Study of Cryostructuration of Polymer Systems. XVII. Poly(vinyl alcohol) Cryogels: Dynamics of the Cryotropic Gel Formation

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ABSTRACT: Freeze-thaw treatment of concentrated (>5 g/dL) aqueous solutions of poly(vinyl alcohol) (PVA) (MW 115,000; DD ≈100%) resulted in the formation of opaque gels. The extent of such a cryostructuration process was exhibited in the rheological properties of similar PVA cryogels. The gels' strength depended on the initial polymer concentration in the solution to be frozen and on the conditions of a cryogenic influence. The key factor was the defrostation dynamics: the slower the thawing rate, the stronger the cryogel sample formed, provided other parameters of the process were identical. The observation for the kinetics of the freeze-thaw–induced gel formation revealed the extreme character of the temperature dependence of the efficacy of PVA cryotropic gelation, the maximum point being in the vicinity of −2°C. It was shown that the effect of the strengthening of PVA cryogels prepared by means of a single-cycle cryogenic treatment could be reached either with use of as slow as possible thawing regimes, or by the prolonged frozen storage of the samples at "high" subzero temperatures. © 2000 John Wiley & Sons, Inc. J Appl Polym Sci 77: 2017–2023, 2000

Key words: poly(vinyl alcohol) (PVA); freeze-thaw–induced gelation; dynamics of the cryotropic gel formation

INTRODUCTION

Poly(vinyl alcohol) cryogels—cryoPVAGs—are the most well-known cryogenically produced gels, which are formed upon the freeze-thaw influence on the initial solutions of gelling agents (for the review on PVA cryotropic gelation see references).[1–3] The resultant PVA-based gel materials are, for the recent years, attracting considerable interest in biotechnology (carriers of immobilized enzymes, antibodies, whole cells),[1,3–6] in medicine (protective covers on wounds and burns, drug delivery systems, artificial cartilage tissue, materials for ophthalmology, etc.),[1,2,7] in material science (gel basis of chemomechanical actuators)[8] and in other applied areas. The procedure for the preparation of cryoPVAGs is rather simple: the aqueous (or DMSO) polymer solution (its concentration is determined by the PVA molecular weight and amount of residual O-acyl groups) without or with the addition of other solutes or fillers is frozen at moderate negative temperatures (routinely, the range from −10 to −40°C is used) and, after storing frozen for several hours, thawed off. CryoPVAGs are the thermoreversible gels, heating up to 70–90°C can melt them, and repeated freeze-thaw treatment gives rise to the cryogels again. The physical properties and structure of cryoPVAGs depend, along with the characteristics of the polymer used, on the cryogenic

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treatment conditions (freezing rate and temperature, duration of the storage frozen, thawing rate, the number of freezing-thawing cycles), as well as on the kind and concentration of additives if they are present. All these points were thoroughly investigated and described in numerous publications, and the current status of the knowledge in this field was recently reviewed.[3] However, one important problem was not so far studied in detail. This is the problem of kinetic features of PVA cryotropic gelation.

Thus, it was earlier shown[9–11] that increase in the frozen storage duration gave rise to the increase in the gels' strength and their fusion temperatures provided, as it was recognized somewhat later,[12] that the samples were thawed out under identical conditions. In fact, thawing regimes have been demonstrated in this latter work to be the key parameters controlling the properties of cryoPVAGs: the faster the defrostation process, the weaker and less thermoresistant gel species formed, and, if the thawing rate exceeded approximately 10°C/min, no PVA cryogels were obtained at all. Hence, these data testified that the gel formation itself occurred virtually in the course of the thawing stage. In addition, in our previous study of the cryostructuration of dilute PVA aqueous solutions (their freeze-thaw treatment resulted in the fabrication of microgel matter—PVA cryoprecipitates), the importance of the duration of residence of the still-frozen samples at "high" subzero temperatures for the efficiency (yield) of similar cryoprecipitation, thus pointing to the significance of the temperature-dependent kinetic experiments for the understanding of fine mechanisms of cryotropic gelation phenomena, in general, has been revealed.[13] That is why the goal of this work was to research the dynamics of the formation of cryoPVAGs and to find the temperature range, where such a cryostructuration proceeded with the highest efficacy.

EXPERIMENTAL

Materials

Poly(vinyl alcohol) (Aldrich Chemical Co., Inc., Milwaukee, WI), 100% hydrolyzed, average MW 115,000 (the manufacturer data) was used without an additional purification. This PVA batch had the viscosity-average molecular weight of 138,000 ± 2,100 as it was determined by capillary viscometry with a Ubbelohde-type viscometer (25°C, water, $[\eta] = 5.95 \times 10^{-4} M_{\eta}^{0.63}$).[14] All the solutions were prepared with deionized water.

Methods

Initial solutions of the polymer (PVA concentration in the range of 5–10 g/dL) were prepared by the suspending of the weighed amount of dry polymer powder in the required volume of deionized water, storing these mixtures for a night at room temperature to swell the polymer followed by its dissolution by heating on a boiling water bath for 30 min with stirring of the sample placed in the glass beaker. The loss of the liquid due to its evaporation was determined by weighing the samples and then was compensated by the addition of the required amount of pure water. Similar cycles (heating—weighing—addition of water) were repeated twice again, so that total heating time was 90 min. The polymer solutions thus obtained were, after cooling to a room temperature, poured into the duralumin cylindrical moulds with 15-mm inner diameter and 10-mm height. The moulds were tightly closed and placed into the chamber of a precision cryostat FP 45 HP (Julabo, Germany), where the preassigned negative temperature (−20°C) was already maintained. After the incubation of the samples under these conditions for the desired time, the defrostation with the thawing rates of 0.03 or 0.3°C/min was carried out using the microprocessor-controlled facilities of the cryostat. A faster defrostation of the samples was conducted by the immersion of frozen specimens into the thermostat at 40, 50 or 60°C, where they had been kept for 10, 8, or 6 min, respectively,. In these cases, as it was determined in the preliminary tests, thawing rates were of the order 3, 4, and 5°C/min, correspondingly. The temperature of the samples did not exceed 20°C at the end of the above-indicated time periods (this was measured with a microthermocouple frozen in specimens). The thawed-out samples usually presented as opaque gels (cryoPVAGs), in which rheological properties were evaluated by the penetration method[15] essentially in accordance with the procedure used in our previous research for the investigation of various PVA cryogels (for details see refs.[10,12,16]) using the modified Kargin-Sogolova dynamometric balance.[17] The measurements were done at 22 ± 1°C, the load was 4.9 mN, the punch had a spherical top 5 mm in diameter. The rheological parameter calculated from the creep data was an apparent instantaneous shear modulus, G_0. Its

Table I Influence of the Thawing Conditions on Rheological Properties of cryoPVAGs

Initial Polymer Concentration (g/dL)	Conditions of Cryogenic Treatment			G_0, (kPa)
	Freezing Temperature (°C)	Freezing Duration (h)	Thawing Rate (°C/min)	
3.0	−20	18	0.30	[a]
			0.03	[a]
5.0			0.30	[a]
			0.03	1.73 ± 0.15
7.0			0.30	1.80 ± 0.10
			0.03	3.93 ± 0.19
10.0			0.30	4.94 ± 0.26
			0.03	10.55 ± 0.57

[a] A very weak cryogel ($G_0 < 0.3$ kPa), impossible to measure its shear modulus with the instrument used.

values were further used throughout the studies for the sake of comparison of the properties of cryoPVAGs formed under various conditions.

The investigations of the dynamics of PVA cryotropic gelation were performed using the following temperature/time protocol. The closed moulds with the polymer solution were put into the cryostat chamber having a temperature of −20°C. After an 18-h storage, the temperature in the cryostat was increased (the rate of the heating was approximately 1.5°C/min) up to the predetermined levels within the range from −10 to −1.5°C and maintained constant for the subsequent 24 h. During this period, three samples were removed from the cryostat after definite time intervals and heated to room temperature rapidly (≈3°C/min) followed by the rheological measurements. The use of similar temperature profiles allowed us to follow the dynamics of the formation of cryogels at each of the above-indicated subzero temperatures. Separate experiments were performed no less than three times for every incubation temperature; the results obtained were averaged.

RESULTS AND DISCUSSION

Rheological parameters of PVA cryogels are very convenient characteristics of these materials for the elucidation of influence of different factors on the cryotropic gel formation, since these parameters, even measured with a simple instruments, are sufficiently indicative of the variations in the gel structure caused by the properties of PVA brand used and freezing-thawing regimes.[9,10,18–20] Thus, Table I summarizes the data on the effects of initial concentration of the polymer under study and thawing conditions on the values of shear modulus (G_0) for the resultant cryoPVAGs, and Figure 1 shows the dependence of the gel's strength on the more broad, as compared with Table I, range of thawing rates (0.03–5°C/min) for the cryogels prepared from the 7-g/dL PVA solution. In the latter case, because of such an expanded range of the thawing rate values, which overlap more than two orders of magnitudes, the abscissa axis is given in the logarithm scale.

One may see a gradual increase in G_0 values with increasing the polymer concentration, when comparing the thawed-out samples with equal rates (Table I). For instance, in the case of moderate-rate (0.3°C/min) thawing, the cryogels prepared from the 10-g/dL PVA solution were 2.74 times as strong as the specimens fabricated from

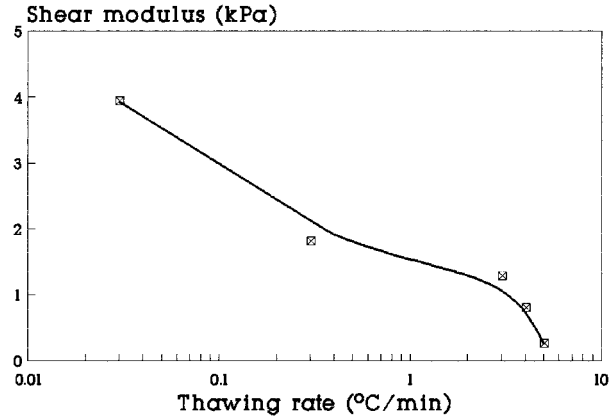

Figure 1 Dependence of the G_0 values of cryoPVAGs prepared from the 7-g/dL polymer water solutions on the thawing rate of the samples initially frozen for 24 h at −20°C.

the 7-g/dL polymer solution. This was an evident trend well known for PVA cryogels.[1–3] At that time, use of the 10-times slower thawing regime (0.03°C) strengthened the samples significantly, e.g., the same 7-g/dL cryogels were reinforced by a factor of 2.2. Quite due to similar strengthening influence of a slow thawing it was possible to form cryoPVAGs from the 5-g/dL polymer solution, which possessed virtually identical shear moduli as the samples prepared from the 7-g/dL system, but thawed faster (0.3°C/min). Besides, when the defrostation of the 10-g/dL system was conducted with the rate of ≈5°C/min (not presented in Table I), we obtained very weak cryogels with shear modulus <0.3 kPa. In other words, these samples were drastically (more than 35 times) weaker than cryogels prepared from the equi-concentrated polymer solution using the slow thawing conditions (0.03°C/min). In addition, similar quickly thawed samples were also considerably (about 6 times) weaker than cryogels formed from the 5-g/dL (two-fold less concentrated) initial system, when a slow thawing was used. Hence, one may conclude that by the variation of thawing rates it was possible to regulate the properties of cryoPVAGs over a very broad range. In fact, the above-discussed data have very well confirmed the earlier observations[12,13] on the principal character of the conditions of thawing stage for occurrence of PVA cryotropic gelation.

In this regard, it was of interest to trace for the kinetic peculiarities of such a gel formation at those temperatures at which the generation of intermolecular links, resulting in the building of 3-D polymer network of cryoPVAGs, occurred with the highest intensity. The important question to be answered was as follows: At what negative temperatures of the still-frozen water PVA system do the conditions arise that are the most favorable for the gel formation? To obtain this answer we used the specific profile of the temperature variation: freezing at a relatively low temperature (−20°C), quick heating until the preassigned subzero temperature, incubation of the samples at these thermal conditions and, at last, a fast thawing (≈3°C/min); for details see Experimental. The studies were performed with the 7-g/dL PVA solutions at the incubation temperatures over the range from −10 to −1.5°C. At somewhat higher temperatures (−1.0 and, especially, −0.5°C) ice melting often proceeded, since it was difficult to provide reliable thermoinsulation of the frozen specimens; therefore, the results obtained were poorly reproducible.

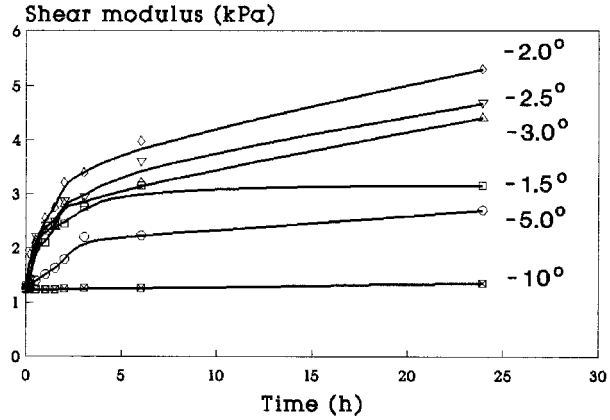

Figure 2 Dynamics of the variation of gel strength of cryoPVAGs during the incubation at subzero temperatures of 7-g/dL polymer water solutions initially frozen for 24 h at −20°C.

Figure 2 depicts the variation of the G_0 values for the cryoPVAG samples in the course of their storing for definite time intervals at each of the temperatures over their above-indicated range. The "zero" time in Figure 2 corresponds to the points when the temperature in the cryostat chamber reached the incubation temperature, as it was seen on the digital display of the instrument's microprocessor. The experimental error in the determination of G_0 values was of the same order as indicated for this parameter in Table I.

The curve "−10°" demonstrates that during at least 24-h incubation virtually no marked changes of the gel's strength were detected, thus testifying that at this temperature the processes of PVA cryotropic gel formation occurred at a very slow rate. In fact, cryoPVAGs possessing the values of shear modulus in the vicinity of 1.2–1.3 kPa were apparently formed during the heating (3°C/min) of the frozen samples from −10°C up to the completion of their thawing, because if the quicker defrostation regimes (e.g., ≈ 5°C/min) were used, no gels were formed at all on the basis of this 7-g/dL system. Such results showed that the formation of the "primary" cryogels proceeded rather rapidly, inasmuch as under the conditions of 3°C/min heating only around 5 min were required for the completion of the ice melting within the bulk of the samples studied, when the heating was commenced from −10°C. However, the strength of these "primary" cryostructurates was rather low. The initial section of the kinetic curve "−5°," reflecting the "development" of gel formation at this higher negative temperature, shows that during the first 2–3-h incubation the gel's

strength of the resultant cryoPVAGs has increased by a factor of ≈2. Then, during the subsequent 20-h storage, similar increasing was insignificant, namely, from 2.2 kPa (3-h sample) to 2.7 kPa (24-h sample). Hence, one may conclude that the additional strengthening of the cryogels kept at −5°C as compared with the samples stored for the same time at −10°C was mainly associated with the 5-degree rise of temperature. For the cryoPVAGs formed at −3, −2.5, and moreover at −2°C, such strengthening effects were all the more pronounced, and the rate of increase in G_0 values with time was higher, as well.

Similar effects were as if the reciprocal in their character comparing those commonly recognized for the processes of formation of usual thermoreversible gels such as gelatin or agar–agar, in which the increase in gelation temperature always results in the fabrication of weaker samples.[21,22] However, similar temperature-dependent regularities inherent in the chilling-induced gelation of homogeneous polymer solutions are not the same for the cryotropic gel formation. The reason is that in the unfrozen gelling systems the formation of the spatial network occurs under the conditions of a constant polymer concentration. This takes place at least until the beginning of syneresis phenomena, if they occur at all. In the case of cryostructuration processes, after freezing of an initial solution, the frozen system at moderate negative temperatures is a heterophase one. It consists of the solid phase (solvent crystallized) and the so-called[23–25] unfrozen liquid microphase, i.e., the portion of the solution remaining unfrozen in the macrofrozen samples. For the systems water–PVA the existence of the liquid inclusions is well detected with differential scanning calorimetry, nuclear magnetic resonance, and electron spin resonance at subzero temperatures.[11,26,27] The solutes are concentrated in such an unfrozen microphase. The polymer concentration in similar unfrozen regions is determined by the temperature: the closer to the melting point of the solvent crystals, the higher the volume of liquid microphase. Respectively, the higher the volume of an unfrozen solvent, the lower the concentration of solutes in it. The cryotropic gelation events proceed just here because of the cryoconcentrating phenomena; the polymer–polymer interactions are strengthened as compared with the initial liquid solution. The particular temperature of the system controls the manifestation of similar interactions, by the polymer concentration and the viscosity of unfrozen inclusions. If the polymer concentration is high enough for the gelation, but the great viscosity of unfrozen inclusions at low enough temperatures interferes, the effective intermolecular interactions capable of causing the gel formation, its rate is insignificant despite similar cryoconcentrating effects. In fact, one deals with definite competition of various factors promoting and inhibiting PVA cryotropic gelation. On the one hand, the lower the temperature of the frozen system, the higher amount of the solvent turned out to be crystallized and, consequently, the higher the solute concentration in the unfrozen inclusions (this increase in polymer concentration should facilitate the gel formation). On the other hand, low mobility of chains and their segments because of great viscosity should restrict such a gelation. Nuclear magnetic resonance measurements showed[26] that unfrozen water presented in these systems at least until the temperatures of 50–60° as low as 0°C, but the mobility of PVA chains in similar frozen systems upon their heating from low temperatures began to increase markedly only commencing from the temperatures around 7–5° below the melting point of such samples. Obviously this was the main reason for the low-rate increase in G_0 values for the specimens being incubated at −10°C and rise of the gelation efficiency at the temperatures in the range from −5 to −2°C. Naturally, that at a certain "high" negative temperature the volume of unfrozen microphase can exceed a boundary between the accelerating influence of increased chain mobility and decelerating influence of the decrease in solutes concentration with the rise of temperature and melting of additional portions of solvent crystals (ice in this case), when such an additional liquid performs as diluent in unfrozen microphase of the still macrofrozen system.

It turned out that in the case under consideration this boundary temperature was over the region between −2 and −1.5°C, inasmuch as at the latter temperature the decrease in the gelation efficiency was already observed. Curve "−1.5°" in Figure 2 shows that the strength of cryoPVAGs formed in the course of frozen samples incubated at this temperature was markedly lower as compared with the PVA cryogels prepared through the incubation at −2°C. Surprisingly, the difference of the gelation temperature only at 0.5° resulted in the samples (24-h incubation) whose shear moduli were 3.15 ± 0.22 and 5.29 ± 0.35 kPa, correspondingly, i.e., the gel strength of these cryoPVAGs differed by a factor

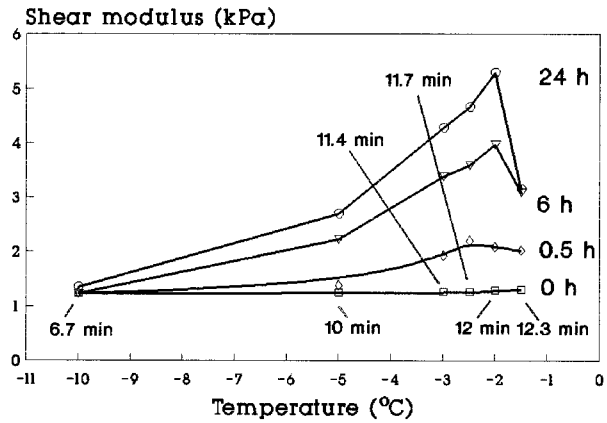

Figure 3 Temperature dependences of the G_0 values of cryoPVAGs formed at different stages of incubation of the frozen samples at subzero temperatures.

of ≈1.7. Hence, the data on the PVA cryotropic gelation over the temperature ranges from −10 to approximately −5°C, then from approximately −5 to −2°C and, at last, from −2°C to the melting point of a sample, revealed three regions where different factors affecting the cryotropic gelation efficiency were dominant. In the first range, this was the low mobility of PVA chains, therefore the gel formation proceeded with an insignificant rate despite the high polymer concentration. In the second range, in contrast, significant increase in the gelation rate took place because of the rise of the chain mobility, and the PVA concentration in the unfrozen liquid microphase was still high enough. In the third range, the effect of the decrease in polymer concentration began to prevail over the increased chain mobility, and the gel-formation efficacy diminished.

Figure 3 presents the dependences of the rheological characteristics of cryoPVAGs on the temperature of the incubation of frozen samples for various periods of time. The digits near the right side of respective curves show the duration of such an incubation. The bottom curve "0 h" accords to the points when the temperature in the cryostat chamber reached the incubation temperature, and the digits with arrows directed to the corresponding points show how many minutes elapsed from the beginning of heating of the frozen samples from −20°C (freezing temperature) to the particular incubation thermal conditions.

In these coordinates, the extreme character of the PVA cryotropic gelation efficiency on the process temperature is well seen. If the short-term (≈1.5–2.5 min) heating of the frozen samples from −10 to −3. . .−1.5°C did not markedly affect the G_0 values of resultant cryoPVAGs, already after 0.5 h of the frozen samples incubation at temperatures over the range from −3 to −1.5°C the shear moduli of the cryogels obtained were about twice as high compared with the shear modulus of a "primary" cryogel (line "0 h"). More prolonged frozen storage of the samples at these subzero temperatures resulted both in the considerable growth of the gel strength and in the more pictorial exhibition of the above-mentioned extreme dependence. These data well explain the strengthening influence of a slow thawing on the physico-mechanical properties of PVA cryogels. When a fast thawing regime (e.g., 3°C/min) was employed, the samples resided at the favored temperatures from approximately −6 to −1.5°C for a very short time (≈1–1.5 min), therefore virtually no strengthening effects were reached. In the case of a moderate-rate thawing, similar time of residence was prolonged until ≈10–15 min, and the gels' strength somewhat increased (Table I; the data for the 7-g/dL samples thawed at the rate of 0.3°C/min). And, at last, the implementation of a slow thawing has provided the 1.5–2.5-h duration of the residence of frozen samples at these favored temperatures thus resulting in the considerable increase in the gel strength. In this latter case, the cryoPVAGs with G_0 equal to ≈4 kPa (Table I) were obtained, and when the same frozen 7-g/dL samples were kept at −2°C for 24 h, the shear moduli of the resultant cryogels rose to ≈5.3 kPa (Fig.3; curve "24 h"). Hence, one may conclude that for the preparation of as strong as possible cryoPVAGs by means of a single-cycle freezing-thawing procedure it is necessary either to use the high-concentrated PVA solutions (but in such a case the initial systems are very viscous and difficult in manipulation), or to perform the samples defrostation as slow as possible, or to store the frozen PVA solutions in the vicinity of −2°C for the prolonged time. The latter variant is the simplest one, but it is necessary to take into account that at similar "high" subzero temperatures a rather reliable thermoinsulation of the frozen specimens is required to prevent a premature thawing.

CONCLUSIONS

The studies of the dynamics of the freeze-thaw–induced gelation of PVA concentrated water solutions revealed the existence of a rather narrow

temperature range, where such a gel formation proceeded. In this regard, the temperatures in the vicinity of −2°C may be considered as the thermal conditions of the highest efficiency of PVA cryotropic gelation. It is of interest that virtually the same result was obtained in our previous study of cryostructuration of dilute PVA water solutions[13]: the formation of the dispersed cryoprecipitates from the 0.5-g/dL system (analogous to the formation of cryoPVAGs from the 7-g/dL system) proceeded with the highest efficacy (yield) in the same narrow temperature range, thus showing the universal character of the regularities established, which turned out to be inherent in the PVA water solutions over a wide polymer concentration range.

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