STUDY ON DIFFUSION PROCESSES IN REACTIVE HYDROGELS FROM MACRO- AND MICROSCOPIC VIEW OF PAPER

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Abstract

This contribution is focused on study on transport properties of selected probe (Rhodamine 6G) in reactive hydrogels. Hydrogels represent important material either from scientific point of view, as well as from the view of possible applications. In present work, hydrogels based on thermoreversible biopolymer agarose were used. This non-reactive agarose hydrogel matrix can be filled with additional homogeneously distributed molecules (e.g. polyelectrolytes). For these purposes we have selected sodium alginate, hyaluronic acid, carrageenan, sodium polystyrene sulfonate, dextran and chitosan. This type of model reactive hydrogels was used as a medium for subsequent transport experiments. Two types of experimental settings of transport experiments were used in experimental part of this work (both based on diffusion process). The first method was based on the simple macroscopic study on diffusion of Rhodamine 6G from solution into cuvettes containing individual agarose-based reactive hydrogels (diffusion model of constant source). The second used technique was based on Rhodamine 6G self-diffusion measurement (method of fluorescence correlation spectroscopy). Both used methods showed to be valuable for deeper description and characterization of interactions and mobility of selected probe in reactive agarose-based hydrogel matrices. The results are indicating that the transport and barrier properties of individual agarose-based reactive hydrogels are significantly affected by polyelectrolyte charge and its charge density. The results of present work in connection with deep meta-analysis of literature can significantly contribute to further applied research and development in the area hydrogels and carrier materials based on complexes with different biopolymers and polyelectrolytes.

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

1. INTRODUCTION

Gels represent outstanding materials, which can be found both in nature and in natural processes, as well as have many applications in human driven processes such as food industry, medicine or chemistry of detergent [1]. From physicochemical point of view, gel can be described as dispersion system, in which the dispersion phase is connected into three-dimensional network. Inside of this interconnected network the dispersion medium is closed. According to the type of interactions between particles or chains of the dispersion phase, gels can be divided on physical or chemical gels. Moreover, according to the used dispersion medium in the gel, we can distinguish hydrogels (medium is water) or oleogels (medium is oil) [2, 3]. Generally, 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). All these findings result in some specific advantageous properties of hydrogels such as easy manipulation with samples, preparation at defined size and shape, easy mathematical description, almost no effects of convection, comparable speed of diffusion in comparison with liquids etc. [3, 4]. Above mentioned properties are highly attractive especially toward the possible applications of these extraordinary systems.

The beneficial properties of hydrogels can be used also in the area of drug delivery systems. Hydrogels (or generally gels) consist of highly porous structure, which can be easily tuned (the density of cross-linking, the degree of swelling of gels…) according to the needs of the application. Moreover, hydrogels are generally also

highly biocompatible [5]. The high porosity of three-dimensional hydrogel networks also tender free cavities for incorporation of additional substances such as drugs or other active compounds. For the area of hydrogel based carrier systems, the knowledge of drugs loading kinetics as well as a rate of release of the drug or other active compound from the system, its stability at different conditions, diffusion coefficient of the small molecule or macromolecule through the gel network seems to be crucial [6]. Most of hydrogel-based drug carriers belong to the group of swelling-controlled drug delivery systems [7].

For purposes of present work, the carrier systems were modelled by agarose hydrogel. 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]. Agarose hydrogel represents non-reactive three-dimensional network, containing free pores. These pores can be used as free cavities, which are available for loading with additional substances (drugs or other active compounds). In present work we have used polyelectrolytes. The addition of polyelectrolytes into non-reactive agarose structure provides free reactive centres for binding of oppositely charged species. These hydrogels containing available reactive centres as well as original pure agarose hydrogel were used in transport experiments. Generally, the knowledge of transport properties of such systems can shed a new light on the phenomenon of mobility and barrier properties and controlled release of different species in studied system. All this findings can be beneficial mainly for better prediction of behaviour 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

All the materials used in experimental part of present work were purchased from Sigma-Aldrich (p.a. purity grade). For preparation of agarose hydrogels solid agarose powder was used (Type I, low EEO, Sigma Aldrich). Subsequently, these agarose-based non-reactive hydrogels were modified by incorporation of polyelectrolytes into the hydrogel matrix during the initial step gelation process. More details on the way of hydrogels preparation and incorporation of polyelectrolytes into hydrogels can be found in chapter 2.1.1. The polyelectrolytes used in this work were sodium alginate, hyaluronic acid, carrageenan, sodium polystyrene sulfonate, dextran and chitosan. All used polyelectrolytes were characterized through determination of their molecular weight and hydrodynamic radius using the method of SEC-MALS (combined instrumental settings from Agilent and 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

Agarose based hydrogels studied in present work were prepared by simple dissolving of accurate weight of agarose powder in exact volume of distilled water (milli-Q purity grade). The temperature was increased up to 85 °C. At this temperature, agarose stars to be soluble in water. After decreasing of the temperature of this mixture back to the normal laboratory temperature, the agarose chains are getting involved into the final 3D structure of agarose hydrogel. For preparation of agarose-based hydrogels containing individual incorporated polyelectrolytes the procedure of agarose hydrogel preparation was similar with previously listed one used for preparation of pure agarose hydrogels, the only difference was in the fact, that defined part of distilled water was substituted by polyelectrolyte solutions. 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 FCS determination of self-diffusion coefficient of Rhodamine 6G 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 into the mixture of water and polyelectrolyte before heating up of the samples. The remaining procedure of agarose hydrogels preparation was the same as was described in previous paragraph.

2.1.2. Macrodiffusion experiments

Macroscale diffusion processes in agarose-based hydrogels were in this work represented by simple observation of time development of in-depth diffusion of selected probe (Rhodamine 6G) from its the source diffusion solution (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 duration from the beginning of the diffusion experiments (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, 13]. For the comparison of different hydrogel samples as well as for the effect of the concentration of incorporated polyelectrolyte and also for mutual correlation with microscale diffusion approach, the values of effective diffusion coefficient were determined.

2.1.3. Microscale self-diffusion measurements by fluorescence correlation spectroscopy (FCS)

Microscale observation of diffusion phenomenon of used probe (Rhodamine 6G) in individual 1 wt. % agarose-based hydrogel samples (both pure agarose hydrogels as well as hydrogels with different incorporated polyelectrolyte) was performed 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). 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

Reactive hydrogels can be listed as an example of carrier systems with possible applications in different areas of human driven processes such as in medicine, cosmetology etc. The knowledge of transport properties of these carrier systems represents one of most crucial parameters necessary for their proper description and for prediction response of these systems after application of external stimuli. Generally, the knowledge of transport properties of these systems can shed a new light on different phenomenon such as release or sorption of specific molecules or nanoparticles, swelling…etc. The experimental works of present paper focused on the study of transport properties of selected probe (Rhodamine 6G) in model systems based on agarose hydrogels with incorporated polyelectrolytes. For these purposes two different approaches based of diffusion were used. Firstly, the macro-diffusion approach was applied (results shown in chapter 3.1). For better description of internal transport of used probe (Rhodamine 6G) inside of all studied hydrogels, the observation of self-diffusion coefficient by means of fluorescence correlation spectroscopy (FCS) was used (results shown in chapter 3.2).

3.1. Macro-diffusion experiments

Firstly, the classical macroscale approach on the study of transport of selected probe (Rhodamine 6G) was used. The performed diffusion experiments were processed 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, 13]. The data shown in **Figure 1a** illustrate the dependence of obtained value of effective diffusion coefficient (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) on the content of sodium alginate in the sample. Similar results were obtained for diffusion in hydrogels containing also other negatively charged polyelectrolytes (carrageenan, polystyrene sulfonate, 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 interactions that are formed during the process between positively charged solute (Rhodamine 6G) and oppositely charged polyelectrolytes in hydrogels. These interactions significantly slow down the diffusion process. Subsequently the lower value of effective diffusion coefficient is observed. These findings are supported by data shown on **Figure 1b** respectively by the picture of the cuvettes after 24 hours diffusion of Rhodamine 6G in hydrogels (**Figure 2a**). The determined ratios of Rhodamine 6G concentrations at the interface gel /solution (**Figure 1b**) are indicating high affinity of agarose-based hydrogels towards the diffusing solute (Rhodamine 6G). This phenomenon is significantly increased with the increased content of incorporated negatively charged polyelectrolytes in hydrogels. Moreover, the picture shown on **Figure 2a** is in good agreement with both above listed findings. It indicates, that higher content of polyelectrolyte in hydrogel caused higher determined concentration at the interface (or up-concentration of Rhodamine 6G in comparison with its concentration in solution) but on the other hand, the distance, where the probe diffused from the interface with increasing content of oppositely charged polyelectrolyte in hydrogel, decreased.

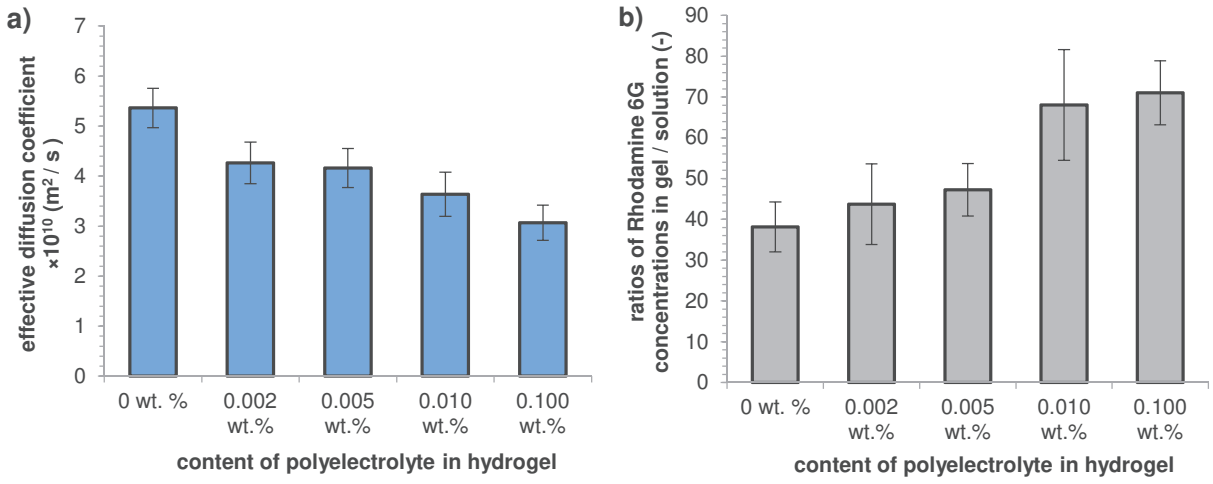

Figure 1a) Dependences of effective diffusion coefficients and **b)** Ratios of Rhodamine 6G concentrations at gel / solution interface on content of polyelectrolyte in hydrogels (here shown data for sodium alginate)

From the comparison of agarose-based hydrogels containing the same amount of incorporated polyelectrolytes shown on the **Figure 3a** is obvious, that the most significant decrease of effective diffusion coefficient of Rhodamine 6G was observed in hydrogels with negatively charged incorporated polyelectrolytes (PSS, sodium alginate, hyaluronic acid, carrageenan). For this phenomenon, the density of the charge of polyelectrolyte seems to be important parameter. On the other side for dextran and chitosan, there was almost no difference between obtained values of effective diffusion coefficient in pure agarose hydrogel and in hydrogels containing dextran respectively chitosan. As was already listed earlier, the explanation of this

findings is connected with electrostatic interactions, which are not formed between positively charged Rhodamine 6G and dextran respectively positively charged chitosan.

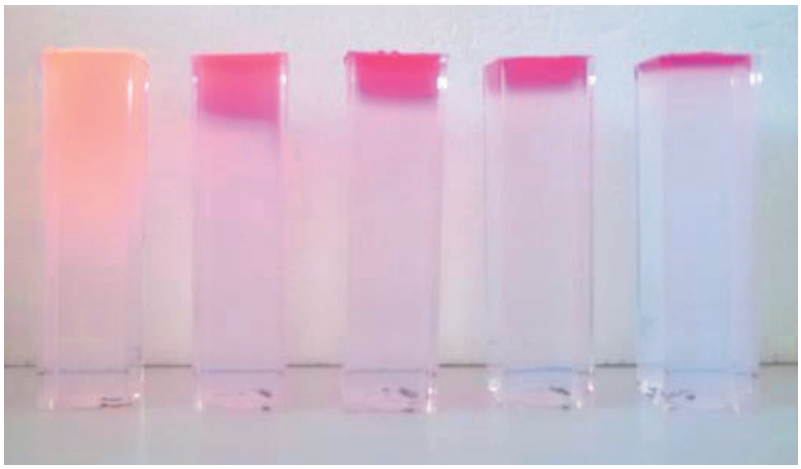

Figure 2 Picture of development of Rhodamine 6G diffusion in agarose hydrogels with variable content of polystyrene sulfonate (increasing content from left to right), results shown here are for 24 hours diffusion

3.2. Microscale self-diffusion measurement by fluorescence correlation spectroscopy (FCS)

For better description of internal transport of selected model probe (Rhodamine 6G) inside utilized reactive hydrogels, the method of FCS was used. This method is based on observing 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 this consequence can be quantified by high diffusion coefficient. Because diffusion coefficient of Rhodamine 6G in water solution is known, it is easy to compare values of Rhodamine 6G diffusion coefficient measured in agarose hydrogel matrix with 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 on **Figure 3b**.

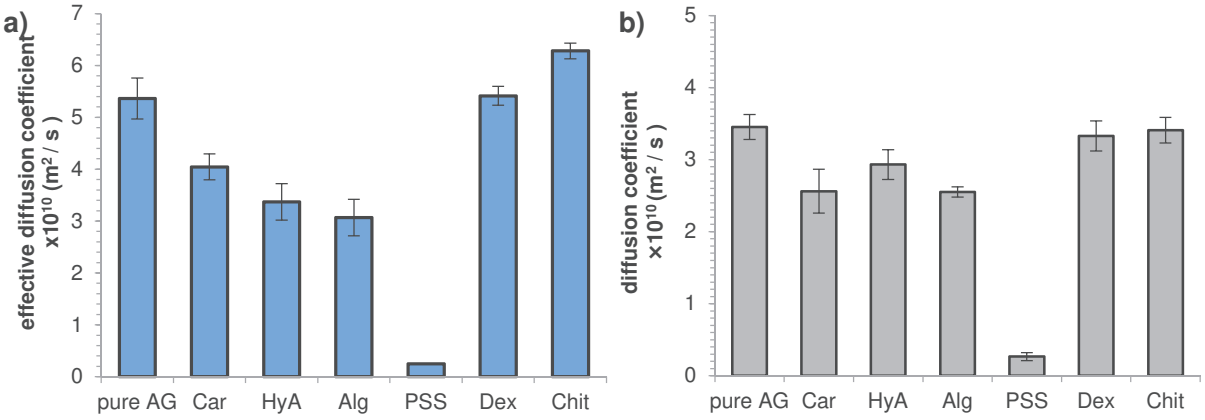

Figure 3a) Effective diffusion coefficients of Rhodamine 6G obtained from macro-diffusion and **b)** Diffusion coefficients of Rhodamine 6G obtained from FCS measurement, both figures for diffusion in agarose hydrogels with 0.1 wt. % of added polyelectrolytes (AG = agarose, Car = carrageenan, HyA = hyaluronic acid, Alg = sodium alginate, PSS = polystyrene sulfonate, Dex = dextran hydrochloride, Chit = chitosan)

The observed changes of diffusion coefficients of Rhodamine 6G after additions of individual polyelectrolytes inside hydrogels confirmed results from macro-diffusions. The decrease in diffusivity of Rhodamine was observed for hydrogels containing oppositely charged polyelectrolytes (=negatively charged polyelectrolytes). The density of the charge of incorporated polyelectrolyte seems to have also significant effect on absolute value of diffusion coefficient. On the other hand, FCS measurements confirmed, that in the case of added dextran or chitosan, there were almost no changes of diffusivity of Rhodamine 6G in samples.

4. CONCLUSION

This work was focused on the study of transport properties of reactive hydrogels from macro and microscopic point by means of measuring diffusivity of selected probe - Rhodamine 6G inside of individual hydrogel samples. From the obtained experimental data it is obvious, that both experimental approaches were in good correlation. The most significant outcome from the measurement was that the electrostatic interactions, which can be formed between the charged probe and oppositely charged functional groups of polyelectrolytes incorporated inside hydrogels, can significantly decrease the mobility of the probe inside the gel matrix. Moreover the charge density of polyelectrolyte was defined as second important parameter. To sum up, above mentioned methods seem to be suitable for deeper characterization of transport properties of complex systems such as hydrogels with incorporated polyelectrolytes, which is highly desirable mainly for their possible future applications as carriers systems of various substances.

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REFERENCES

[1] GULREZ, S.K.H., AL-ASSAF, S., PHILIPS, G.O. Hydrogels: *Methods of Preparation, Characterisation and Applications*. InTech: CC BY-NC-SA, 2011. 150 p.

[2] FERRY, J.D. *Viscoelastic Properties of Polymers*, 3rd ed. New York: Wiley, 1980. 672 p.

[3] KOPECEK, J. Hydrogel biomaterials: A Smart future, *Biomaterials*, 2007, vol. 28, pp. 5185-5192.

[4] KALINA, M., KLUCAKOVA, M., SEDLACEK, P. Utilization of fractional extraction for characterization of the interactions between humic acids and metals. *Geoderma*, 2013, vol. 207-208, pp. 92-98.

[5] SUTTON, CH. Adhesions and their prevention. *The Obstetrician & Gynaecologist*, 2005, vol. 7, pp. 168-176.

[6] HOARE, T.R., KOHANE, D.S. Hydrogels in drug delivery: Progress and challenges. *Polymer*, 2008, vol. 49, pp. 1993-2007.

[7] OSTROHA, J., PONG, M., LOWMAN, A., DAN, N. Controlling the collapse/swelling transition in charged hydrogels. *Biomaterials*, 2004, vol. 25, pp. 4345-4353.

[8] SMILEK, J., SEDLACEK, P., KALINA, M., KLUCAKOVA, M. On the role of humic acids' carboxyl groups in the binding of charged organic compounds. *Chemosphere*, 2015, vol. 138, pp. 503-510.

[9] KALINA, M., MICHALICOVA, P., SEDLACEK, P., KRATOCHVIL, Z., SMILEK, J., KLUCAKOVA, M., PEKAR, M. Research on stability of biopolymers by means of light scattering techniques. In *NANOCON 2015: 7th International Conference on Nanomaterials - Research and Application.* Ostrava: TANGER, 2015, pp. 660-665.

[10] SIMULESCU, V., MONDEK, K., KALINA, M., PEKAR, M. Kinetics of long-term degradation of different molar mass hyaluronan solutions studied by SEC- MALLS. *Polymer Degradation and Stability*, 2015, vol. 111, pp. 257-262.

[11] KALINA, M., CHYTILOVA, A., KLUCAKOVA, M. Aggregation and Degradation Processes of Biopolymers and Biocolloids Studied by Light Scattering Techniques. In *NANOCON 2014: 6th International Conference on Nanomaterials - Research and Application*. Ostrava: TANGER, 2014, pp. 844-849.

[12] SEDLACEK, P., SMILEK, J., KLUCAKOVA, M. How the interactions with humic acids affect the mobility of ionic dyes in hydrogels-2. Non-stationary diffusion experiments. *Reactive &Functional Polymers*, 2014, vol. 75, pp. 41-50.

[13] CUSSLER, E.L. *Diffusion - Mass Transfer in Fluid Systems*, 2nd ed. Cambridge University Press, 1997. 580 p.