CHARACTERIZATION OF HYDROGELS BY MACRO AND MICRORHEOLOGICAL TECHNIQUES

LASTUVKOVA Marcela[1], SMILEK Jiri[1], KALINA Michal[1], KRACALIK Milan[2], KRATOCHVILOVA Romana[1], PEKAR Miloslav[1]

[1]Brno University of Technology, Faculty of Chemistry, Brno, Czech Republic, EU
[2]Johannes Kepler University Linz, Institute of Polymer Science, Linz, Austria, EU

Abstract

The main aim of this contribution is the study of mechanical properties of hydrogels systems by classical rheological techniques (oscillation measurements) and further correlation of mechanical properties with inner structural and mechanical properties determined by novel microrheological techniques (dynamic light scattering, fluorescence correlation spectroscopy). As a model hydrogel medium; agarose as a representative polysaccharide will be used. The main advantage of the agarose hydrogel is their thermoreversibility and non-reactivity. Therefore the agarose hydrogels can be prepared with defined properties. As the studied samples will be agarose hydrogels with different concentrations in mixture with biopolymers. As the suitable biopolymers; chitosane, alginate, hyaluronate will be used, and for comparison also polystyrenesulfonate will be used. The main emphasis will be taken on time and temperature dependence on mechanical properties determined by macro- and microrheological techniques. Further fluorescence labeling hydrogels samples will be studied and the ability of probes penetrated into the hydrogels.

Keywords: Microrheology, hydrogels, rheology, polyelectrolytes

1. INTRODUCTION

Gels are the dispersion systems formed by particles (dispergum) with the size about 1 - 1000 nm and by dispersion medium (dispergens), which occupies majority part of gels. Gels are the semi-solid materials with the 3D network, which are formed for example by cross-linking of linear polymers [1]. The gel formation depends on the number of the created nodes between the long chains and the more of nodes create the rigid material. The gels are portioned by according to behavior of dispergens as lyogels and xyrogels. Lyogels are the gels, which obtained the solution (organic solution, water, etc.) and xerogels are the gels without solution (so called dry gels). For our experiments we used agarose gels with the water solution, and then are called hydrogels. Agarose is the natural biopolymer; it is polysaccharide with the gelation properties, which is descripted in chapter 2.1.1.

Hydrogels belong to one of the most used drug forms in pharmacy especially for drug delivery, tissue engineering, bioseparation or biosenzoring [2 - 5]. The functions of drug forms are depended on the many factors (structure, addition of other compounds, inner structure, dissolution etc.). Their properties and behavior in human body are based on the right function of drug delivers, dissolution or releasing of drugs. The mean properties have the effect on the diffusion of drugs and the compounds from outer side into the hydrogels [6 - 10], which have further effects on their functions.

The function of hydrogels is based on mechanical properties, too and then the viscosity determines the behavior of hydrogels. For the characterization of mechanical properties is possible used to rheology, which belongs to common method for determination of visco-elastic properties [11-12]. This method is used for the macroscopic observation of mechanical hydrogel properties, but some changes must not be observed immediately. The microscopic mechanical properties are possible to measure by microrheology. It is the new young method based on the dynamic light scattering (DLS) and fluorescence correlation spectroscopy (FCS).

This instrumentation has big potential for the characterization of mechanical properties in micro scale. The principle of methods is description in chapter 2.1.3 and 2.1.4.

2. EXPERIMENTAL

2.1 Materials and Methods

2.1.1. Preparation agarose hydrogel with addition of biopolymers

Agarose is a natural biopolymer isolated from seaweeds (*Floridae* and *Gelidium*). Our experiments are based on polysaccharides - agarose - formed by linear units D-galactose and 3,6-anhydro-L-galactopyrane galactose with the molecular weight 120 000 Da. Agarose represents thermoreversible polysaccharide, which can at specific conditions form hydrogel. Agarose is insoluble in water at laboratory temperature, on the other side at high temperature (approximately 85 °C) it starts to be soluble. The long chains of agarose are untwisted at high temperature and after decrease of temperature of sample (30 - 40 °C) are the chains intertwined. Our experiments are based on the optically clear 1 wt. % agarose hydrogel with the addition of 0.1 wt. % of polyelectrolyte. The procedure of the preparation of agarose hydrogels with different content of polyelectrolyte is follows: the accurate weight of powder agarose (Type I, low EEO, Sigma Aldrich) was placed into the beaker with exact volume of pure distillated (mili-Q) water. The mixture was heated up to 85 °C, when the agarose became soluble. Then melting agarose was allowed at laboratory temperature. When the temperature decreases, the agarose chains are getting involved into the final structure of hydrogels (30 °C).

2.1.2. Macro rheology (REO)

Agarose hydrogels with different addition of polyelectrolytes were prepared according preparation procedure mentioned in the section 2.1.1.

The mechanical properties of agarose hydrogels with addition of different polyelectrolytes (alginate, hyaluronate, chitosan and polystyrene sulfonate) were determined by classical macrorheological experiments (realized on Rheometer AR-G2, TA Instruments). As suitable experiments - two types of oscillatory measurements were chosen. Firstly, the linear viscoelastic region (LVR) was determined by amplitude deformation test (strain sweep). The experimental parameters of the strain sweep experiments were set as follows: constant frequency of oscillation - 1 Hz, temperature - 25 °C, conditioning step - 3 minutes, dynamic range of deformation amplitude - 0.01-100 %, measuring gap - 1000 μm. The main aim of these experiments was determination of linear viscoelastic region. From this region, the constant amplitude of deformation must be chosen for further experiments (frequency sweep).

Frequency sweep measurements were realized on the same instrument as above mentioned strain sweep tests. Parameters of these experiments were set as follows: constant amplitude of deformation (chosen from the linear viscoelastic region) - 0.5 %, temperature - 25 °C, dynamic range of oscillation frequency - 0.1-20 Hz, measuring gap - 1000 μm.

Titanium sensor plate-plate with diameter 40 mm and Peltier plate for maintenance of accurate temperature was used for all mentioned experiments.

2.1.3. Dynamic Light Scattering (DLS)

For deeper discussion of influence of polyelectrolytes content on viscoelastic properties of studied samples two micro-scale rheological methods were utilized. Firstly, the method of dynamic light scattering with further extension to DLS microrheology was used. This method is based on observing of the movement of tracer particles with defined particle size (polystyrene monodispersed particle size standard with nominal particle size

100 nm) in the samples. The second utilized micro-scale rheological method was fluorescence correlation spectroscopy (FCS). More details about FCS measurements can be found in following chapter 2.1.4.

To perform DLS microrheological experiments firstly the method was optimized on series of pure agarose hydrogel with variable concentration of agarose ( ). Subsequently, the individual samples of hydrogels with different incorporated polyelectrolytes (fixed concentration 0.1 wt. %) were used. More details about way of sample preparation can be found in chapter 2.1.1.

DLS microrheology experiments on both these groups of samples (pure hydrogels with variable concentration of agarose as well as hydrogels with different incorporated polyelectrolytes) were carried out on Zetasizer Nano ZS instrument (Malvern Instruments). For DLS analysis the samples with additionally homogeneously dispersed tracer particles (polystyrene monodisperse, 100 nm, ratio: 20 µl of tracer solution on 5 ml of total volume of hydrogel) were prepared directly into glass cuvettes (classical cuvettes for routine UV-VIS spectroscopy). The cuvettes with individual samples were tempered at 25 °C (for 30 minutes) and subsequently analyzed by DLS microrheology method. From obtained raw data (autocorrelation function of tracer movement in studied samples) the main outcome from the method, the dependences of mean square displacement (MSD) of tracer particles in individual hydrogel samples on observation time were determined.

2.1.4 Fluorescence Correlation Spectroscopy (FCS)

Fluorescence correlation spectroscopy measurements were carried out using a time-resolved confocal fluorescence microscope MicroTime 200 (PicoQuant) equipped with an inverted microscope (Olympus IX 71) containing a water immersion objective (Olympus UPlansApo, 60×1.2/NA). An excitation light of 470 nm was applied to excite the samples.

The method of FCS is able to determine the viscoelastic properties of studied samples by means of observing of movement of fluorescently labeled tracer particles in sample. The utilized measuring method is called microrhelogy. For purposes of this type of measurement two different fluorescently labeled tracer particles were used. For pure agarose hydrogel and hydrogels containing negatively charged (PSS, sodium alginate, hyaluronic acid) and neutral polyelectrolytes (dextran) sulphonated polystyrene tracer particles with nominal particle size 30 nm were used. On the other side for hydrogel containing positively charged polyelectrolyte (chitosan) amino-modified polystyrene tracer particles with nominal particle size 100 nm were used. From observed number of particles flowing through the confocal volume during the measurement, the viscosity of studied material is calculated. Subsequently, the obtained data were fitted by SymphoTime64 software. The main outcome from the method was again as from DLS microrheology the dependence of MSD of tracer particles in studied hydrogels on the observation time.

3. RESULTS AND DISCUSSION

3.1. Macro rheology (REO)

The main aim of the strain sweep experiments was the determination of linear viscoelastic region of all agarose hydrogels with different addition of polyelectrolytes. All experimental data are summarized in **Figure 1**.

From the graphical dependence (**Figure 1a**)) is obvious, that all agarose hydrogels independently of addition of polyelectrolytes have very similar viscoelastic behavior. The linear viscoelastic region is in the range from 0.01 % to 0.5 % amplitude of deformation. Therefore 0.5 % amplitude of deformation was chosen as a suitable value for further oscillation experiments (frequency sweep). From the macro rheological point of view, there are no significantly changes in the mechanical properties of agarose hydrogels with addition of polyelectrolytes according to strain sweep amplitude experiments.

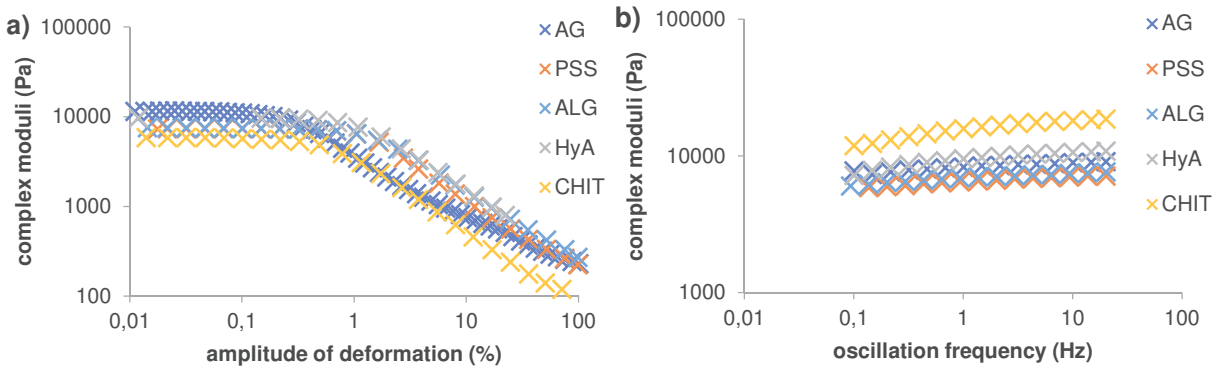

Figure 1 a) Strain sweep amplitude test and **b)** frequency sweep test for agarose hydrogels with different polyelectrolytes (× represents pure agarose hydrogel with 1 wt. % of agarose, × represent 1 wt. % of agarose hydrogel with 0.1 wt. % of polystyrene sulfonate, × represents 1 wt. % of agarose hydrogel with 0.1 wt. % alginate, × represents 1 wt. % of agarose hydrogel with 0.1 wt. % of hyaluronate and finally × represents 1 wt. % of agarose hydrogel with 0.1 wt. % of chitosan

As was mentioned above, the mechanical properties of agarose hydrogels were studied also by second oscillation test (frequency sweep experiment). All experimental data obtained from this type of experiments are summarized in **Figure 1b**). It is obvious, that there are only slight differences in values of complex moduli for different agarose hydrogels. If we will compare pure agarose hydrogel with the same concentrated agarose hydrogel with addition of chitosan, we can take into account slight increase of complex moduli for agarose hydrogel with chitosan. On the macrorheological scale, this increase is not so significant in comparison with microrheological point of view (see chapters 3.2. a 3.3.) because all agarose hydrogels are viscoelastic materials with a predominance of elasticity (elastic modulus is higher in comparison with viscous modulus in all cases for whole range of chosen oscillation frequency.

The inner mechanical properties from the microrheological point of view were studied more deeply in further chapters (3.2. - DLS microrheology and 3.3. - FCS microrheology). From the comparison of results obtained from macrorheological and microrheological experiments is obvious, that changes in mechanical properties, which are noticeable in microrheological scale, are not observed in macrorheological point of view.

3.2. Dynamic light scattering (DLS)

The first part of DLS microrheological experiments was focused on optimization of settings of this method for purposes of hydrogel sample analysis. In principle DLS method is based on observing of differences in scattered light intensity caused by moving particles in measured sample. Moreover, the method is highly sensitive on presence of big particles/aggregates of particles, which scatter light with significantly higher intensity in comparison with smaller particles and sometimes the scattering of small particles can be completely hidden by scattering of big particles/aggregates of particles. Finally another limitation of DLS is connected to the concentration effect of analyzed sample. To be able to collect good experimental data the initial laser light beam must be able to penetrate through the sample (sample must be optically clear). Sometimes even ostensibly optically clear sample can provide wrong results (=data with low signal to noise ratio) due to multiple scattering caused by too much high concentration of particles in sample. To sum up, all these effects are significantly limiting the application of DLS microrheology for characterization of hydrogel samples.

The data (**Figure 2a**)) describing comparison of MSD curves of tracer particles for pure agarose hydrogels with variable concentration of agarose in hydrogels shows typical and also expected shape. The effect of elasticity of sample is increasing with increasing concentration of agarose in the hydrogels.

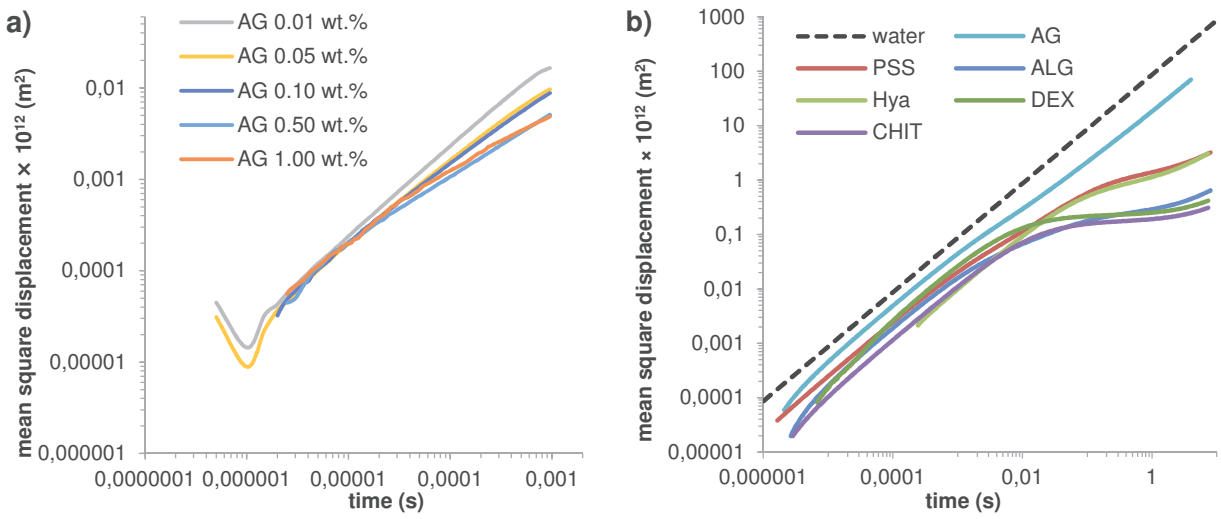

Figure 2 a) MSD dependences on observation time for pure agarose hydrogels with variable concentration of agarose obtained from DLS microrheology; **b)** MSD dependences on observation time for agarose hydrogels with different incorporated polyelectrolytes obtained from FCS microrheology

The second part of the DLS microrheology experiments was focused on comparison of 1 wt. % agarose hydrogels containing 0.1 wt. % of different polyelectrolyte. Unfortunately, the results from this part of experiments showed that method of DLS microrhelogy is not suitable for such a type of samples. The polyelectrolyte content caused significant increase of polydispersity, which reflected in much higher multiple scattering of gel matrix. Consequently these undesirable effects cannot be filtered from the signal of tracer scattering even at high concentration of tracer particles in sample. By these reasons for further description of microrheological properties of studied samples the method of FCS was used.

3.3. Fluorescence Correlation Spectroscopy (FCS)

For further description of viscoelastic properties of individual samples of agarose hydrogels on microscopic scale the method of fluorescence correlation spectroscopy was used. The measurement on FCS is in comparison with DLS not affected by undesirable effects of gel matrix. The obtained comparison of basic outcome from this method - MSD curves of traces particles in individual hydrogel samples - is showed on **Figure 2b)**. The displayed MSD curves for individual hydrogels with different incorporated polyelectrolytes are compared with pure agarose hydrogel as well as with the results obtained from the measurement of MSD of tracer in water. Experimental MSD curve obtained for movement of tracer in pure agarose hydrogel showed almost linear dependence with minor effect of elasticity of gel matric. The observed shift towards lower values of MSD in comparison of tracer movement in water was expected. It indicated higher viscosity of agarose matrix in comparison with water. From the comparison of MSD curves for pure agarose hydrogels and the data obtained for samples containing different incorporated polyelectrolytes is obvious that two different effects can be distinguished. Firstly, all the MSD curves determined for sample containing individual polyelectrolytes are shifted towards lower values, indicating slightly higher viscosity of these samples. The most significant decrease was observed for hydrogel containing chitosan. The second observation is connected to the shape of MSD curves. The MSD curves obtained for hydrogels with different incorporated polyelectrolytes are showing non-linear dependences with significant turn to the right indicating much higher contribution of elasticity of samples. The elastic behavior is the most significant for hydrogels containing dextran, sodium alginate and chitosan.

The results of this part of the work showed that despite of no observed effect of polyelectrolyte content on macroscopic viscoelastic properties of reactive hydrogels, the internal microstructure of reactive hydrogels can be affected even by such a low content of added polyelectrolytes.

4. CONCLUSION

This work is focused on the study of macro and micro rheological properties of agarose hydrogel with addition of polyelectrolytes. From the obtained experimental data is obvious, that in macrorheological point of view, the small differences in the microstructure of measured samples caused by addition of polyelectrolytes cannot be determined. However, the differences in microstructural properties of hydrogels are able to detect by DLS and FCS methods. FCS method seems to be more sensitive in comparison with DLS method for determination of differences in microstructural properties of agarose hydrogels with addition of polyelectrolytes, because the sensitivity of DLS method decreases with increasing concentration of polyelectrolyte because of higher scattering. Above mentioned methods (FCS and DLS) seem to be very promising in deeper characterization of these complex systems such as hydrogels with polyelectrolytes especially from the microstructural point of view.

ACKNOWLEDGEMENTS

This work has been supported by project AKTION Austria-Czech Republic No. 76p5 "Characterization of hydrogels systems by advanced macro-rheological, micro-rheological and spectroscopic techniques" and by project "Materials Research Centre at FCH BUT - Sustainability and Development" No. LO1211 of the Ministry of Education, Youth and Sports of the Czech Republic.

REFERENCES

[1] LIU, D. J., DURSCH, T. J., TAYLOR, N. O., CHAN, S. Y., BREGANTE, D. T., RADKE, C.J. Diffusion of water-soluble sorption drugs in HEMA/MAA hydrogels. *Journal of Controlled Release*, 2016, vol. 239, pp. 244 - 248.

[2] DURSCH, T. J., TAYLOR, N.O., LIU, D. E., WU, R.Y., PRAUSNITZ, J. M., RADKE, C.J. Water-soluble drug partitioning and adsorption in HEMA/MAA hydrogels. *Biomaterials*, 2014, vol. 35, pp. 620-629.

[3] PEPPAS, N.A. Hydrogels and drug delivery. *Curr. Opin. Colloid Interf. Sci.,* 1997, vol. 2, pp. 531-537.

[4] PEPPAS, N. A., BURES, P., LEOBANDUNG, W. ICHOKAWA, H. Hydrogels in pharmaceutical formulations. *Eur. J. Pharm. Biopharm.*, 2000, vol. 50, pp.27-46.

[5] GUPTA, P., VERMANI, K., GARG, S. Hydrogels: from controlled release to pH-responsive drug delivery. *Drug Discov* Today, 2002, vol. 7, pp. 569-579.

[6] STRINGER, J. L., PEPPAS, N. A. Diffusion of small molecular weight drugs in radiation-crosslinked poly (ethylene oxide) hydrogels. *J. Control. Release*, 1996, vol. 42, pp. 195 - 202.

[7] AM ENDE, M. T., PEPPAS, N.A. Transport of ionizable drugs and proteins in crosslinked poly (acrylic acid) and poly (acrylic acid-co-2-hydroxaethyl methacrylate) hydrogels. II. Diffusion and release studies. *J. Control. Release, 1997, vol. 48, pp. 47 - 56.*

[8] AMSDEN, B. Solute diffusion in hydrogels: an examination of the retardation effect. *Polym. Gels Network, 1998, vol. 6, pp. 13 - 43.*

[9] TONG, J., ANDERSON, J. L. Partitioning and diffusion in swollen membranes: Part I. A new theory. *J. Membr. Sci.*, 1983, vol. 15, pp. 275-287.

[10] SMILEK, J., SEDLACEK, P., KLUCAKOVA, M. New approach for characterization and study on reactivity of biomaterials. In *NANOCON 2014:* 6th *International Conference on Nanomaterials - Research and Application.* Ostrava: TANGER, 2014, pp. 808-813.

[11] LASTUVKOVA, M., SMILEK, J., KLUCAKOVA, M., SEDLACEK, P. Characterization of hydrogels for diffusion experiments. In *NANOCON 2015: 7th International Conference on Nanomaterials - Research and Application.* Ostrava: TANGER, 2015, pp. 342-347.

[12] HNYLUCHOVA, Z., BJALONCIKOVA, P., KARAS, P., MRAVEC, F., HALASOVA, T., PEKAR, M., KUBALA, L., VITECEK, J. A simple microviscometric approach based on Brownian motion tracking. *Rev Scie Instrum*, 2015, vol. 86, pp. 1 - 5.