

Cryostructuring of Polymer Systems. XXX. Poly(vinyl alcohol)-Based Composite Cryogels Filled with Small Disperse Oil Droplets: A Gel System Capable of Mechanically Induced Releasing of the Lipophilic Constituents

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ABSTRACT: Composite heterophase poly(vinyl alcohol) (PVA) cryogels containing entrapped small droplets of Vaseline oil have been prepared and studied. Such oil-filled cryogels were formed via freeze–thaw treatment of freshly prepared oil-in-water emulsions containing varied volume fraction of lipophilic phase, and the influence of the amount of this phase, as well as the effects of freezing conditions on the physicochemical (shear moduli) and thermal (gel fusion temperature and fusion enthalpy) characteristics of resulting composites have been explored. It was shown that over certain range of PVA concentrations in aqueous phase and a range of volume fraction of the hydrophobic phase its microdroplets performed as “active” fillers causing an increase in both the gel strength and the heat endurance of composites. The light micros-

copy data on the morphological features of such filled PVA cryogels revealed the effect of diminution in size of oil droplets entrapped in the gel matrix as compared with the initial emulsions. This effect can be explained by the disintegrating action of crushing and shear stresses arising upon the system freezing and growth of ice crystals. The oil-filled PVA cryogels were found to be capable of gradually releasing the lipophilic constituents (the Rose hips oil, in this case) in response to the cyclic mechanical compression. © 2010 Wiley Periodicals, Inc. *J Appl Polym Sci* 117: 1332–1349, 2010

Key words: PVA; composite cryogels; freezing–thawing; liquid filler; vaseline oil; rose hips oil; mechanically induced oil release

INTRODUCTION

Poly(vinyl alcohol) cryogels (PVACGs) are known to be formed via freeze–thaw treatment of concentrated aqueous or DMSO solutions of the polymer.^{1–5} The conditions of such cryogenic processing, either in a single or multiple cycles of freezing – frozen storage – thawing, strongly affect the structure and properties of the final cryogels. Macroporosity, i.e., formation of a system of interconnected pores with cross-section up to several micrometers, is a characteristic feature of PVACGs. These large pores are appeared upon melting of the solvent crystals, ice in the case of aqueous systems, so the crystals act as porogens in the course of PVA cryotropic gel-formation.^{3,6–8} Physicochemical properties and structural features of PVA cryogels have been studied thoroughly, and their dependence on various factors, including both the characteristics

of the initial polymer and the regimes of cryogenic treatment, has been established.^{1–8}

PVACGs have attracted considerable attention for recent years in applied areas, especially in biotechnology as carriers of immobilized enzymes and cells, matrices for immunosorbents, etc.^{3,5,9–13} and also as materials of biomedical interest, e.g., gels for controlled drug release, covers on wounds and burns, artificial cartilages, components of model heart implants, phantoms for calibration of NMR tomographs, and ultrasound equipment.^{1–5,14–18}

Along with PVACGs originating simply from the polymer solutions without any additives, complex and filled (composite) PVA cryogels were prepared, investigated, and described.^{3,5,12} The former contain various soluble compounds, both of low- and high-molecular weight, whereas the latter include such dispersed matter, as solid particles, semi-solid gel granules, microbial cells, air bubbles, etc., entrapped (immobilized) in the matrix of continuous phase—macroporous PVA cryogel. At that, several interesting effects of the fillers on the integral properties and morphology of filled PVACGs have been established. For instance, it was shown in our previous studies of this series that the rigidity of respective composite PVA cryogels depends not only on the

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amount and the particle size of the discrete phase added, but also on the porosity characteristics of the filler. Its pore size governs the possibility for PVA macromolecules to penetrate into the particle bulk and thus to form the “network-in-network” structures, and the latter phenomenon results in a significant increase in the gel strength.^{19–21} If the polymeric chains of the continuous phase are cross-linked with auxiliary agents by additional stable hydrogen bonds, apart from their “own” PVA-PVA H-bonding, this causes a significant increase in the toughness of composite materials. This effect was recently observed for organic–inorganic hybrid PVA cryogels prepared via combination of cryotropic gelation and sol-gel techniques.²² It was also revealed that the rigidity of composite PVACGs grew considerably when ion-exchange resins in a strong acidic or alkaline form were used as fillers, since such resins are capable of ionizing PVA hydroxyl groups thus facilitating the formation of tight ionic contacts between the continuous (PVA cryogel) and discrete (filler) phases.^{23,24} On the contrary, a decrease in the gel strength took place when solid hydrophobic fillers were used, whose particles were incompatible with aqueous PVA solution and thus unable of forming efficient adhesion contacts with the hydrophilic phase of the resulting cryogel.^{20,21} At last, for the gas-filled (foamed) PVA cryogels, which were formed upon the freeze–thaw treatment of whipped PVA solutions and contained small air bubbles entrapped in cryogel matrix, it was found that the properties and structure of such composite PVACGs depended crucially on the stability of the initial liquid PVA foam²⁵ and also on the presence of solutes capable of manifesting surfactant properties.²⁶

At the same time, rather limited data are only available on the composite PVACGs containing liquid disperse fillers. It is clear that such fillers can be either water-immiscible, i.e., hydrophobic, or hydrophilic liquids formed upon phase separation owing to thermodynamic incompatibility of PVA and the other water-soluble polymer in a common medium—a system similar to the well-known Albertson’s aqueous two-phase polymer systems.²⁷ Whereas the latter type of composite PVA cryogels, e.g., those prepared from mixed solutions of PVA and oligoethyleneglycols,^{28,29} was obtained and explored, the former type was not, to the best of our knowledge, so far reported in scientific literature. It is thought that similar filled PVACGs containing liquid hydrophobic discrete phase can be of both fundamental and applied interest, for instance, as potential wound healing or cosmetic dressings carrying medicinal or cosmetic oils, respectively.³⁰ Therefore, this study was aimed at preparing PVA-cryogel-based composites filled with small dispersed droplets of Vaseline oil emulsion (as model liquid

hydrophobic dispersed particles) and studying the influence of such filler on the properties and morphological features of the resulting Vaseline oil-filled PVA cryogels (vof-PVACGs).

EXPERIMENTAL

Materials

PVA with molecular weight of 86 kDa and deacetylation degree of 99–100% (Acros Organics, Belgium) was used in the work for preparing both nonfilled (reference) and composite PVA cryogels.

Medicinal Vaseline oil with density 0.86 g/cm³ and viscosity 0.135 ± 0.007 Pa s (23°C) was purchased from Tatkhimfarmpreparaty Ltd. (Kazan, Russian Federation). This oil solidified over the temperature range of –8 to –10°C. Rose hips oil with density 0.93 g/cm³ and viscosity 0.043 ± 0.002 Pa s (23°C) was purchased from Altaivitaminy Ltd. (Biisk, Russian Federation). This oil solidified over the temperature range of –15 to –18°. The Congo red dye (Aldrich Chemical Co., USA), gelatine (photo quality), phenol (pure for analysis), glycerol (pure for analysis) (all Reakhim Co., Russian Federation) were used without additional purification. All aqueous solutions were prepared using deionized water.

Methods

PVA solutions were made as follows. The calculated amount of PVA was suspended in the required volume of water; the mixture was stored for 15 h at room temperature for the polymer to swell. Then the suspension was heated for 1 h under stirring in a boiling water bath for complete PVA dissolution. The sample was weighed before and after heating, and the amount of evaporated water was compensated. The obtained solution was filtered; warm (ca. 50°C) PVA solution was then used for making compositions with Vaseline or Rose hips oils. Oil was introduced in amounts of 5–45 wt % accounting for the total mixture (in the terms of oil volume fraction (φ) the range of 0.058–0.523). Then the composition was subjected to intense stirring (ZMIB homogenizer, Kiev, former USSR) at 1600 rpm for 2 min followed by the ultrasonic treatment (UM-1 ultrasonic bath, UNIMA, Poland) for 20 min before freezing of the resulting emulsion.

Dynamic viscosities of PVA solutions and respective emulsions were measured with Type BH2 Hoesppler falling ball viscometer (MLW, former GDR) at 23.0 ± 0.1°C (U10 Ultrathermostat; MLW, former GDR).

The PVACG and vof-PVACG samples for measuring their physicomechanical characteristics were formed in sectional duralumin moulds with inner

diameter 15 mm and height 10 mm. The cryogels used for fusion temperature measurement were prepared in transparent poly(ethylene) test tubes with inner diameter 10 mm. The test tubes were filled with 5 mL of respective liquid compositions and a stainless steel ball with diameter of 3.5 mm and weight of 0.275 ± 0.005 g was placed on the bottom of each test tube. The containers and the test tubes were placed into the chamber of precision programmable cryostat FP 45 HP (Julabo, Germany), where the samples were frozen and kept at the desired sub-zero temperature for 12 h. Further the temperature was raised to $+20^\circ\text{C}$ at the rate of $0.03^\circ\text{C}/\text{min}$ controlled by the cryostat microprocessor.

Apparent instantaneous shear modulus (G_0 ; characterizes the elastic properties of the material) and shear modulus for the 30-min-loading (G_{30} ; reflects the plastic behaviour of the material) were determined with the technique used for the characterization of rheological behavior of diverse nonfilled and filled PVA cryogels and described in detail in Refs. ^{6,19-22,31}. The measurements were performed at $21 \pm 1^\circ\text{C}$ by penetration of a spherical punch (5 mm dia.) into a gel sample at constant load of 4.9×10^{-3} N. To avoid drying, the samples were covered with a thin layer of silicone oil. The values of G_0 and G_{30} were computed from respective creep curves with the method by Lee and Radok³² using the formula:

$$G = \frac{3}{16} \cdot \frac{P}{R^{0.5} \times h^{1.5}},$$

where P is load (N), R is the punch radius (m), and h is the depth of punch penetration into the gel (m).

Gel fusion temperatures (T_f) were determined as described elsewhere.^{7,8,22,23} In brief, the sealed polyethylene tube, where cryogel with metal ball at the bottom has been formed, was placed upside down into a water bath. The temperature was increased with the rate of $0.4 \pm 0.1^\circ\text{C}/\text{min}$. The gel fusion point was determined as the temperature when the ball fell down on the stopper of the test-tube after passing through the fusing gel.

Shear moduli and cryogel fusion temperatures were measured for four parallel samples; the samples were obtained in three to five independent experiments (this also relates to morphological studies, X-ray analysis, and mechanically induced oil release tests). The results obtained were averaged.

The cryogels' morphology was studied with optical microscope Axiolab Pol (Carl Zeiss Jena GmbH, Germany) equipped with video-recording system (Sony, Japan). Thin (6–8 μm) sections of PVACGs and vof-PVACGs were prepared using cryomicrotome Shandon Cryotome (Thermo Electric Corp., UK). Each section was placed on the microscope glass, which was then immersed for 10 s into 1%

aqueous solution of Congo red for staining. The excess of the dye was rinsed with water; the excess of water was removed with filter paper. Then one drop of "fixing medium" (solution of 1 g of gelatine in 12 mL of 50% aqueous glycerol and 0.2 g of phenol as a bacteriostatic agent) was applied on to the section and the latter was sealed with a cover glass. Prior to the studies the samples were stored at 4°C in a closed vessel.

The dimensions of the oil droplets (number average and weight average diameters – D_n and D_w , respectively) in the o/w emulsions were determined with optical microscopy. The following formulas were used for calculations:

$$D_n = \left(\frac{\sum(N_i \times D_i^3)}{\sum N_i} \right)^{1/3}, \quad D_w = \left(\frac{\sum(N_i \times D_i^6)}{\sum(N_i \times D_i^3)} \right)^{1/3};$$

where $\sum N_i$ values ranged from 100 ± 5 to 200 ± 10 (depending on the number of droplets in the image area). The polydispersity index was determined as $k = D_w/D_n$.

X-ray diffraction studies were carried out using D8 Advance diffractometer (Bruker, Germany) ($\lambda[\text{CuK}\alpha] = 1.5418 \text{ \AA}$) in transmission mode at room temperature. To prevent drying of the hydrogels during X-ray diffraction experiments, the samples were placed into hand-made containers (the contributions of the latter were subtracted from the total intensities). Besides, the diffraction curves for pure H_2O and pure PVA were recorded under the same conditions. The background was approximated by straight baseline.

The experiments on the mechanically induced release of lipophilic constituents from the oil-filled PVACGs were performed as follows. The composites were formed as cylindrical samples (diameter 15 mm, height 10 mm) originating from the o/w-emulsions based on 80 g/L PVA solution and 20 vol % ($\varphi = 0.2$) of the Rose hips oil. Each gel cylinder was submerged in 10 mL of water, and then the gel sample was either incubated there without any handling (reference sample), or was subjected to a cyclic (~ 1 Hz) mechanical compression with a load of about 0.1 N for 10 or 30 min. After that the liquid phase was decanted, filtered through a paper filter, and UV-spectrum of the solution was recorded using Specord M40 (Carl Zeiss Jena, former GDR) UV-VIS spectrophotometer.

RESULTS AND DISCUSSION

Characterization of emulsions prepared by dispersing of vaseline oil in aqueous PVA solutions

It is well known that incompletely deacylated poly(vinyl alcohols), i.e., those containing 10–30 molar % of residual O-acyl, routinely O-acetyl groups, are

widely used for the stabilization of o/w emulsions, e.g., in emulsion polymerization processes, whereas the more hydrophilic highly deacylated PVAs possess poorer emulsifying abilities.³³ At the same time, PVA cryogels can be fabricated from the latter type of polymer only, and sufficiently rigid cryogels are formed via the freeze–thaw treatment of rather concentrated, usually >50 g/L, solutions of such highly deacylated PVAs.^{1–5,31} Therefore, creation of vof-PVACGs required finding the conditions for preparing stable enough emulsions consisting of small droplets of Vaseline oil dispersed in aqueous PVA solutions. Preliminary experiments showed that vof-PVACGs with physicochemical properties suitable for the subsequent rheological measurements were formed when the polymer concentration in initial aqueous solution was not lower than 60 g/L (for PVA with molecular weight of 86 kDa), otherwise the final cryogels were rather weak. On the other hand, polymer solutions with concentration of PVA more than 120 g/L had so high viscosity that it caused serious experimental difficulties for reproducible preparation of the respective emulsions. Therefore, all our further studies were accomplished using solutions with PVA concentration within the range of 60–120 g/L. At the same time, preparing such emulsions was found to be possible only for Vaseline oil fractions below a certain value, which depended on PVA concentration in the continuous phase. Above such boundaries, this polymer has coagulated upon mixing the respective aqueous PVA solution with the liquid oil. It is well known³⁴ that inversion of emulsions, e.g., the inversion of o/w into the w/o, commonly occurs in the disperse systems containing low-molecular-weight emulsifiers, e.g., surfactants, above a certain ratio of hydrophilic and hydrophobic constituents. On the contrary, in case of such high-molecular-weight emulsifier as virtually completely deacylated PVA, the coagulation process occurred instead of phase inversion. We have found that such coagulation ensued for the emulsions based on 60, 80, and 100-g/L PVA solutions when ϕ exceeded 0.523, 0.349, and 0.233, respectively. For the more concentrated PVA solutions (120 and 140 g/L) such boundary ϕ values were smaller still, namely, 0.174 and 0.116. In other words, a “coagulation ability” of Vaseline oil regarding to the highly deacylated PVA grew with the increase in polymer concentration in aqueous continuous phase. We suppose that this effect was induced by the hydrophobic interactions of the surface of hydrocarbon oil droplets and the carbochain core of PVA. Such interactions can change somewhat the conformation and therefore the hydration of PVA coils near the interface,³⁵ thus affecting the solubility of this amphiphilic polymer and causing its partial coagulation.

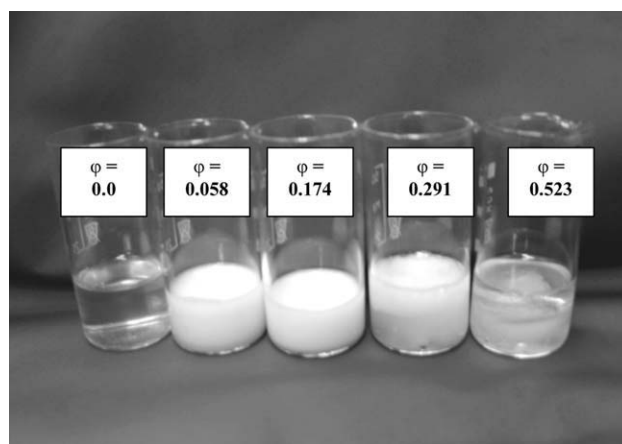


Figure 1 The effect of volume fraction (ϕ) of lipophilic phase in the composition of o/w-emulsions prepared from 100-g/L PVA aqueous solution and Vaseline oil additives under the conditions indicated in “Methods.”

Photograph in Figure 1 demonstrates this phenomenon for the case of 100-g/L PVA solution. The second and the third, from left to right, beakers contain milky-white stable emulsions with the oil volume fraction ϕ of 0.058 and 0.174, respectively. The fourth sample ($\phi = 0.291$) is the “boundary” system, where a certain amount of separated bottom transparent phase of PVA solution can be seen. The rightmost beaker contains heterogeneous system ($\phi = 0.523$) consisting of the transparent oil phase separated from the hydrated PVA coagulum. It is clear that vof-PVACGs with uniform enough distribution of Vaseline oil droplets entrapped within the hydrophilic continuous gel phase can be prepared only from emulsions like the second and third ones in this figure.

In view of the necessity of reproducible preparation of vof-PVACGs originating from such o/w emulsions, the stability of the latter is an important issue, as the emulsions should be stable for the time required to freeze the samples. We found that emulsions prepared under the conditions described in the experimental part were stable for at least 1–2 h, i.e., no visible formation of separated liquid layers was detected. This time was absolutely sufficient for pouring these fluid dispersions into moulds, placing them into the cryostat and completing the samples’ freezing at desired sub-zero temperature. Otherwise, in case of emulsions with low stability, systems of ambiguous morphology, namely, a mixture of macroscopically split oil and aqueous phases additionally containing dispersed oil microdroplets, will be frozen, and this will lead to the formation of undesired extremely heterogeneous cryogels. The 1–2-h “time of stability” in our case allowed measuring the dynamic viscosities of the emulsions, and these values served as the physicochemical characteristics of

TABLE I
Dynamic Viscosities^a of the Emulsions "Vaseline Oil – Aqueous PVA Solution"

Volume fraction (ϕ) of Vaseline oil in an emulsion ^b	Viscosity (Pa s) of the emulsions prepared by Vaseline oil dispersing in aqueous PVA solutions of various polymer concentration (g/L) ^c		
	60	80	100
0	0.090 ± 0.003	0.260 ± 0.007	0.650 ± 0.006
0.058	0.110 ± 0.002	0.320 ± 0.005	1.090 ± 0.009
0.116	0.125 ± 0.005	0.370 ± 0.002	1.380 ± 0.004
0.174	0.150 ± 0.005	0.425 ± 0.005	1.590 ± 0.008
0.233	0.160 ± 0.009	0.480 ± 0.004	1.819 ± 0.031
0.291	0.205 ± 0.011	0.530 ± 0.005	CP
0.349	0.259 ± 0.010	0.843 ± 0.013	CP
0.407	0.365 ± 0.007	1.161 ± 0.018	CP
0.465	0.482 ± 0.012	CP	CP
0.523	0.699 ± 0.021	CP	CP

^a Measured at $23 \pm 0.2^\circ\text{C}$; the viscosity of Vaseline oil at this temperature was equal to 0.135 ± 0.007 Pa s.

^b The values correspond to the weight % series: 0, 5, 10, 15, 20, 25, 30, 40, and 45%.

^c CP—The coagulation of the polymer was observed.

the colloid systems under consideration. Table I summarizes the obtained data, when the 60–100-g/L aqueous PVA solutions were used as continuous phase for the preparation of emulsions. The systems with higher polymer concentrations were too viscous for using the viscometer at our disposal. However, as it was required to reveal only the character of PVA concentration influence on the rheology of respective emulsions, the viscometry data obtained for the systems based on the 60–100-g/L PVA solutions turned out to be enough for demonstrating this character.

The trend observed was as follows: the higher the oil fraction in the system, the higher the viscosity of an emulsion. Similar effect of increase in viscosity of concentrated emulsions with the increase in the amount of discrete phase is known,³⁴ and it is explained by the growth of attractive interactions between the droplets.³⁶ For the emulsions studied, the rise in the viscosity with the increase of ϕ was more pronounced for continuous phases with higher PVA concentrations. For instance, the viscosities of emulsions with $\phi = 0.116$ were higher as compared with the viscosities of respective (60, 80, and 100 g/L) homogeneous PVA solutions by the factors of 1.39, 1.42, and 2.12, respectively; then a twofold increase in the filler's fraction ($\phi = 0.233$) gave the following series of such factors: 1.78, 1.85, and 2.80. So, a smaller amount of oil was required to cause stronger effect for the o/w emulsions with polymer-rich aqueous phase.

A sharp rise in the viscosity growth with PVA concentration can be seen in Figure 2 at ~ 90 -g/L polymer concentration for disperse systems ($\phi = 0.116$ and 0.233) compared with the case of oil-free PVA solutions ($\phi = 0$). Therefore, the formation of PVA adsorption layers, which are known^{33,37} to be arranged at the droplets' interfaces in o/w emul-

sions, can also be responsible for the effect of viscosity growth. As the thickness of such ordered layers grows with the increase in concentration of polymeric emulsifier,³⁷ thus resulting in an increase of the volume fraction of the regions with reduced fluidity, the viscosity of disperse system rises, too.

The average size and size distribution of discrete phase particles, i.e., oil droplets in the case of o/w-type emulsions, are certainly also significant factors influencing the physicochemical properties of emulsions.³⁴ The micrographs in Figure 3 show the patterns observed with a light microscope for the "Vaseline oil + aqueous PVA solution" emulsions

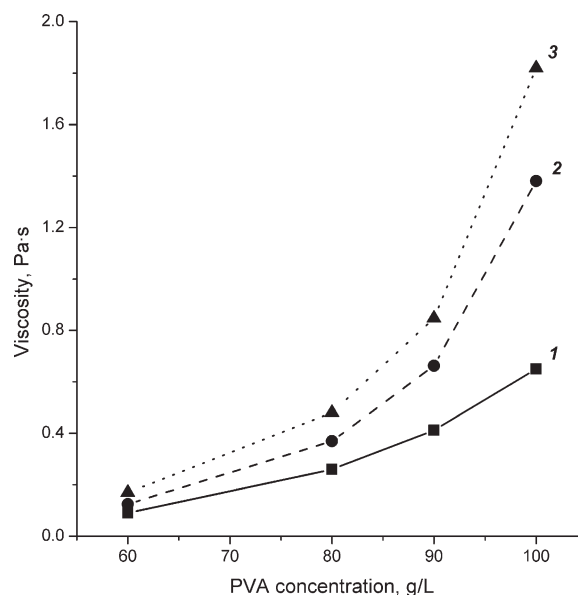


Figure 2 Dependence of viscosity of PVA solutions (1) and o/w emulsions with different Vaseline oil fraction (2: $\phi = 0.116$, 3: $\phi = 0.233$) on the polymer concentration in aqueous phase (experimental errors for the viscosity values are given in Table I).

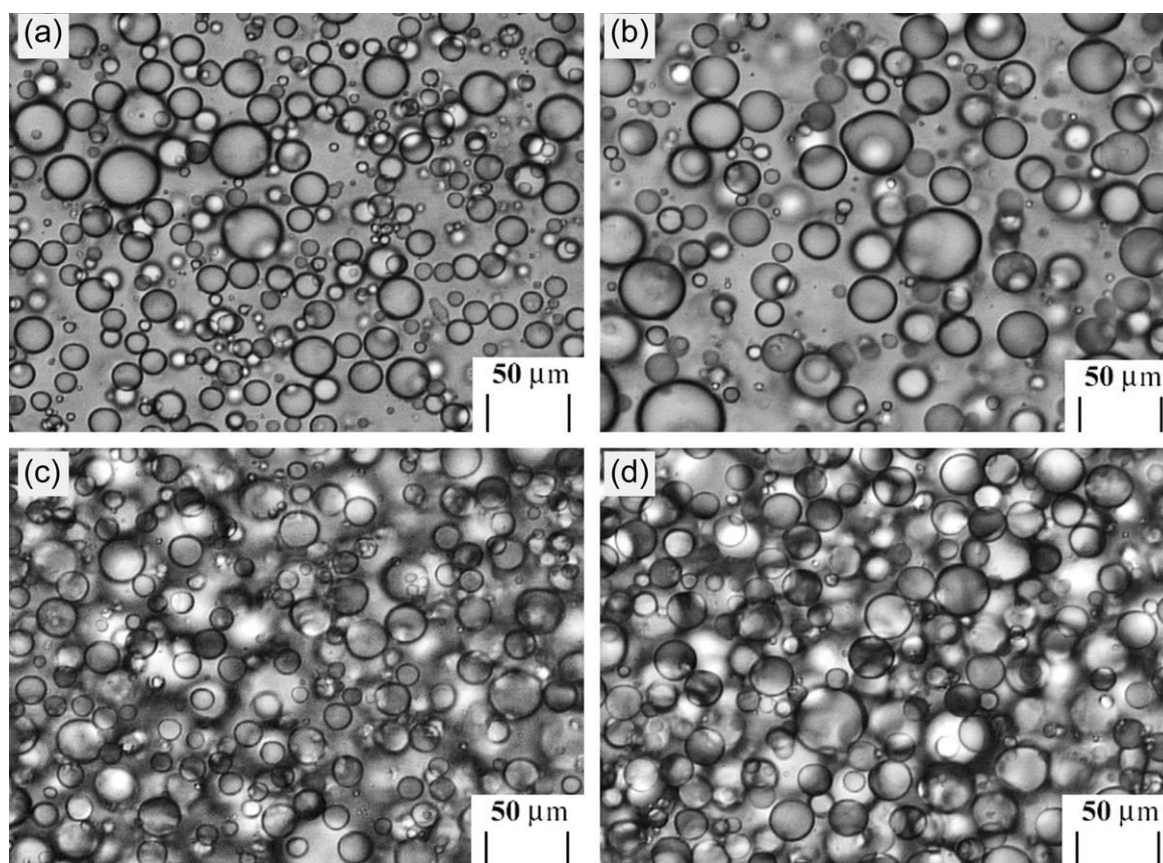


Figure 3 Optical microscopy images of the Vaseline oil emulsified in PVA aqueous solution: (a) [PVA] = 60 g/L, $\phi = 0.058$; (b) [PVA] = 80 g/L, $\phi = 0.058$; (c) [PVA] = 60 g/L, $\phi = 0.291$; (d) [PVA] = 80 g/L, $\phi = 0.291$.

prepared under the conditions described earlier, when both the stirring rate and stirring duration were the same for all the emulsions thus fabricated.

In the case of [PVA] = 60–100 g/L, the emulsions were rather polydisperse ones, and the size of oil droplets did not exceed 50–55 μm . Larger droplets (on the average) were observed in emulsions with higher PVA concentration in the continuous phase, given the same oil fraction. Thus, at $\phi = 0.058$ the size of droplets was within the range of 3–45 μm and their weight average diameter D_w was 27 μm at [PVA] = 60 g/L [Fig. 3(a)], whereas for the case of [PVA] = 80 g/L [Fig. 3(b)] the droplets' size was over the range of 5–55 μm and D_w was 37 μm . In other words, formation of smaller oil droplets was somewhat suppressed in the more concentrated and, hence, more viscous aqueous media, as compared with the continuous phase of lower viscosity. When it was possible to perform "manual" measurements of the droplets' size (namely, at $\phi = 0.058$; Fig. 3), we found that the size distribution was narrower (the polydispersity index k was 1.71) in the less concentrated continuous phase ([PVA] = 60 g/L) as compared with the more concentrated system ([PVA] = 80 g/L), where k was 3.74. This is illustrated by the diagrams in Figure 4 reflecting the

influence of PVA concentration in the continuous phase on the character of particle size distribution in the emulsions studied.

The emulsion with less concentrated continuous phase [Fig. 4(a)] mainly contained smaller Vaseline oil droplets in contrast to emulsion with higher PVA concentration [Fig. 4(b)], for which wider droplet size distribution was found. Note also that such a trend is known for PVAs used as emulsifying agents in emulsion polymerization, where higher PVA concentration in the continuous phase resulted in a wider size distribution of the resulting polymer particles, e.g., the final product of divinylbenzene polymerization.³⁸

As for the influence of oil phase concentration in the systems studied, it was found that increasing the oil fraction in these emulsions has led to the growth of droplets' amount and to a decrease in their average size. Thus, at $\phi = 0.291$ the droplet size was over the range of 3–30 μm at [PVA] = 60 g/L [Fig. 3(c)] and of 5–50 μm for the case of [PVA] = 80 g/L [Fig. 3(d)], and the variations of the particle size with ϕ increase were not so drastic as to affect significantly the stability and rheological properties of similar polydisperse emulsions. Evaluation of the droplet size at yet higher oil fraction using light

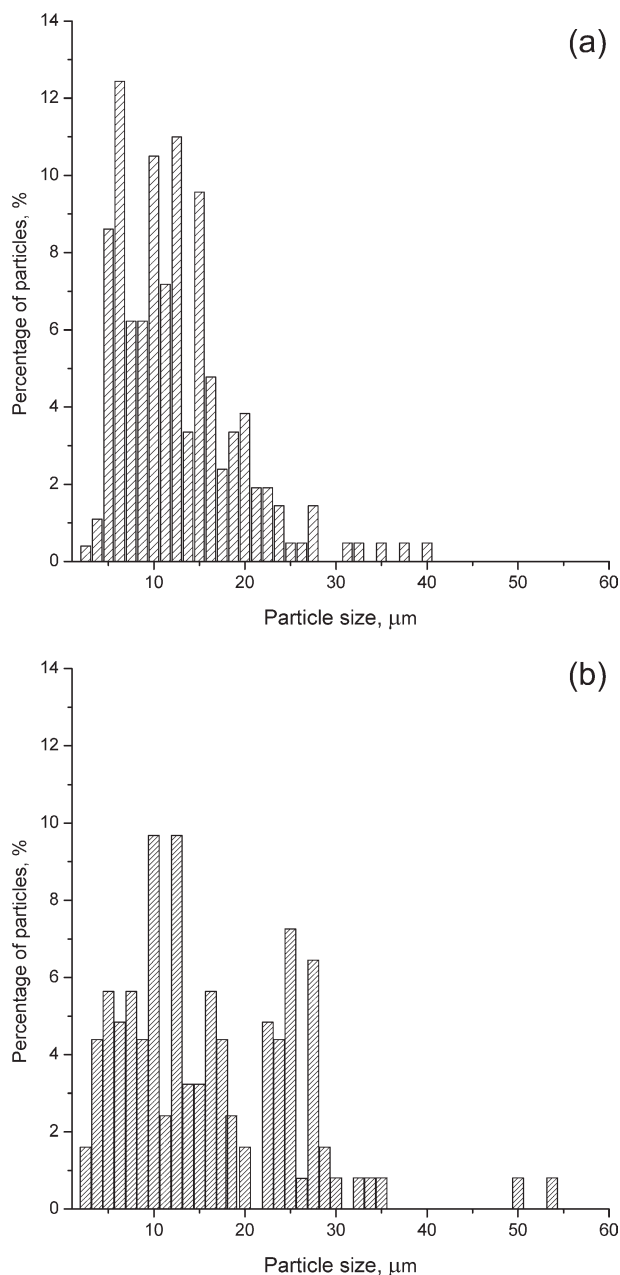


Figure 4 Diagrams of particle size distribution in the o/w emulsions with different PVA concentration in aqueous phase: (a) [PVA] = 60 g/L, $\phi = 0.058$; (b) [PVA] = 80 g/L, $\phi = 0.058$.

microscopy was impossible because of huge amount of the particles and strong overlapping of their images in the microscope field of view.

Physicomechanical properties of composite PVA cryogels filled with disperse droplets of vaseline oil

The o/w emulsions, whose preparation and some characteristics were described in the previous section, have been used for the formation of respective vof-PVACGs. The influence of the emulsion composition and freezing temperature (T_{fr}) on the proper-

ties and morphological features of the resulting filled PVA cryogels has been traced. There were several variables ([PVA], ϕ , T_{fr}), and the amount of characteristics measured was large, therefore these are given first together in the plots of Figure 5, and after that we shall discuss the influence of each of the factors separately. All the plots in Figure 5 are presented at identical scale.

The effects of the variables listed earlier on the physicomechanical properties of the resulting vof-PVACGs have been evaluated. The data for the respective nonfilled PVACGs prepared under identical regimes of cryotropic gelation were used for comparison. Figure 5 shows that both the rise of the initial PVA concentration from 60 to 120 g/dL and lowering the freezing temperature from -10 to -30°C resulted in the growth of gel strength for the non-filled ($\phi = 0$) PVA cryogels, thus confirming the known^{1-7,31,39-41} regularities for the water-PVA systems gelled cryogenically. So, possible deviations from such regularities to be met for vof-PVACGs can be attributed to the effects caused by the oil droplets entrapped in the PVA cryogel matrix.

Thus, when dealing with vof-PVACGs fabricated from the o/w emulsions with PVA concentration of 60 g/L, a rather weak variation of elastic and plastic properties of the composites was observed up to high filling extent irrespective of the freezing temperature used in the process [curves 1 in Fig. 5(a,c,e) for G_0 and Fig. 5(b,d,f) for G_{30}]. In case of systems based on 80-g/L PVA solutions, an increase in the filler fraction resulted in a more pronounced growth of gel strength, especially for vof-PVACGs formed at -20°C (curves 2 in Fig. 5), but, naturally, up to the boundary ϕ values, when PVA coagulation began to occur already upon the preparation of the initial emulsions (Table I).

However, if 100- or 120-g/L PVA solutions were used as emulsifiers for Vaseline oil, the character of dependences of physicomechanical properties of the oil-filled cryogels on ϕ changed, and the tendencies found were influenced not only by the filling ratio and the PVA concentration but also by the freezing temperature. Thus, in case of cryogels formed at -10°C from the emulsions based on 100-g/L polymer solutions G_0 increased from ~ 5.2 kPa ($\phi = 0$) to ~ 8.8 kPa ($\phi = 0.233$), i.e., by a factor of 1.7 [curve 3 in Fig. 5(a)]; for the samples frozen at -20°C the increase in rigidity was smaller (by a factor of ~ 1.2) [curve 3 in Fig. 5(c)], and for vof-PVACGs prepared by freezing at -30°C we observed a slight decrease in G_0 from 15.1 kPa ($\phi = 0$) to 13.9 kPa ($\phi = 0.233$) [curve 3 in Fig. 5(e)]. The variations of G_{30} modulus were qualitatively similar to the changes of G_0 for vof-PVACGs fabricated at -10°C [curves 3 in Fig. 5(b,a)], but differed for the samples frozen at -20°C [curves 3 in Fig. 5(d,c)] and at -30°C [curves 3 in Fig. 5(f,e)]. At last, in case of the composite

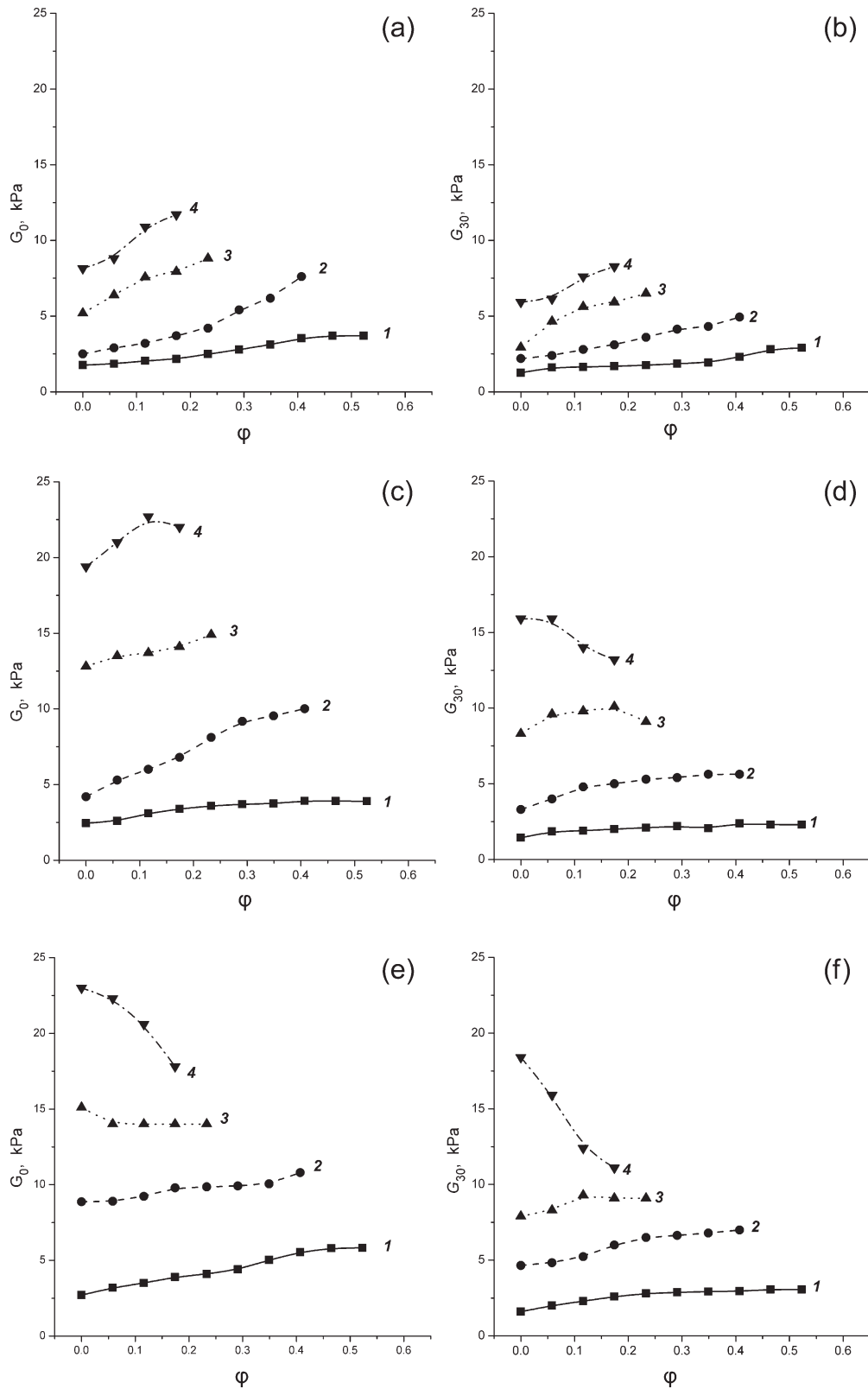


Figure 5 Apparent instantaneous shear modulus – G_0 and shear modulus for 30 min loading – G_{30} of PVACGs and vof-PVACGs versus filling extent in the cryogel samples prepared by freezing at -10°C (a, b), -20°C (c, d), and -30°C (e, f) of PVA aqueous solutions ($\phi = 0$) and o/w emulsions with varied PVA content: 60 g/L (curves 1), 80 g/L (curves 2), 100 g/L (curves 3) and 120 g/L (curves 4). Experimental error in the measurements of G_0 and G_{30} values did not exceed 10%.

systems with the highest PVA concentration, we registered an increase in both G_0 and G_{30} values for the oil-filled cryogels formed by freezing at -10°C [curves 4 in Fig. 5(a,b)], a bell-like shape of G_0 on ϕ dependence and a descending G_{30} versus ϕ curve for the samples prepared at -20°C [curves 4 in Fig. 5(c,d) respectively], and an abrupt decrease in both moduli for the vof-PVACGs formed at -30°C [curves 4 in Fig. 5(e,f)].

As a marked increase in viscosity of respective o/w emulsions also occurred at PVA concentration in the continuous phase higher than ~ 80 g/L (Fig. 2), we assumed that this phenomenon can be one of the factors affecting the efficacy of PVA cryotropic gel-formation. Indeed, an increase in PVA concentration in the solutions to be frozen-thawed causes a growth of gel strength for the resulting PVACGs, as it was many times described elsewhere^{3-9,25,31,39-41} and was observed in this study as well (the data in Figure 5 for the cases of $\phi = 0$). On the other hand, suspending small oil droplets in PVA aqueous solutions causes a very strong growth of viscosity of the resulting heterophase systems (Table I), hence the mobility of the polymeric chains and their segments is hindered significantly. Such effect, as we assume, can be capable of interfering with the formation of intermolecular links in the junction knots of arising polymeric 3D network. These noncovalent physical junctions are known to be the microcrystallinity zones.^{3,4,42-46} It is also known that cryotropic gelation of PVA solutions occurs in the so-called unfrozen liquid microphase⁴⁷—the regions remaining unfrozen as the greater part of the initial solvent is crystallized, where solutes, PVA macromolecules in this particular case, are concentrated and the viscosity is very high.^{3,5,48} Therefore, an additional increase in viscosity induced by the presence of dispersed oil droplets (Table I and Fig. 2) necessarily hampers the polymer-polymer interactions. As the cryoconcentrating effect is stronger and, as a consequence, viscosity of unfrozen liquid microphase is higher at lower temperatures (a greater amount of the solvent is frozen out), this hindering influence is stronger exhibited upon lowering the freezing temperature. It follows from the comparison of curves 3 and, especially 4, in Figure 5(a,b) ($T_{\text{fr}} = -10^\circ\text{C}$), 5(c,d) ($T_{\text{fr}} = -20^\circ\text{C}$), and 5(e,f) ($T_{\text{fr}} = -30^\circ\text{C}$) that such processes did affect these gelling systems. Note also that such an influence was somewhat more pronounced for the G_{30} moduli responsible for the plastic characteristics of vof-PVACGs than for the G_0 values characterizing the elastic properties of these gel composites.

The influence of the filler fraction ϕ on the physico-mechanical characteristics of composite PVA cryogels is yet another problem that needs to be discussed. Figure 5 (curves 1 and 2) shows that in the

cases of vof-PVACGs based on 60- and 80-g/L PVA solutions a certain increase in both G_0 and G_{30} moduli was observed with an increase of ϕ . In other words, small liquid droplets of Vaseline oil dispersed within the PVA cryogel continuous phase behave as the so-called⁴⁹ “active filler” capable of reinforcing composite material as compared with the nonfilled one. The same effects of this filler were also registered for the vof-PVACGs formed at -10°C from the more concentrated (100 and 120 g/L) polymer solutions [curves 3 and 4 in Fig. 5(a,b)], as well as somewhat weaker effects were observed for G_0 in case of the composites formed at -20°C using 100 g/L PVA solution [curve 3 in Fig. 5(c)] and for the G_{30} modulus of the same composites prepared at -30°C [curve 3 in Fig. 5(f)]. A bell-like shape of the curves 4 [Fig. 5(c)] and 3 [Fig. 5(d)] obviously indicates a competition between the strengthening and the weakening factors. The former are the reinforcing abilities of the filler, the latter include the increase in viscosity with the rise of both polymer and oil concentrations discussed earlier [curves 4 in Figure 5(c,d,e,f)].

It should be pointed out that the reinforcing influence of the oil-type dispersed hydrophobic fillers on the mechanical properties of composite hydrogels is a well-documented phenomenon, especially for the food-like systems. Thus, similar effects were described for gelatin gels filled with small droplets of vegetable oil,⁵⁰ for the whey protein gels filled with the droplets of grape seed oil,⁵¹ for the milk-protein-based gels filled with small particles of milk fat,⁵² for the soybean protein isolate gels containing entrapped droplets of soybean oil,⁵³ and for several other food composites. The same trend was also found in the case of filled gels based on synthetic polymers, namely, the hydrogels of PVA carried with Congo red dye and containing entrapped microdroplets of paraffin oil.⁵⁴ Such reinforcing effects are commonly explained by the influence of multiple factors, in particular, by that the droplets of similar liquid hydrophobic filler at small deformations of the composite gels behave as non-deformable stiff beads, and also by the gelation process being accompanied by “the formation of an intermediate layer between the filler particles and the gel matrix.”⁵⁴ Depending on the filler amount and rheological properties of these adsorption layers one can obtain composites with increased rigidity as compared with the equi-concentrated (in respect to the gel-forming polymer) nonfilled gel. Most probably, in case of PVA cryogels with entrapped droplets of Vaseline oil we dealt with a similar influence, at least, for the composites based on the 60- and 80-g/L initial PVA solutions (curves 1 and 2 in Fig. 5), when the “deteriorative” effects of the increased viscosity discussed earlier did not yet become

dominant (curves 4 in Fig. 5). In this context, the composites prepared using the 100-g/L PVA solutions (curves 3 in Fig. 5) were in an “intermediate position,” and for the composite gel systems formed from PVA solutions with yet higher concentration (120 g/L) by freezing at -20 and -30°C the influence of the elevated viscosity became predominant, thus resulting in a decrease in gel strength [curves 4 in Fig. 5(d–f)].

Thermal properties of composite PVA cryogels filled with microdroplets of vaseline oil

It is commonly known that fusion behavior of physical (noncovalent) thermoreversible gels is indicative of the gelation efficiency, since the greater the number of intermolecular links formed upon gelling, the higher energy quantity should be spent for thermal dissociation of these links in the resulting gels upon their melting.⁵⁵ Hence, the differences in the gel fusion parameters between the nonfilled PVACGs and corresponding, in terms of PVA concentration, vof-PVACGs prepared under identical conditions can be attributed to the influence of liquid filler on the gelation efficiency. Therefore, we have measured the gel fusion temperatures, T_f for the same samples, whose physicochemical characteristics are illustrated in Figure 5, and then calculated the values of gel fusion enthalpy (ΔH) for such systems using Eldridge-Ferry equation⁵⁶:

$$\ln c = \Delta H/RT_f + \text{const},$$

where c (g/L) is the polymer concentration, ΔH (J) is the fusion enthalpy of physical junction knots of 3D network, and R is the gas constant ($8.324 \text{ J mol}^{-1} \text{ K}^{-1}$).

The ΔH values were found for vof-PVACGs with filling extent in the range of 0.058–0.174, i.e., for the cases when the oil-containing composites could be prepared over the whole range of used PVA concentrations (60–120 g/L). The data obtained on the thermal properties of the respective samples are collected in Figure 6 and in Table II.

The graphs in each left-to-right row in Figure 6 are arranged in pairs: the left plot shows the dependence of T_f values on the filling ratio, and the corresponding right plot gives the lines in the coordinates of Eldridge-Ferry equation for the PVACGs and vof-PVACGs prepared under identical freezing conditions. It was found that, similar to gel strength (Fig. 5), the values of T_f also increased for both nonfilled and oilfilled PVA cryogels with the increase in the initial PVA concentration [Fig. 6(a,c,e)]. For traditional PVACGs such trend is well documented,^{3–9,39–41,55} and this regularity is also true for the composite PVA cryogels filled with “soft” de-

formable and solid nondeformable dispersed particles.^{19,22–24} For the case of vof-PVACGs, the same effect testifies that the polymer concentration in the continuous phase is also the key factor controlling the heat endurance of these gel materials containing liquid hydrophobic filler.

At the same time, the character of the influence of such factor of cryogelation process, as freezing temperature (T_{fr}), on the gel's fusion temperatures for vof-PVACGs was rather complicated. The nonfilled cryogels produced via freezing at the highest sub-zero temperature, -10°C in the present study, had the lowest T_f for all the studied PVACGs. The samples with the same PVA concentration frozen at -20 or -30°C had higher and quite close fusion temperatures. At the same time, thermal properties of composites with entrapped microdroplets of Vaseline oil depended not only on the T_{fr} and PVA concentration in the continuous phase, but also on the filling extent. This dependence was most pronounced for vof-PVACGs prepared by freezing at -10°C , for which the increase in T_f was the most abrupt [Fig. 6(a)] as compared with the respective (regarding the filling extent) composites prepared at lower temperatures -20°C [Fig. 6(c)] or -30°C [Fig. 6(e)]. In other words, the character of T_{fr} influence on the thermal properties of vof-PVACGs was changed in comparison with the “empty” PVACGs. No doubt, this was caused by the presence of oil microdroplets in the matrix of PVA cryogel; moreover, Figure 6(a,c,e) show a remarkable growth of T_f with the increase in filling extent. In turn, the latter effect indicated that upon cryostructuring of the initial o/w emulsions containing increased fraction of hydrophobic discrete phase a higher amount of thermally fusible links was formed than in the nonfilled PVA cryogels. Since such links are the hydrogen bonds within the microcrystallites,^{3–5,39–41,44,45,55,57} we suppose that the processes taking place in the course of emulsification can result in unfolding of the coils of PVA macromolecules thus opening some additional vacancies for H-bonding. Evidently, this assertion concerns those PVA chains that contact immediately with the surfaces of oil microdroplets and participate in the formation of ordered interface layers surrounding the droplets; such layers were already discussed in the section related to the properties of the initial emulsions. Hence, if this “scenario” is real, an increase in T_f should be observed upon increasing the oily filler content, as it was indeed found in our experiments [Fig. 6(a,c,e)].

At the same time, the variation of fusion enthalpy (ΔH) with ϕ increase was qualitatively somewhat different from the variation of T_f . The Figure 6(b,d,f) shows how the slope of the logarithm dependence of [PVA] on $1/T_f$ is changed, thus reflecting, according to the Eldridge-Ferry equation, the

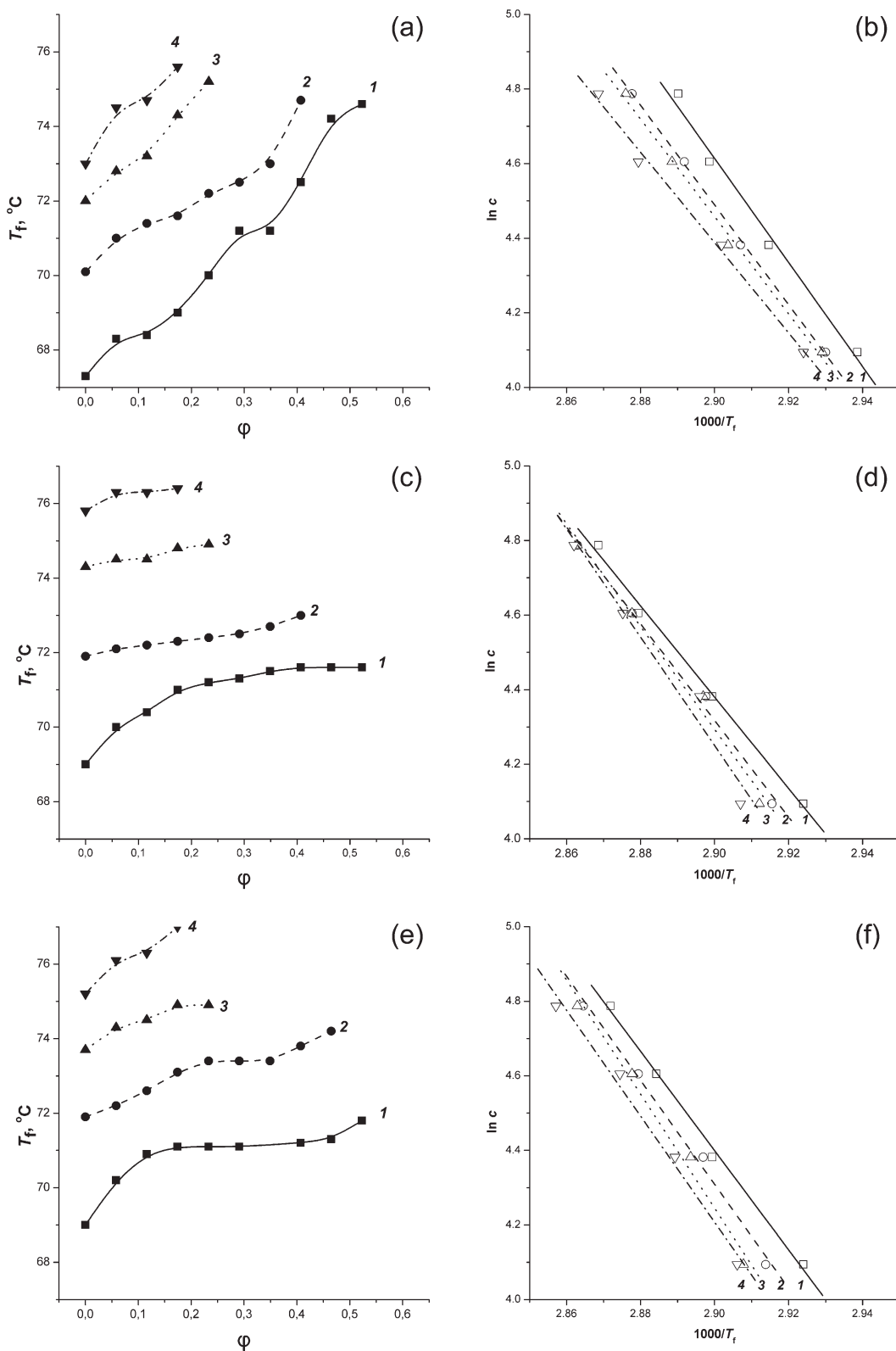


Figure 6 Thermal properties [gel fusion temperature – T_f (a, c, e) and Eldridge-Ferry graphs (b, d, f)] of PVACGs and vof-PVACGs at different filling extent in the cryogel samples prepared by freezing at -10°C (a, b), -20°C (c, d) and -30°C (e, f) of PVA aqueous solutions ($\phi = 0$) and o/w emulsions with varied PVA content: 60 g/L (curves 1), 80 g/L (curves 2), 100 g/L (curves 3) and 120 g/L (curves 4). Experimental error in the measurements of T_f values did not exceed $\pm 0.5^\circ\text{C}$.

TABLE II
Fusion Enthalpy of Nonfilled PVACGs (Reference) and Filled vof-PVACGs Formed at Various Freezing Temperatures

Volume fraction (ϕ) of Vaseline oil in the emulsion to be cryostructured	Freezing temperature (T_{fr}), °C	Fusion enthalpy (ΔH) of PVACGs and vof-PVACGs, kJ/mol	K_D
0	-10	116.1 \pm 3.5	0.9938
0.058		111.1 \pm 1.3	0.9995
0.116		108.6 \pm 2.4	0.9974
0.174		100.6 \pm 2.6	0.9968
0	-20	101.8 \pm 2.6	0.9975
0.058		107.8 \pm 2.4	0.9966
0.116		114.6 \pm 3.8	0.9935
0.174		120.9 \pm 3.7	0.9938
0	-30	110.7 \pm 1.7	0.9980
0.058		116.0 \pm 2.7	0.9968
0.116		127.2 \pm 3.6	0.9937
0.174		118.3 \pm 3.3	0.9940

tendencies of fusion energy variation, and the ΔH values themselves along with the correlation coefficients (K_D) computed for the approximating straight lines are given in Table II. As the ΔH values are indicative of the cooperativity extent in the fusion of junctions in physical gels, one can trace the influence of oily filler on the structural perfection of microcrystallites in vof-PVACGs with different filling ratio.

First, it was found that $\ln c$ versus $1/T_f$ dependences in Figure 6(b,d,f) fitted well the straight lines, as the correlation coefficients were very high, more than 0.99 (Table II). This indicated that the entrapment of Vaseline oil microdroplets in the matrix of PVA cryogel over the range of ϕ from 0.058 to 0.174, at least, did not interfere with the mechanisms of gel formation. However, second, certain variations in ΔH values for different vof-PVACGs prepared by freeze treatment at -10 , -20 , or -30°C testified on the obvious influence of the used liquid filler on the structure of fusible junction knots. This most probably concerns the junctions neighboring the droplets of hydrophobic discrete phase. Although the differences between the experimental ΔH values did not exceed 15–20%, the tendencies observed were rather evident, moreover, these ones depended on the freezing temperatures used for gel producing. Thus, the increase in filling extent from $\phi = 0$ to $\phi = 0.174$ resulted in the $\sim 15\%$ decrease of the fusion enthalpy (from ~ 116 to ~ 101 kJ/mol) in case of cryogels formed at -10°C , whereas for the samples prepared at -20°C this trend was reversed, namely, ΔH increased by about 20% (from ~ 102 to ~ 121 kJ/mol). At last, a bell-like ΔH versus ϕ dependence with a maximum point ($\Delta H \approx 127$ kJ/mol) at $\phi = 0.116$ was obtained for the respective composites fabricated at $T_{fr} = -30^\circ\text{C}$. In other words, the cooperativity of heating-induced dissociation of PVA microcrystallites in the junction knots of supramolecular network of matrix gel matter decreased in the first

case, grew in the second one, and had an extreme character in the third case. The latter fact pointed to a competition between some promoting and inhibiting factors. The exact reasons for such an influence of the freezing conditions on ΔH for vof-PVACGs are not yet clear. However these data evidently indicate that the oil microdroplets interact with different intensity with the neighboring PVA chains within the near-interface layers during the cryogenic treatment of the initial emulsions under different thermal conditions. At that several significant factors act simultaneously and oppositely on the intensity of intermolecular interactions in the unfrozen liquid microphase of moderately frozen systems. Thus, lowering the freezing temperature causes a drastic increase in viscosity of this microphase and, as a consequence, suppresses the interactions; whereas the smaller volume of liquid inclusions at lower temperatures is the reason for an increase of the solute concentration, thus facilitating the polymer–polymer interactions. Therefore, the results presented in Table II can be interpreted as a manifestation of such a competition, when depending on the temperature of freeze treatment the one or the other factor becomes predominant.

To reveal the possible contribution of the ordered interface discussed earlier, PVA layers neighboring the oil microdroplets on the variation of thermal properties of composites with increase in filling extent, we performed X-ray diffraction studies of the gel samples with $\phi = 0$ (nonfilled PVACG) and $\phi = 0.407$ (vof-PVACG). The idea was to try obtaining experimental evidences on the additional ordering of PVA chains near the water-oil interfaces caused by the presence of disperse lipophilic phase. The methodology described for PVA cryogels by Ricciardi et al.⁴⁵ has been applied to analyze the diffraction data (Fig. 7). According to this approach, the diffraction intensity can be considered as a sum of contributions from crystalline parts, free water in

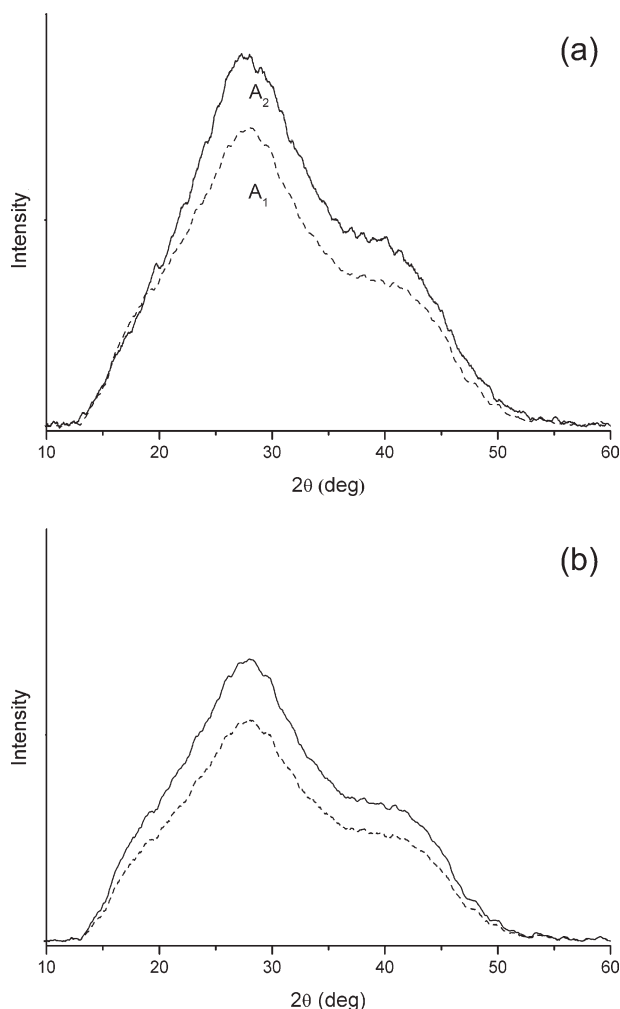


Figure 7 X-ray diffraction patterns of PVACG (a; $\phi = 0$) and vof-PVACG (b; $\phi = 0.407$) samples prepared by freezing of the respective initial systems ($[PVA] = 80$ g/L) at -20°C (the areas that correspond to the swollen amorphous part and free water are shown as A_1 and A_2 , respectively).

macropores, and swollen amorphous parts containing both water and PVA.

The diffraction curve for PVACG sample [Fig. 7(a)] shows virtually no diffraction peak over the 2θ range of $18\text{--}21^{\circ}$ answering the $\{101\}$ direction, so the contribution of the crystalline parts was rather small likewise the results already reported for PVA cryogels formed via a single freeze-thaw cycle;^{42,45} when only extremely precise measurements were able to give the value of the crystallinity degree of about 0.4%, and the size of microcrystallites did not exceed $\sim 30\text{\AA}$.⁴⁵ Hence, the fraction of swollen amorphous parts can be estimated as:

$$f_{sw} = A_1 : (A_1 + A_2),$$

where A_1 and A_2 are the areas that correspond to the free water and the swollen amorphous parts, respectively, as it is shown in Figure 7(a).

For the examined samples of PVACG and vof-PVACG, the estimated f_{sw} values were 18.6 and 21.4%, that is, filling PVA cryogels with Vaseline oil microdroplets caused an increase in the fraction of swollen amorphous parts. At the same time, a little “additional” shoulder over the 2θ range of $18\text{--}21^{\circ}$ could be distinguished in the diffraction pattern for the vof-PVACG case [Fig. 7(b)], as compared with the non-filled gel sample. Such effects with approximately the same magnitudes were observed elsewhere⁴⁵ for the case of PVACG formed using triple freeze-thaw procedure, which resulted in a slight increase (up to 0.8%) of the crystallinity degree with a parallel 1.6% increase in the fraction of swollen amorphous regions. In other words, we observed, at a semi-quantitative level at least, that the trend caused by the oily filler entrapment in PVA cryogel was very similar to the effect caused by multiple cryogenic treatments. The latter is known to result in the growth of crystallites’ size and degree of crystallinity.^{4,42–46} Therefore, we suppose that the adsorption layers of PVA macromolecules near the water-oil interfaces can make an additional contribution to the integral ordering of polymeric chains in such composites. Certainly, we understand the necessity of further studies for substantiation of this assumption.

Morphological features of composite PVA cryogels filled with disperse droplets of vaseline oil

The morphology of vof-PVACGs was studied using light microscopy. This technique was shown earlier^{5–8,21–26,42,58–62} to be informative regarding the macroporous structure of PVA cryogels, both the filled and the usual nonfilled ones, and the character of the filler particles distribution within the continuous phase of respective composites. Micrographs in Figures 8 and 9 exemplify the images of thin sections of vof-PVACGs prepared from the emulsions, whose pictures are given in Figure 3. The images in Figure 8 with a small-scale magnification shows the general character of the samples’ morphology, and the micrographs in Figure 9 at a higher magnification (by a factor of 5) present the finer structural features of the same composites. The dark areas in these pictures are the gel phase proper stained with Congo red, the clear areas are the pores, and the opaque rounds with light transparent periphery well-seen in Figure 9 are the oil droplets. It is necessary to point out that the latter are not the “primary” droplets entrapped in PVA cryogel matrix: the droplets observed in these micrographs have appeared due to a different mechanism. They were formed upon the fabrication of thin sections of the already prepared oil-filled cryogels: after sectioning with microtome their fine slices placed onto the

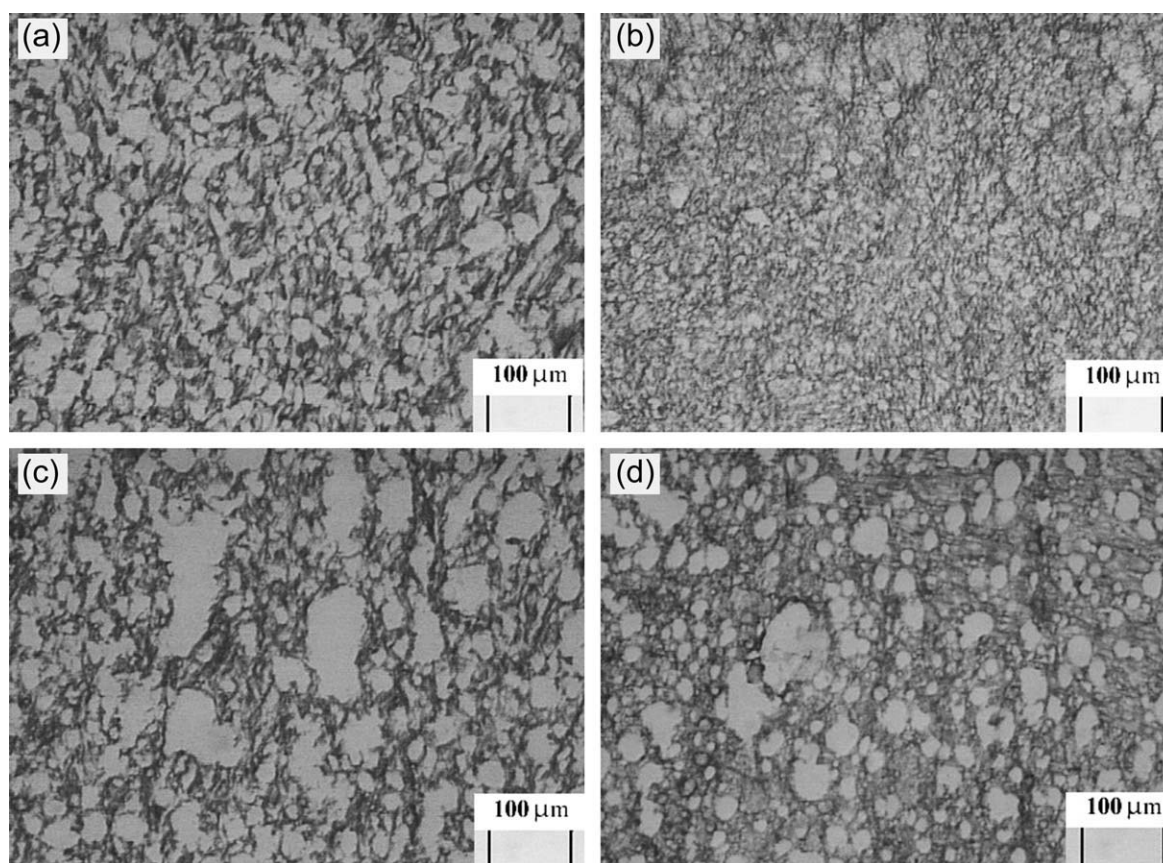


Figure 8 Optical microscopy images (small-scale magnification) of the stained with Congo red thin sections of vof-PVACGs prepared by freezing at -20°C of initial emulsions of the following compositions: (a) [PVA] = 60 g/L, $\phi = 0.058$; (b) [PVA] = 80 g/L, $\phi = 0.058$; (c) [PVA] = 60 g/L, $\phi = 0.291$; (d) [PVA] = 80 g/L, $\phi = 0.291$.

microscope glasses were immersed in an aqueous staining solution and then were rinsed with water. At that, the most of the liquid oily filler did float from the sections thus leaving respective cavities in the gel matrix visible in Figures 8 and 9 as clear holes; whereas only a minor amount of oil remained within the sections and assembled in the secondary droplets, which are the ones observed in the images under discussion.

It was found that relatively large (in comparison with the size of pores in the gel phase proper) cavities in the thin sections of vof-PVACGs were not precisely round although they could have been, *ex facto*, as if replicas of spherical oil microdroplets entrapped in the matrix of PVA cryogel. At least two possible factors capable of causing such shape deformation can be suggested. First, this could be the mechanical stresses arising in the course of ice crystallization, the magnitudes of similar stresses are known to be rather high.⁶³ Water in concentrated aqueous PVA solutions is crystallized at about -1°C ,³ and Vaseline oil is solidified at the temperatures from -8 to -10°C . Therefore, we suppose that such mechanical stresses affect the shape of oil droplets mainly in the course of ice crystallization within

this temperature interval, i.e., at the dynamic stage of freezing process commencing from the onset of ice formation till the oil solidification. The next possible reason for the distortion of the cavities' shape can be deformations of the very thin ($6\text{--}8\ \mu\text{m}$) sections of vof-PVACGs during their handling. Since such sections of wide-porous gel matter have low mechanical strength, they can easily be deformed when placing onto the microscope glass and subsequent staining, rinsing, etc. Although these operations were performed very carefully, it was hardly real to avoid completely distorting influence on the thin sections. Nonetheless, we believe the first reason, i.e. the deformation of oil droplets during ice crystallization in the course of cryostructuring of initial emulsions, to give the greater contribution to shape distortion of these cavities, whereas the second factor, i.e., effects of manipulation with thin sections, to be a minor one.

An interesting observation can be made when examining by and large the images of vof-PVACGs in the following pairs: Figure 8(a–d), as well as Figure 9(a–d), which correspond to the samples with equal filling ratio. Larger (on average) pores, i.e., the cavities left by the separated oil droplets, were

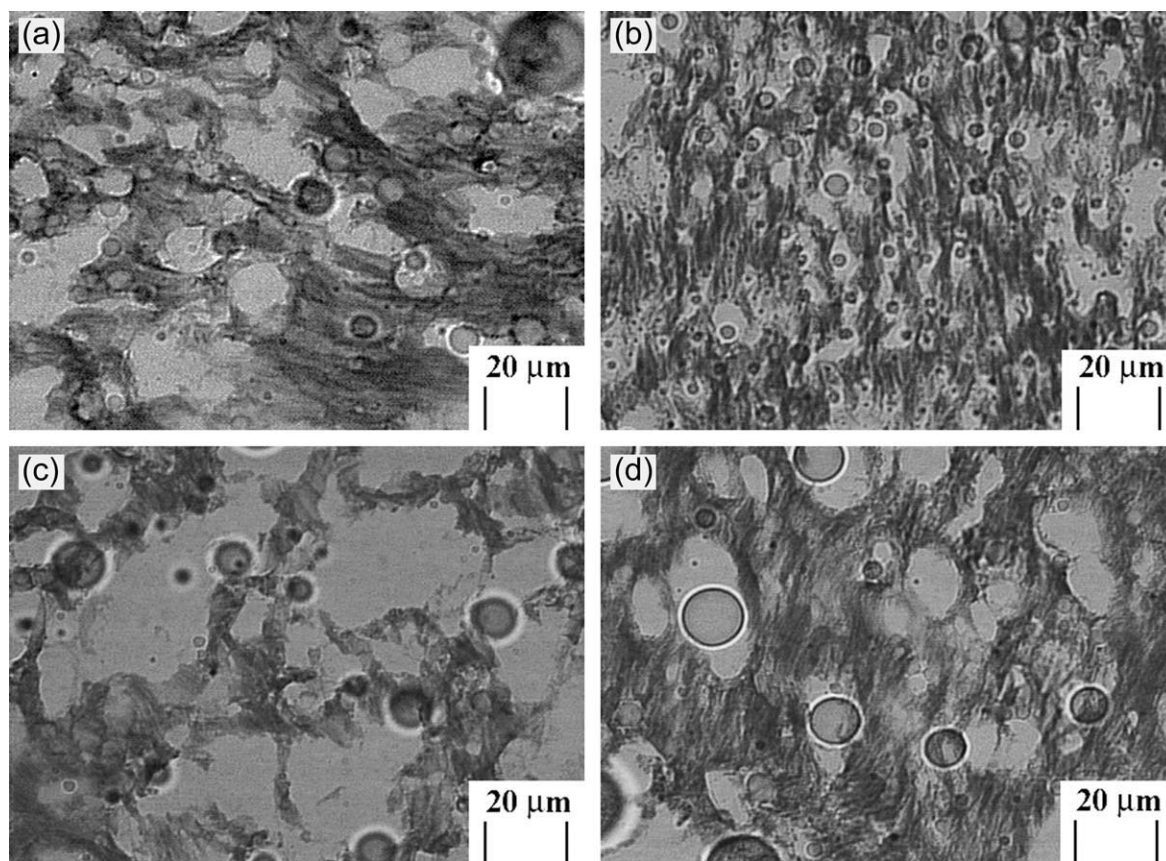


Figure 9 Optical microscopy images of the stained with Congo red thin sections of the same as in Figure 8 vof-PVACG samples at higher (by a factor of 5) magnification.

found in the composites with lower PVA concentration, whereas in case of the initial emulsions an opposite tendency took place, namely, larger droplets were formed with the more concentrated PVA-containing aqueous phase (see Figure 4 and discussion thereof). For instance, in case of $[PVA] = 80 \text{ g/L}$, the size of oil droplets decreased from 5–55 μm in the initial emulsion [Fig. 3(b)] to around 2–15 μm in the vof-PVACG [Fig. 9(b)]. Hence, we conclude that a certain diminution of oil droplet size occurs in the course of freeze-thaw-induced gelation of the respective o/w emulsions, and this phenomenon becomes especially evident with the increase in PVA concentration in aqueous phase. From our viewpoint, the most probable mechanism responsible for this “diminution effect” can be the disintegration of liquid oil droplets by significant crushing and shear stresses arising upon the growth of ice crystals. Such stresses are well known to be capable of causing the so-called freeze-damage of diverse objects including disruption of integrity of semi-solid bioparticles like the blood cells upon freezing respective dispersion systems.⁶⁴ In other words, the stresses acting on the oil droplets suspended in the frost-solidifying aqueous PVA solution can operate as a peculiar kind high-pressure homogenizer on a microscopic scale.

Since the vectors of crushing and shear stresses are directed to the oil droplets under different angles, this will, as we believe, change the droplets’ size and shape, and subsequent solidification of the Vaseline oil itself upon chilling the freezing system below $-8 \dots -10^\circ\text{C}$ will “fix” the deformed shape of the lipophilic phase particles. Further cryotropic gelation of PVA will “imprint” such diminished in size and distorted in shape spheroids in the bulk of the continuous gel phase. A rather similar effect, excluding solidification of the deformed filler particles, was detected in the case of gas-filled PVA cryogels prepared via freeze-thaw treatment of whipped aqueous solutions of the polymer,^{25,26} when small air bubbles entrapped in the resulting composites were not the exact spheres as in the initial liquid foam: the bubbles within such cryogels were disfigured by ice crystallization.

The suppressing influence of PVA on ice crystallization was reported,⁶⁵ and one of the manifestations of this effect is lowering the supercooling level with the increase in PVA concentration in the solution to be frozen.⁶ So the diminution, discussed earlier, in the size of oil droplets with the increase of PVA concentration in the continuous phase of the composite vof-PVACGs can indicate greater magnitudes of the

stresses in the more concentrated (in respect of the polymer) systems upon their freezing. The growth of shear stresses is connected with the supercooling level, since the higher the polymer concentration in the freezing system, the deeper the PVA solution or emulsion, as in our case, is supercooled. Consequently, the ice formation rate upon the temperature jump from the supercooling bottom point up to the crystallization plateau increases. This, in turn, results in a greater speed of crystallization front propagation, and therefore causes a higher shear stress in between the individual "needles" of branched ice crystals.⁶⁶

As for the morphological features of the continuous gel phase proper, its own porosity, as can be better seen in micrographs of Figure 9, is typical for PVA cryogels in general.^{3-5,7-11,31,42,58-62} The pores in these cryogels are usually long and anisotropic, with the cross-section of the order of 1–2 μm ,^{7,8} Such key feature of PVA cryogels is the ground for their excellent diffusional properties. This makes possible practical application of the usual nonfilled, as well as complex and composite PVACGs as, e.g., carriers of immobilized enzymes and cells, macroporous supports of immunosorbents, drug delivery matrices, etc.^{3-5,9-14} Such pores do exist in the structure of vof-PVACGs, i.e., this general morphological feature of macroporous PVA cryogels is retained in the oil-filled composites, too.

Mechanically induced release of lipophilic constituents from the oil-filled PVA cryogels

We have already pointed out in the "Introduction" that oil-filled PVA cryogels have perspective applications as biomedical and cosmetic materials. It was, therefore, necessary to examine the oil-releasing abilities of such gel composites. Corresponding experiments were carried out using PVA cryogels filled with dyed oil, Rose hips oil in this case, in order to employ spectrophotometry for evaluating oil release from the gel matter. The UV-spectra in Figure 10 show the results obtained in these experiments.

Natural Rose hips oil contains UV-absorbing substances, mainly terpenoids. In spite of small solubility of the substances in water, their absorbance at the wavelength of maximum (~ 280 nm) turned out to be sufficient for a reliable demonstration of the effects of interest. It was found that nonfilled PVA cryogel subjected to cyclic mechanical compression for 30 min in aqueous medium did not release (trace 1) any matter capable of absorbing the UV light at the wavelengths characteristic of Rose hips oil (trace 5), and this was, of course, predictable. Then, when the composite gel material containing this dyed oil was immersed in an excess of water for the same 30 min, some release of UV-absorbing substances was

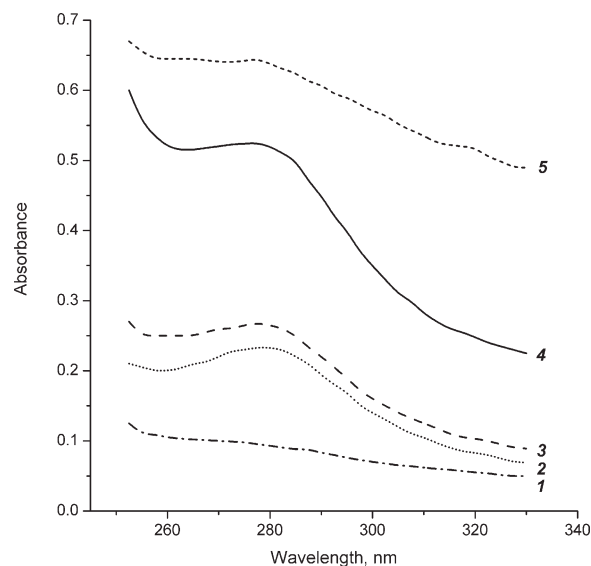


Figure 10 UV spectra of aqueous solutions obtained after: 30-min cyclic mechanical compression of the non-filled PVACG in an excess of water followed by the filtration (filter paper) of aqueous phase from possible small solid impurities (1); 30-min immersion of the Rose-hips-oil-filled PVA cryogel in an excess of water followed by the filtration (filter paper) of aqueous phase (2); 10-min cyclic mechanical compression of the Rose-hips-oil-filled PVA cryogel in an excess of water followed by the filtration (filter paper) of aqueous phase (3); 30-min cyclic mechanical compression of the Rose-hips-oil-filled PVA cryogel in an excess of water followed by the filtration (filter paper) of aqueous phase (4); preparation of the "Rose hips oil water" emulsion, subsequent phase separation upon the 30-min standing followed by the filtration (filter paper) of aqueous phase from possible small oil droplets (5).

detected (trace 2). This indicated a small-scale diffusion-driven leakage of such substances from the vof-PVACG. Then a similar composite sample was subjected to cyclic compression for 10 min, and after that we registered some additional increase in absorption intensity (trace 3). At last, when the duration of cyclic compression was 30 min, the release value was almost doubled (trace 4). These data indicate that mechanical influence induces release of the lipophilic constituents from the oil-filled PVA cryogels. Note also that in spite of filtering of the aqueous layer separated from the o/w emulsion (see legend for Fig. 10), the aqueous phase used for recording trace 5 was a little turbid. Therefore, because of certain light scattering, this spectrum had poorly exhibited peak as compared to traces 2–4 recorded after the oil release from composite cryogel. The latter, apparently, acted in this case as a certain filter material capable of "letting outside" only very fine oil droplets, so the turbidity of the respective aqueous phase was negligible.

One of the examples of "forced" release of oily filler from gel composites was recently described for

the thermo-responsive supermacroporous poly (N-isopropylacrylamide) cryogels that contained entrapped tetradecane-based o/w emulsion.⁶⁷ Such “smart” composites were able to exude the lipophilic components through the system of capillary-size channels, whose cross-section was from tens to hundreds of micrometers, as a consequence of heat-induced collapse of wide-porous polymeric matter. In this study, on composite PVA cryogels possessing significantly smaller pores than the aforementioned supermacroporous cryogel, we have demonstrated the possibility to cause the release of hydrophobic oil from the hydrophilic gel material with the aid of a different external stimulus, namely, by the cyclic mechanical impact. To the best of our knowledge, this is the first example of such an effect.

CONCLUSIONS

Composite heterophase PCACGs containing entrapped small droplets of Vaseline oil were prepared via freeze–thaw treatment of oil-in-water emulsions. It was shown that over certain range of PVA concentrations in aqueous phase and a range of volume fraction of the hydrophobic phase its microdroplets caused an increase in both the gel strength and the heat endurance of composites. The effect of diminution in size of oil droplets entrapped in the gel matrix as compared with the initial emulsions was observed, and this effect was explained by the disintegration action of crushing and shear stresses arising upon the system freezing and growth of ice crystals. Such oil-filled PVA cryogels are capable of gradually releasing the lipophilic constituents in response to the cyclic mechanical compression.

Diverse nonfilled, complex, and filled composite PVA cryogels are known from the early seventies of previous century,^{1–5,68–71} and many of them are of biomedical interest. In this respect, the oil-filled PVA cryogels could, from the authors’ point of view, be considered as prospective materials of the same purpose, e.g., in the cases when along with definite mechanical function, like the artificial cartilages,⁷² such composites could perform as the drug depositaries capable of releasing appropriate medicinal oil upon the cyclic compression. Therefore, the data obtained in our study concerning properties of similar polymer systems and influence of the preparation conditions on these properties, as well as on the porous morphology of such heterophase gels, could be of significance in both scientific (for better understanding of mechanisms governing the gel-formation processes) and applied (as a basis for manufacturing procedures) aspects.

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