m^2/s. The effective diffusion coefficient based on the intercept was determined as (8.44 ± 0.36) × 10^{-11} m^2/s. The first value is higher in comparison with D_{eff} determined for agarose hydrogel enriched by humic acids by method of diffusion cells [30], the second one is lower. Both values determined in this work are simultaneously lower than the diffusion coefficient of diclofenac in water (1.1×10^{-9} m²/s) [33] because of a deceleration of movement of diclofenac in hydrogel caused by humic network. The difference between both values determined in this work indicated relatively strong interactions between humic acids and diclofenac (the difference in magnitude). Simultaneously, the diffusion coefficient is general concentration dependent and therefore obtained results differ for the method of instantaneous planar source (where only narrow pulse of diffusing substance is added) and methods using more concentrated solutions. As mentioned above, the value based on intercept using total amount *n* which includes both free movable and immobilized diclofenac. Therefore, the D_{eff} based on the slope can be considered as a real effective diffusion coefficient including the influences of hydrogel network and interactions of diclofenac with humic acids.

In order to investigate interactions in more detail, adsorption/desorption experiments were performed. Adsorption isotherm obtained by means of traditional batch experiments was fitted by well-known Langmuir model [7]

$$a = a_{max} \frac{bc}{1+bc} \tag{3}$$

where *a* is adsorbed amount, a_{max} is adsorption capacity, and *b* is the ratio between adsorption and desorption rate constants *b* ($b = k_{ads}/k_{des}$). The adsorption capacity was determined as 2.41 ± 0.11 mg/g and parameter *b* as 0.43 ± 0.02 dm³/mg. Desorption experiments provided results shown in **Figure 1b**. As can be seen the mobile fraction increases slightly with increasing initial diclofenac concentration (from 48 to 56 %). It means that in the case of higher adsorbed amounts only lower part can be strongly bound and more diclofenac particles can remain free and movable. This finding corresponds with the results of diffusion experiments.



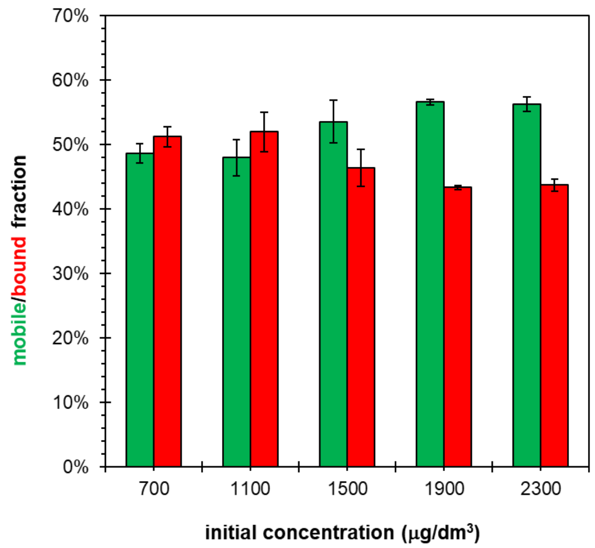


Figure 1 (a) Concentration profile of ibuprofen in humic hydrogel after 24 (red), 48 (blue) and 72 h (black); (b) Mobile (green) and bound (red) fractions of diclofenac in humic acids.

4. CONCLUSION

In this contribution, the diffusion of diclofenac in humic hydrogel was studied. Diffusion was completed by adsorption/desorption experiment to obtain a comprehensive description of migration ability and bioavailability of diclofenac in model humic gel. Differences obtained for the diffusion coefficients based on slope and intercept of linearized mathematical model (**Equation 2**) indicated strong interactions between humic acids and diclofenac which was confirmed by adsorption/desorption experiments where the leachability of diclofenac was around 50%.

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