

# Study of Cryostructuration of Polymer Systems. XX. Foamed Poly(vinyl alcohol) Cryogels

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**ABSTRACT:** Foamed poly(vinyl alcohol) (PVA) cryogels, which are formed as a result of freeze–thaw treatment of whipped PVA water solutions (polymer with MW of 69,000 Da and DD ~99 mol % was used), were obtained and their properties were studied. The rheological characteristics and macrostructure of these gel materials were controlled by the same factors as for the ordinary nonfoamed PVA cryogels (initial polymer concentration and freezing–thawing regimes) and also by the conditions of generation of fluid PVA foams. The study of the kinetics of the freeze–thaw-induced gel formation of these foams revealed that the temperature dependence of the efficiency of cryotropic gelation showed a maximum at about  $-1.5^{\circ}\text{C}$ . The presence of low molecular weight admixtures in the initial polymer solution appears to be a rather important factor because the admixtures were capable of decreasing the stability of fluid PVA foams and weakening both foamed and nonfoamed cryogel samples. © 2001 John Wiley & Sons, Inc. *J Appl Polym Sci* 82: 1609–1619, 2001

**Key words:** foamed poly(vinyl alcohol) cryogels; PVA foams; rheological properties; structure

## INTRODUCTION

Poly(vinyl alcohol) cryogels (cryoPVAGs) are, apparently, the mostly well-known gel materials produced by the freezing–thawing technique. Numerous fundamental and applied studies on these interesting heterogeneous gels have been published, and the knowledge obtained in those works was thoroughly reviewed.<sup>1–3</sup> CryoPVAGs are of considerable practical interest, especially as biomedical and biotechnological materials.<sup>1,4,5</sup> In this regard, not only empty cryogels (i.e., those without any foreign additives), but also cryogels containing various solutes (e.g., electrolytes, cryoprotectants, biopolymers, etc.)<sup>6–12</sup> and fillers (e.g.,

microbial cells, collagen fibers, gel particles, powdered solids, etc.)<sup>13–16</sup> are under study in many laboratories. However, one type of filled PVA cryogels, namely gas-filled or foamed cryoPVAGs, has not been investigated so far at all. Therefore, it was of interest to explore the formation of this kind of cryogenically produced gel materials and investigate their basic properties.

## EXPERIMENTAL

### Materials

Atactic PVA of trade mark 16/1 (NPO “Azot”, Severodonetsk, Ukraine) was used in the work. The technical grade polymer was purified by the following procedure: The polymer powder was dispersed in a 6-fold excess of deionized water and

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left to stand at room temperature for 3 days with periodical manual stirring. Every morning, the supernatant liquid was discarded and the necessary amount of fresh water was added. Then, the water-swollen polymer was pressed-off between four layers of cotton cloth to remove free liquid. The "cake" obtained (containing  $\sim 24$  wt % dry matter) was stored in tightly stoppered glass container and used for further preparation of PVA solutions. This washed PVA had a viscosity average molecular weight of  $\sim 69$  kDa and saponification degree of 99 mol %.

Congo red (Aldrich, Milwaukee, WI), base-processed gelatine, phenol, and glycerol (all from Reakhim, Moscow, Russia) were of analytical grade and used without additional purification.

## Methods

The polymer solutions were prepared by dispersing the weighed amount of swollen PVA cake in the necessary volume of deionized water and heating on a boiling water bath for 30 min with stirring. The loss of the liquid due to evaporation was determined by weighing the samples and then compensated by the addition of the required amount of pure water. The final solutions were cooled to room temperature and then whipped with a blender (ENVO MVR-203M; Energiya, Voronezh, Russia) to foams of required foaming capacity ( $\beta$ , the ratio between the volume of resultant foam and initial volume of the polymer solution before the foaming treatment).<sup>17</sup>

Foam stability was determined as described in the known protocol<sup>17</sup> by placing the whipped fluid PVA foams in graduated cylinders and recording the times elapsed (i) between the end of whipping up and the appearance of liquid layer at the bottom of the cylinder ( $T_1$ ), and (ii) between the end of whipping up and the moment when the foam volume was a half of the initial volume ("the foam half-life",  $T_{1/2}$ ).

The foamed PVA cryogels (f-cryoPVAGs) were prepared as follows. The whipped polymer fluids were gently poured into cylindrical duralumin molds (15-mm diameter and 10-mm height), sealed, and placed in the chamber of an FP 45 HP (Julabo, Seelbach, Germany) precision programmable cryostat (the accuracy of the temperature control was  $\pm 0.01^\circ\text{C}$ ). The samples were frozen and incubated at one of the following temperatures:  $-10$ ,  $-20$ , or  $-30^\circ\text{C}$  for 18 h. They were then thawed at the rates of 0.3 or  $0.03^\circ\text{C}/\text{min}$

using the microprocessor-controlled facilities of the cryostat or at the rate of  $\sim 3^\circ\text{C}/\text{min}$  in an air thermostat set to  $+38^\circ\text{C}$ . In all the thawing regimes, the final temperature of the cryostructures reached during heating did not exceed  $+20^\circ\text{C}$ . The rheological measurements with the prepared f-cryoPVAGs were also performed at this temperature.

Studies of the dynamics of cryotropic gelation of foamed PVA solutions were carried out using the following temperature/time protocol. The sealed molds with the whipped polymer fluid were placed into the cryostat chamber at  $-20^\circ\text{C}$ . After 18 h of storage, the temperature in the cryostat was raised (at the rate of  $\sim 1.5^\circ\text{C}/\text{min}$ ) to the predetermined levels ( $-5$ ,  $-2$ ,  $-1.5$ , or  $-1^\circ\text{C}$ ) and maintained constant for the subsequent 24 h. During this period, three samples were removed from the cryostat at specified time intervals and heated rapidly ( $\sim 3^\circ\text{C}/\text{min}$ ) to room temperature; the rheological measurements were conducted with these samples. The use of similar temperature profiles allowed us to follow the dynamics of the formation of cryogels at each of the previously indicated sub-zero temperatures. Separate experiments were carried out at least three times for each incubation temperature, and the results obtained were averaged.

Rheological measurements with cryoPVAGs and f-cryoPVAGs were conducted by the penetration method<sup>18</sup> using the modified Kargin-Sogolova dynamometric balance<sup>19</sup> essentially as described in detail for the investigation of filled and nonfilled PVA cryogels.<sup>13,20,21</sup> During the measurements, the load was 4.9 mN and the punch had a spherical top that was 5 mm in diameter. The creep data were used to calculate apparent instantaneous shear modulus,  $G_0$ . These values were further used throughout the studies to compare the properties of cryoPVAGs or f-cryoPVAGs prepared under various conditions.

Thin sections of cryoPVAGs and f-cryoPVAGs for light-optical microscopy were cut with a cryomicrotome MZ-2 (Medical Equipment Plant, Kharkov, Ukraine). A small sample ( $\sim 5 \times 5$  mm) of foamed cryogel was placed onto a metal probe table of the microtome and flash frozen ( $\sim 20$  s) in a stream of injected  $\text{CO}_2$ . Thereafter, several sections ( $7\text{--}8\text{-}\mu\text{m}$  thick) were cut and submerged into degassed distilled water to remove the sol-fraction, which is known to be present in PVA cryogels.<sup>21,22</sup> Then, the sections were stained by immersing for 5 s in a 1% aqueous solution of

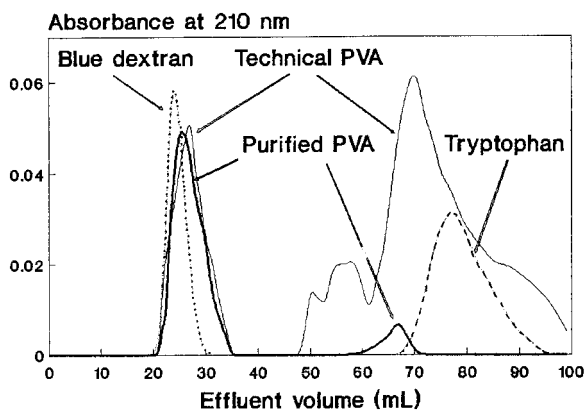
Congo red. The excess of the dye was washed out with distilled water, each section was mounted on a microscope slide, and excess water was removed gently with a cotton cloth. A drop of "sealing solution" (1 g of gelatine dissolved in 12 mL of 50% (v/v) water glycerol with 1 drop of melted phenol added as bacteriostatic agent) was applied to a section, which was then covered with a cover glass. The preparations were stored at 4°C before the investigation. Light-optical microscopy studies were accomplished with an Axiolab Pol microscope (Carl Zeiss Jena GmbH, Jena, Germany).

Gel chromatography of 0.2-g/dL PVA solutions was carried out on a column (15 × 450 mm) packed with Sephadex G-75 resin (Pharmacia Fine Chemicals, Uppsala, Sweden). The ultraviolet (UV) absorption of the effluent was recorded at 210 nm with a Model 200-10 flow-through spectrophotometer (Hitachi, Tokyo, Japan).

## RESULTS AND DISCUSSION

### Foaming Behavior of PVA Water Solutions

To be able to reliably prepare f-cryoPVAGs of the same quality, it was necessary to identify the foaming conditions that would give fluid PVA foams that were stable for at least the time needed to freeze the samples. Otherwise, that is, if the foam stability is insufficient, one would be freezing the system consisting of both a fluid PVA foam and an already separated nonfoamed solu-



**Figure 1** Gel-chromatography data for the solutions of technical-grade and water-rinsed PVAs [for experimental conditions see Methods; traces for Blue dextran (MW 2000 kDa) and tryptophan (MW 0.2 kDa) show the working volume of the column used].

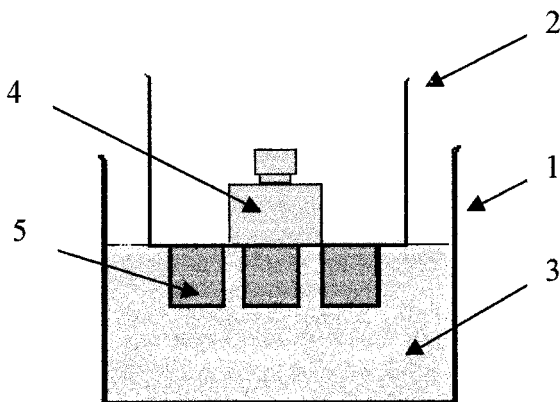
**Table I** Parameters of Stability of Fluid Foams<sup>a</sup> Prepared from the Technical Grade and Water-Rinsed PVAs

Initial PVA Concentration (g/dL)	Technical Grade Polymer		Water-Rinsed Polymer	
	$T_1$ (min)	$T_{1/2}$ (min)	$T_1$ (min)	$T_{1/2}$ (min)
7	9 ± 1	~180	15 ± 2	~300
8	11 ± 2	~210	17 ± 2	~360
9	17 ± 3	~270	33 ± 2	~390
10	29 ± 3	~330	62 ± 7	~500
11	56 ± 4	~420	76 ± 9	>600
12	114 ± 10	>600	135 ± 11	>900

<sup>a</sup>  $\beta = 2.0$ .

tion of the polymer. Preliminary experiments have showed that ~10 min was enough to freeze a whipped PVA fluid in a duralumin mold under the freezing conditions employed. Therefore, only PVA foams with  $T_1 > 10$  min were subsequently used.

It turned out that the technical grade polymer from the batch used in this work contained a variety of oligomeric and low-molecular-weight admixtures (curve "Technical PVA" in Fig.1). Note that these substances with molecular weight as low as 15–10 kDa had chromophoric groups that assured significant UV adsorption of the effluent with  $V_e = 50$ –100 mL in the course of chromatographic analysis. However, the content (weight portion) of these admixtures in the technical grade PVA did not correspond to the UV pattern and was relatively low (several wt %). However, these admixtures had a strong effect on the foaming behavior of PVA water solutions. The foams obtained from the 7–12-g/dL solutions of technical grade polymer were 1.5–2 times less stable (Table I) than the foams prepared from the equiconcentrated solutions of the purified PVA, from which such admixtures were largely removed by washing (curve "Purified PVA" in Fig.1 shows only minor amount of the oligomeric substances in the water-rinsed PVA). In this regard, the data in Table I evidently demonstrate that the impurities existing in the technical grade PVA have a pronounced foam-destabilizing effect. One may suggest that methanol and sodium acetate, routine admixtures in PVAs of a technical grade, have the major foam-destabilizing effect. Therefore, if not indicated otherwise, the f-cryoPVAGs



**Figure 2** Schematic diagram of the “buoyancy test”. Key: (1) large glass beaker; (2) small glass beaker; (3) water; (4) weight; (5) cylindrical f-cryoPVAG.

examined here were prepared from the water-rinsed purified polymer.

Because comprehensive investigation of fluid PVA foams was not the goal of this particular work that was mainly devoted to the gas-filled PVA cryogels, no additional studies of the foams prior to freezing were conducted.

#### Properties of Foamed PVA Cryogels Prepared Under Various Freezing–Thawing Conditions

At the beginning of these studies we deemed it necessary to show the very possibility of making foamed PVA cryogels because it was not clear whether the cryogenic treatment (freezing - frozen storage - thawing) would retain the foamed system or the ice crystallization within the liquid walls of air bubbles would destroy (partially or completely) the intactness of these partitions between the bubbles, thus resulting in the disappearance of foamed morphology in the final cryogels. The starting phenomenological experiments demonstrated that f-cryoPVAGs could be successfully prepared from the whipped PVA fluids, and the properties of resultant cryogels were controlled by both the characteristics of initial foams and the regimes of the cryogenic process. Hence, it was shown that the freeze–thaw cycle transformed fluid PVA foam into a viscoelastic air-filled gel-like material. This result certainly was due to the cryotropic gelation of PVA solution within the partitions between the air bubbles, when the liquid sol turned into a gel. In other words, the freeze–thaw treatment fixed the PVA foams, transforming them from the transient (Ta-

ble I) whipped fluids to highly stable (for at least 1 month) foamed systems.

This quality (i.e., stability) was evaluated in the “buoyancy test” (schematic diagram of the experiment is shown in Fig. 2). Three cylindrical f-cryoPVAG samples formed in the molds for rheological measurements (the volume of each cylinder was  $\sim 1.77 \text{ cm}^3$ ; see Experimental) were placed into a glass beaker filled with water, another beaker was mounted on the tops of these floating gel samples, and a small weight was placed into the top beaker so that the foamed cylinders were floating (1–2 mm under the water surface). The total weight of the top beaker was  $\sim 4 \text{ g}$ ; hence, the Archimedes’ buoyant force acting per each foamed cylindrical buoy was  $1.3 \times 10^{-2} \text{ N}$ . The system was kept at room temperature for 1 month, and no significant changes in the buoyancy of these f-cryoPVAGs were observed. This result evidently meant that the air entrapped inside the air bubbles of the foamed cryogels was not replaced by water (for instance, via the simple diffusion mechanism) in such a static-mode experiment.

Subsequent studies revealed some basic factors capable of affecting the rheological properties of f-cryoPVAGs. The influence of the characteristics of fluid PVA foams on the values of instantaneous shear modulus ( $G_0$ ) of the respective foamed cryogels prepared from the 10-g/dL water polymer solution whipped to different foaming capacities is shown in Table II. The data on usual nonfoamed cryoPVAG are also presented for the sake of comparison to show that f-cryoPVAGs were significantly weaker. This quality of foamed cryogels was absolutely evident because the air bub-

**Table II** Influence of Characteristics of the PVA Foams on the Rheological Properties of Resultant f-cryoPVAGs<sup>a</sup>

$\beta$	Foam Stability (min)		$G_0$ (kPa)
	$T_1$	$T_{1/2}$	
0 <sup>b</sup>	—	—	$9.1 \pm 0.6$
$1.3 \pm 0.1$	$13 \pm 1$	$40 \pm 7$	$8.3 \pm 1.2$
$1.6 \pm 0.1$	$18 \pm 2$	$\sim 200$	$7.0 \pm 0.5$
$2.0 \pm 0.1$	$62 \pm 7$	$\sim 500$	$5.9 \pm 0.9$
$2.5 \pm 0.2$	$185 \pm 15$	$>1800$	$6.2 \pm 0.5$

<sup>a</sup> Initial PVA concentration: 10 g/dL; freezing temperature:  $-20^\circ\text{C}$ ; freezing duration: 18 h; thawing rate:  $0.03^\circ\text{C}/\text{min}$ .

<sup>b</sup> Reference nonfoamed PVA cryogel.

**Table III Effect of the Initial Polymer Concentration on the Rheological Properties of Foamed and Nonfoamed PVA Cryogels<sup>a</sup>**

Initial PVA Concentration (g/dL)	Instantaneous Shear Modulus ( $G_0$ , kPa) of Cryogel Samples Prepared from:			
	Technical Grade PVA		Water-Rinsed PVA	
	f-cryoPVAG	cryoPVAG	f-cryoPVAG	cryoPVAG
7	1.0 ± 0.2	1.2 ± 0.1	1.1 ± 0.1	1.5 ± 0.2
8	1.3 ± 0.2	1.8 ± 0.1	1.5 ± 0.2	2.0 ± 0.2
9	1.8 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.6 ± 0.2
10	2.3 ± 0.2	2.8 ± 0.3	2.7 ± 0.3	3.4 ± 0.5
11	2.9 ± 0.1	3.1 ± 0.1	3.1 ± 0.4	4.2 ± 0.2
12	3.6 ± 0.4	4.4 ± 0.2	4.1 ± 0.2	6.2 ± 0.3

<sup>a</sup>  $\beta = 2.0$ ; freezing temperature:  $-20^\circ\text{C}$ ; freezing duration: 18 h; thawing rate:  $0.3^\circ\text{C}/\text{min}$ .

bles entrapped in the gel bulk have performed as weakening fillers,<sup>23</sup> in the same way as in other foamed polymeric materials (foamed polyurethanes, rubbers, etc.).<sup>24</sup> Indeed, the increase in  $\beta$  values resulted in an increase in the volume fraction occupied by air bubbles in the fluid PVA foams and, as a consequence, in a decrease in  $G_0$  values of the gas-filled cryogels prepared from these fluid foams. However, this was observed until  $\beta$  reached  $\sim 2$ ; thereafter the f-cryoPVAGs of rather close strength were obtained (last two examples in Table II).

Further experiments also showed that, for the initial PVA water solutions over the studied concentration range (from 7 to 12 g/dL), f-cryoPVAGs with the most reproducible characteristics were obtained when  $\beta$  values of the whipped systems to be frozen and thawed were near 2. On the basis of this result, all subsequent studies of the influence of cryostructuring regimes on the properties of cryogels were conducted with fluid PVA foams of the foaming capacity  $\beta = 2.0 \pm 0.1$ . These PVA foams were formed by whipping the initial PVA solutions in a graduated vessel for the time required to achieve this  $\beta$  value. In other words, we compared the properties of f-cryoPVAGs fabricated on the basis of foams with virtually identical initial ratio between the gas and liquid phases.

The data of Table III demonstrate the effect of the initial polymer concentration on the rheological properties of the resultant foamed PVA cryogels. One may see the evident trend: the higher the initial polymer concentration in the solution to be whipped–frozen–thawed, the higher the

strength of cryostructured gel samples. This result certainly was due to the increase in rigidity of the polymer phase within the partitions between the air bubbles as the PVA concentration increases, which is a well-known fact for ordinary nonfoamed cryoPVAGs<sup>1–3,19,25</sup> and seen also from the comparative data for foamed cryogels. In Table III we also present the data on the rheological properties of cryoPVAGs and f-cryoPVAGs fabricated on the basis of technical-grade gelling polymer. One may clearly see the “deteriorative” influence of the admixtures existing in the nonpurified PVA on the gel strength. Note that such influence was somewhat higher for the foamed cryogels than for the nonfoamed ones. The weakening effect of the low molecular weight admixtures may obviously be explained as follows: the higher the content of these monomeric and oligomeric fractions in the initial gelling polymer, the lower its average molecular weight and, hence, the weaker (at the equal initial polymer concentration) the PVA cryogels formed, as repeatedly reported previously.<sup>1–5,19,20,25,28,31</sup> This relationship means that the admixtures existing in the technical-grade polymer have an adverse effect not only on the stability of fluid PVA foams, but also on the strength of resultant foamed and nonfoamed cryoPVAGs.

With regard to the influence of cryogenic treatment regimes on the properties of foamed PVA cryogels, we focused on two factors: (i) freezing temperature and (ii) thawing rate. The first factor controls the freezing rate and the phase state (the ratio between the frozen and unfrozen portions of the system undergoing freezing at a particular

**Table IV Influence of Freezing Temperature on the Rheological Properties of Foamed and Nonfoamed PVA Cryogels<sup>a</sup>**

Freezing Temperature (°C)	$G_0$ Values (kPa) of the Samples	
	f-cryoPVAG	cryoPVAG
-10	1.8 ± 0.2	2.0 ± 0.1
-20	2.8 ± 0.1	3.2 ± 0.3
-30	2.6 ± 0.3	3.2 ± 0.2

<sup>a</sup> Initial PVA concentration: 10 g/dL;  $\beta = 2.0$ ; freezing duration: 18 h; thawing rate: 0.3°C/min.

temperature), and the second factor has been earlier recognized<sup>3,21,22,26</sup> to be the key factor determining the main structural and physicochemical characteristics of cryoPVAGs.

The influence of freezing temperature over the range -10--30°C on the values of  $G_0$  for the foamed and ordinary nonfoamed PVA cryogels, when a "moderate" thawing rate (i.e., 0.3°C/min), was used is shown in Table IV. One can see that the samples frozen at -10°C were somewhat weaker than the respective f-cryoPVAGs and cryoPVAGs prepared at lower negative temperatures, whereas the samples frozen at -20 and -30°C showed very close strengths. In principle, one may expect an increase in the gel strength of f-cryoPVAGs with a decrease in freezing temperature. This expectation could be due to the following reasons: the lower the temperature, the higher the freezing rate and the higher the dynamic physical stresses arising in the course of solvent crystallization in a viscous polymer solution,<sup>27</sup> and, as a consequence, the more opportunities for the destruction of thin partitions between the air bubbles and the ensuing compactization of the heterogeneous structure of foamed cryogels. In reality, a similar marked increase in the values of shear modulus for f-cryoPVAGs was observed only on decreasing the temperature from -10 to -20°C, and freezing, at least to -30°C, no longer had an effect on the rheological properties of the foamed cryogels. These results are in good agreement with those reported elsewhere<sup>20</sup> for other PVA cryogels fabricated in a similar freezing temperature range. Those earlier results were explained<sup>28</sup> on the basis of nuclear magnetic resonance data about different extent of polymer concentrating in the unfrozen regions

(so-called unfrozen liquid microphase)<sup>29</sup> of macrofrozen samples. Thus, the trends inherent in the freezing/frozen storage temperature effects for the air-filled PVA cryogels were the same as for ordinary nonfoamed cryoPVAGs. Also the freeze-induced mechanical destruction of PVA foams, even if this phenomenon occurred, did not affect significantly the dependence of gel strength on freezing temperature.

Considerably more pronounced was the influence of thawing regimes. The experimental data summarized in Table V show a marked growth of  $G_0$  values for both foamed and nonfoamed PVA cryogels obtained by slower thawing. Such an effect, as already noted, was known for ordinary cryoPVAGs and, in this work, was found to be also true for f-cryoPVAGs. An especially high strengthening effect took place when very slow thawing (0.03°C/min) was used. In such a way we were able to prepare foamed cryogel samples ( $G_0 = 5.9$  kPa) that were even more rigid than the nonfoamed cryoPVAGs ( $G_0 = 3.2$  kPa) thawed-off at a 10-times faster rate (0.3°C/min) and, obviously, the rapidly (3°C/min) defrosted cryoPVAGs ( $G_0 = 1.4$  kPa). These results suggest an experimentally simple approach to the preparation of tough foamed PVA cryogels; namely, the thawing regimes should be as slow as possible.

#### Dynamics of the Formation of Foamed PVA Cryogels

In our previous research dealing with the dynamics of the freeze-thaw-induced gelation of water-PVA solutions we found that similar gel-formation proceeded in the course of thawing of frozen samples.<sup>21</sup> Such a cryotropic gel formation mainly occurred over the range of "high" negative tem-

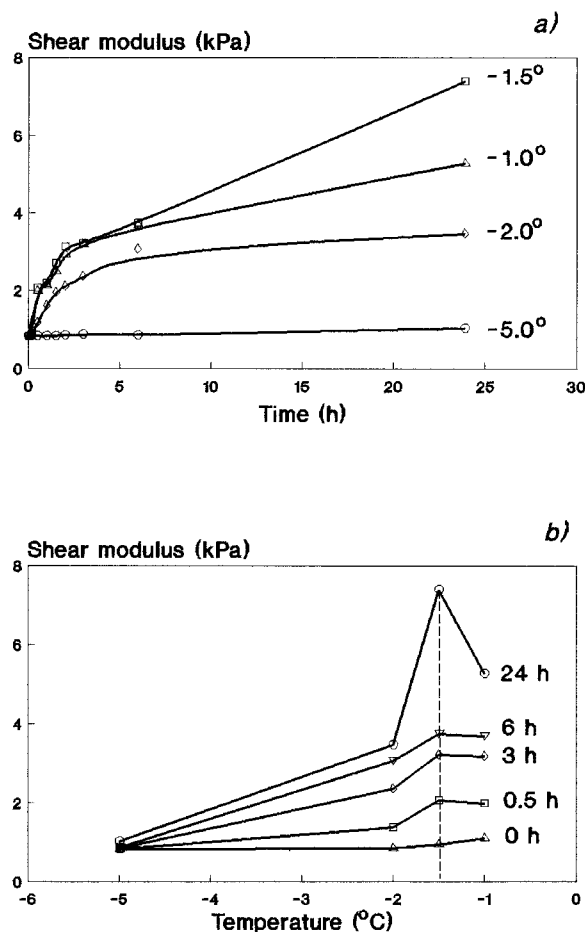
**Table V Effects of Thawing Rate on the Rheological Properties of Foamed and Nonfoamed PVA Cryogels<sup>a</sup>**

Thawing Rate (°C)	$G_0$ Values (kPa) of the Samples	
	f-cryoPVAG	cryoPVAG
~3	1.0 ± 0.2	1.4 ± 0.2
0.3	2.8 ± 0.1	3.2 ± 0.3
0.03	5.9 ± 0.9	9.4 ± 0.5

<sup>a</sup> Initial PVA concentration: 10 g/dL;  $\beta = 2.0$ ; freezing temperature: -20°C; freezing duration: 18 h.

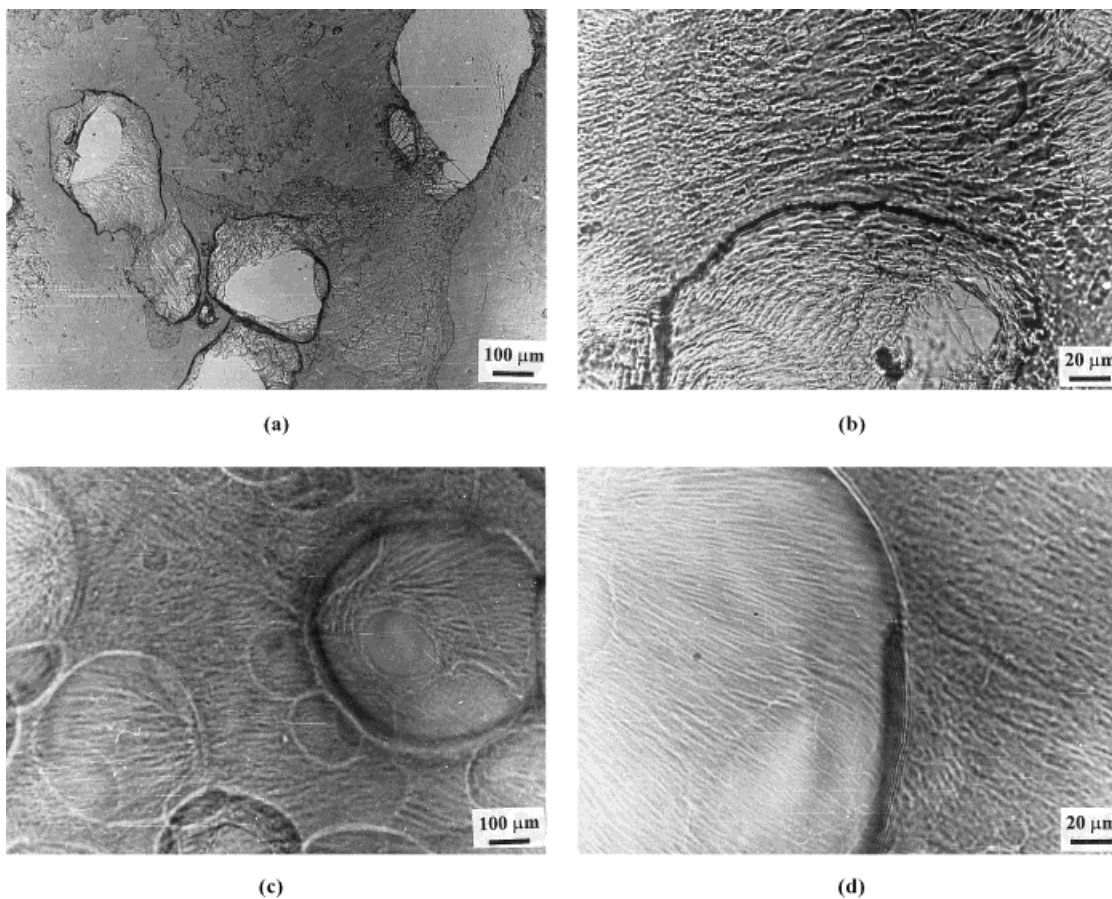
peratures (starting at about  $-5^{\circ}\text{C}$  the rate of gelation increased significantly), the extreme character of the temperature dependence was an inherent feature characteristic of the cryotropic gelation, and the maximum efficiency of gel formation was observed around  $-2^{\circ}\text{C}$ .<sup>26</sup> Based on these data, we implemented the same procedure as earlier to evaluate the rates of formation of foamed PVA cryogels; that is, freezing of the samples at  $-20^{\circ}\text{C}$  and their further incubation at one of the “high” negative temperatures already mentioned (see Experimental). The temperatures at which the kinetic runs were performed ranged from  $-5$  to  $-1^{\circ}\text{C}$ ; below this range the gelation processes were very slow, and above  $-1^{\circ}\text{C}$ , the samples began to thaw.

The kinetic curves obtained in those experiments are shown in Figure 3. A slow increase in the  $G_0$  values with time is seen for the samples incubated at  $-5^{\circ}\text{C}$ , whereas only  $3\text{--}4^{\circ}\text{C}$  higher, the gel strength grows many times faster. Extreme temperature dependence was an inherent feature of the dynamics of the process of interest (as in the case of ordinary nonfoamed PVA cryogels<sup>21,26</sup>), and the highest growth rate was observed at  $-1.5^{\circ}\text{C}$ . Similar temperature dependences of the efficiency of the formation of respective f-cryoPVAGs after incubation of the frozen samples for certain periods of time at sub-zero temperature within the studied range are shown in Figure 3b. The curve “0 h” corresponds to the samples that were removed from the cryostat chamber at the moment when the corresponding incubation temperature was reached; that is, either  $-5$  or  $-2$ , or  $-1.5$ , or  $-1^{\circ}\text{C}$ . One may see the extreme character of these dependences gradually progressing in time to a maximum point at about  $-1.5^{\circ}\text{C}$  (dashed vertical line in Figure 3b points to this temperature). Indeed, after 24 h of incubation of frozen samples at this temperature and subsequent fast thawing, the foamed PVA cryogels were about 1.4 times stronger than f-cryoPVAGs stored for the same time at  $-1^{\circ}\text{C}$  and  $\sim 2.1$  times stronger than the samples kept at  $-2^{\circ}\text{C}$ . These results showed that in the region of maximum gelation efficiency, even the difference in the incubation temperature as small as  $0.5^{\circ}\text{C}$  had a great impact on the properties of the gel materials under discussion. Note that the extreme temperature dependence was most pronounced when the cryostructuration was allowed to proceed for a sufficiently long period of time, 24 h in this particular case (cf., for instance,



**Figure 3** Dynamics of the formation of foamed PVA cryogels. (a) Variation of the gel strength as a function of the incubation time of frozen samples at subzero temperatures (initial PVA concentration: 10 g/dL;  $\beta = 2.0$ ; freezing temperature:  $-20^{\circ}\text{C}$ ; freezing duration: 18 h). (b) Temperature dependences of the  $G_0$  values for f-cryoPVAGs formed at different stages of incubation of the frozen samples at subzero temperatures.

traces “3 h”, “6 h”, and “24 h” in Fig. 3b), giving another evidence of that cryotropic gelation of PVA is a rather slow process even under the optimum temperature conditions. In general, we conclude that the major characteristic features of the dynamics of f-cryoPVAGs formation are virtually the same, with a slight ( $\sim 0.5^{\circ}\text{C}$ ) differences in optimum temperature, as for the nonfoamed PVA cryogels. In other words, cryotropic gelation of fluid PVA foams is controlled by the same regularities that were previously recognized<sup>21, 26</sup> for the freeze–thaw–induced gelation of homogeneous water PVA solutions.



**Figure 4** Light optical micrographs of the thin slices of f-cryoPVAGs prepared from the fluid PVA foams of different initial polymer concentration in the solutions to be whipped [8 g/dL (a, b) and 12 g/dL(c, d);  $\beta = 2.0$ ; freezing temperature:  $-20^{\circ}\text{C}$ ; freezing duration: 18 h, thawing rate:  $0.03\text{ Z}^{\circ}\text{C}/\text{min}$ ].

### Structure of Foamed PVA Cryogels

The morphology of the f-cryoPVAGs was examined by light optical microscopy. For better visualization of the structure, the thin sections of the gel samples were stained with Congo red (see Experimental), which is well known<sup>30</sup> to form stable dyed conjugates with PVA. The main goal of these studies was to trace the cellular texture of the foamed gel materials of our interest, although it turned out that under the microscopy conditions employed we were even able to distinguish some features of the porous “architecture” of the partitions between the air bubbles.

The micrographs in Figure 4 demonstrate the structure of f-cryoPVAGs formed on the basis of 8- and 12-g/dL water PVA solutions (rheological characteristics of these samples are presented in Table III). Each specimen is given at two magni-

fications to better show its cellular texture (Figs. 4a and 4c) and the porosity of the gel phase proper (Figs. 4b and 4d). Thin sections of foamed PVA cryogels prepared under the same and other cryogenic conditions from the polymer solutions of other concentrations (Table III) were also examined. However, the most marked distinctions were observed for the samples obtained from solutions of considerably different initial PVA concentration; therefore only the structure of these specimens is discussed here.

In the micrograph of f-cryoPVAG prepared from an 8-g/dL solution of the polymer (Fig. 4a) one can see significantly fewer number of gross pores produced by the air bubbles than in the case of the sample formed from 12-g/dL PVA solution (Fig.4c), even though both foamed systems had virtually equal foaming capacities of  $\beta \approx 2$ ; that

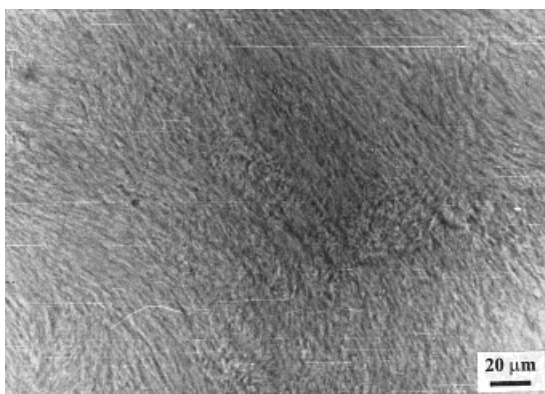


is, the volumes of liquid and gas phases in the initial fluid PVA foams were very close. The decrease in the number of these large pores in the less concentrated cryogel could suggest that the foam underwent partial destruction during the freeze–thaw treatment. The deformed nonspherical shape of the bubbles in Figure 4a adds validity to this explanation. At the same time, for the 1.5-times more concentrated f-cryoPVAG, we observed a considerably higher number of the bubbles, which had a virtually nondeformed spherical geometry (Fig. 4c). In both cases, however, the bubble-size distribution was very broad (from  $\sim 50$  to  $\sim 250$   $\mu\text{m}$  and larger). We believe that the rather weak gel phase in the partitions of the low-concentrated (in respect to the gel-forming polymer) f-cryoPVAG lacks elasticity sufficient to maintain the spherical shape of very large pores, which therefore appears deformed. In contrast, as the increase in the initial PVA concentration led to the strengthening of the gel phase between the air bubbles, the elasticity of these partitions became high enough (beginning with the 10-g/dL specimens) to maintain the nondeformed shape of the large pores. Such sufficiently elastic foamed PVA cryogels entirely retained long-term “buoyancy” when placed in water (see the results of the “buoyancy test”).

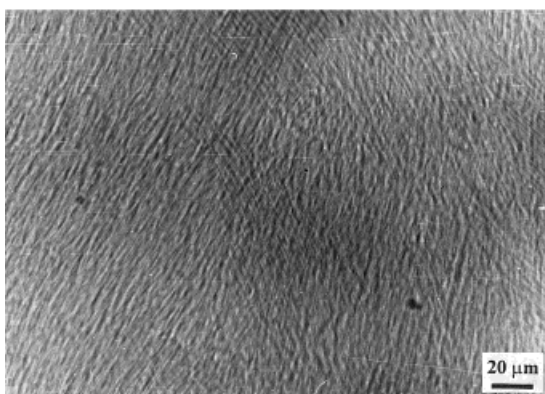
The first data of light-optical microscopy investigations of PVA cryogels were published 15 years ago in the work by Yokoyama et al.,<sup>31</sup> who studied the effect of the number of iterative freeze–thaw cycles on the pattern observed in polarization microscope. The authors found that the amount and the size of PVA microcrystallites increased with cryogenic cycling; however, no visible structural elements were distinguished after the first freezing–thawing cycle. All other available significant information on the porous structure of PVA cryogels was obtained with scanning and transmission electron microscopies, when the samples were prepared by various techniques and always dehydrated, thus affecting to various extent the “reality” of the observed pattern and pore size.<sup>1,13,14,16,20,31–35</sup> The microscopy studies carried out in this work allowed us to reveal (using the Congo red staining of the gel sections) the character of porosity of the water-swollen gel phase of cellular f-cryoPVAGs and to measure the size of some structural elements in such heterogeneous gels. These elements are seen in the micrographs of Figure 4, especially at higher magnification (Figs. 4b and 4d), as a combination of

alternating dark (stained polymer phase) and light (water-filled pores) strands of various length. The thickness of dark strands (gel matter) was evaluated to be from  $\sim 0.5$  to  $2$   $\mu\text{m}$ , and the width of the respective pores was observed to be  $1$ – $4$   $\mu\text{m}$ . It should be noted that the polymer strands of very close size were also found.<sup>20</sup> in the structure patterns obtained with scanning electron microscopy, when the samples for observation were prepared using critical point drying technique; it is noteworthy that prior to drying, those samples were fixed by chemical cross-linking with an excess of glutaric aldehyde. However, the morphology of the pores (with respect to the gel phase they could be considered as macropores) in the scanning electron microscope pictures was somewhat different. These pores were mainly oval in shape, and their size was, on the average, larger. Hence, one may conclude that the drying procedures used in the SEM studies just described affected the architecture of the macropores rather than that of the supramolecular polymer strands.

Such a highly porous structure of PVA cryogels is also seen in the light optical patterns of the nonfoamed cryoPVAGs, when the same sectioning/staining preparative procedure was used. Micrographs in Figure 5 show the morphology of 12-g/dL PVA cryogels frozen under identical conditions ( $-20^\circ\text{C}/18$  h), but thawed at either moderate (a) or slow (b) rates. We found that the first of them possessed a rather irregular structure in respect to pore size and shape (Fig. 5a), whereas the slowly-thawed PVA cryogel had a more regular structure of alternating gel strands and the pores similar (in size and shape) to those constituting the gel partitions between the air bubbles in foamed cryogels (Fig. 4). Note that the latter ones were also thawed with a slow rate of  $0.03^\circ\text{C}/\text{min}$ . Hence, one may draw a conclusion that in the course of a slow thawing, when the still-frozen system resides at sub-zero temperatures for a long time, a more “perfect construction” of the PVA cryogel arose apparently due to the ice recrystallization phenomena. Such a more perfect structure undoubtedly facilitated, along with other factors discussed above, the higher mechanical strength of the slowly thawed cryoPVAGs as compared to the faster-thawed ones.



(a)



(b)

**Figure 5** Light optical micrographs of the thin slices of nonfoamed cryoPVAGs prepared from the 12-g/dL water polymer solution (freezing temperature:  $-20^{\circ}\text{C}$ ; freezing duration: 18 h) and thawed with different rates: (a) 0.3 and (b)  $0.03^{\circ}\text{C}/\text{min}$ .

## CONCLUSIONS

To the best of our knowledge this work was the first study dealing with the foamed poly(vinyl alcohol) cryogels. Here we found that the rheological properties and macrostructure of these gel materials were controlled, on the one hand, by the same factors as for the ordinary nonfoamed PVA cryogels (i.e., initial polymer concentration and freezing–thawing regimes) and, on the other hand, by the particular conditions of generation of fluid PVA foams. The presence of low-molecular-weight admixtures in the initial polymer solution appeared to be a rather important feature, since the admixtures were capable of decreasing the stability of fluid PVA foams and weakening the resultant foamed as well as nonfoamed cryogels.

In this regard, the influence of the foam-stabilising additives (surfactants, in particular) on the properties of foamed PVA cryogels is the target of our further studies.

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