Comparative analysis of the influence of urea additives on the properties of poly(vinyl alcohol) cryogels formed from the polymer solutions in water or dimethylsulfoxide media

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Abstract. Various poly(vinyl alcohol)-based gels are known for a long time and they are related to the gel materials of significant scientific and applied interests. Among different poly(vinyl alcohol) (PVA) hydrogels those formed via the so-called cryotropic gelation approach, i.e. PVA cryogels, attract special attention owing to, firstly, simplicity of their preparation and, secondly, very good properties of such matrices. The aim of this work was to compare the physicomechanical properties of PVA cryogels formed from the urea-containing aqueous and dimethylsulfoxide solutions of the polymer. It was shown that not only the solution’s nature influences greatly on the properties of the resultant cryogels, but also the low-molecule additives in the initial polymer solutions lead to the opposite effect in cryogels characteristics. The results of our investigation can expend the area of PVA cryogels usage in many fields.

1. Introduction.
Poly(vinyl alcohol) (PVA) cryogels are the macroporous heterophase gels formed as a result of freezing of concentrated solutions of this polymer, their further incubation in a frozen state and subsequent thawing [1]. In this case, a prerequisite for the formation of such heterogeneous gels is the crystallization (freezing) of the bulk of the low molecular weight liquid (solvent). The physicochemical properties and porous structure of PVA cryogels depend on many factors, such as the characteristics of poly(vinyl alcohol) itself (its molecular weight, chain tacticity, the amount of unsaponificated O-acyl groups), polymer concentration, cryogenic treatment regimes, the number of freeze-thaw cycles [2]. Most often, aqueous media are used as initial solvents in the preparation of initial PVA solutions subjected to further cryogenic treatment. However, the literature describes PVA cryogels formed from polymer solutions in organic solvents, in particular in dimethylsulfoxide (DMSO) [3].

The physicomechanical as well as thermophysical properties of PVA cryogels formed from the DMSO solutions differ noticeably from the properties of PVA cryogels obtained in aqueous media. In particular, PVA cryogels formed in DMSO medium have lower rigidity and heat endurance than the samples formed from aqueous polymer solutions [4]. Since DMSO is a thermodynamically better solvent for poly(vinyl alcohol) than water, that is, the affinity of the polymer for DMSO is higher, this reduces the gelation efficiency due to competition between polymer-solvent and polymer-polymer interactions.

In addition, it is known that the introduction of low molecular weight substances into the initial PVA solution has an effect on cryotropic gelation and the properties of the resulting cryogels. For example, the effect of chaotropic and cosmotropic additives on the thermophysical and rheological properties of PVA cryogels formed aqueous media has been studied [5, 6], but only a few cases are known about the effect of low molecular weight additives on samples formed from the DMSO solutions of PVA [7]. Thus, the aim of this work was a comparative analysis of the effect of solvents
and urea additives (Figure 1) on the properties of PVA cryogels formed from a polymer solution in water and DMSO containing urea in various concentrations.

![Chemical structures](image)

**Figure 1.** Chemical structures of: 1- urea, 2- dimethylsulfoxide, 3- poly(vinyl alcohol), 4- water.

### 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), urea (ultra pure grade; Sigma, USA), the Congo red dye (Aldrich, USA), as well as gelatine (photo quality), phenol (pure for analysis) and glycerol (pure for analysis) (all from Reakhim Co., Russian Federation).

#### 2.2 Preparation of Feed PVA Solutions

The dissolution of PVA in DMSO or in water was performed as described elsewhere [8]. 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 additive 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 [8]. 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 [8]. 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.

2.5 Macroporous morphology of PVACG samples

The macroporous morphology of PVA cryogels was examined using an Eclipse 55i (Nikon, Japan) optical microscope equipped with digital photocamera as it was described in the previous study [9]. DMSO in the respective PVACGs has been replaced for pure water prior to the thin sections preparation. Such sections of the 10 µm thickness were cut in the direction orthogonal to the axis of cylindrical samples using a cryomicrotome SM-1900 (Leica, Germany). Each section was placed on the microscope glass, which was then immersed into 1% aqueous solution of Congo red for staining for 10 seconds and then rinsed with pure water. The excess of liquid was removed with filter paper. Then the section was poured with one drop of “fixing medium” (solution of 1 g of gelatin in 12 mL of 50% aqueous glycerol and 0.2 g of phenol as a bacteriostatic agent) and sealed with a cover glass. Prior to studies, the samples were stored at 4°C in a closed vessel.

3. Discussion

3.1 Features of the influence of urea on the properties of PVA cryogels in an aqueous medium.

Urea is a classical chaotropic agent; in an aqueous medium it inhibits intermolecular hydrogen bonding of the OH groups of the polymer, which leads to a decrease in the stiffness and heat endurance of the resulting cryogels. With an increase in the urea concentration in the initial polymer solution, the elastic moduli (E) and the melting temperature (T_m) of the resulting PVA cryogels decrease.
Figure 2. Dependences of the elastic modulus $E$ and the fusion temperature $T_f$ of PVA cryogels formed in the aqueous medium at -20°C on the urea concentration in the initial polymer solution.

3.2 Features of the effect of urea on the properties of PVA cryogels in dimethylsulfoxide.
Since the crystallization temperature of DMSO is $T_0 = 18.4^\circ$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 -21.6°C, thus $\Delta T = -40^\circ$. PVA cryogels were formed under the following conditions: a concentration of PVA of 10 g/dL, a freezing temperature of -21.6°C, a freezing time was 12 h, a heating rate upon the system thawing was 0.03°C/min.

When studying the effect of urea on the properties of PVA cryogels formed in its presence, we conducted a series of experiments varying the concentration of a low molecular weight agent; moreover, it should be noted that the range of working concentrations of urea in dimethylsulfoxide was significantly higher than in an aqueous medium, and ranged from 1 to 4 mol/L.
Figure 3. Dependences of the elastic modulus $E$ and the fusion temperature $T_f$ of PVA cryogels formed in DMSO at -21.6°C on the urea concentration in the initial polymer solution.

As follows from the diagrams in Figure 3, in the case of PVA cryogels formed from DMSO polymer solutions, with an increase in the urea concentration in the samples, in contrast to the effects in the aqueous medium (Fig. 2), the values of both the fusion temperature and the elastic modulus...
increase. So, when the urea content in the sample was 4 mol/L, the melting temperature increased by 20°C compared with the PVA cryogel formed without additives, and the elastic modulus $E$ increased by 26 times. That is, for PVA cryogels formed from DMSO polymer solutions, we discovered the anomalous nature of the effect of urea additives when it acted as a cosmotropic agent.

In order to study the effect of solvents on the properties of PVA cryogels, the organic solvent from the samples was replaced with water. For this, the samples were placed in a large volume of water for a week; water was replaced with fresh water daily. Next, the elastic modulus and the melting temperature of PVA cryogels were measured. So, it is worth noting that the values of the elastic moduli and the fusion temperature of the samples increased to 210±5 kPa and 83±0.5 °C, respectively. Thus, the replacement of the thermodynamically better solvent of dimethyl sulfoxide (for poly(vinyl alcohol)) with the worst - water - leads to a partial replacement of polymer-solvent interactions with polymer-polymer interactions, which, in turn, affects the strength and thermophysical characteristics of PVA cryogels.
3.3 Peculiarities of the morphology of PVA cryogels formed in aqueous and organic media.

Figures 5 and 6 show microphotographs of thin sections of PVA cryogels equiconcentrated with respect to the polymer and formed in aqueous and organic media both without urea and in its presence. The urea concentration was 0.1 or 0.3 mol/L for aqueous solutions of poly(vinyl alcohol), or 1 and 3 mol/L for a solution of PVA in dimethyl sulfoxide. Microphotographs of thin sections perpendicular to the heat transfer gradient during freezing of the samples show a pattern of alternation of dark (gel phase stained with Congo red) and light (macropores) structural elements of various shapes and sizes.

Figure 5. Microphotographs of PVA cryogels formed in an aqueous medium.
4. Conclusion

Thus, the strong influence of dimethylsulfoxide in the presence of urea on the properties of the obtained PVA cryogels can be explained by hydrogen bonding between urea and dimethylsulfoxide molecules, which makes the latter a thermodynamically poorer solvent for poly(vinyl alcohol).

A study of the influence of the nature of the solvent on the physicochemical characteristics of PVA cryogels formed from polymer solutions in DMSO with urea additives of various concentrations showed that heterophase cryogels with higher rigidity and heat endurance than cryogels of DMSO – water composition are formed. Since various PVA cryogels are currently being widely used, primarily as materials of biotechnological and biomedical applications, the discovered features of the properties of PVA cryogels formed in an organic medium can serve as the basis for the development of such gel materials, but with new characteristics useful for practice.

5. Acknowledgments

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6. References

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