Cryo-SEM imaging of reactive and non-reactive agarose hydrogels

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Gels are ubiquitous materials with broad applications in both scientific [1-3] and industrial [3] field. They can be described as dispersion systems, containing a solid dispersion phase interconnected into three-dimensional network. Inside this network, a liquid dispersion medium is entrapped. From mechanical point of view, gels are often described as materials with properties overlapping between solids and liquids. This means that at some specific conditions, gels can have properties of liquids (viscosity). Contrarily, under different conditions, they can act as solids (elastic response). These specific properties, together with their simple preparation procedure, biocompatibility, 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, make hydrogels a highly attractive material. Gels can be divided according to the type of used dispersion medium to hydrogels (where the liquid medium is water) and oleogels (the medium is oil).

In recent years, there has been an increasing interest in various hydrogels, a material considered to have a significant potential with regard to the range of its possible applications. Hydrogels (or generally gels) can be assumed as highly porous structure, which can be easily tuned (the density of cross-linking, the degree of swelling etc.) according to the needs of the application. This contribution is focused on structure investigation of hydrogels with modulated chemical reactivity with the use of cryo-SEM imaging techniques. The hydrogels used in this research are based on biopolymer agarose. Generally, agarose is an example of thermoreversible polysaccharide with the ability of forming, under certain conditions, a non-reactive hydrogel matrix, in which additional specific substances (e.g. polyelectrolytes) can be incorporated in order to change its final properties from non reactive to reactive. The cryo-SEM method was selected for being particularly useful in studying hydrated samples, since their native structure remains preserved thanks to its frozen state.

Two methods of sample preparation for cryo-SEM imaging were performed on the agarose hydrogels - freezing by plunging into slushy nitrogen and liquid ethane, and by far more effective high-pressure freezing method (HPF). Subsequently, the frozen samples were processed in the cryo-vacuum chamber (ACE600, Leica Microsystems) and transferred into the cryo-SEM (Magellan 400L, FEI) via the cryo shuttle system (VCT100, Leica Microsystems) and imaged at the temperature of -120 °C without any coating. The sublimation process was performed in several steps, represented by sequential decreasing of the temperature inside the microscope. Having undergone the sublimation process, the hydrogel samples started to reveal their structure, as can be seen in Figure 1.

References:

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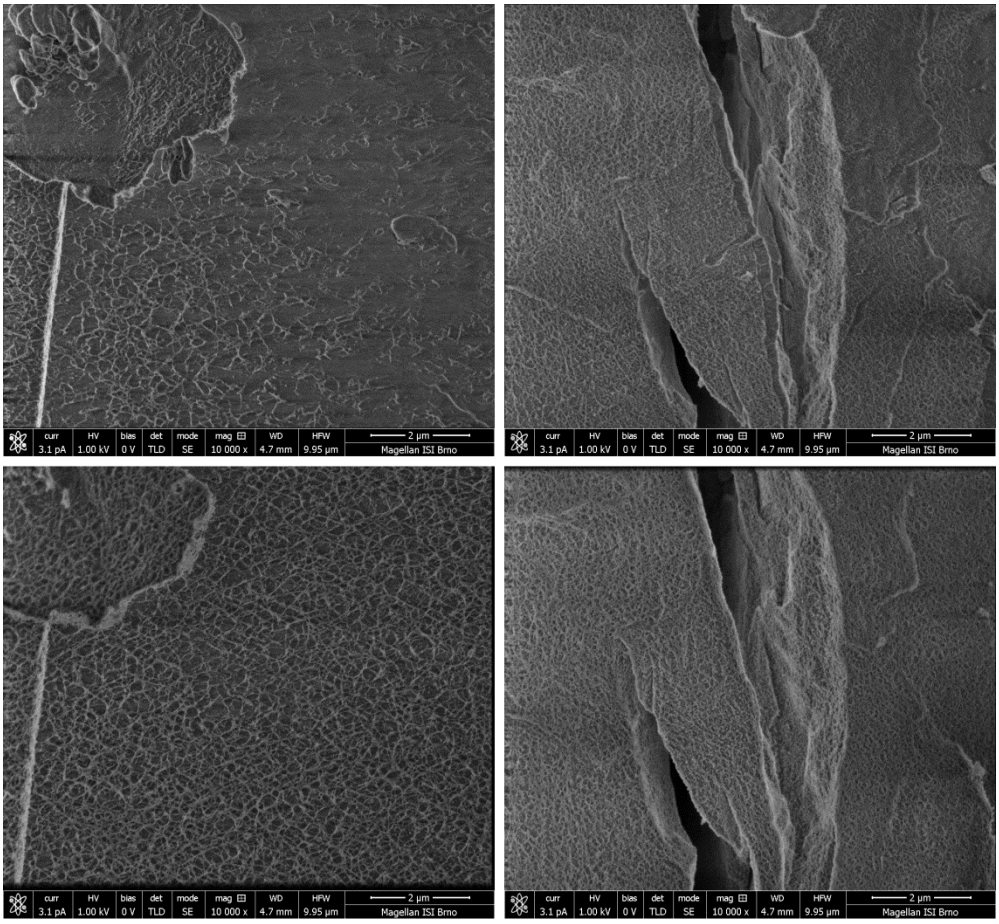

Figure 1: Cryo-SEM images of HPF frozen agarose hydrogels (2wt.% on the left and 4wt.% concentration on the right, without addition of polyelectrolyte component) showing the mesh-like structure of the physically cross-linked polysaccharide chains. Sublimation times at the temperature of -100 °C: just rising to the temperature (top) and 4 minutes (bottom).