How the interactions with humic acids affect the mobility of ionic dyes in hydrogels – 2. Non-stationary diffusion experiments

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ABSTRACT

Non-stationary diffusion of two cationic dyes (Methylene Blue and Rhodamine 6G) was studied in hydrogels with different content of agarose and humic acids (HA). A simple spectrophotometrical method was utilized in the *in situ* measurement of dye concentration in the gel samples at different distances from the boundary. The effect of temperature, pH and ionic strength was investigated. The results confirmed the considerable partitioning of both dyes in agarose gels as well as the strong immobilization of dyes caused by their sorption on HA. The apparent diffusion coefficients of both dyes decreased with increasing solid content in gels. In the case of agarose gels without the addition of HA, this decrease was attributed to increased tortuosity of diffusion caused by denser agarose network. The apparent equilibrium constant of the sorption of dyes on HA in agarose/HA gels was calculated from their apparent diffusion coefficients. The value of the equilibrium constant increased with the content of HA in gel and, surprisingly, also with decreasing pH inside gel.

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1. Introduction

Humic substances form the key organic component of soils, sediments and young coals. From the chemical point of view, they represent complex heterogeneous mixtures of polydispersed materials with the complicated structural skeleton and can be divided operatively into three main fractions: humic acids (HA), fulvic acids and humin. Humic and fulvic acids are extracted from soil and other solid phase sources using a strong base. HA are insoluble at low pH, and they are precipitated by adding strong acid. Humin cannot be extracted with either a strong base or a strong acid [1]. Although they are well known to stand behind the crucial environmental phenomena (*e.g.* the carbon sequestration or self-detoxification of soils), even after more than two centuries of substantial research, the basic chemical nature, biosynthetic pathways, and the reactivity of humic substances and soil organic matter are still poorly understood [2].

The key feature of natural behavior and of function of humics lies in their outstanding ability to bind compounds of diverse chemical nature. This process is of an exceptional biological, environmental and even industrial importance. In soils, sediments and in water aquifers, binding on solid or dissolved humic substances determines the local concentrations and the fluxes of bound compounds, which crucially affects the dynamics of essentially all other components of the systems. Hereby, the presence of humic substances controls the ecotoxicity of harmful pollutants and the bioavailability of essential nutrients in soils at the same time.

Therefore, a considerable experimental effort has been directed towards describing the interactions of humic substances with the diverse model pollutants – *e.g.* heavy metals [3], radionuclides [4], pesticides [5] or pharmaceuticals [6] – and as well with some typical nutrients [7]. Moreover, the development of some humics – based sorbents and artificial barriers for various environmental and industrial applications has become a subject of vast concern [8,9].

Practically all the above referenced reviews summarize the studies which focus on the common batch sorption experiments, aiming at the detailed description of the sorption equilibrium and the sorption kinetics in the solute–humics systems. The experimental procedures are always similar and the individual studies usually differ just in the preferred combination of solute and humics and often also in the level of complexity of the mathematical model used for the interpretation of sorption data (compare various models reviewed in [10]).

On the other hand, in our recent works, simple diffusion studies were put forward as the reasonable experimental alternative which better describes the actual effects of the humics–solute interactions on the transport of a solute in humics-containing matrices [11–16]. In these papers, a hydrogel form of humic acids is utilized both as a reasonable model of native humic environments and also because a semi-solid hydrogel sample provides

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better feasibility of the diffusion experiments. In the most recent work [16], the diffusions of Methylene Blue (as a model cationic organic dye) in aqueous solutions and in agarose gels with and without the addition of humic acids were studied by the method of diffusion cells. The method is based on the measurement of time needed by the solute to penetrate through the studied porous specimen and, after the penetration, of the steady-state flux of the solute. The results of these experiments clearly showed the barrier effect of humic acids on the transport of Methylene Blue in gels. The experimental results were processed using the comprehensive theoretical model, summarized by Shackelford and Moore [17] for the description of the diffusion of solutes in the porous media, in order to calculate some diffusion and interaction parameters of the studied systems. Nevertheless, apart from the obvious illustration of reactivity and barrier properties of humic acids, the experiments in the diffusion cells proved the insufficiency for an adequate separation of the two independent effects, acting simultaneously in the systems: (i) of the partitioning (*i.e.* unequal distribution caused by a phase-equilibrium) of free solute at the solution–gel boundary and (ii) of the immobilization of solute in gel caused by some specific solute – HA interactions (for details, see [16]).

To address the two effects independently, the non-stationary diffusion studies could provide an improved experimental tool (a comprehensive handlist of the non-stationary diffusion models with the basic experimental layout can be found *e.g.* in [18]). In the non-stationary experiments, the actual concentration of diffusing solute is measured in the studied material at different times and different distances from the solute source. The diffusion coefficients of solute are then calculated either from the time change of the solute concentration profile in the sample or from the solute total diffusion flux. Diverse sophisticated analytical techniques were applied previously in the measurement of solute concentration profiles in gels, *e.g.* fluorescence microscopy [19], nuclear magnetic resonance techniques [20] or ultrasonic acoustics [21]. Some of the uncomplicated non-stationary diffusion techniques were also utilized in our preliminary study on the transport of cupric ions in the model humic matrices [11–15].

The experiments presented here improve the previous studies of humic matrices by introducing a direct, non-destructive, spectrophotometric method of determination of the solute concentration profiles in the supporting hydrogels loaded with different amounts of humic acids. It utilizes the characteristic visible light absorption of the solutes (charged organic dyes) in order to measure their concentration when diffusing in the appropriately selected hydrogel materials which allow the transmission of light. This method features the advantage of great availability – UV–VIS spectroscopy represents the routine laboratory method with low equipment demands. Moreover, it can be used for a wide range of model hydrogels and solutes. Direct *in situ* imaging of the concentration profile of solutes in hydrogels was already used as a powerful tool in the diffusion studies; Dunmire et al. [22] developed and evaluated automated UV spectrophotometric method for analyzing molecular transport of several test molecules into gels with a relevance to the design of controlled drug-delivery systems, similar techniques for UV or visible imaging of a solute diffusion in optical transparent hydrogels were utilized also in other pharmaceutical [23–25] or food engineering studies [26,27]. The experiments presented in this paper focus on the diffusion of two solutes – Methylene Blue and Rhodamine 6G – in agarose hydrogels loaded with different amounts of lignite-derived humic acids. Both selected solutes represent positively charged organic dyes with well-known affinity to bind on the humic substances [28,29]. Methylene Blue was included, *inter alia*, to provide a basis for the comparison with results of our previously published diffusion-cell experiments, Rhodamine 6G was added because its diffusion in hydrogels have been extensively studied by several authors

[30–32]. Experimentally determined concentration profiles of solutes were subjected to the least-square regression with a suitable mathematical model in order to calculate the diffusion and partition coefficients of solutes. Moreover, the influence of pH and ionic strength on the diffusion process was analyzed as well.

2. Materials and methods

2.1. Chemicals

Agarose (routine use class, <10 wt.% moisture content), Methylene Blue hydrate (C.I. Basic Blue 9, dye content, $\geqslant$95 wt.%) and Rhodamine 6G (C.I. Basic Red 1, dye content, $\geqslant$95 wt.%) were purchased from Sigma–Aldrich and used without further purification. Humic acids were isolated by alkaline extraction from South-Moravian lignite [11,33]. The details on the chemical structure of both the original lignite matrix and isolated HA (total and carboxylic acidity, elemental and spectroscopic analysis), can be found in previously published papers [16,33,34].

Phosphate buffer saline (PBS), used for the adjustment of pH of the dye solution and of inner pH of hydrogels (Section 5.3), was prepared by the dissolution of accurate amount of $Na_2HPO_4 \cdot 2H_2O$ and $NaH_2PO_4 \cdot 2H_2O$ (p.a., Sigma–Aldrich) in deionized water. Three different pH values (3, 7 and 11) and two buffer ionic strengths (10 mM and 200 mM) were used.

2.2. Preparation of hydrogels

All hydrogels, utilized in subsequent diffusion experiments, were prepared via the same method of thermoreversible gelation of aqueous solution of agarose as in previous work [16]. Agarose hydrogels (without the addition of HA, dry agarose content in gel: 0.5 wt.%, 1 wt.%, 2 wt.% and 4 wt.%) gelatinized from the solution of agarose in water (Section 5.1) or in the respective buffer solution (Section 5.3), while agarose/HA gels did from the solution of both agarose (1 wt.%) and HA (0.002 wt.%, 0.005 wt.% and 0.010 wt.%) in water (Section 5.2) or in the buffer solution (Section 5.3).

A simple gelation procedure was applied: accurately weighted amount of agarose powder was dissolved in deionized water or in the buffer solution (preparation of agarose gels) or in the solution of HA of the corresponding concentration (preparation of agarose/HA gels), respectively. The mixture was slowly heated when stirring continuously to 80 °C and maintained at the temperature until the occurrence of transparent solution. The solution was degassed in ultrasonic bath for 1 min. (at 80 °C) and slowly poured into the PMMA spectrophotometric cuvette (inner dimensions: $10 \times 10 \times 45$ mm). The cuvette 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.

2.3. Diffusion experiments

The non-stationary diffusion experiments with hydrogels were performed as follows: Pre-prepared hydrogel samples in the PMMA cuvettes were immersed in horizontal positions in 0.01 g dm^{-3} aqueous solution of the respective dye (Methylene Blue or Rhodamine 6G, four cuvettes in one container filled with 250 cm^3 of the dye solution). The dye solution was stirred continuously by the magnetic stirrer and the dye was left to diffuse from the solution into the gel samples through the square orifices of the cuvettes. Each experiment was duplicated. In selected time