CHARACTERIZATION OF HYDROGELS FOR DIFFUSION EXPERIMENTS

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Abstract

Our research is focused on foliar fertilizers and their impacts on plant grow. These experiments are realized in agarose hydrogels (blank agarose hydrogels and agarose hydrogel with addition of potassium lignohumate). Used materials (hydrogels and isolated plant cuticles) were characterized by basic physical chemical instrumentations (rheology, scanning electron microscopy, mercury intrusion porosimetry and stereomicroscopy). Mentioned methods are able to characterize behavior of hydrogels. Rheology enables to determine the mechanical properties of hydrogels. Hydrogel materials must be modified for some trials; otherwise it wouldn't possible to measure it. Lyophilisation was used for modification of hydrogel, because dry hydrogels (xerogels) are more appropriate for aforementioned instruments. Scanning electron microscopy and stereomicroscopy were used to determine the surface of hydrogels. Mercury intrusion porosimetry was used for determination of hydrogels pores for both types. It was considered, from obtained experimental data, that this material doesn't significantly affect the diffusion processes.

Keywords: Hydrogels, agarose, potassium lignohumate, lyophilisation

1. INTRODUCTION

The plant uptake is discussed theme for many years over the world [1], [2]. The numerous of works are focused on the uptake of mineral ions, fungicides, pesticides etc. Root absorptions of nutrients were replaced by foliar fertilizers, which become the new method uptake of plant and it becomes one of the most widespread applications of fertilizers in the world. Absorption by living leaf cells of any foliar applied chemical (mineral nutrients, growth regulators, pesticides, antibiotics) must be processes through transcuticular penetration [3].

Leaves uptake is studied through an isolated top and/or bottom part from plant leaves - cuticles. It is the first limiting barrier, which it has the many important functions (respiration, regulation of water, regulation of ions, penetration of nutrients, etc.). The permeability of cuticle membrane for ions, herbicides, and pesticides has been discussed in several papers [4]-[6]. Foliar uptake of active ingredients is a complex process and it is depending on leaf surface characters of plant, physicochemical properties of the chemicals, types going to change of structure or properties of cuticles [7]. Important role for nutrients absorption plays concentration of the additives as well as environmental conditions. Wang and Liu f7] summarized the major progress of foliar fertilization especially during the last 15 years and they wanted to clarify pesticide uptake into plant foliage and influence of adjuvants.

Two isolation methods - chemically and enzymatically - were used to obtain of plant cuticles. Enzymatically isolation methods (EIM) were demonstrated by Chayen [8] and Hohl [9]. They have used pectin enzymes as macerating agents in anatomical and cytological studies. Further, Orgel work [10] tried to develop a simplified cuticle isolation procedure, which it is based on the usage of commercially available pectin enzymes. Chemically isolation methods (CHIM) were demonstrated by Holloway and Baker [11], where they used zinc chloride-hydrochloric acid solution. Solel and Edgington [12] were inspirited by these isolation methods and they used isolated cuticles for his transcuticular movement of fungicides.

Our research is focused on the study of diffusion of potassium lignohumate through plant cuticles and determination of diffusion coefficients. These experiments can be realized in agarose hydrogels and their using in different transport models - stationary or non-stationary diffusion, diffusion pair, Stokes diaphragm cells etc. Stokes diaphragm cells were used by work Smilek, Sedláček and Klučáková [13]-[16], when they studied of

mobility of ionic dyes with humic acids in hydrogels and role carboxyl groups in humic acids and their binding of charged organic compounds.

Diffusion experiments, which we use for our studies, are focused on agarose hydrogels. For our purpose it is very important, if hydrogels materials have not influence on the rate of diffusion processes. For the characterization of agarose hydrogels we used rheology, scanning electron microscopy (SEM), mercury intrusion porosimetry (MIP) and stereomicroscopy.

2. EXPERIMENTAL

2.1. Materials and methods

2.1.1. Agarose, preparation and lyophilisation

Agar is a nature material, which it is obtained from seaweeds (*Floridae* and *Gelidium*). Structure of agar is formed by polysaccharides agarose and enzymes agaropektinase. Our hydrogels which are used for diffusion experiments are based on this polysaccharide **-** agarose. Agarose is the nature polysaccharide formed by linear repeats units D-galactose and 3,6-anhydro-L-galactopyrane galactose [17]. Behavior of agarose is characterized by high ability of gelation and his molecular weight is about 120 000 Da. Agarose has very good gelation behaviors and it enables to form three dimensions net. Agarose is very good soluble in hot water (approximately 85 °C). During cooling of agarose occurs its solidification (30 **-** 40 °C) to the formation of semi-solid gel material [18]. Our experiments are based on clear 1 wt. % agarose gel and 1 wt. % agarose gel with lignohumate 1 wt. %. For same methods we need dry form of hydrogels (xerogels), which is prepared by lyophilisation by **-**115°C.

2.1.2. Rheology

Rheology characterize mechanical and viscoelastic behaviors of materials For determination of mechanical behaviors of agarose hydrogels, it was used two simple oscillation test (frequency sweep and strain sweep). Geometry for the measurement was used plate-to-plate system with diameter 40 mm (titanium). Tests were realized by Rheometer AR-G2 (TA Instruments), Inc. Frequency sweep test was set following parameters: frequency of oscillation between 20 **-** 0.01 Hz and amplitude of deformation was constant on the value 0.1 %. Strain sweep was set on the parameters: amplitude of deformation was change in range 0.01 % to 200 % and frequency of oscillation was constant on the value 1 Hz. Experiments were realized at 25 °C. Maximal force applied on the sample during compression was 5 N.

2.1.3. Mercury intrusion porosimetry

Mercury intrusion porosimetry (MIP) is the method based on injection of mercury in pores and cavities of material and determination of pores distribution. This method enables to determine size of pores, total pore volume, apparent density or specific surface. Lyophilized agarose hydrogel was measured by Poromaster 60 by pressure between 0.2 **-** 50 psi.

2.1.4. Scanning electron microscopy

Scanning electron microscopy (SEM) is used for observation of object surface. Great advantage is large depth of focus. Lyophilized sample of hydrogels were measured on ZEISS EVO LS 10 and input current has been set on the value 10 kV. Images were made for the enlargement 23 **-** 6 000 multiple.

2.1.5. Stereomicroscopy

Stereomicroscopy enables to observation samples with different enlargement in three dimensions. This microscope works with lower magnification and large working distance. It is enable to perceive depth and contrast of sample structure. This method enables to obtain new observation on the xerogels surface.

3. RESULTS AND DISCUSSION

3.1. Rheology

For comparison of mechanical properties, it was used rheology, 1 wt. % agarose hydrogel and 1 wt. % agarose hydrogel with 1 wt. % lignohumate. From different results would be possible to deduce that the change of mechanical properties of hydrogels influence the transport properties. Mechanical properties were evaluated from complex elastic module G' and loss module G''. Frequency sweep test (**Fig. 1 left**) indicates that 1 wt. % agarose hydrogel with lignohumate has more rigid structure in comparison with clear agarose hydrogel, but the differences aren't significant. Both agarose materials held by them viscoelastic character with predominance of elasticity (G' > G'') in whole measurement frequency range. It is typical for hydrogel materials. Difference in elastic and viscose module for agarose hydrogel without lignohumate is about 3 000 Pa and for agarose hydrogel with lignohumate is 6 000 Pa. Lignohumate has influence on mechanical properties of prepared hydrogels. Structure of hydrogel from mechanical properties is a more rigid and earlier subject to degradation. This fact can influence value of measured diffusion coefficients, because we can wait less of diffusivity soluble compounds in hydrogel.

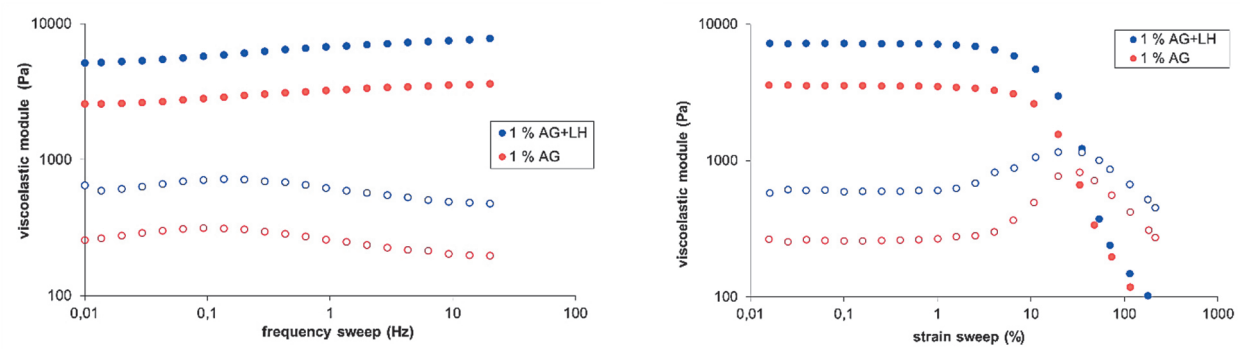

Fig. 1 Frequency sweep test (left) and strain sweep test (right)

Oscillation test (**Fig. 1 right**), at lower strain deformation of internal net of agarose hydrogel able to resist applied stress - modules are independent on strain deformation. For 1 wt. agarose hydrogel without lignohumate is linear viscoelastic area in range 0.01 - 4 % strain deformation and for 1 wt. % agarose hydrogel with 1 wt. % lignohumate is linear viscoelastic area in range 0.01 - 1 %. We can say that force of binding is stronger for clear agarose hydrogel than for agarose hydrogel with lignohumate.

3.2. Mercury intrusion porosimetry

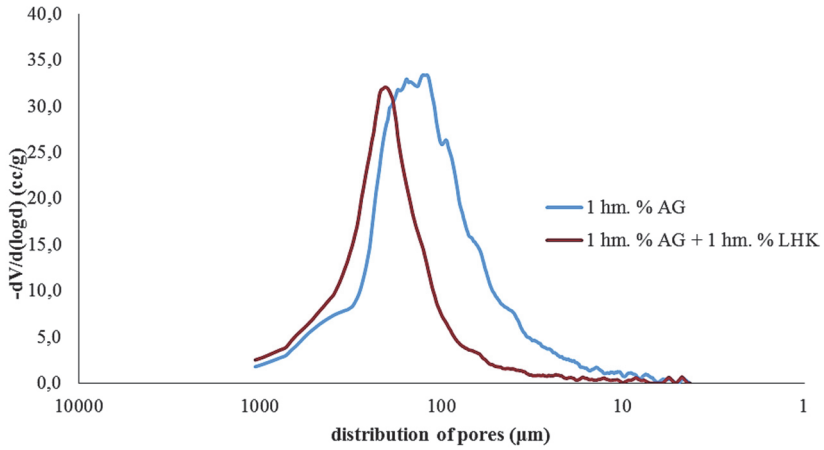

Fig. 2 Distribution of size pores of agarose hydrogel without lignohumate and with lignohumate

This method enables to determine of distribution of pores size of 1 wt. % agarose hydrogels without lignohumate and with 1 wt. % lignohumate. Values of size of pores in diffusion materials play important role in transport processes. Different of pore sizes would result different rate of active material transport in hydrogels. On the graph (**Fig. 2**) is illustrated of running of distribution curve **-** blue curve shows running of pores for clear agarose hydrogels and brown curve of pores distribution shows us running for agarose hydrogels with lignohumate. A shift is insignificant from the scale. From measurement we can claim, that size of pores is same for both types of hydrogels, also diffusion of lignohumate isn't influence steric effect of net hydrogel.

3.3. Scanning electron microscopy

Hydrogel materials were characterized with scanning electron microscopy (SEM) and this method enable to obtained differences in morphology in both types of hydrogels. This method should illustrated internal structure of agarose materials without respectively with lignohumate.

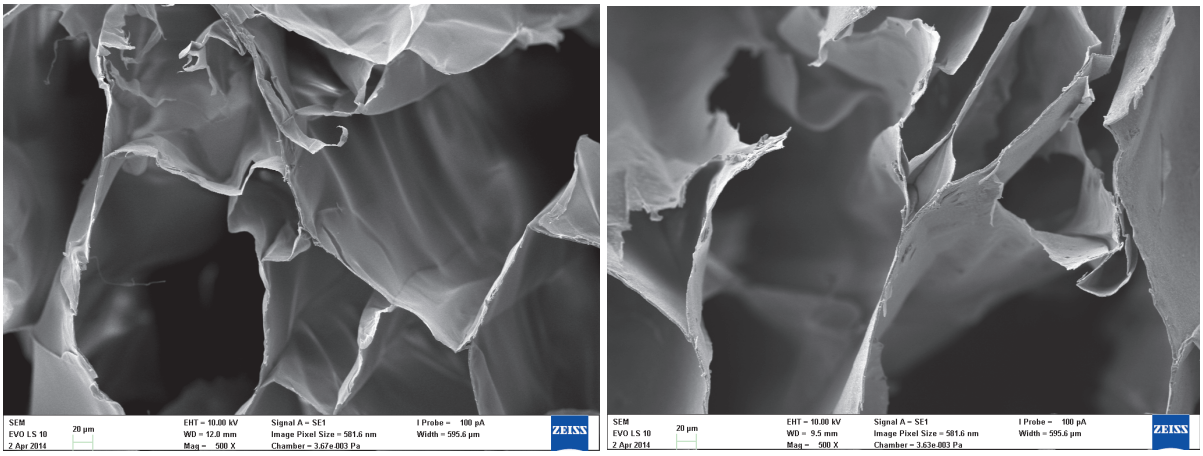

Fig. 3 Images for 1 wt. % agarose hydrogels (left) and for 1 wt. % agarose hydrogel with 1 wt. % lignohumate (right). A picture taken with 500-fold magnification and measure 20 μm

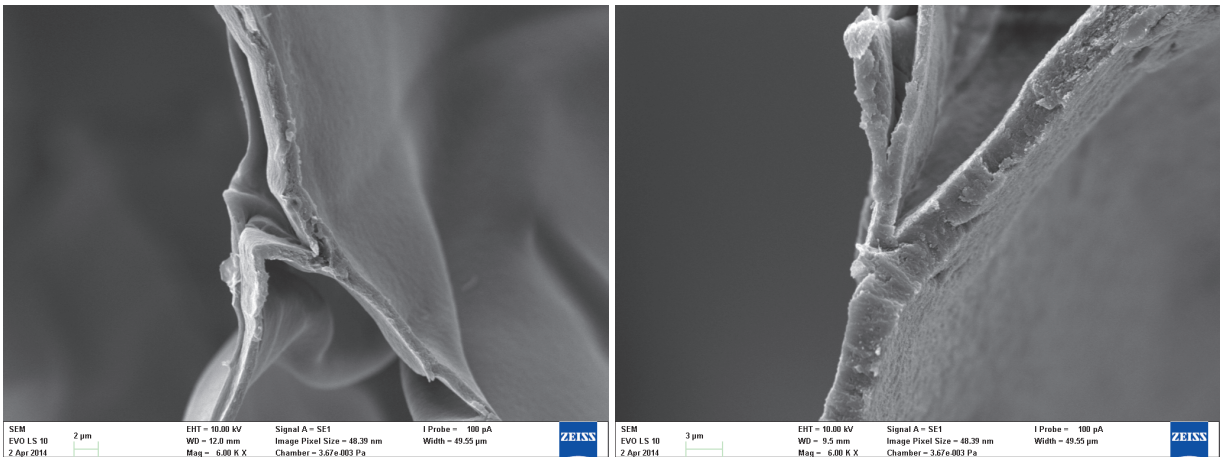

Fig. 4 Cross section for clear agarose hydrogel (left) and agarose hydrogel with lignohumate (right). A picture taken with 6 000-fold magnification and measure 2 μm (left) and 3 μm (right)

From the pictures (**Fig. 3**) is evidently any difference in internal structures, but their difference in cross section is characterized by **Fig. 4**, where it is seen rough structure for agarose hydrogel with lignohumate and smooth surface for agarose hydrogel without lignohumate. On the cross section of hydrogels we can see, that structure of agarose hydrogel with lignohumate is fragile than clear agarose hydrogel. This method demostrated very important information about differences of material, but in terms diffusion processes play any role.

3.4. Stereomicroscopy

Lyophilized and cuted agarose samples were studied on steremicroscopy, when was obtained pictures of 1 wt.% agarose hydrogel and 1 wt. % agarose hydrogel with 1 wt. lignohumate. Results of measurement were three dimension pictures ilustrated of morphology of xerogel surfaces. Hydrogels have differences in their surface, it is possible see in **Fig. 5**. Hydrogels prepared from 1 wt. % agarose have smoother surface and no more ragged than hydrogel with 1 wt. % lignohumate.

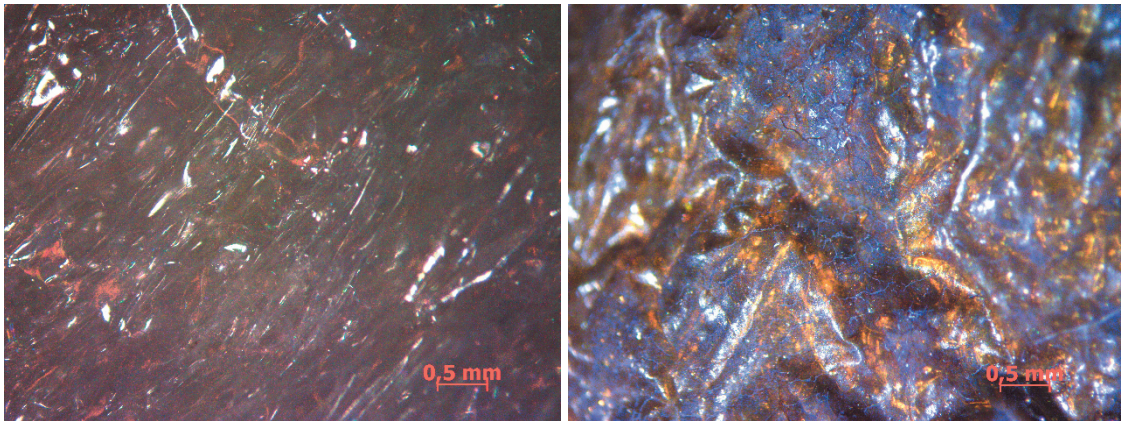

Fig. 5 Three dimensions images of hydrogels, 1 wt. % agarose hydrogel (left) and 1 wt. % agarose hydrogel with 1 wt. % lignohumate (right)

4. CONCLUSION

Diffusion experiments are based on transport medium on the base agarose hydrogels, when we studied their properties and differences. For the characterization we used four methods and thought them we obtained important and interesting knowledge. Method of rheology revealed the mechanical properties of hydrogels and dates point out rigid structure for agarose hydrogels with lignohumate. These data point out influence of diffusion soluble matters. Other measurements showed, than difference in structure isn't so great to influence of diffusion processes. From mercury intrusion porosimetry was obtained, than pore size is very similar and structure of surface is almost same. Greater differences were on surface, it was showed on scanning electron microscopy and stereomicroscopy. From SEM was found, that agarose hydrogel with lignohumate have rough surface than clear hydrogel and his surface is quite ragged. These properties haven't any influence on diffusion properties.

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