MACRO- AND MICROSCALE APPROACH IN THE STUDY ON TRANSPORT PROPERTIES OF REACTIVE AGAROSE HYDROGELS

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Abstract

Hydrogels represent nowadays important material either from scientific point of view, as well as for the applications. This contribution is focused on the study of transport properties of reactive hydrogels based on agarose. This polysaccharide can at certain conditions form hydrogel matrix, which represent ideal carrier matrix for additional substances (e.g. polyelectrolytes). Incorporation of specific modifiers allows tuning the final properties (e.g. reactivity) of these hydrogel in respect to the needs of the application. The experimental part of present work was focused on the observation and quantification of the transport of Rhodamine 6G in individual reactive agarose-based hydrogels. Two basic approaches were used (both based on diffusion processes). Firstly, the simple macroscopic observation of diffusion of Rhodamine 6G from solution into cuvettes containing individual agarose-based reactive hydrogels (diffusion model of constant source) was applied. The second used technique was based on Rhodamine 6G self-diffusion measurement (using the method of fluorescence correlation spectroscopy). Both methods showed to be valuable in deeper description and characterization of the interactions and mobility of selected probe in reactive agarose-based hydrogel matrices. The obtained data from diffusion experiments were correlated with several basic structural characteristics of studied hydrogels (internal pH value, content of water, wettability…) as well as their mechanical properties (mainly rheological). The results indicated that the transport and barrier properties of individual agarose-based hydrogels are significantly affected by charge of the polyelectrolyte, which is incorporated in hydrogels matrix, as well as by its charge density.

Keywords: Diffusion, fluorescence correlation spectroscopy, hydrogels, polyelectrolyte, reactivity

1. INTRODUCTION

Gels are ubiquitous materials with broad application both from scientific [1-3] as well as industrial [3] point of view. Gels can be described as dispersion systems, containing the dispersion phase interconnected into three-dimensional network. Inside this network, the dispersion medium is entrapped. Gels can be divided according to the type of used dispersion medium to hydrogels (medium is water) or oleogels (medium is oil). Moreover, according to the type of interactions between particles or chains of the dispersion phase, gels can be divided on physical or chemical gels [2,3]. From mechanical point of view, gels are often described as materials on the border between solids and liquids. This means that at some specific conditions gels can have properties of liquids (viscous properties, can flow). On the other side, under different conditions, they can behave as solids (elastic response). These specific properties together with their simple preparation procedure at defined size and shape, experimentally easy way of manipulation, usually simple mathematical description of shape and basic parameter, almost no effects of convection on internal transport and comparable speed of diffusion in comparison with liquids create from hydrogels highly attractive material especially in respect toward the possible applications [3].

The above described properties of hydrogels are highly beneficial also for the application in the area of drug delivery systems. For this specific application, important parameter - biocompatibility must be also considered and deeply accessed [4]. Hydrogels (or generally gels) can be assumed as highly porous structures, which can be easily tuned (the density of cross-linking, the degree of swelling of gels…) according to the needs of

the application. The high porosity of three-dimensional hydrogel networks also tender free cavities for incorporation of additional substances such as drugs or other active compounds [5]. For the area of hydrogel based carrier systems, the knowledge of drug loading and releasing kinetics as well, its stability at different conditions, diffusion coefficient of the small molecule or macromolecule through the gel network seems to be crucial [6]. Especially with respect to the fact, that most of the hydrogel-based carrier systems belong to the group of swelling-controlled drug delivery systems [7].

The study on the transport properties of carrier systems based on thermoreversible agarose hydrogel is object of present contribution. Generally, agarose is an example of thermoreversible polysaccharide, which can at specific condition form hydrogel. More details on the way of agarose hydrogel preparation can be found in [8]. In our work, agarose hydrogel represents non-reactive three-dimensional carrier matrix containing free pores, which can be assumed as available cavities for loading with additional substances (drugs or other active compounds). For purposes of experimental works of present contribution as active substances incorporated in agarose hydrogels, polyelectrolytes were used. The addition of polyelectrolytes into non-reactive agarose structure provides to agarose hydrogels reactive centers for binding of oppositely charged species. These hydrogels containing available reactive centers as well as original pure agarose hydrogel were used in our work in subsequent study on their transport experiments. Basically, the knowledge of transport properties of carrier system represent a crucial parameter, which can significantly contribute in the knowledge of mobility, barrier properties and controlled release of different species in such a system. All this findings can be beneficial mainly for better prediction of behavior of hydrogel-based carrier systems and their response on external stimuli (e.g. change of pH, ionic strength, temperature).

2. EXPERIMENTAL

2.1. Materials and Methods

Solid agarose powder (Type I, low EEO, Sigma Aldrich) as well as all the polyelectrolytes (sodium alginate, hyaluronic acid, carrageenan, sodium polystyrene sulfonate, dextran and chitosan) used in present work were purchased in p.a. purity grade and were used in work without further purification. More details on the way of hydrogels preparation and incorporation of polyelectrolytes into hydrogels can be found in chapter 2.1.1. All the individual polyelectrolytes were characterized by determination of their molecular weight and hydrodynamic radius using the method of SEC-MALS-dRI (combined instrumental set-up based on size exclusion chromatography - Agilent and Multiangle Light Scattering and Differential Refractive index detection - WYATT). More details on the way of determination of both molecular weight and hydrodynamic radius as well as on the settings of the instruments can be found in our previous publication [9-11].

2.1.1. Preparation of agarose hydrogels

For preparation of basic agarose hydrogel, the accurate weight of agarose powder was dissolved in exact volume of distilled water. The temperature was increased up to 85 °C. At this temperature, agarose becomes soluble in water. Subsequently, the sample was immersed at the same temperature in ultrasound bath for 1 minute. The sample was transferred into form to create desired hydrogel shape (spectrophotometric cuvettes) for transport experiments. For preparation of agarose-based hydrogels containing individual incorporated polyelectrolytes, the procedure was similar. The only difference was in the initial step, where solid agarose powder was dissolved in the exact volume of water + appropriate amount of stock solution of individual polyelectrolytes. All the agarose based hydrogels in present work were prepared with fixed content of agarose 1 wt. %. The concentrations of individual polyelectrolytes, which were incorporated into agarose structure, were following: 0, 0.002, 0.005, 0.010 and 0.100 wt. %.

For the study of self-diffusion of Rhodamine 6G using fluorescence correlation spectroscopy (FCS), the procedure of individual hydrogels preparation was similar. The only difference was in the fact, that the samples for FCS already contained homogenously dispersed constant concentration of diffusion probe (Rhodamine

6G, concentration in the hydrogels ≈ 5×10^{9} M). The probe was added into the initial mixture of agarose powder and water (respectively water and exact volume of polyelectrolyte stock solution) before heating up of the sample. The remaining procedure of agarose hydrogels preparation was the same as was described in previous paragraph.

2.1.2. Macroscale diffusion approach

The macroscale diffusion approach was in present work observed by simple observation and quantification of time development of in-depth diffusion of selected probe (Rhodamine 6G) from its source diffusion solution (initial concentration 0.01 g / dm^3) into classical spectroscopic cuvettes filled with individual hydrogel samples (both pure agarose hydrogels as well as hydrogels with different incorporated polyelectrolytes), which were during the diffusion experiments immersed inside the diffusion solution. More details about the sample preparation procedure can be found in chapter 2.1.1. At defined time (24, 48 and 72 hours) the cuvettes with individual agarose-based hydrogels were taken out from the solution, carefully dried and the concentration of Rhodamine 6G in dependence on the distance from the solution/hydrogel interface was determined by means of UV-VIS spectrometry (the method of calibration curve; for measurements used cuvette holder with adjustable measuring position). More details on mathematic description of used diffusion model as well as on settings of the diffusion experiment can be found elsewhere [8,12-14]. The effective values of diffusion coefficients were determined as main parameters used for deeper comparison of transport phenomenon in different hydrogel samples as well as for assessment the effect of the concentration of incorporated polyelectrolyte.

2.1.3. Microscale diffusion approach

For better description of the diffusion of used probe (Rhodamine 6G) in individual studied 1 wt. % agarose-based hydrogel samples (both pure agarose hydrogels as well as hydrogels with different incorporated polyelectrolyte) as well to be able to get information on the internal processes and inhomogeneities in individual studied samples, the microscale observation of self-diffusion of probe using FCS on MicroTime 200 instrument (PicoQuant, Germany) equipped with fluorescence microscope Olympus IX71 (used setup of system: laser wavelength 510 nm, dichroic mirror 514 / 640 nm, emission filter 550 / 49, laser intensity 6.6 μW) was also utilized. Moreover during FCS measurements 2 SPAD detectors were used, which allowed us to use cross-correlation for data evaluation. To maintain uniform measurement conditions, at the beginning of the experiment the xz scan was performed and the position of glass-gel interface was identified. Afterwards, xy scan was performed 5 μm above the glass surface and 3 points were chosen for measurements for each sample. Subsequently, for FCS analysis each individual hydrogel sample was prepared in five replicates. The main outcome from FCS analysis is coefficient of self-diffusion of Rhodamine 6G in each individual hydrogel sample.

3. RESULTS AND DISCUSSION

The present contribution is dealing with the study on the transport properties of reactive carrier systems based on thermoreversible agarose hydrogel. The knowledge of transport properties of such carrier systems represents the one of the most crucial parameters, which must be taken into account considering their proper description (e.g. release or sorption of specific molecules or nanoparticles, swelling…etc.) as well as for prediction of response of these systems after application of external stimuli. In our work, we focuses on the study of transport of selected probe (Rhodamine 6G) in model systems based on agarose hydrogels containing incorporated polyelectrolytes. The experimental works can be divided on two parts. The first part was focused on the study and quantification of macroscopic diffusion process (results shown in chapter 3.1). For better description of internal transport phenomenon, fluorescence correlation spectroscopy (FCS) was used to determine the self-diffusion coefficient of used probe (Rhodamine 6G) inside of all studied hydrogels (results discussed in chapter 3.2).

3.1. Macroscale diffusion approach

The first part of experimental works of present contribution was focused on the macroscale approach in the study on the transport of selected probe (Rhodamine 6G) in individual used hydrogels. The obtained experimental data were subsequently mathematically described according to the diffusion model of constant source. More details on the mathematical model, description of data evaluation or necessary conditions of the model can be found in [12-14]. The example of obtained experimental data for diffusion in hydrogels containing incorporated variable amount of sodium alginate is shown in **Figure 1a**). The calculated values of effective diffusion coefficients (the value of diffusion coefficient in which the influence of formation of the interactions in the system as well as of torturous movement in porous structure of gel matrix is hidden) indicate direct relation to content of polyelectrolyte in agarose hydrogel. These results are also in good correlation with direct visual observation (**Figure 1b**) - example for 24 hours duration of diffusion). It is obvious, that with increasing concentration of polyelectrolyte in the agarose hydrogel, the transport of Rhodamine 6G into gel is slowed down and on the other hand, the concentration on the interface is regularly increasing (resulting in more intense color of probe on the gel/solution interface in **Figure 1b**). Similar results were obtained for hydrogels containing incorporated other negatively charged polyelectrolytes (carrageenan, PSS, hyaluronic acid). On the other hand, for hydrogels containing dextran and chitosan, we have observed no differences respectively small increase of effective diffusion coefficient. These findings illustrates importance of electrostatic interaction that is formed during the process between positively charged solute (Rhodamine 6G) and oppositely charged polyelectrolytes in hydrogels on its transport.

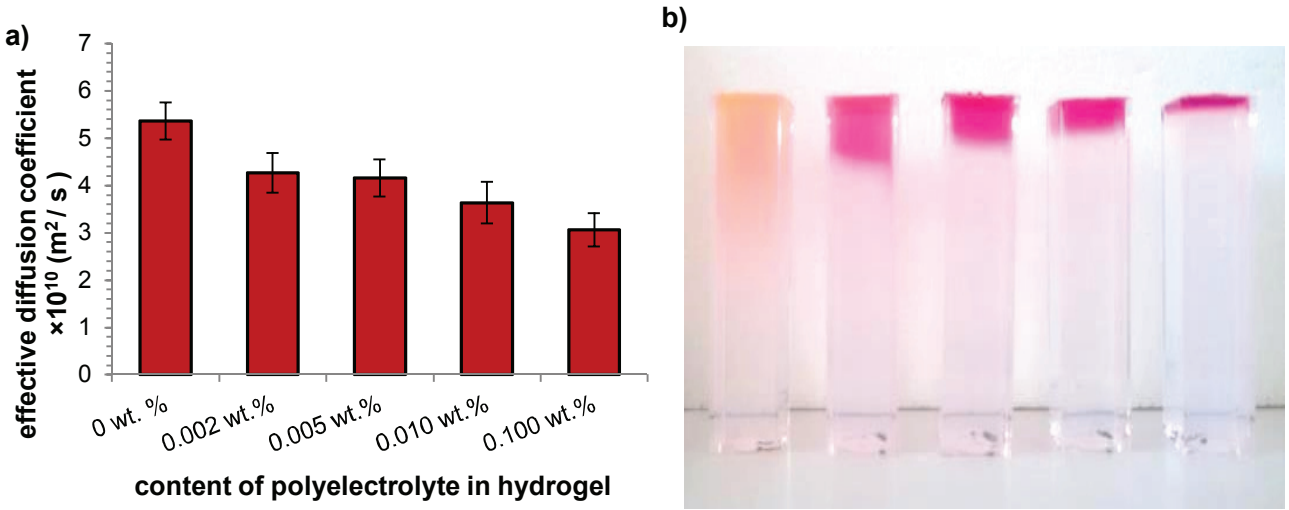

Figure 1 a) Dependences of effective diffusion coefficients of Rhodamine 6G and b) Photo of individual cuvettes contacting variable concentration of incorporated polyelectrolyte (increase in direction from left to right) - in both shown data for diffusion in hydrogels containing sodium alginate

Moreover, the data comparing effects of individual used polyelectrolytes incorporated in agarose hydrogels on the transport of Rhodamine 6G are shown in the **Figure 2a**). The most significant decrease of effective diffusion coefficient of Rhodamine 6G was observed for hydrogels containing incorporated negatively charged polyelectrolytes (PSS, sodium alginate, hyaluronic acid, and carrageenan). For this phenomenon, the density of the charge of polyelectrolyte is obviously another important parameter. In the case of dextran and chitosan, there was almost no difference between obtained values of effective diffusion coefficient in pure agarose hydrogel and in hydrogels containing these polyelectrolytes. This confirms no interaction between positively charged Rhodamine 6G and both polyelectrolytes (dextran as well as chitosan) possessing also positive charge.

3.2. Microscale diffusion approach

The results from macroscopic diffusion were in present work compared with microscale observation of internal transport of selected model probe (Rhodamine 6G) inside individual utilized reactive hydrogels. For these purposes the method of FCS was used, enabling observation of the fluctuations of fluorescence signal resulting from random motion of Rhodamine 6G in and out of the confocal volume, which is created in the sample, by focused laser-beam. Stronger fluctuations (for definite temperature and viscosity of the solvent) mean, that the molecule spends less time in the confocal volume, its diffusion is therefore faster and as an consequence also higher diffusion coefficient is measured. Because diffusion coefficient of Rhodamine 6G in water solution is known, it is easy to compare the values of Rhodamine 6G diffusion coefficient measured in agarose hydrogel matrix with the values for free diffusion in water. From this comparison, suggestions about influence of agarose gel matrix and addition of reactive polymer into the agarose gel on Rhodamine 6G diffusion can be made. Obtained diffusion coefficients of Rhodamine 6G inside pure agarose hydrogels as well as hydrogels with incorporated 0.1 wt. % of individual polyelectrolytes are listed in **Figure 2b**).

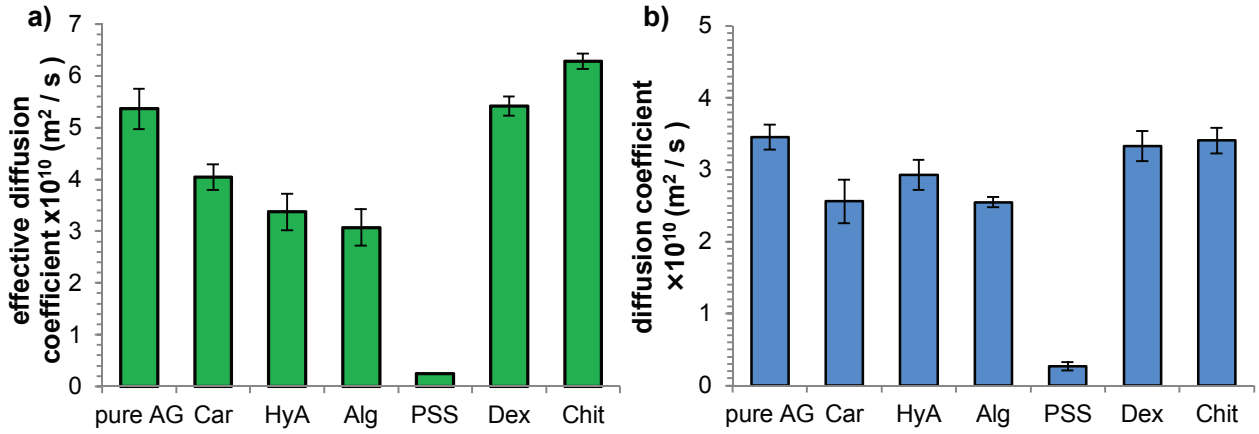

Figure 2 a) Determined values of effective diffusion coefficients of Rhodamine 6G from macrodiffusion experiments and b) Self-diffusion coefficients of Rhodamine 6G obtained by FCS; both for diffusion in agarose gel containing 0.1 wt. % of polyelectrolytes (AG = agarose, Car = carrageenan, HyA = hyaluronic acid, Alg = sodium alginate, PSS = polystyrene sulfonate, Dex = dextran hydrochloride, Chit = chitosan)

The microscopic observation of self-diffusion coefficients of Rhodamine 6G in agarose hydrogels containing individual polyelectrolytes confirmed results from macro-diffusions. The decrease in diffusivity of Rhodamine was observed for hydrogels containing oppositely charged polyelectrolytes (=PSS, sodium alginate, hyaluronic acid, carrageenan). The results also indicated the importance and the impact of density of polyelectrolyte charge on absolute value of diffusion coefficient. Moreover, FCS measurements also confirmed, that in the case of incorporation of dextran or chitosan (possessing the consistent charge as the probe) into agarose hydrogel matrix, there were almost no changes in Rhodamine 6G diffusion coefficients.

4. CONCLUSION

This contribution provides unusual combination of macro- and microscale approach in the study on transport and barrier properties of reactive hydrogels based on agarose. From the obtained experimental data it is obvious, that results obtained from both experimental approaches were in good correlation. The experimental outcomes confirmed electrostatic interaction of observed probe with polyelectrolytes incorporated in agarose hydrogels as the main parameter affecting the transport of the probe. Moreover, the charge density of polyelectrolyte was defined as second important parameter. To sum up, the proposed combination of both macroscopic and microscopic approaches in the observation of probe movement and transport through

agarose hydrogels proved to be suitable for deeper characterization of transport properties of complex systems such as hydrogels, which is highly desirable mainly for their possible future applications in the area of carriers systems.

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