Influence of trehalose additives on the properties of poly(vinyl alcohol) cryogels formed in aqueous as well as in organic media

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Influence of trehalose additives on the properties of poly(vinyl alcohol) cryogels formed in aqueous as well as in organic media

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Abstract. Physical (non-covalent) macroporous cryogels of poly(vinyl alcohol) (PVACGs) are formed as a result of the consecutive freezing of concentrated PVA solutions, their incubation in a frozen state and then thawing. In this study the influence of the additives of trehalose and the solvent used on the PVA cryotropic gel-formation, as well as on the properties of the respective PVACGs was explored. It was shown that both the rigidity and the heat endurance of cryogels formed in the DMSO medium in the presence of trehalose progressively increased with increasing the additive concentration. It turns, in the case of cryogels prepared in the aqueous medium in the presence of trehalose the dependences of the physicomechanical characteristics on the additive concentration were of a bell-like (concave) type, while the heat endurance of the samples grew monotonically.

1. Introduction.

Polymeric cryogels are macroporous gel materials that are formed as a result of the consecutive freezing of molecular or colloidal solutions of certain precursors, subsequent incubation of the system in a frozen state, and then thawing [1–4]. The macroporosity of cryogels is generated by the polycrystals of the frozen solvent, while the gel formation itself proceeds within the volume of the remaining unfrozen inclusions (so-called unfrozen liquid microphase) [5] where the precursors are concentrated [1,4]. One of the most attractive cryogels are those based on poly (vinyl alcohol) (PVA), since these cryogenically-structured gel materials are of great applied interest in many fields, especially in biomedicine and biotechnology [6]. PVA cryogels possess by various favorable qualities, including excellent physico-mechanical and thermal characteristics, interconnected macroporosity, biocompatibility and non-toxicity PVACGs were successfully examined as artificial cartilages [7], wound and burn dressings, cardiovascular devices and drug vehicles [8].The physicochemical properties and porous structure of PVA cryogels depend on many factors, in particular, on the characteristics of the polyvinyl alcohol itself (the molecular weight, the amount of residual O-acyl groups, the chain tacticity), the PVA concentration in its solution prior to the freeze–thaw gelation, the solvent used, the conditions of cryogenic processing, namely, the rate of cooling upon the freezing of the polymer solutions, the temperature and duration of the freezing and frozen storage, the rate of heating upon defrosting the samples, and the number of freeze–thaw cycles [1]. And, certainly, the presence of low-weight additives in the initial solution of PVA influences on the properties of the resultant cryogels. Among such additives the kosmotropes are of especial

significance because of their ability to promote hydrogen bonding [9] thus affecting positively on the efficience of PVA cryotropic gel-formation, and therefore natural approved for biomedical application kosmotropes can potentially be used upon the elaboration of various PVA-cryogel-based biomedical materials. Trehalose is one of such natural kosmotropes; it is a disaccharide formed by a 1,1-glycosidic bond between two α-glucose units (Figure 1). Trehalose was named after its source, trehalamanna, a sugar solution obtained from the brood and / or cocoon of some insects (Larinus species). Trehalose has found application in cosmetology as the face skin care agent [10]

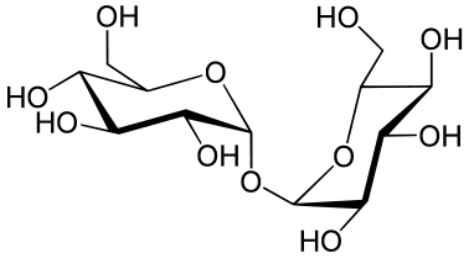

Figure 1. Structure of trehalose.

Most often, aqueous media are used as a solvent in the preparation of the initial PVA solutions for further cryogenic processing. However, there are few studies where PVA cryogels were formed from polymer solutions in organic solvents, in particular, in dimethyl sulfoxide (DMSO). Since DMSO is a thermodynamically better solvent than water for high-deacylated PVA samples, the PVA gelation in DMSO occurs poorly. Therefore, the PVA/DMSO cryogels are always weaker and have lower fusion temperatures than the PVACGs prepared from aqueous solutions of the same polymer concentration [11].

In this regard, it was reasonable to improve the mechanical properties and heat endurance of PVA cryogels formed in the DMSO medium using the trehalose additives, as well as to compare their influence on the PVA cryotropic gel-formation in both aqueous and DMSO milieus.

2. Materials and Methods

2.1. Materials

The following compounds were used: poly(vinyl alcohol) (molecular weight of ca. 86 kDa, the deacetylation degree of 100%; Acros Organics, USA), dimethyl sulfoxide (>99.8%; Komponent-Reaktiv, Russian Federation), trehalose (>98%; Panreac, Spain).

2.2. Preparation of Feed PVA Solutions

The dissolution of PVA in DMSO or in water was performed as described elsewhere [12]. Briefly, a known amount of dry polymer was dispersed in a calculated volume of DMSO to reach a PVA concentration of 100 g/L. The mixture was incubated for 18 h at room temperature for swelling of the polymer, and then, the system was heated for 1 h on a boiling water bath under stirring until the completion of PVA dissolution. When preparing the feed solutions that should contain trehalose the required amounts of additive were dissolved in the polymer solution at room temperature. The final solutions were sonicated for 20 min at room temperature in an UNITRA ultrasonic bath (Unitra, Poland) to remove air bubbles.

2.3. Preparation of PVA Cryogels

The samples of PVA cryogels were prepared analogously to the earlier described techniques [12]. In these experiments, samples for physicomechanical tests were formed in sectional duralumin molds (inner diameter 15 mm, height 10 mm). To determine T_f values, cryogels were formed in transparent polyethylene test tubes (inner diameter 10 mm), 3-mL portions of the polymer solution were poured,

and a stainless steel ball (diameter 3.5 mm, weight 0.275 ± 0.005 g) was placed on the bottom of each tube. The containers and the tubes were put into the chamber of an FP 45 HP precision programmable cryostat (Julabo, Germany), and the samples were frozen and incubated at a preset temperature for 12 h. Then, the temperature was raised to 20°C at a rate of 0.03 °C/min controlled by the cryostat microprocessor.

2.4. Characterization of PVA cryogels samples

The compression Young's modulus (E) and the fusion temperature (T_f) of PVACG samples were evaluated in accordance with the protocols described elsewhere [11,12]. The E and T_f values were measured for three samples; the samples were examined in three to five independent experiments. The results obtained were averaged.

In brief, the E modulus of the PVACG samples was determined from the linear portion of the stress–strain dependence that was found using a TA-Plus automatic texture analyzer (Lloyd Instruments, UK) at a loading rate of 0.2 mm/min. The tests were performed until reaching a 30% deformation. The measurements were accomplished either for PVA cryogels prepared in water medium, either for the PVACGs prepared in DMSO and also for the samples in which the organic liquid phase (that is, DMSO and the dissolved solutes) was replaced by pure water. In the latter case, the cryogel samples were incubated for seven days at room temperature in glass beakers that each contained deionized water (100-fold excess relative to the volume of the PVACG sample); the water was replaced with a fresh portion every day. The fusion temperatures of the PVACG samples were measured by placing the tightly corked polyethylene tube containing cryogel with the stainless steel ball at the bottom upside down into the water bath. The bath temperature was increased at a rate of 0.4 °C/min. The gel fusion point was determined as the temperature when the ball fell down onto the stopper of the test tube after passing through the fused gel.

3. Discussion.

3.1. Physicomechanical properties of PVACGs prepared in aqueous medium with the addition of trehalose

Trehalose is a typical kosmotropic agent which promotes hydrogen bonding. Such effect on the physicochemical characteristics of PVA cryogels is similar to the influence of other kosmotropic agents, for example, sulfate or phosphate anions. So, we could expect that the higher concentration of trahalose is the stronger cryogels will be formed. But it was observed experimentally that with increasing in the trehalose concentration the rigidity of PVA cryogels initially lowered (Figure 2a), passed through a some minimum, and only then commenced to grow. When the concentration of trehalose in initial polymer solution reached 1 mol/L, the value of the Young's modulus for the resultant cryogel was of 19.5±0.5 kPa. At the same time, the fusion temperature of cryogels monotonically heightened with an increase in the trehalose concentration, and the value of fusion temperature raised by 7 °C for a sample with a trehalose concentration of 1 mol/L as compared with a sample formed without additives.

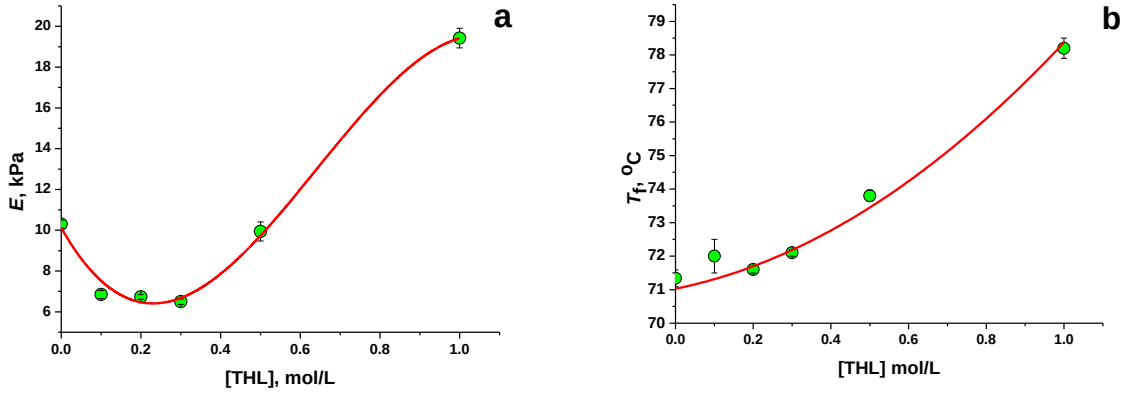

Figure 2. The dependence of the Young's modulus (a) and the fusion temperature (b) of PVA cryogels formed in aqueous medium on the trehalose concentration in the samples.

3.2. Physicomechanical properties of PVACGs prepared in DMSO medium with the addition of trehalose

It should be noted that the physicomechanical as well as the thermophysical properties of PVA cryogels formed from DMSO polymer solutions are very different from the properties of PVA cryogels obtained in aqueous medium under similar cryogenic processing conditions. Thus, PVA cryogels formed from the initial solutions of PVA in DMSO turned out to be less rigid and less heat resistant than the samples formed from aqueous solutions of the polymer [13]. Since DMSO is a thermodynamically better solvent for polyvinyl alcohol than water, that is, the affinity of the polymer for DMSO is higher, this reduces the efficiency of gelation due to the competition between interactions of the polymer-solvent and the polymer-polymer.

Since the crystallization temperature of DMSO is T_0 = 18.4 °C, describing the cryogenic treatment mode of such systems, it is more convenient to use not the absolute value of the temperature, but the difference value $\Delta T = T_i - T_0$, where T_0 and T_i are, respectively, the crystallization temperature of the pure solvent and the temperature of a specific experiment (expressed in the Celsius degrees). In our case, the cryogenic treatment temperature was -21.6ºC, thus ΔT = -40º. PVA cryogels were formed under the following conditions: a concentration of PVA of 10 g/dL, a freezing temperature of -21.6ºC, a freezing time was 12 h, a heating rate upon the system thawing was 0.03ºC/min.

As it was noted earlier, PVA cryogels formed in DMSO medium have lower elastic modulus values E compared to PVA cryogels equiconcentrated with respect to the polymer, but formed from aqueous solutions of PVA. Thus, the value of the Young's modulus of a sample formed without additives was 3.35 ± 0.3 kPa (3 times less as compared to the PVA cryogel formed in the aqueous medium). However, an increase in the trehalose concentration in the samples led to a significant growth in the values of elastic modulus and reached 75.5 ± 3 kPa (at trehalose concentration of 1 mol/L).

A similar pattern was observed in the case of the fusion temperature of the samples. Thus, the fusion temperature increased from 42.5±0.5°C (sample without trehalose) to 83.0±0.2°C (PVA cryogels with the concentration of trehalose 1 mol/L).

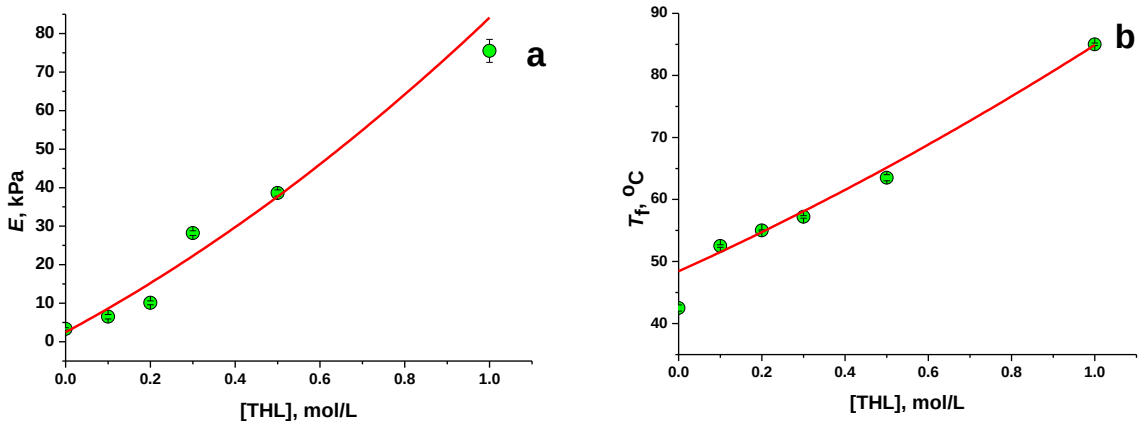

Figure 3. The dependence of the Young's modulus (a) and the fusion temperature (b) of PVA cryogels formed in DMSO medium on the trehalose concentration in the samples.

The next step of our study was to evaluate the changes in the physicomechanical and thermophysical properties of PVA cryogels after replacing an organic solvent for water. After rinsing with water of the cryogels formed in DMSO medium, the values of elastic moduli of cryogels and their fusion temperature were measured again.

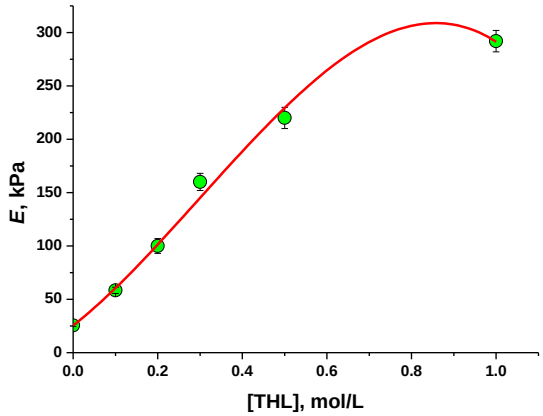

Figure 4. The dependence of the Young's modulus of PVA cryogels formed in DMSO medium and rinsed with water on the trehalose concentration in the samples.

The plots in the Figs. 4 and 5 show that the value of elastic modulus increased up to 292±5kPa, and the fusion temperature to 85±0.5°C. So, the change of a more thermodynamically good solvent (for PVA) DMSO with a less good one — water — resulted in the replacing of a part of the polymer-solvent interactions with polymer-polymer interactions, which, in turn, gave rise to a significant increase of the rigidity and fusion temperature of the cryogel samples.

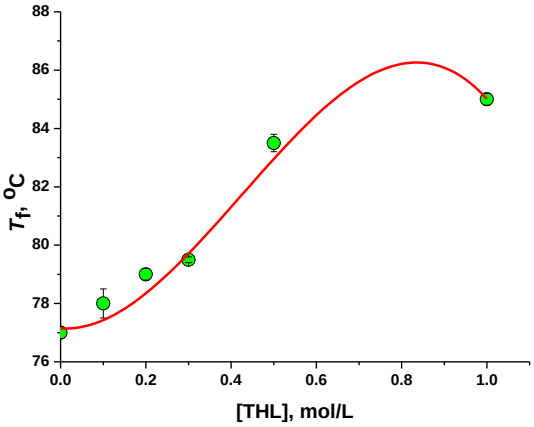

Figure 5. The dependence of fusion temperature of PVA cryogels formed in DMSO medium and rinsed with water on the trehalose concentration in the samples.

4. Conclusions

Taking in account a wide area of application of PVA cryogels [14] and, on the other hand, a rather simple technology of their preparation [15,16], it is reasonable to know well different approaches for varying and reliable control of the properties of these gel materials. So, the first, the introduction of the available low-molecular-weight additives (in our case trehalose) to the initial PVA solutions influence on the physicochemical characteristics of the PVA cryogels. And the second, the variation of the initial solvent for poly (vinyl alcohol) leads to the formation of cryogels with higher rigidity and heat resistance.

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