

Study of Cryostructuration of Polymer Systems.

XI. The Formation of PVA Cryogels by Freezing–Thawing the Polymer Aqueous Solutions Containing Additives of Some Polyols

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SYNOPSIS

The influence of the presence of some polyols in initial poly(vinyl alcohol) aqueous solutions on rheological and thermal properties of the cryogels, which were produced by the freezing–thawing method, was investigated. The polyol additives (cosolvents) were as follows: glycerol, propylene glycol, mono-, di-, and triethylene glycols, and oligoethylene glycols (PEG-400 and PEG-1000). It was shown that the introduction of glycerol, propylene, or ethylene glycols into the polymer solution resulted in a decrease of cryogel's shear modulus and fusion temperature, whereas the use of triethylene and higher ethylene glycol oligomers as cosolvents gave rise to an increase in the gel's strength and thermostability, and diethylene glycol exhibited a transitional influence. The possible mechanism of the action of reinforcing cosolvents is discussed. © 1995 John Wiley & Sons, Inc.

INTRODUCTION

Poly(vinyl alcohol) cryogels—cryoPVAGs—are thermoreversible highly elastic heterogeneous macroporous gels, which are formed by the freezing–thawing of concentrated aqueous solutions of the polymer.^{1–5} These gel materials were thoroughly studied in respect to their properties, structure, and mechanisms of cryotropic gelation. CryoPVAGs are known to be applied in medicine and biotechnology.^{6,7}

In addition to PVA cryogels prepared by the freezing–thawing of water solutions of the polymer, some examples, where DMSO or DMSO–water mixtures were used as initial solvents, were also described.^{8–10} In the latter case the properties of the obtained cryoPVAGs have depended on the fraction of above cosolvent, which, certainly, determined the freezing point of a PVA solution, too.

The formation of cryoPVAGs using another mixed (water/organic liquid) solvent has not so far been reported, although similar systems are of definite interest, as ordinary thermoreversible PVA gels prepared either by increasing the portion of organic cosolvent (pooring the mixed solvent quality) or by chilling (but without freezing) of the solutions—water/cosolvent/PVA—are well known.^{11–16} Thus, for instance, transparent microporous gels have been obtained originating from PVA solutions, where, in addition to water, a series of organic low-molecular-weight liquids were utilized as cosolvents, and gel formation occurred on the system cooling below 0°C with subsequent replacement of an organic moiety with water in the gel obtained. Among these solvents some polyols (glycerol, ethylene glycol, and triethylene glycol) have been described, and PVA gels thus formed have been proposed for the creation of soft contact lenses.¹⁶

In the present communication we report on the cryotropic gelation of PVA-containing solutions, when glycerol, propylene glycol, ethylene glycol, or its oligomers were in the composition of mixed solvents for the polymer. To characterize the cryogels

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prepared and to compare them with each other, the rheological and thermal properties of cryoPVAGs were studied.

EXPERIMENTAL

Materials

Atactic PVA of trade mark 20/1 (NPO "Azot," Severodonetsk, Ukraine) was used in the work. The technical grade polymer was additionally purified by the reprecipitation from an aqueous solution into the methanol excess, as described elsewhere.⁵ Thus prepared PVA had a viscosity average molecular weight of about 82 kDa and saponification degree of 98 mol %.

The glycerol (GL; bp 150–151°C at 8 mm Hg), propylene glycol (PG; bp 53–55°C/8 mm Hg), ethylene glycol (EG; bp 87–89°C/8 mm Hg), diethylene glycol (DEG; bp 114–116°C/8 mm Hg), triethylene glycol (TEG; bp 140–143°C/8 mm Hg) (all Reakhim, Russia) were purified by vacuum distillation. Ethylene glycol oligomers were PEG-400 and PEG-1000 species (Ferak, Germany); they were used without additional refining.

Methods

The PVA solutions were prepared as follows. The particular mixed solvent with preassigned water/cosolvent weight ratio was added to the known amount of dry polymer powder, stirred, and after swelling for 18 h at room temperature, was heated (with periodical shaking) on a boiling water bath up to the polymer dissolution. The duration of the samples heating in all cases was 40 min. PVA solutions thus obtained were filtered through the sintered glass filter to remove possible mechanical impurities.

CryoPVAGs for rheological tests were formed in duralumin cylindrical molds, and the samples for fusion temperatures measurements were prepared in transparent polycarbonate test tubes.¹⁷ PVA cryogels in the shape of uniform spherical beads (diam. ~ 3 mm) were obtained with the aid of special cryogranulating installation.¹⁸ In all cases the freezing temperature was –20°C, duration of storage frozen was 18 h, and thawing rate was equal of around 0.2°C/min.

The methods of rheological parameters determination (shear deformation measurements) and of gels' fusion temperatures detection were reported in detail in a previous publication.¹⁹

The variation of freezing rates for the systems under study was performed with a set of thermoinsulating covers on duralumin molds; the quantitative evaluation of the freezing rates was performed with the thermocouple connected to a recording potentiometer; both procedures were fulfilled according to Lozinsky et al.²⁰

For the cryoPVAG beads the apparent instantaneous elastic modulus (E_0) was determined by the method of uniaxial compression on a Kargin–Sogolova dynamometric balance²¹ at constant load of 4.9×10^{-3} N. The values of E_0 were calculated from the following formula:²²

$$E_0 = (F : h^{3/2}) \times [3(1 - \sigma^2) : (2R)^{1/2}]$$

where F is load (N), h is deformation (decreasing of bead diameter, m), σ is the Poisson's ratio (assumed equal to 0.45), and R initial bead radius (m). The measurements of the cryogel's mechanical strength at every point were carried out for 5 beads; the obtained E_0 values were averaged.

RESULTS AND DISCUSSION

Low- and medium-molecular-weight polyols are well known as cryoprotectants, which are widely applied in cryobiology and cryomedicine,²³ i.e., these substances are able to depress freezing temperatures of aqueous solutions, to reduce a size of ice polycrystals, and even to result in the glass formation of respective water systems at their fast low temperature cooling. Hence, in the present case, that is, preparation of cryoPVAGs in media of mixed solvents containing similar polyols, it was necessary to find suitable cosolvent concentrations and regimes of cryogenic processing capable of supplying conditions for solvent crystallization, as only upon PVA solution freezing could a characteristic macroporous structures of cryogels be produced.

In the preliminary test experiments it was shown that up to 20 wt % content of the polyols used (with the exception of EG) a temperature of –20°C was enough to freeze firmly the initial PVA solution (polymer concentration, 10 g/dL), and that after storing the system frozen for 18 h at this temperature and subsequent thawing, the cryoPVAGs were obtained. At the same time, shear moduli of the samples appeared to be dependent on the freezing rate of an initial polymer solution. Thus, Table I demonstrates the influence of the freezing dynamics on the rheological properties of PVA cryogels formed

Table I Influence of Freezing Rate on Rheological Properties of CryoPVAGs Obtained after System Thawing

Sample No.	Freezing Duration ^a (min)	Freezing Rate ^a (°C/min)	Shear Modulus (kPa)
1	35	0.14	1.2
2	22	0.23	1.5
3	10	0.49	2.1
4	1	5.88	2.4

^a Freezing duration and freezing rate are as follows¹⁷: time of the system cooling from 0 to -5°C (including water crystallization plateau) and dynamics of the temperature variation in this temperature range, respectively.

from the aqueous polymer solution containing 2.5 wt % of DEG. Contrary to PVA solutions without any cosolvent additives, where insignificant influence of the freezing rates in a wide range ($0.1\text{--}17^{\circ}\text{C}/\text{min}$) on the cryogel's strength was observed,¹⁷ the twofold increase of shear modulus took place under a freezing rate variation from 0.14 to $5.88^{\circ}\text{C}/\text{min}$.

Low values of shear moduli for cryoPVAGs obtained under slow cooling were due to the system's "ordinary" gelation, yet prior to the beginning solvent crystallization—for rather prolonged residence of PVA solution at low positive temperatures. In this case, the freezing factors did not act to a polymer solution, but to a weak "ordinary" PVA gel, resulting in the disturbance of its structure. Moreover, higher oligomers of ethylene glycol were able to induce the system gelation at room temperature. For instance, 10 g/dL PVA aqueous solutions containing 15 and 20 wt % of PEG-400 have formed similar gels possessing fusion temperatures of 27.0 and 60.3°C , respectively. Shear modulus of the latter gel was equal to only about one-third of the modulus of the cryoPVAG prepared by the freezing-thawing technique from the polymer solution without an organic cosolvent present and was 7 times less than the modulus of PVA cryogel obtained from the same concentration polymer solution, but including just 5 wt % of the above oligoethylene glycol.

Therefore, to prevent at positive temperatures the gelation of PVA due to poor solvent quality and to form, when it was possible, the "real" cryogel, it was necessary to freeze the system—water/cosolvent/PVA—sufficiently quickly, "outstripping" the kinetics of the ordinary thermotropic gel formation. Thereafter, under cryoPVAGs preparation, freezing rates of about $5\text{--}6^{\circ}\text{C}/\text{min}$ were used throughout this

work. They were achieved by immersing the stoppered molds with PVA solutions into the coolant (ethanol, -20°C) in a cryostat chamber. The system including 20 wt % of EG did not freeze at given conditions; with concentrations of PEG-400 higher than 15 wt % or PEG-1000 more than 7.5 wt %, they did form gels rather rapidly at room temperatures; therefore we were unable to prepare cryogels in these cases.

Figure 1 shows the generalized picture of influence of the polyol additives used on a relative variation of rheological properties of cryoPVAGs. For convenience of comparison, the shear modulus of the PVA cryogel prepared from the 10 g/dL polymer water solution (G_w) was assumed equal to 100, and the rheological parameters determined for cryoPVAGs formed in the media of mixed solvents (G_{ms}) were expressed in relative values: $G_{rel} = G_{ms}/G_w \times 100\%$. As reinforcing effects of ethylene glycol oligomers were manifested to a very considerable extent (for example, higher than 1700% in the case of 15 wt % PEG-400), the data of Figure 1 are presented in logarithmic scale of the ordinate axis.

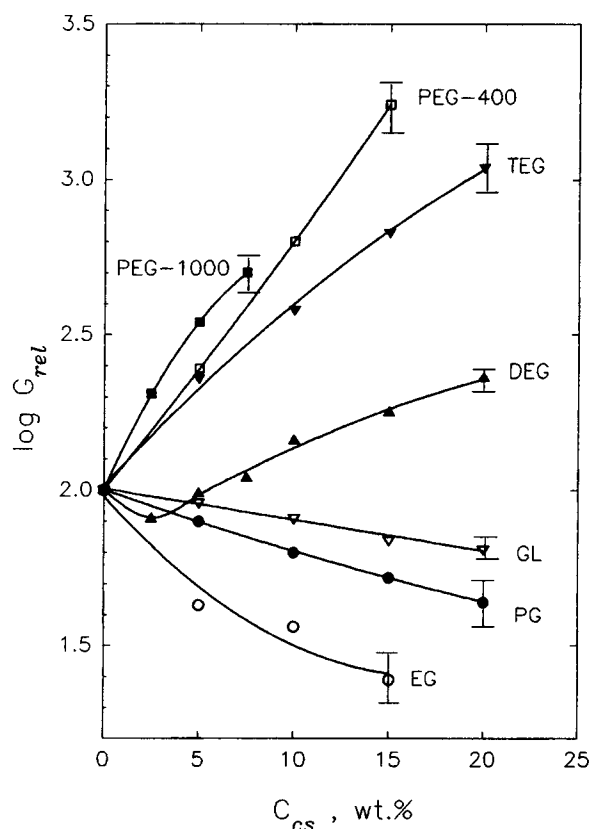


Figure 1 Influence of cosolvent concentration (C_{cs}) in the initial solution of PVA on the relative shear modulus of cryoPVAGs obtained.

One can see that the weakest cryoPVAGs were obtained when EG or PG has been applied as co-solvent. Thus, a PVA cryogel sample containing 15 wt % of EG possessed a shear modulus of about 25% as compared with G_w , and the use of 20 wt % PG solution in the capacity of mixed solvent for PVA resulted in a cryoPVAG with G_{ms} constituting the 41.3% of G_w . To a lesser extent the weakening influence was also a characteristic feature of the glycerol additives. Hence, one can conclude that low-molecular-weight polyols such as EG, PG, or GL are related to ones interfering with the PVA cryotropic gelation, whereas at positive temperatures the increase in concentration of these substances in PVA aqueous solutions has promoted the ordinary gelation of the polymer,¹¹⁻¹⁶ thus indicating the definite differences between the mechanisms of two similar kinds of PVA gel formation processes.

In contrast to EG, tri- and higher oligomers, being introduced into the initial polymer solution, contributed to the growth of cryoPVAGs' strength. At those concentrations of TEG, PEG-400, and PEG-1000, where it was possible to prepare cryogels in the regimes employed, the reinforcing ability of such oligoethylene glycols was positively correlated with their polymerization degree, namely the larger the amount of EG units contained in the corresponding PEG molecules, the higher the shear modulus of cryoPVAG prepared in the presence of a given additive. In this context, DEG occupied a "transitional" position between the weakening and reinforcing polyols. The influence of this substance on cryoPVAGs' properties will be detailed further later.

Besides the rheological parameters, the introduction of various polyols into the initial polymer solutions affected the thermal characteristics of the cryoPVAGs obtained. Thus, Figure 2 demonstrates the dependence of PVA cryogels' fusion temperatures on the polyol amount in the systems under study.

It is seen that the main tendency was as follows: those polyol substances that were capable of reducing the gel's strength (Fig. 1) have decreased T_f values for the same cryoPVAG preparations. And if PVA cryogel containing 15 wt % of EG had fusion temperature of about 3°C lower than the gel sample without polyol additives, cryoPVAG prepared in the presence of 15 wt % PEG-400 possessed a T_f about 9°C higher than above reference cryogel.

So great an increase in the heat resistance of thermoreversible noncovalent gels was rather surprising, as similar significant temperature effects are usually inherent only after considerable growth of

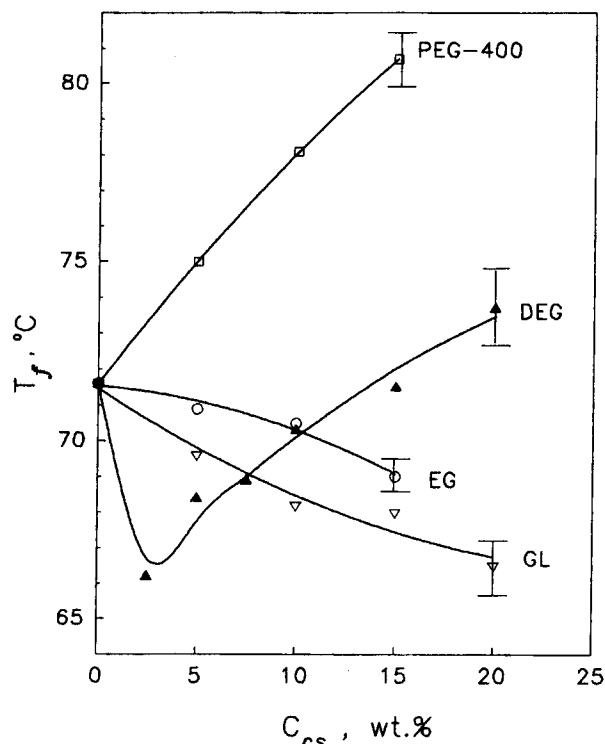


Figure 2 Dependence of cryoPVAGs' fusion temperatures on the amount of some polyols in the initial polymer solution.

a polymer concentration in the gel formed. For instance, in the classic work of Eldridge and Ferry²⁴ it was shown that the fusion temperature of gelatin gel has risen from 27.0 to only 29.9°C under the polymer content variation by a factor of about three—from 2.03 to 5.96 g/dL. But in the case of cryoPVAGs the twofold increase in PVA concentration from 5.96 to 12.16 g/dL resulted in the growth of T_f from 61.4 to 70.6°C.²⁰ Therefore, we have assumed that polyols capable of reinforcing cryoPVAGs and of increasing their fusion temperatures can, due to some mechanisms, promote the rise of PVA concentration in the areas of the proper gel formation.

One possible mechanism would be the following. When freezing the system, water/cosolvent/PVA, a pure water is first frozen-out as ice crystals. This leads to the solutes concentrating in the parts of a sample's volume still being unfrozen. The formation of similar heterophase "composites" consisting of a solvent polycrystals (solid phase) and so-called "liquid microphase" was described for numerous moderate-frozen multicomponent solutions.²⁵ Concerning the freeze-crystallized PVA aqueous solutions, some properties of their unfrozen fractions, where

gel formation does occur, were studied by NMR²⁶ and ESR²⁷; they showed rather high translational mobility of unfrozen water molecules and segmental mobility of PVA chains. In the case of freezing PVA solutions containing polyol cosolvents, ice crystallization causes the alterations of polymer and additive concentrations, which could cause a shift in equilibria in the above mentioned liquid microphase, giving rise to liquid-liquid phase separation with the emergence of two new liquid phases—one enriched with oligoethylene glycol and the other with PVA. Similar phase segregation because of limited thermodynamic compatibility of the polymeric ingredients in solutions is well known for various multicomponent aqueous polymer systems²⁸ and for the mixtures—water/PEG/PVA, in particular.²⁹ In the present case, i.e., upon moderate freezing of the initial solution, this liquid-liquid phase separation in liquid microphase must increase the polymer concentration in the resultant PVA-rich “microscopically unfrozen regions of the macroscopically frozen object,” as compared to the polymer content in similar liquid microphase of a frozen water/PVA system, but without oligoethylene glycol additives. As poly(vinyl alcohol) is a gel-forming agent, and under cryotropic gelation more rigid and less fusible cryoPVAGs are obtained from the more concentrated polymer solutions,^{4,5} the growth of G_{rel} and T_f values, observed in experiments would be a consequence of the mechanism discussed.

If such assumed additional PVA concentrating phenomena do take place, the increased gel strength of cryoPVAG prepared in the presence of a reinforcing polyol could remain after washing-off of the additive from the obtained cryogel. To check this, we prepared cryoPVAGs in the form of spherical beads and evaluated their deformability in the course of prolonged bead rinsing with a pure water. The measurements of elastic moduli revealed (Fig. 3) that cryogel produced with PEG-400 addition has retained elevated E_0 values, instead of those decreasing to the level inherent for cryoPVAG prepared simply from the aqueous polymer solution. These data, in our opinion, attest to the formation of a more concentrated gel framework in macroporous cryoPVAG in the presence of reinforcing polyols, rather than participation of given molecules in interactions with PVA's OH groups (for example, by hydrogen bonding) capable of building up the strength of the cryogel as a whole.

We would now like to consider more widely the influence of DEG additives on rheological and thermal characteristics of cryoPVAGs produced by

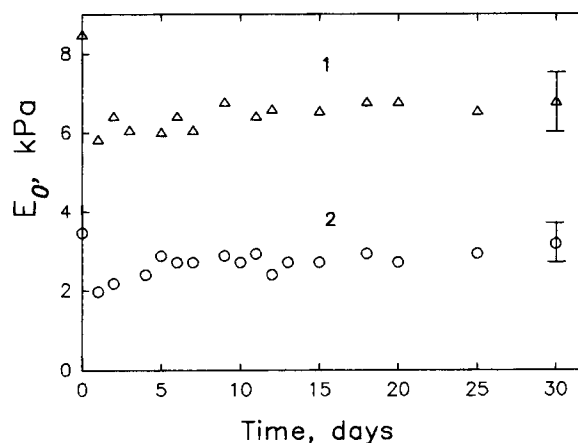


Figure 3 Variation of the elastic modulus for cryoPVAG beads in the course of their prolonged rinsing with a pure water (1, beads formed in the presence of 10 wt % of PEG-400; 2, PVA cryogel beads prepared from the polymer aqueous solution without polyol additives). Some decline in E_0 values for the first day is due to the washing-off of the polymer sol fraction.¹⁷

freezing-thawing the solutions—water/DEG/PVA. As was pointed out above, this cosolvent in relation to cryoPVAGs' properties has exhibited a transitional action compared to that of EG and TEG. In Figure 4 the effects of increasing DEG concentration are presented.

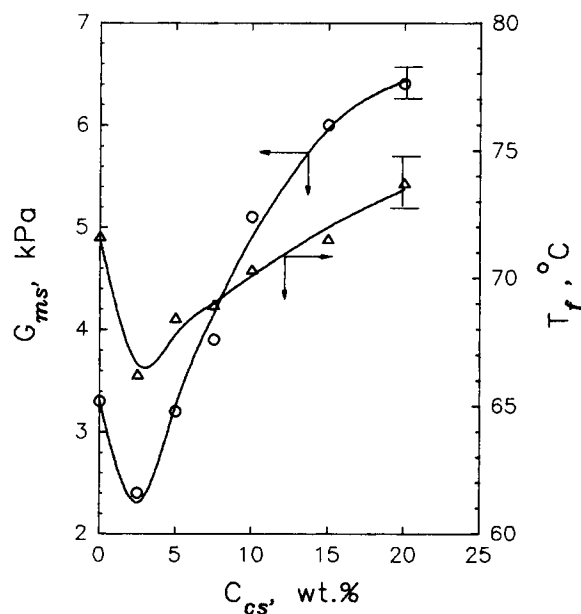


Figure 4 Comparison of variation in the rheological and thermal characteristics of cryoPVAGs formed with addition of various quantities of DEG.

One can see a practically parallel variation (with an extreme manner) of both G_{ms} and T_f values with the growth of this polyol content. The minimal magnitudes of shear modulus and fusion temperature were observed when 2.5 wt % of DEG was in the initial polymer solution. Such a cosolvent in the 0–5 wt % concentration range in respect to cryo-PVAG's properties acted as the weakening additive; furthermore, with the growth of the DEG fraction, the reinforcing factors began to predominate. Taking into account the earlier discussed liquid–liquid phase separation in unfrozen microphase, it is possible to conclude that at low initial DEG concentrations this phenomenon did not proceed at freezing conditions used, and by increasing the polyol content additional concentrating of the gel-forming polymer (due to the arising two-phase liquid systems) would be included into a cryogelation process.

CONCLUSION

Inasmuch as cryoPVAGs are taken up in biotechnology as carriers for cell entrapment within these macroporous gel matrices, in the course of preparation the cell suspension of such immobilized biocatalysts in PVA solution is frozen. During cryogenic treatment a viable biomass is subjected to hazardous freezing stresses capable of lowering significantly the biological activity of the cells. In spite of the definite cryoprotective properties of PVA itself, which are manifested in a considerable decrease of ice crystal size,²⁹ some cultures withstand the freeze action very poorly. Therefore, in the latter cases, it is desirable to use cryoprotectants, for instance, oligoethylene glycols, relating to the penetrating (into the cell's inner volume—cytoplasm) ones. On the other hand, it is also necessary to know how these cryoprotective substances can influence the gel matrix formation processes and the physical characteristics of the cell carrier obtained. The experiments performed in this work have supplied information concerning the effects of the addition of GL, PG, EG, DEG, TEG, PEG-400, and PEG-1000 on an initial PVA solution in terms of the rheological and thermal parameters of cryoPVAGs. These data show that beginning with TEG (and then proceeding to higher oligoethylene glycols) one cannot “be afraid” of the deteriorative influence of the presence of these additives on cryogelation and on the cryogel's properties. On the contrary, the use of the above substances will enhance gel strength and thermostability. As regards the reinforcing mechanisms, it was assumed that

liquid–liquid phase separation phenomena take part in the increase in PVA concentration in those areas of the unfrozen microphase where cryotropic gelation proceeds. Now we perform experiments to support additionally the mechanism proposed both for the temperature conditions described here and others.

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