

Study of Cryostructurization of Polymer Systems.

IX. Poly(Vinyl Alcohol) Cryogels Filled with Particles of Crosslinked Dextran Gel

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SYNOPSIS

The influence of deformable disperse filler on rheological and thermal properties of poly(vinyl alcohol) cryogels (obtained by freezing–thawing of concentrated aqueous solutions of the polymer) was studied. Spherical particles of crosslinked dextran gel—the Sephadex beads of two types: G-25 and G-100—were used as filler. It was found that an increase in the filler concentration from 0 to 20 wt % produced an increase in the composite cryogel strength; the greater reinforcement effect was observed with large-pore Sephadex beads into the cryogel matrix. An increase in the fraction of disperse phase also leads to an increase in fusion temperatures of these composites, such effect being determined mainly by the filler type. The filling of cryogels with G-100 beads improves the thermostability of composite samples to a higher extent as compared with the use of G-25 particles. Several possible reasons for the trends observed in rheological and thermal properties of Sephadex-filled PVA cryogels were revealed.

INTRODUCTION

Poly(vinyl alcohol)-based cryogels (PVA cryogels) were obtained by freezing the concentrated solutions of the polymer, keeping them frozen for a definite time, and subsequently thawing them. When the gel-like material formed in this way was heated to 80–100°C, it melted, yielding a solution. The next freezing–thawing cycle restored the cryogel structure; hence these structurized polymeric systems can be classified as thermo-reversible gels.

The gelation produced by freezing and thawing an aqueous PVA solution appears first to have been described by Kukharchik and Baramboim,¹ and later was described by Peppas.² In subsequent publications, the mechanisms of gel formation, the properties of PVA cryogels, and the effect of cryogenic treatment conditions on the gel structure—its rheological and thermal characteristics—were considered.^{3–25} The possibility of obtaining thermo-revers-

ible cryogels starting not only from aqueous PVA solutions, but also using DMSO¹⁰ or water–DMSO mixtures²³ as the initial solvent, was demonstrated.

The investigations revealed that the properties of PVA cryogels depend on the characteristics of the gel-forming compound itself (molecular weight and degree of deacetylation)^{5,6,10,14,20}, on its concentration in the initial solution,^{1,3,5,10,14,21,23,49} on the solvent nature,^{10,23,25} and on the conditions of cryostructurization process. The conditions of the process include the temperature and the duration of freezing,^{4–10,13,14,18–23} the number of freezing–thawing cycles,^{5,6,11,12,16,19} and the regime of thawing^{18,22,25,48} or ice sublimation from the frozen samples.^{7–9,13}

Along with the fundamental investigations, practical studies were carried out. At present the PVA cryogels are widely used, for instance, as biomedical materials, frozen food coatings, gel-base of solid microbiological media, etc. (patent information is available). In particular, one promising practical application of PVA cryogels is revealed by the development of immobilized biocatalysts containing microbial cells entrapped in a matrix of this highly porous hydrophilic gel.^{26–31}

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Unfortunately, there are actually no investigations concerning the effect of various additives (the cell biomass in particular) on the properties of gel-like composites obtained by cryostructurization techniques.

Recently, we have found that the entrapment of yeast cells in a PVA cryogel produces a considerable increase in the gel strength.³¹ However, to elucidate the main features of the effects of filling on various characteristics of gel objects, it is necessary to perform experiments with disperse additives that are simpler than the microbial cells. It should be noted that filled polymeric gels have not yet been sufficiently studied from the viewpoint of physico-chemistry of high molecular weight compounds. Such systems, however, especially of the hydrogel origin, widely occur in nature (e.g., animal tissues) and serve as a basis of many traditional and fabricated (artificial) foodstuffs. Only in recent years have the above composites, that is, the gels filled with disperse additives, been studied in more detail. Some examples can be found in publications concerned with the properties of gelatin gels containing an inorganic filler^{32,33} or a hydrogel filler,^{34,35} and of chemically crosslinked PVA gels or thermocoagulated casein gels filled with microdroplets of fat.³⁶ In the above works, the factors increasing the composite gel strength were shown to include the growth in the fraction of a disperse phase (up to some limiting level), adhesion of the gel matrix to the filler, and the mechanical properties (deformability) of the filler.

The purpose of the present work is to study how the introduction of a well-defined filler would affect the properties of PVA cryogels. Of special interest were the fillers whose properties could model, to

some extent, the physico-mechanical characteristics of microbial cells. In our experiments, such "model" particles were represented by spherical granules of a water-swollen, chemically crosslinked dextran gel—Sephadex. In contrast to the solid inorganic additives, each microbead of Sephadex possesses viscoelastic properties, which resembles in this respect a microorganism cell. These microbeads, however, have quite different dimensions, structure, and chemical composition. The choice of Sephadex was also favored because of the availability of Sephadex fractions with various grain sizes and of grades with various porosities determined by the extent of crosslinking of the polysaccharide chains of dextran in a spatial network. This last circumstance was important for the characterization of PVA cryogels formed in the presence of a gel filler that was either permeable or nonpermeable for the PVA molecules.

EXPERIMENTAL

Materials

Poly(vinyl alcohol) with the trademark 20/1 (NPO "Azot," Severodonetsk, USSR) was used in this work. The technical grade polymer was additionally purified by reprecipitation from a 7% aqueous solution into a 4-fold excess of methanol. The resultant polymer had a viscosity-average molecular weight of about 81.7 kDa and a deacetylation degree of 98%.

Sephadex beads, produced by Pharmacia Fine Chemicals (Uppsala, Sweden), were utilized as filler material. Their notation and characteristics are given in Table I. Prior to use, dry Sephadex powders were soaked in excess distilled water until equilib-

Table I Characteristics of Crosslinked Dextran Gels Used as Fillers for PVA Cryogels

Type	Notation Used in Text	Dextran Mol wt Excluded* (kDa)	Particle Size of Dry Filler* (μm)	Bead Size of Swollen Filler† (μm)	Dry Content in Swollen Sephadex** (%)	Relative Deformability of Filler‡
Sephadex G-25 coarse	G-25c	≥ 5	100–300	200–350	26.0	28.9
Sephadex G-25 fine	G-25f		20–80	50–150		
Sephadex G-100	G-100	≥ 100	40–120	100–200	6.3	2.3
Sephadex G-100 superfine	G-100sf		10–40	30–100		

* Data of Sephadex manufacturer.

† Measured on an MBI-3 light microscope (USSR), swollen in water.

** Measured gravimetrically after drying the swollen Sephadex to the constant weight state.

‡ According to data in Ref. 35, relative to the rigidity of swollen Sephadex G-200 taken as 1.

rium-swollen, and then were washed at least ten times with fresh water on a porous glass filter. The excess water was then filtered out and the swollen gel beads were stored in a desiccator at 100% humidity. The water content of Sephadex gel remained in a constant state during the time required to carry out studies.

Methods

Viscometric Measurements

Reduced viscosities (η_{red}) of aqueous PVA solutions were evaluated at 25°C using an Ubbelohde-type capillary viscometer. The values of $[\eta]$ were calculated by the least squares method from the linear dependence $\eta_{\text{red}} = f(C_{\text{PVA}})$. The viscosity-average molecular weight was estimated by the following equation³⁷:

$$[\eta] = 5.95 \times 10^{-4} \times \bar{M}_{\eta}^{0.63} \text{ (dL/g)}$$

Rheological Studies

These were performed on both filled and nonfilled PVA cryogels prepared in duralumin cylindrical molds with 15 mm inner diameter and 10 mm height. The initial samples were prepared by mixing a definite amount of swollen Sephadex beads with the calculated quantity of freshly prepared PVA solution of known concentration. The suspension obtained was held for 18 h at room temperature, heated for 15 min on a boiling water bath, cooled to 25–30°C, and remixed to provide a uniform filler distribution. The suspension was then cast in the molds and stored for 18 h in a freezing chamber at $-15 \pm 2^\circ\text{C}$. Prior to rheological measurements, the samples were kept for 5 h at $+4^\circ\text{C}$ (thawing) and then, before the test, for 1 h at room temperature. The creep curves were obtained at $22 \pm 1^\circ\text{C}$ and a constant load of 4.9 mN using a Kargin–Sogolova dynamometric balance.³⁸ The procedure had been used earlier for the investigation of PVA cryogels.^{14,21} The creep data were utilized, as recommended in Ref. 39, for the calculation of an apparent instantaneous shear modulus (G_0) and a total (for 90 min load application) compliance of the gel sample (J_{90}).

Cryogel Fusion Temperature (T_f)

This parameter was determined using the method of Braudo et al.⁴⁰ Each sample of a filled or nonfilled cryogel was formed in a transparent polycarbonate test tube (inner diameter 9 mm). One drop of perfluorodecalin was layered on top of the gel obtained. The test tube was then placed into a glass thermo-

stated water bath, of the type conventionally used in capillary viscometry, that allowed direct visual observation of the gel melting. The samples were heated at a rate of $0.2^\circ\text{C}/\text{min}$. When gel fusion temperature was reached, the drop of perfluorodecalin drowned. This moment was considered to be fusion temperature, which was measured by a thermometer with scale accuracy of 0.1°C .

PVA Concentration in Solution (C_{PVA})

These values were obtained by weighing the non-volatile residue after drying aliquots of polymer solutions to constant weight. PVA concentrations were determined both in the initial solutions and after their contact with the filler. For this purpose, Sephadex suspension in a gel-forming solution was centrifuged for 15 min at 6000 rpm in a T-52.1 centrifuge (MLW, GDR), and an aliquot of supernatant was used for the determination of the dry weight. In what follows, PVA concentration in solution without filler is denoted by C'_{PVA} and that after contact with the filler and its removal, by C''_{PVA} .

Scanning Electron Microscopy (SEM)

The investigations were carried out on an S 405A electron microscope (Hitachi, Japan). The PVA samples preparation procedure for SEM is described elsewhere.¹⁴

RESULTS AND DISCUSSION

The preswollen Sephadex beads that were used as a filler for the matrix hydrogel (here—PVA cryogel) exhibit several specific features.

First, the swollen beads of Sephadex are themselves a polymeric hydrogel, that is, a structured polymer–solvent system containing a large amount of immobilized liquid. The viscoelastic and osmotic properties of the Sephadex beads depend on the degree of crosslinking between polymeric chains in the spatial network of the gel.

Second, Sephadexes are molecular sieves. For G-25 and G-100 types used in the present work, the excluded molecular weight limits of water-soluble uncharged polymers (for the case of narrow dextran fractions) are presented in Table I.

Finally, the swollen network of Sephadex can be considered as a rather concentrated dextran phase,*

* According to Table I, the polymer contents in G-25 and G-100 equilibrium-swollen Sephadexes were 26.0 and 6.3%, respectively.

and it is quite reasonable to expect that this will exhibit only a limited thermodynamic compatibility with the concentrated aqueous PVA solutions (the two-phase, aqueous, dextran-PVA polymer systems are known⁴¹).

It is also known that disperse fillers in polymers can be subdivided, with respect to their effect on the mechanical properties of the resulting composites, into three groups: the strengthening (active), low-efficient (inactive), and loosening (strength-reducing) ones.⁴²⁻⁴⁴ According to our data, as a filler in PVA cryogels, Sephadex beads can be ascribed to the first group. However, before discussing the effects of these fillers on the physico-mechanical and thermal properties of composite PVA cryogels, it is necessary to make two additional remarks.

The first remark concerns the method of calculating the concentration of the disperse filler. Conventionally, the disperse filler is expressed as a fraction of the total composite volume, or in volumetric percents.⁴² However, in the system studied, it is difficult to specify the real volume occupied by the porous Sephadex granules mixed with a PVA solution. As is shown below, part of PVA macromolecules can diffuse into the filler phase. For this reason, the concentrations of Sephadex beads are expressed here in weight percents. Taking into account the observation that the densities of PVA solutions and of the resulting cryogels are close to those of water-swollen dextran gels, as well as the observation that the filler concentrations in the system are not too high (not exceeding 20 wt %), the volume and weight fraction of Sephadex in composite gels seem to differ only slightly.

The second remark is closely related to what has just been noted and concerns the concentration of PVA in a continuous phase after mixing the starting polymer solution with the filler and equilibrating the system obtained (see Experimental). When such suspensions are prepared by merely mixing the initial components at a weight ratio corresponding to the preset content of the filler, that is, the redistribution of the polymer binder and the solvent molecules between the continuous phase and Sephadex gel is neglected, the actual concentration of PVA (in the space between filler particles) appears to deviate from that in a solution containing no molecular sieve additives. Indeed, in a suspension containing 20 wt % Sephadex, the real PVA concentration changes by 17.9% (G-25) and 9.5% (G-100) as compared to the systems where an equal weight fraction of water was added instead of Sephadex gels. Such results indicate, on the one hand, that in both G-25 and G-100 beads not all solvent volume is ac-

cessible for the PVA macromolecules [which is *a priori* evident for G-25 Sephadex and PVA with $\bar{M}_n \sim 81.7$ kDa (see Table I)], and, on the other hand, that during the preparation of suspensions containing various amount of Sephadex beads the ratio between the components should be properly adjusted. In Figure 1(b), circles with arrows pointing to the concentration axis indicate the C_{PVA}^* values in the continuous phase (with an unavoidable experimental error related to the sample preparation) where such a correction has been made. It is seen that at levels of PVA concentrations of about 10.2% (indicated by a dotted line) the C_{PVA}^* values vary within $\pm 0.4\%$ ($\pm 4\%$ of the absolute magnitude).

Figure 1 shows the variation of rheological characteristics of PVA cryogels due to the entrapment of various amounts of G-25 or G-100 particles into their matrices. Besides the comparison of the effects of small-pore and large-pore fillers, the G_0 and J_{90} values for the composite PVA cryogels are presented as a function of the bead size for each Sephadex type.

It can be seen that Sephadex G-100 exhibits a much greater reinforcing effect as compared with Sephadex G-25. As the content of G-100s f^* , introduced in the system, increased from 0 to 20 wt %, the composite cryogel shear modulus increased from 1.9 to 13.0 kPa, approximately seven-fold, while the total compliance of the samples decreased from 8.0×10^{-4} to 1.9×10^{-4} Pa⁻¹. At the same time, the filling of PVA cryogels with the G-25 f^* type Sephadex beads up to the same concentration (20 wt %) increased the composite strength only two-fold, and decreased the sample compliance also by a factor of 2, although the rigidity of G-100 is 12.6 times lower than that of G-25 (see Table I).

This result seems to be rather unusual. Thus, in the case of thermo-reversible gelatine hydrogels, a greater reinforcing effect was produced by a more rigid Sephadex (G-25),^{34,35} in agreement with the well-known fact of increasing the strength of composite by introducing more rigid fillers.⁴²⁻⁴⁴ The opposite pattern of this effect observed in Sephadex-filled PVA cryogels enables us to conclude that the relative deformability of such a disperse additive as the crosslinked dextran hydrogel is not a predominant factor determining the rheological parameters of the composite objects studied.

As to the effect of bead size of the fillers used, a 2-3-fold increase in the particle diameter of G-25 type Sephadex (cf. G-25 f and G-25 c in Table I) did

* The G-100s f and G-25 f types in a swollen state have approximately equal bead size (Table I).

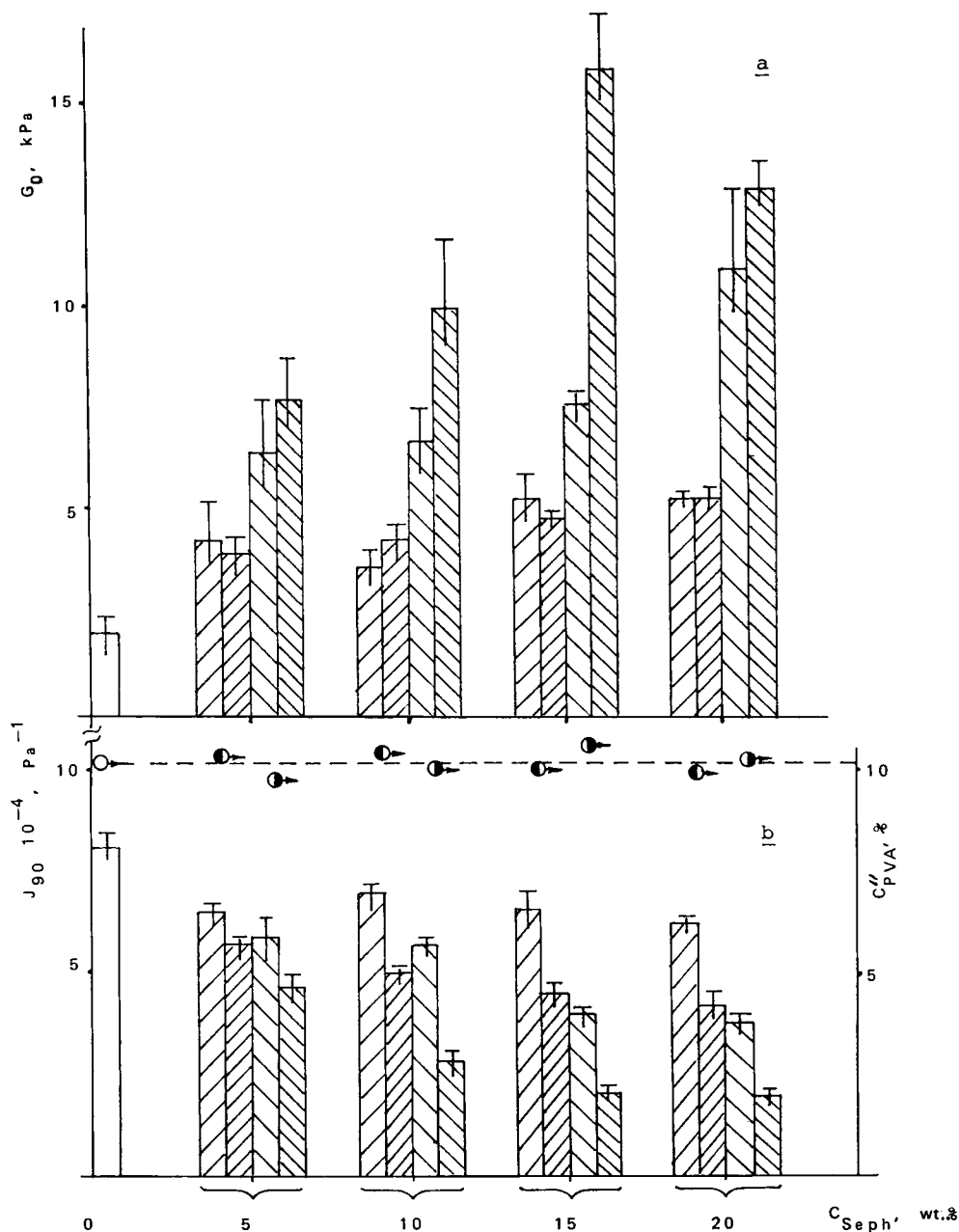


Figure 1 Values of G_0 (a) and J_{90} (b) for the nonfilled and filled PVA cryogels vs. the content of swollen Sephadex beads. Symbols: (\square), Nonfilled; (\square), G-25c; (\square), G-25f; (\square), G-100; (\square), G-100sf.

not affect strongly the G_0 values (Fig. 1a), whereas the compliance (Fig. 1b) of the filled PVA cryogels decreased approximately by 30%. The differences were more pronounced for the G-100 and G-100sf types, where a greater reinforcing effect (sometimes, two-fold) was observed for a disperse additive with the smaller particle size within the whole range of filler concentration studied. However, one should

also bear in mind a rather broad distribution of the swollen Sephadex beads by size (Table I), which might affect to some extent the absolute values of the differences observed, since no special separation of the commercial Sephadex beads with respect to the particle diameter has been made.

It was also interesting to answer the question of whether or not the experimentally observed varia-

tion in the rheological properties of filled PVA cryogels is reflected by their thermal characteristics, such as fusion temperatures, since a nonfilled PVA cryogel with greater strength usually melts at higher temperature.^{11,13,18-20,22,24}

Figure 2 shows the dependence of the PVA cryogel fusion temperature (T_f) on the filler content. It is clearly seen that filled samples melt at higher temperatures as compared to the nonfilled cryogels. This effect is also much more pronounced in the case of G-100sf type filler: the 20 wt % of this additive increased the fusion temperature by 7.6° as compared to a filler-free cryogel, while the increase in T_f produced by the G-25f Sephadex was 2.8°. For the PVA cryogels with practically equal polymer concentration in the binder phase, such a considerable variation in the fusion temperature (particularly in the former case) primarily gives evidence to some changes induced by the presence of disperse additives, affecting the energetics of every PVA intermolecular contact.

For a quantitative estimation of these changes, experiments were performed to determine the enthalpies of the thermal dissociation (ΔH) of the junctions of spatial network for the corresponding cryogels. For this purpose, 10 wt % suspensions of G-25f (or G-100sf) Sephadex in PVA solutions with various polymer concentrations were prepared. One part of each composition was used for the formation of cryogel and determination of its T_f , and the remaining part was used for the analysis of the actual polymer content in the continuous phase. The results obtained are presented in Figure 3 in coordinates of the Eldridge-Ferry equation,⁴⁵ which represents a linear dependence of the logarithm of gel-forming polymer concentration (g/L) vs. the reciprocal fusion temperature of a gel:

$$\ln C = (\Delta H/RT_f) + \text{constant}$$

Since the method of determining T_f used in this work detected the melting of the binder phase gel,

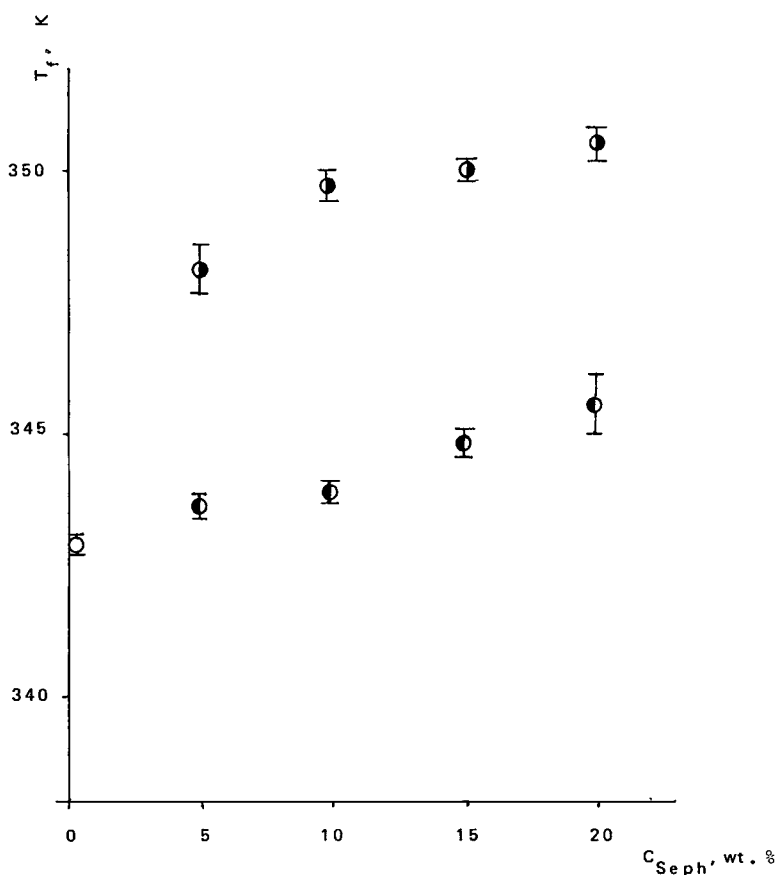


Figure 2 Changes in the T_f of the composite PVA cryogels vs. the content of the filler beads. Symbols: (○), nonfilled; (◐), G-25f; (◑), G-100sf.

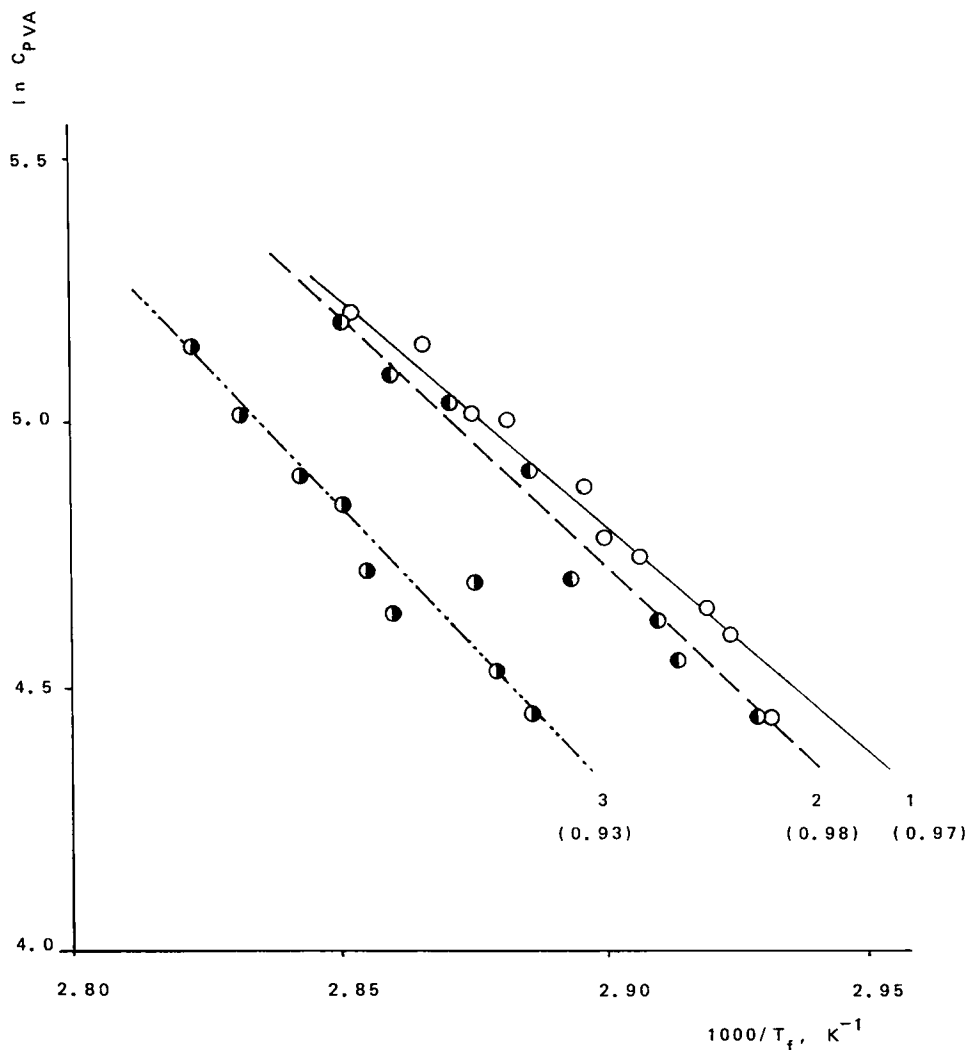


Figure 3 Logarithm of PVA concentration (C , g/L) vs. reciprocal fusion temperature (T_f , K). Data in parentheses give the coefficients of determination calculated for the corresponding straight lines (symbols are the same as in Fig. 2).

the PVA concentration in filled cryogels entering in the above equation was taken to be equal to C_{PVA}'' .

The ΔH values so determined were: 71.7 kJ/mol for nonfilled PVA cryogel (Fig. 3, line 1), 80.8 kJ/mol for the cryogel containing the G-25 *f* Sephadex beads (Fig. 3, line 2), and 86.9 kJ/mol for the cryogel filled with G-100s *f* Sephadex (Fig. 3, line 3). These data suggested differences in the thermal stability of the PVA intermolecular contacts formed (probably manifested in different numbers of hydrogen bonds) between the polymer chains in the junctions of the three-dimensional network of the objects studied.

Thus, analysis of all the above results (Figs. 1-3) allowed us to conclude that the filling of PVA

cryogels with particles of the crosslinked dextran gels not only reinforced the composite materials obtained, but also affected the interactions between PVA macromolecules in the cryogel matrix. This conclusion necessitated a more detailed investigation of the effects arising from the mixing of the starting PVA solution with Sephadex particles.

For this purpose, several cryogel samples were prepared from the PVA solutions pre-equilibrated with Sephadex G-25 *f* or G-100s *f* and subsequently separated from the filler as was done during determination of the C_{PVA}'' values. The cryogel samples so obtained were subjected to rheological tests, and their fusion temperatures were also measured. The results of these experiments are summarized in Ta-

Table II Properties of PVA Cryogels Prepared from Gel-Forming Polymer Solutions with Various Prehistories

No.	Filler Preliminarily Equilibrated with PVA Solution		PVA Conc. in Solution for Cryogel Formation (%)	Rheological Characteristics of Cryogel			Viscosity-Average Molecular Weight of PVA (kDa)
	Type	CSeph (wt %)		G_0 (kPa)	J_{90} (Pa^{-1})	Fusion Temp. (K)	
1	—	0	10.1 ± 0.2	1.9 ± 0.4	8.0 ± 0.1	342.9 ± 0.2	81.7 ± 1.8
2	G-25f	20	9.9 ± 0.2	4.8 ± 0.5	5.4 ± 0.5	343.8 ± 0.4	84.0 ± 1.2
3	G-100sf	20	10.2 ± 0.2	11.3 ± 1.4	1.3 ± 0.2	348.5 ± 0.5	87.7 ± 1.7

ble II. Table II also presents the data on viscosity-average molecular weights of PVA calculated from the intrinsic viscosity values of the corresponding solutions.

It is seen that the PVA solutions, after preliminary equilibration with any of the fillers used, produce cryogels with a higher strength and higher T_f than those obtained from the preparation using the same gel-forming solution, which had not been preliminarily mixed with Sephadex. Also, as in the case with filled cryogels (Figs. 1 and 2), the absolute magnitude of the effects observed is greater for the preliminary mixing of PVA solutions with a large-pore filler, that is, Sephadex of the G-100 type.

In this respect, it is of interest to consider the data given in the last column of Table II. These data show evidence for a definite variation of molecular weight characteristics of PVA due to the equilibration of initial polymer solutions with G-25 and G-100 type fillers. Such equilibration cannot be accompanied by degradation, nor by crosslinking of PVA macromolecules, so that the \bar{M}_n changes are apparently due to the redistribution of PVA fractions that have different degrees of polymerization between the continuous and discrete phases. In other words, these effects are the manifestation of sieve properties of the fillers used.

The above-mentioned redistribution results from a strongly crosslinked dextran gel (of G-25 type) swollen in water producing a "depletion" of the PVA solution with respect to the low-molecular polymer fractions, leading to a change in the solution viscosity and, hence, in the values of \bar{M}_n calculated. At the same time, only extremely high-molecular PVA fractions and associated macromolecules (always present in the concentrated PVA solutions) were unable to diffuse into the pores of a weakly crosslinked filler (of G-100 type), which seems to be the

reason for somewhat higher \bar{M}_n values obtained in this case.

Unfortunately, gel permeation chromatography* failed to reveal sufficiently reliable differences between the elution profiles of these three samples (with aliquots taken from the solutions used for the determination of $[\eta]$) in the region of high molecular weights. However, the portion of the chromatographic pattern pertaining to low-molecular polymer-homologues showed their fraction to decrease after mixing with, and then separating from, the G-25 beads, than in the starting PVA solution. Therefore, GPC data confirm qualitatively the fact of "enrichment" of the continuous phase with high-molecular fractions of gel-forming polymer due to the "dilution" of low-molecular fractions because of their redistribution between the PVA solution and hydrogel-filler beads.

Comparison of the rheological characteristics and fusion temperatures of cryogels containing the Sephadex beads of G-25 or G-100 types (Figs. 1 and 2) with those of cryogels prepared from PVA solutions preliminarily equilibrated with a filler and then separated from it (Nos. 2 and 3, Table II), demonstrated that the former cryogels display greater strength and higher T_f than the latter ones. In other words, the total effect of the filler is not confined to the variation of the continuous phase (which is a real process, as shown above), but it is also due to the presence in the composite cryogel of a disperse filler *per se*. Regarding the physico-mechanical properties of cryogels, this conclusion is quite evident because an increase in the concentration of the

* The experiments were performed on a Series 700 HPLC instrument (Bio-Rad Lab., USA) with a 7.5×300 mm column of TSK Gel Type 4000 PW (Varian, USA) as described elsewhere.¹⁵

reinforcing filler to a certain extent will produce, for instance, a growth in the composite strength.⁴² Moreover, the use of a large-pore disperse additive (of G-100 type), which is permeable for the cryogel-forming macromolecules of PVA, may in principle form (in contrast to the small-pore additive) the "network-in-network" type structures within each particle of a weakly crosslinked Sephadex. As a consequence, another factor enhancing the reinforcing effect of fillers is the increase of the "size" of contacts between the discrete phase and the binder,⁴²⁻⁴⁴ which is also one of the possible reasons for the observed differences in the effects of G-100 and G-25 types of Sephadex on the rheological characteristics of the composite PVA cryogels. As to the fusion temperatures of the objects studied, at present it is difficult to give a well-substantiated and correct explanation for the observed increase in the T_f values in the presence of crosslinked dextran gels (Fig. 2) as compared to the samples obtained on the basis of PVA solutions corresponding to Nos. 2 and 3 from Table II.

In beginning the discussion of the results obtained in this work, we noted three specific features of the swollen beads of crosslinked dextran gels used as the fillers of PVA cryogels. The first two features (inherent deformability of Sephadexes and their sieve properties) have been discussed above. The third feature consists of the possibility of a limited thermodynamic compatibility of two concentrated polymer phases—the crosslinked dextran phase and PVA one. It is important to note that the case considered has one essential difference from the process of formation of the liquid two-phase polymer systems. Upon mixing of two aqueous solutions, for example, of the dextran and PVA, the establishment of equilibrium is accompanied by the distribution of all initial components, the solvent and both polymers, between the two liquid phases formed. As a result, each component will be present in both liquid phases; one of these is enriched with dextran and includes some PVA, while the other is rich in PVA and contains some dextran.⁴¹ Upon mixing the covalently crosslinked particles of a swollen Sephadex gel with the PVA solution, only the polyalcohol and water can be redistributed between the discrete and continuous phases. Their compatibility or incompatibility (similar to the thermodynamic incompatibility*) might affect, in our opinion, the structure and properties of the adhesion layer at the boundary

between each filler particle and the cryogel. In other words, in the case of compatibility (perhaps, even at limited compatibility) of the starting PVA solution and swollen Sephadex gel, the cryogel regions adjacent to the filler beads could not be significantly different in properties from those distant from the discrete phase, while the latter could contain cryogel even inside their granules. In the case of incompatibility, well pronounced interphase boundaries are expected to exist, for instance, as was observed in mixed gelatin-agarose gels.^{46,47}

It should be noted that we could not trace any published data concerning the properties of such boundary layers in a filled polymeric hydrogel. However, this problem is well known to be of importance in determining the integral characteristics of the composite materials in general. These are often considered as three-component systems composed of filler, binder, and the boundary layer phase itself.⁴²

Micrographs presented in Figure 4 show the results of SEM investigation of the composite PVA cryogels containing "immobilized" beads of G-25 *f* (Figs. 4a-c) or G-100 (Figs. 4d-f) Sephadex.

At small magnification, both disperse fillers show a pattern of uniform distribution of particles in the matrix of a heterogeneous PVA cryogel (Figs. 4a and 4d), and practically a complete absence of contacts between the beads. This last point is manifested in the lack of aggregation or formation of new spatial structures, for example, of the type of a continuous network of the filler itself. Moreover, a clearly pronounced boundary was observed between the G-25 *f* particles and the cryogel matrix, while in the case of G-100 *s f* filler, such kind of interfaces were not so conspicuous.

At greater magnification, the difference in the surface morphology of the discrete phase particles becomes more visible, depending on the type of a crosslinked dextran gel entrapped into the PVA cryogel body. The surface of G-25 *f* bead contains almost no "foreign material" (Fig. 4b), while the G-100 *s f* bead seems to be covered with a porous PVA shell (Fig. 4e). The thickness of this shell, and a possible penetration depth of the cryogel material inside the particle of a weakly crosslinked dextran gel, are yet unclear. Further studies are necessary in order to establish whether or not an additional network of PVA cryogel is formed within the volume of each G-100 grain.

Interesting information is disclosed in Figures 4(c) and 4(f), showing the cryogel structure in the boundary layers near the G-25 *f* and G-100 *s f* par-

* Brownsey et al.^{35,36} call this a "chemical affinity" of the matrix gel to the gel-filler particles.

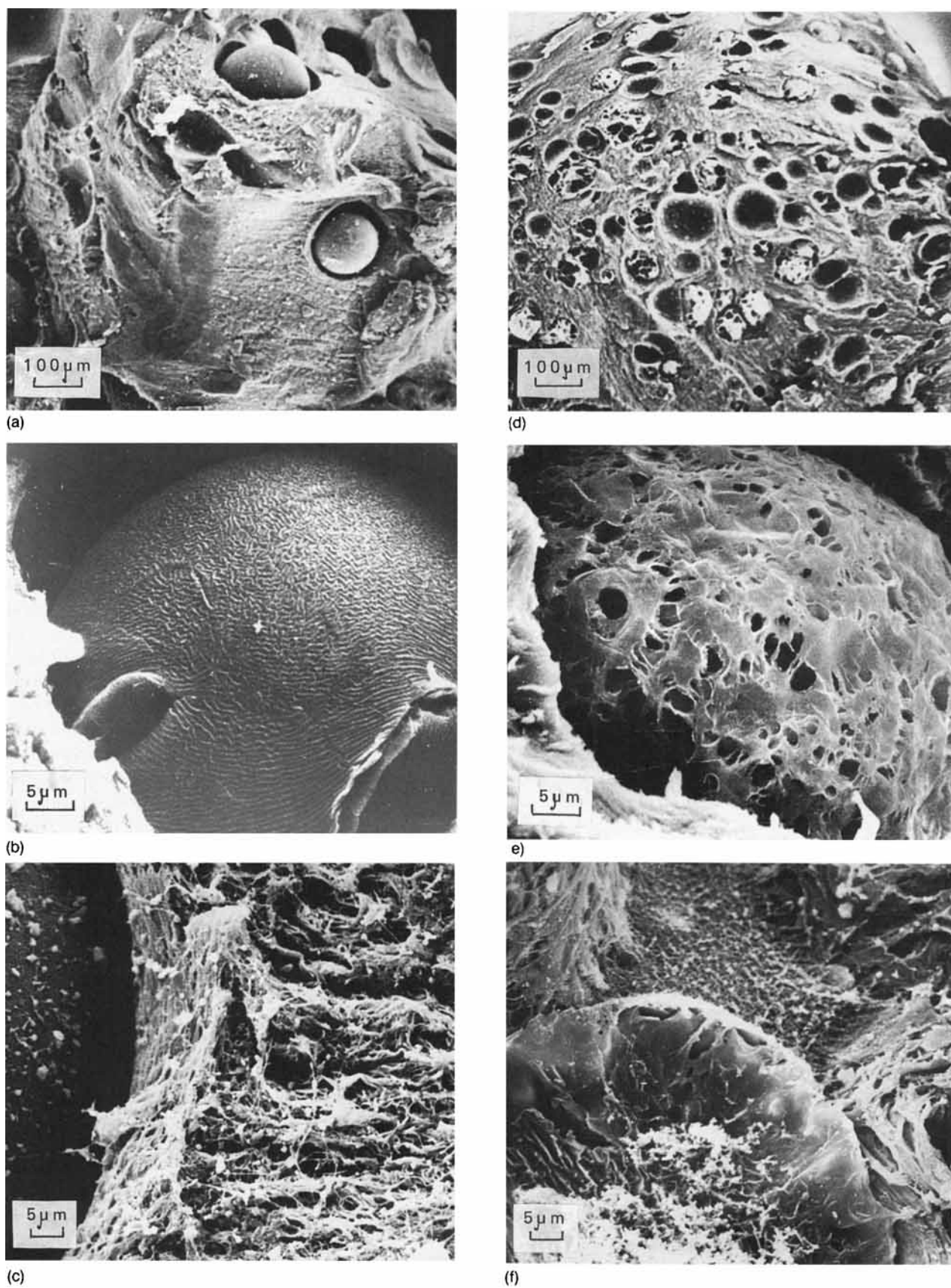


Figure 4 Scanning electron micrographs of 10% PVA cryogels filled with 10 wt % of swollen Sephadex beads: G-25f (a-c) and G-100s (d-f).

ticles, respectively. It is seen that as compared to the bulk of the binder matrix, PVA cryogel regions directly adjacent to the G-25 *f* filler surface are more compact, the pore size is smaller, and the network strands* tend to acquire a different predominant orientation (Fig. 4c). In the latter case with G-100s *f*, such differences between the cryogel structure "far away" from and "near" the filler beads are less pronounced, although this system also exhibits a certain tendency to an increased density of polymer binder in the boundary layers (Fig. 4f).

Such variations in the PVA cryogel structure at the interphase boundaries are not confined to the effects of limited thermodynamic compatibility; apparently they are due to a variety of some other phenomena. These may include, in particular, a local increase in the PVA concentration near a strongly curved surface of Sephadex microspheres, or a difference between the heat conductivities of a gel-forming medium and a disperse filler. The last factor might, in turn, affect the rate of propagation of a crystallization front across the boundary regions during the freezing of the composition and/or affect the thawing dynamics of these zones during defrosting. Note that the structure of PVA cryogels is highly sensitive to the regimes of cryogenic stages of gel formation.^{10,14,16,18,21,22,48}

In our opinion, a greater homogeneity of macrostructure of the PVA cryogel matrix filled with G-100 type Sephadex is another factor responsible for the greater reinforcement of a given composite as compared to the cryogel containing the G-25 type filler. This conclusion is based on the following consideration: since the average PVA concentration in the binder phase is practically the same in both cases (see Table II), compaction of the supermolecular cryogel network in the one region of the composite, that is, near the bead surface, must be accompanied by the "loosening" of the network in other, neighboring regions. This, in turn, will affect the rheological properties of the whole material, because the external stress-induced deformation occurs primary on the weakest elements of structure. SEM data reveals that inhomogeneities of this kind were mostly inherent to the structure of cryogels filled with swollen G-25 type Sephadex particles. Therefore, this factor, together with the points considered above, might lead to a lower strength of the G-25-

filled samples as compared to that of the cryogels containing the G-100 type beads.

Of course, it should be taken into account that the SEM pattern corresponds to the structure of a dried material, but not to the actual structure of the water-swollen hydrogel. In spite of all the precautions taken to retain the cryogel structure in the process of sample preparation for SEM (chemical fixation, gradual substitution of water by a volatile organic solvent, and drying at its critical point¹⁴) the real structure of the objects studied is usually somewhat distorted. For this reason, the bead dimensions, the pore sizes in the matrix of such composite gels, or the thicknesses of the boundary layers can be compared only at a qualitative level, that is, on the relative scale.

CONCLUSION

The study of the structure, rheological, and thermal properties of PVA cryogels containing an entrapped deformable disperse filler, viz., a crosslinked dextran hydrogel (Sephadex beads), revealed that the effects of various factors on the characteristics of the obtained composite materials are quite complex. Already at a stage of preparation of the suspension of filler particles in PVA solution, the molecular weight composition of the gel-forming polymer exhibits some changes due to sieve properties of Sephadex. After the redistribution of mobile components of the initial mixture and establishment of the equilibrium, a thermodynamic compatibility of two concentrated polymeric phases, the discrete and the continuous ones, may affect the adhesion between the filler and the PVA cryogel matrix obtained by the freezing-thawing method. The low-temperature structuring leads to the formation of compacted cryogel regions in the boundary layers near the Sephadex beads, thus increasing the inhomogeneity of the supermolecular network of the binder component in the composite material. The difference in permeability of various Sephadex types for the PVA macromolecules in the case of large-pore filler (of G-100 type) may result in an increase in the volume of regions where the filler particles are in contact with the binder cryogel phase. This might occur due to the possible formation of a "network-in-network" structure inside the beads or in their subsurface layers. Undoubtedly, there are some other, still unknown factors affecting the properties of the objects studied. All these factors, both known and unknown, produce various and sometimes even opposite effects

* In Ref. 14, the network strands have been shown to possess a bead-like construction and to be composed of globular supermolecular aggregates.

on the parameters of composite materials composed of a PVA cryogel with the particles of crosslinked dextran gels entrapped into the cryogel matrix. The resultant effect of the additives used on the physico-mechanical properties of PVA cryogels, for both types of Sephadexes studied (G-25 and G-100), can be classified as "active" fillers, although the latter exhibits a greater reinforcing ability.

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