Short Time Dynamics of Solvent Molecules and Supramolecular Organization of Poly (vinyl alcohol) Hydrogels Obtained by Freeze/Thaw Techniques

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ABSTRACT: The tensile mechanical properties of poly(vinyl alcohol) (PVA) hydrogels obtained by freeze/thaw techniques have been investigated by measuring the stress−strain curves. This analysis has been extended to gels obtained by drying the freeze/thaw PVA hydrogels immediately after their preparation and then rehydrating the so-obtained dried samples. The effect of aging and the drying/rehydration procedure on the viscoelastic behavior of PVA hydrogels in the freshly prepared state has been also analyzed. The stress−strain curves of PVA hydrogels indicate that the stress at any strain and the stress at break of gels increase with increasing the number of freeze/thaw cycles. Rehydrated gels are tougher than as-prepared gels due to the increase of polymer concentration. The shear storage modulus, G', at low frequency and low strain amplitude increases as the number of freeze/thaw cycles (n) increases. It reaches a plateau for $n = 3-5$ cycles; moreover, for a given number of freeze/thaw cycles, aged and rehydrated gels always exhibit lower G' values than as-prepared gels. This indicates that the stiffness of our gels decreases upon aging and rehydration. The gels prepared by imposing a single cycle make an exception. In the latter case, the drying/rehydration procedure produces physical properties that are higher in the resulting gels than in the as-prepared state. A structural parameter related to network mesh size is derived from measurements of the dynamic storage moduli at low values of strain frequency and amplitude. Finally, the intradiffusion coefficient of water in the gels has been measured through ^{1}H PGSE-NMR experiments. The latter experiment indicates that the polymer-poor regions embedded in the network scaffolding (due to interconnected polymer-rich regions) are in turn interconnected. Therefore, the network structure of freeze/thaw PVA gels may be described in terms of two bicontinuous phases.

Introduction

Poly(vinyl alcohol) (PVA) hydrogels prepared by repeatedly freezing and thawing diluted PVA aqueous solutions have attracted much attention in the past years for their many attractive properties as for instance high water content, dimensional stability at room temperature, high mechanical strength, rubberlike elasticity, lack of toxicity, and biocompatibility.[1−5]

The outstanding physical properties of freeze/thaw PVA hydrogels are closely related to their complex structure which is made of PVA chains and water molecules organized on different length scales.[1,2,6−8]

The setting up of the complex structure which characterizes freeze/thaw PVA hydrogels involves the formation of a cryogel at the low temperatures generally used during the freezing step (≈−20 °C).[2,6] Cryogels typically have interconnected macropores (or supramacropores) allowing unhindered diffusion of solutes of practically any size as well as mass transport of nanoparticles and even microparticles.[6,9]

The porous structure of freeze/thaw PVA hydrogels, along with their chemical and mechanical stability, makes freeze/thaw PVA hydrogels attractive matrices in a large variety of biotechnological applications as, for instance, in bioseparation chromatography or for immobilization of biomolecules and cells.[1,2,6] In addition their use for biomedical applications is largely documented.[10−15]

It is now accepted, although never directly confirmed, that the porous structure of these hydrogels is imprinted during the first freeze/thaw cycle and involves different phenomena as phase separation in a polymer-rich and polymer-poor phase and formation of ice crystals.[16−19] Upon thawing the gel to room temperature, the ice crystals melt but the gel structure does not collapse and leaves the porous structure of the hydrogel unaltered. At room temperature or during the thawing step, indeed, the porous structure of these gels is further stabilized by formation of PVA crystallites, acting as the junction points of the network scaffolding. PVA crystallites do not form at low temperatures, probably because the polymer is in the glassy state. During the successive cycles, the structure of the polymer scaffolding progressively acquires a higher stability.[16]

Therefore, the structure of freeze/thaw PVA hydrogels may be described in terms of a porous polymer network originating from the presence of two separated phases, one poorly concentrated in polymer (essentially water) and the second one with a high polymer concentration.[2,16,17] The polymer-rich phase is segregated into closely interconnected regions, which constitute the

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macroscopic network forming pores which are filled by the polymer-poor phase. The size of the polymer-poor domains is of the order of microns, consistent with the observation that these gels are opaque.[17,20]

Moreover, the polymer-rich phase is itself organized and consists of small micellar crystalline aggregates of PVA chains (of the order of nanometers) and amorphous domains. The PVA chains in the amorphous domains are swollen by the solvent and connect as tie chains the fringed micelle-like crystals. The presence of crystalline knots ensures a high dimensional stability of the gel and induces high strength and elastic properties in these materials.[16]

The rubber elasticity and high strength of these materials derive from their complex structural organization on different hierarchical scale lengths. The size of the different phases and microphases present in the gels is controlled by a number of factors as, for instance, polymer microstructure, degree of hydrolysis, concentration of mother solution, number of freeze/thaw cycles, and processing conditions (i.e., duration and temperatures of freezing and thawing steps).[1,18]

The possibility to control the size of the different phases and microphases present in these gels and their relative arrangement is limited by the fact that these systems may be subjected to large changes upon aging, even under conditions that reduce the water evaporation.[16,21]

It is possible to reduce, at least in part, the negative effects of aging by drying the freeze/thaw gel samples immediately after the preparation and then restoring the gel in the swollen state when needed, upon rehydration of the dried samples.[16] A structural analysis of freeze/thaw PVA hydrogels in the fresh, aged, and rehydrated states has been recently performed.[16] The relative amount of the different phases (crystals, swollen amorphous, and "free" water) present in the gels has been determined. It has been observed that the porous structure, formed during freeze/thaw cycles in PVA hydrogels, is not greatly altered upon drying and during the successive rehydration step; rehydrated gels, indeed, almost completely recover volume and shape of the as-formed freeze/thaw PVA hydrogels. It has been also noticed, but not directly proved, that rehydrated gels recover the outstanding mechanical properties of the gels in the fresh state used for their preparation. Moreover, in the case of gel samples obtained by imposing a single freeze/thaw cycle, apparently, the mechanical properties of rehydrated gels are improved with respect to those of the gel immediately after its preparation.

In ref 22, the viscoelastic properties of fresh freeze/thaw PVA hydrogels have been studied by measuring the dynamic shear modulus as a function of the polymer concentration of the mother solution used for their preparation and the number of freeze/thaw cycles. The degree of crystallinity of the PVA-rich phase has been also determined by ^{1}H free induction decay experiments. This analysis indicates that the PVA hydrogels become more fragile as the number of freeze/thaw cycles and the concentration of the initial solution increase; the storage modulus and the degree of crystallinity first increase with increasing the number of freeze/thaw cycles and then tend to a limiting value after six cycles. A detailed comparison of NMR and rheological data leads to the conclusion that the storage modulus is mainly controlled by the PVA crystallinity while the hydrogen bond interactions have a much smaller contribution.[22]

The first aim of this paper is to analyze the role of the different phases (polymer-poor and polymer-rich regions) and microphases (crystalline and swollen amorphous domains), which characterize the structure of PVA hydrogels, in determining their outstanding mechanical properties. The change in mechanical and viscoelastic properties of freeze/thaw PVA hydrogels due to the drying/rehydration procedure will be quantified by measuring the stress−strain curves of gels in the fresh and rehydrated states. A structural parameter related to the average length of the network strands will be derived from measurements of the dynamic storage modulus at low values of strain frequency and amplitude.

Diffusion of small molecules in polymer solutions is important in a variety of processes, including polymer processing, polymerization kinetics, drug delivery, and drying of coatings. From a basic point of view, it gives information concerning (i) the interaction between the diffusing molecules and the polymer chains and (ii) the polymer structuring in solution, which determines the path followed by the diffusing molecules. Whereas previous studies of as-prepared freeze/thaw PVA hydrogels show only a slight decrease of intradiffusion value of water in gel samples with respect to that one of water in mother solution[23] or no variation at all,[24] the effects of the number of freeze/thaw cycles and of a drying/rehydration procedure on the water diffusion within the gel samples have not been studied in detail. Therefore, in the second part of the paper, we will use the pulsed gradient spin echo−^{1}H NMR method (^{1}H PGSE-NMR) to determine the intradiffusion coefficient of water molecules within the hydrogels. Data thus obtained will then be discussed in conjunction with results obtained from mechanical and viscoelactic techniques to deduce further information on the supramolecular organization of PVA chains and water molecules in PVA hydrogels at the mesoscopic scale.

Experimental Section

Materials. All experiments utilized commercial grade PVA (Aldrich, ref 36,315-4) with an average molecular weight, $\bar{M}_w$, of about 115 000 and a degree of hydrolysis of 98−99%. The ^{13}C NMR spectrum analysis of PVA in deuterated water solution showed that the percentages of *mm*, *mr*, and *rr* configurational triads are 22.1, 50.1, and 27.8%, respectively.

PVA Hydrogel Preparation. Aqueous solutions of PVA with 11% w/w concentration were prepared by dissolving the PVA polymer in deuterated water at 96 °C, under reflux and stirring, for about 3 h. The polymer was entirely dissolved, and the obtained homogeneous solutions were slowly cooled to room temperature. We checked that the solutions do not jelly and remain transparent when left at room temperature in a sealed text tube for more than 1 month.

The freshly prepared PVA solutions were kept for one night to eliminate air bubbles and then poured between glass slides with 1 mm spacers, at room temperature.

Strong physical PVA hydrogel films were obtained by subjecting the polymer aqueous solutions to several repeated freeze/thaw cycles, consisting of a 20 h freezing step at −22 °C followed by a 4 h thawing step at 25 °C. In the following sections, the as-formed PVA hydrogels obtained by 1−9 freeze/thaw cycles are denoted as GEL-1 to GEL-9 samples.

Aged freeze/thaw PVA hydrogel samples were obtained by storing the as-prepared samples for 2 months at room temperature in sealed vials in order to minimize the loss of solvent.

Dried PVA hydrogel specimens were obtained by keeping in air, at room temperature, the as-formed PVA GEL-*n*

Table 1. Degree of Crystallinity Determined by ^{1}H Free Induction Decay Experiments, f_c(NMR), and Polymer Weight Concentration, Q_w, of PVA GEL-*n* Samples

PVA GEL-*n* state	no. of freeze/thaw cycles	f_c(NMR) (%)[a]	Q_w (% w/w)[b]
as-prepared	1	2.2	12.0
	3	5.31	12.7
	5	6.5	13.4
	9	7.1	14.9
aged	1	4.5	14.4
	3	6.6	18.7
	5	7.3	21.3
	9	7.7	20.0
24 h rehydrated	1	6.1	16.6
	3	9.9	22.1
	5	10.1	22.3
	9	10.6	23.0
14 days rehydrated	1	7.0	17.2
	3	9.9	23.6
	5	10.9	23.3
	9	11.9	23.3

[a] Determined from ^{1}H NMR experiments. [b] Determined by gravimetric measurements.

immediately after the last *n*th freeze/thaw cycle. The drying procedure was performed until the PVA hydrogel samples achieve a constant weight.

Rehydrated PVA hydrogel films were obtained by dipping the so obtained "dried gels" in deuterated water for 1 day (24 h) or 2 weeks (14 days).

PVA Hydrogel Specimens for ^{1}H Pulsed Gradient Spin-Echo NMR. In the case of ^{1}H pulsed gradient spin-echo NMR (^{1}H PGSE-NMR) experiments, a solvent mixture D_2O (80 vol %)/H_2O (20 vol %), rather than pure D_2O, was used both for preparing the starting 11 wt % PVA solution and for rehydrating the dried PVA hydrogel films, with the above-mentioned procedure. The use of this mixture ensures the best conditions for NMR lock stability and OH proton signal detection in the ^{1}H PGSE-NMR experiments.

Gravimetric and Crystallinity Measurements. The polymer content and the degree of crystallinity in the polymer-rich regions of the fresh, aged, and rehydrated GEL-*n* samples have been evaluated according to the procedure set up in refs 21 and 22. Polymer weight concentrations of aged and rehydrated PVA hydrogels were determined by weighting each sample in the swollen and in the corresponding dried state by using an analytical balance. The degree of crystallinity in the polymer-rich regions has been determined as the percentage of rigid protons of PVA in the gels by measuring the percentage of protons that relaxes during the first 20 μs in ^{1}H free induction decay experiments. According to the procedure set up in ref 22, solid-state ^{1}H NMR experiments were carried out at 300 MHz, using a Bruker AVANCE 300 WB spectrometer. ^{1}H free induction decay experiments were performed on static samples by using a single pulse sequence with a $\pi/2$ pulse duration of 3 μs and a delay time of 60 s. The error on each measurement was estimated to be of the order of $\pm$0.5%.

The so-determined polymer content and degree of crystallinity in the gels in the fresh, aged, and rehydrated states are listed in Table 1. It is apparent that the degree of crystallinity and polymer concentration increase with *n*, aging and under the drying/rehydration procedure.

It is worth noting that the degrees of crystallinity of these gels evaluated by solid-state ^{1}H free induction decay experiments are in good agreement with the degrees of crystallinity evaluated using other techniques.[16,21] In particular, the small values reported in Table 1 measured using the NMR method are consistent with the results of X-ray diffraction analysis of these gels, indicating that only a small fraction of PVA chains are involved in the formation of crystals.[16,21] Furthermore, in ref 21, it has been shown that the degrees of crystallinity obtained from ^{1}H NMR experiments provide the most accurate measurement of the degree of crystallinity of these systems.

Stress−Strain Experiments. Rectangular gel strips, of approximately 4 mm length and 2−3 mm width, were cut from PVA hydrogel sheets with a thickness ranging between 0.25 and 0.48 mm. To minimize the effect of water evaporation, gel samples were stored in sealed vials before measurement and each experiment was performed in a short time (less than 5 min, for a weight loss less than 5%).

Stress−strain curves for fresh and rehydrated PVA hydrogels were recorded using a miniature mechanical tester apparatus (Minimat by Rheometrics Scientific), at room temperature. The ratio between the drawing rate and the initial length was equal to 10 min^{-1}. The specimens were stretched up to the break.

The reported values of the mechanical properties were averaged over at least five independent experiments.

The determination of the whole stress−strain curves of our gels allows an easy evaluation of differences in the mechanical behavior in terms of stress at any strain and stress and strain at break of rehydrated gels with respect to the gels in the fresh state. However, the differences in stiffness of our gels upon rehydration (Young modulus) could not be determined with enough accuracy using our tensile apparatus because the values of stress at low deformation measured by our tensile apparatus would be affected by a too large error.

Rheological Measurements. Oscillatory dynamic mechanical measurements were performed on aged and rehydrated PVA hydrogels by using a strain-controlled Rheometrics RFSII rheometer equipped with parallel plate geometry (diameter 25 mm).

The experiments were carried out at 25 °C. Disk-shaped samples (diameter 26 mm, thickness $\sim$ 0.5−1 mm) of PVA gels were placed between the tools. The samples were protected from drying by a homemade cover which prevented the water from evaporating. This protection ensured the sample stability over a time period long enough (i.e., 1 h) to perform the measurements of the shear mechanical properties. In all experiments, a weak normal force was applied on the surface of the sample disks in order to avoid the sweeping of the gel from the tool plates. This force ensured a slight compression of the sample. In the frequency sweep experiments, the shear loss (G'') and elastic (G') moduli were measured in the linear viscoelastic regime, for frequencies ranging from 1 to 50 rad/s, at a maximum strain, γ_0, of 0.1−0.6%, depending on the sample. The γ_0 value was determined by preliminary strain sweep experiments, in which the storage and loss modulus were measured as a function of strain at a fixed frequency value of 1 Hz, to check whether the deformation imposed to the gel structure by the rheological experiment is entirely reversible.

Each measurement was repeated at least twice, on two different disk specimens from the same sample. The relative error on the storage modulus was of the order of 15%.

^{1}H PGSE-NMR Measurements. Intradiffusion coefficients of water in PVA solution and hydrogels were measured by the ^{1}H pulsed gradient spin-echo NMR technique.

A spin-echo ^{1}H NMR signal is generated from a sequence consisting of a $\pi/2$ radio-frequency (rf) pulse, followed by a π rf pulse. The intensity of the signal is modulated by two magnetic field gradient pulses. The magnetic field gradients of duration δ and strength G are applied between the initial $\pi/2$ and the refocusing π pulses of the spin-echo sequence and after the π pulse.[25] A delay time of 0.01 s was inserted between the offset of each rf pulse and the onset of magnetic field gradient, in order not to induce distortions of rf pulses. The duration between the onsets of the two gradients is Δ. T_E is the echo time. ^{1}H PGSE-NMR experiments were performed by varying the gradient width, δ, and keeping the gradient strength, G, and all other timing parameters constant.

If only one kind of diffusing molecules is responsible of the NMR signal, the quantitative relationship between echo attenuation and the intradiffusion coefficient is given by

$$A = A_0 \exp\left[-\frac{T_E}{T_2} - D_i\gamma^2G^2\delta^2\left(\Delta - \frac{\delta}{3}\right)\right] \quad (1)$$

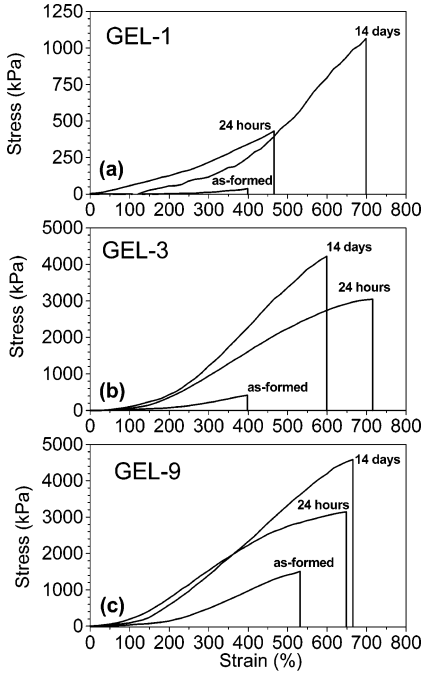

Figure 1. Stress−strain curves recorded at room temperature for as-formed, 24 h rehydrated, and 14 days rehydrated PVA hydrogels, GEL-*n*, obtained after different numbers of freeze/thaw cycles, *n*: (a) GEL-1; (b) GEL-3; (c) GEL-9.

where D_i stands for the intradiffusion coefficient of the *i* species responsible for the NMR signal, γ for the gyromagnetic ratio of the ^{1}H nucleus, T_2 for the spin−spin relaxation time, *A* for the spin-echo signal amplitude, and A_0 for the spin-echo amplitude without gradients. If more than one kind of diffusing molecules were responsible for an NMR signal, eq 1 does not hold anymore, and a multiexponential decay is usually observed.

From a series of spectra with increasing δ, the intradiffusion coefficient, D_i, can be determined by fitting the data with eq 1, if the strength of the applied gradient, *G*, is known. To establish the *G* value, ^{1}H PGSE-NMR measurements are performed on a reference sample with a known intradiffusion coefficient (D_{ref}).

In the present study, ^{1}H PGSE-NMR experiments were performed on as-formed and rehydrated PVA hydrogels at room temperature. A Varian FT80 ^{1}H NMR spectrometer operating at 80 MHz was employed. It was equipped with an AUTODIF/9010 pulsed magnetic field gradient unit, made by Stelar (Mede, Italy).

For each sample, the amplitude of spin-echo signal, *A*, was measured as a function of δ. The time between the $\pi/2$ and π pulses, Δ, was kept constant, and $T_E/2$ was fixed equal to Δ. $\Delta = 0.12$ s was used for PVA solution and $\Delta = 0.08$ s for GEL-*n* samples. The $\pi/2$ and π pulse durations were 5 and 10 μs, respectively, for a magnetic field of strength 18 800 G. The length of the two gradient pulses, δ, was varied between 0.002 s and 0.045 s to observe the decay of the spin-echo signal *A*. The measured values of *A* as a function of δ were fitted by using eq 1, through a nonlinear least-squares analysis.

Measurements to establish *G* value were performed by using D_2O with trace amounts of H_2O as the reference sample ($D_{ref} = D_{HDO} = 1.872 \times 10^{-9}$ m^2 s^{-1}).[26] The experimental error on the intradiffusion coefficients was generally less than 3%. Each measurement was repeated at least three times on the same sample, leading to intradiffusion coefficient values with a standard deviation lower than 2%.

Results and Discussion

Tensile Mechanical Properties. The stress−strain curves of as-formed and rehydrated (24 h and 14 days) PVA GEL-*n* samples are shown in Figure 1. They show that, for as-formed PVA GEL-*n*, in agreement with literature data,[17] the values of the stress at any strain and of the stress at break increase with increasing the number of freeze/thaw cycles while the values of the strain at break remain nearly constant. Moreover, for 24 h rehydrated GEL-*n*, the stress at any strain and the stress and strain at break increase with respect to those of the corresponding as-formed gel-sample. The stress−strain curves of 14 days rehydrated GEL-*n* samples are close to those of 24 h rehydrated samples, except that they present a higher value of stress at break and therefore of toughness.

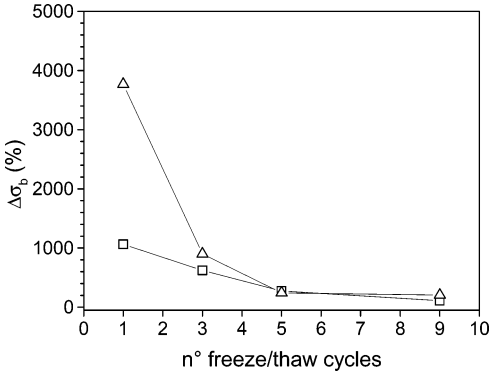

Figure 2. Relative increase of the stress at break, $\Delta\sigma_b$, for (□) 24 h rehydrated and (△) 14 days rehydrated GEL-*n* calculated with respect to the stress value at break of the corresponding as-formed samples.

Therefore, for a given number of freeze/thaw cycles, the toughness of rehydrated GEL-*n* samples is always higher than the toughness of as-prepared gels, and the very likely reason for this difference is that the polymer concentration increases upon application of the drying/rehydration protocol (see Table 1).

The major changes in the mechanical properties of GEL-*n* samples upon rehydration occur for $n = 1$. The relative increase of the stress at break, $\Delta\sigma_b$, for 24 h and 14 days rehydrated GEL-*n* samples with respect to that of as-formed GEL-*n* is reported in Figure 2. $\Delta\sigma_b$ is evaluated as $[(\sigma_b(\text{rehydr}) - \sigma_b(\text{fresh}))/\sigma_b(\text{fresh})] \times 100$, where σ_b(rehydr) is the stress at break of rehydrated samples and σ_b(fresh) the stress at break of freshly prepared samples. The percentage of stress increase in rehydrated GEL-1 is always higher than that of gel obtained by higher numbers of freeze/thaw cycles.

It is worth noting that the stress increases for as-formed GEL-1 after 24 h and 14 days drying/rehydration procedure are of about 1000% and 4000%, respectively (Figure 2). The structure of GEL-1 appears more susceptible to changes of physicomechanical properties than samples submitted to higher number of cycles.

Rheological Behavior. Aged PVA Hydrogels. Rheological measurements of GEL-*n* samples in the freshly prepared state have already been reported in ref 22. The effect of 2 months aging on the rheological behavior of these systems is analyzed here. As an example, the frequency dependence of G', performed at the low strain amplitude of 0.2−0.4%, is plotted in Figure 3 for 2 months aged PVA hydrogels obtained after different numbers of freeze/thaw cycles. For all the aged PVA GEL-*n* samples, G' and G'' do not depend on the test frequency in the range between 1 and 50 rad/s.

Figure 4 compares the strain dependence at 1 Hz of the storage G' and loss G'' modulus determined for as-

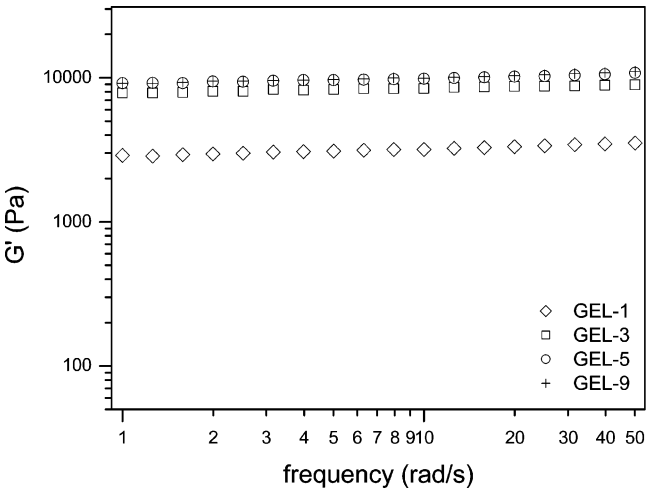

Figure 3. Storage modulus, G', as a function of frequency for 2 months aged PVA GEL-n samples.

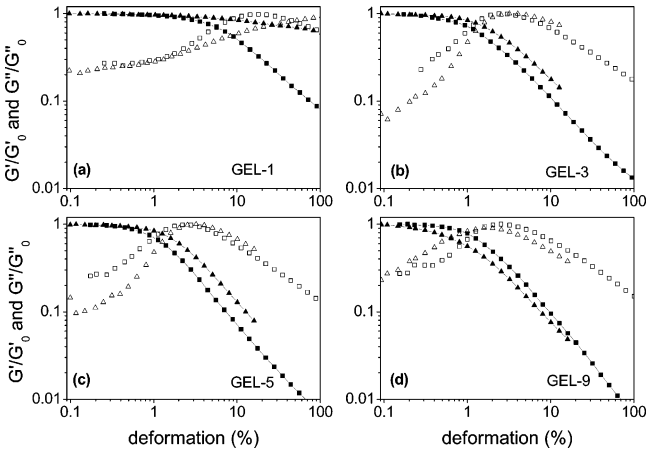

Figure 4. G' (full symbols) and G'' (open symbols) modulus at 1 Hz as a function of strain for as-prepared (△, ▲) and 2 months aged (□, ■) PVA GEL- n hydrogels: (a) GEL-1; (b) GEL-3; (c) GEL-5; (d) GEL-9. The G' and G'' moduli have been normalized with respect to their maximum values G'_0 and G''_0.

formed and 2 months aged PVA GEL-n samples. The values of G' and G'' have been normalized by their respective maximum value, G'_0 and G''_0. The behavior of the storage, G', and loss, G'', modulus in aged PVA GEL-n is close to that of GEL-n samples in the as-formed state, although some difference can be evidenced.

In aged GEL-1, the constant regime for G' appears shorter than that of as-formed GEL-1. The same phenomenon, even if less pronounced, is present in GEL-3 and GEL-5. In the case of GEL-9 samples, instead, aging seems to determine a slight extension of the linear regime of G'.

For GEL-1 sample, aging induces the major changes in the fragility of the network structure, consistent with the apparent high coherence induced by aging.

In Figure 5, the values of the storage modulus, G', determined at 1 Hz for the as-formed PVA gel samples[22] are compared to those of the corresponding 2 months aged PVA specimens as a function of the number of freeze/thaw cycles, n. The storage modulus, G', for aged GEL-n increases with increasing the number of freeze/thaw cycles and reaches a plateau value after the first 3−5 cycles. Moreover, G' values of aged PVA GEL-n samples with $n > 1$ are slightly lower than those of as-

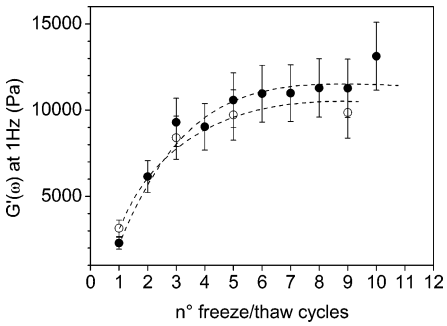

Figure 5. Storage modulus, G', at 1 Hz, as a function of the number of freeze/thaw cycles, for as-formed (●) and 2 months aged (○) PVA hydrogels. Fixed strain amplitude of 0.2−0.4% for aged gel samples and of 0.1% for as-formed samples. Data for GEL-n samples in the fresh state have been taken from ref 22.

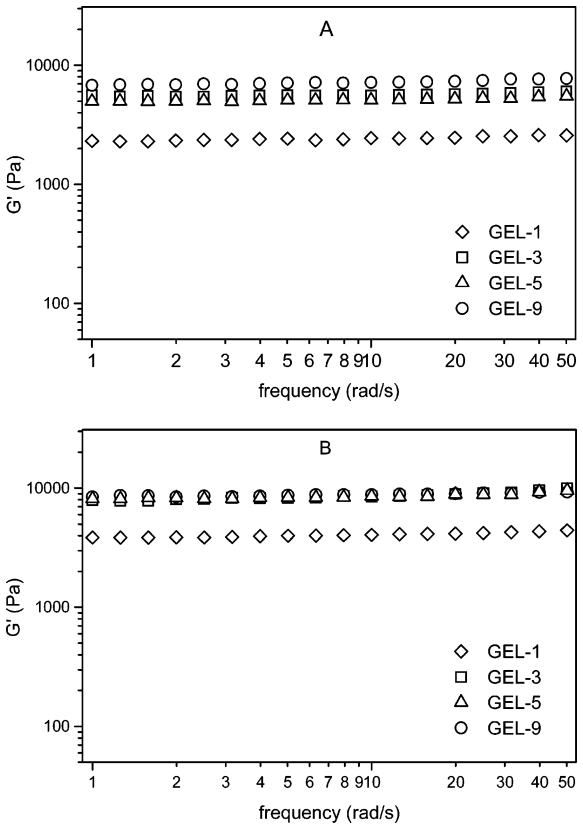

Figure 6. Storage modulus, G', as a function of frequency for dried PVA GEL-n samples: (A) 24 h rehydrated; (B) 14 days rehydrated.

formed gel samples, indicating that stiffness decreases upon aging.

Rehydrated PVA Hydrogels. Rheological experiments have been performed on dried PVA hydrogels rehydrated in D_2O for 24 h and 14 days.

All the frequency sweep experiments for rehydrated GEL-n were performed at the low strain amplitude of 0.3−0.6%, i.e., in the constant regime for G' and G''.

The frequency dependence of G' for 24 h and 14 days rehydrated PVA hydrogels, obtained after different numbers of freeze/thaw cycles, is reported in Figure 6A,B as an example.

For all the rehydrated PVA GEL-n samples investigated, G' and G'' (not shown in Figure 6) do not depend on the test frequency in the range between 1 and 50 rad/s, indicating that gels keep their elastic behavior.

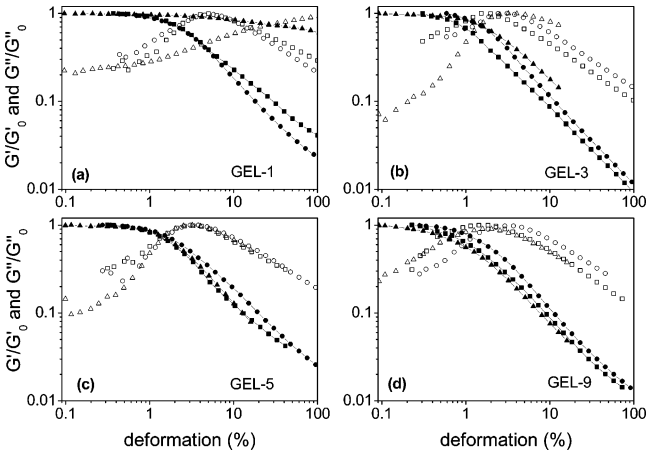

Figure 7. G' (full symbols) and G'' (open symbols) modulus at 1 Hz as a function of different strain values for as-prepared (△, ▲), 24 h rehydrated (□, ■), and 14 days rehydrated (○, ●) GEL-*n* samples: (a) GEL-1; (b) GEL-3; (c) GEL-5; (d) GEL-9. The G' and G'' moduli have been normalized with respect to their maximum values G'_0 and G''_0.

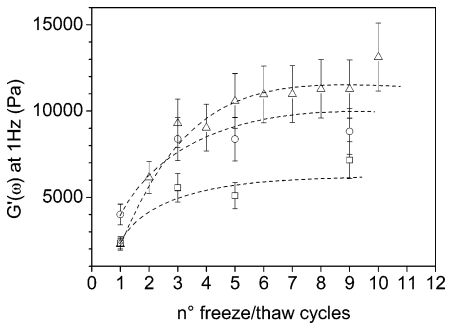

Figure 8. Storage modulus, G', at 1 Hz, as a function of the number of freeze/thaw cycles, for PVA hydrogels: as-formed (△); 24 h rehydrated in D_2O (□); 14 days rehydrated in D_2O (−). Fixed strain amplitude of 0.3−0.6% for rehydrated gel samples and of 0.1% for as-formed samples. Data for GEL-*n* samples in the as-formed state have been taken from ref 22.

The storage, G', and loss, G'', moduli determined for as-formed samples and for 24 h and 14 days rehydrated PVA GEL-*n* samples are compared in Figure 7 as a function of the strain at a fixed frequency of 1 Hz.

Figure 7 shows that rehydration does not significantly affect the strain amplitude which characterizes the end of elastic regime of the sample in all cases, except for GEL-1 sample.

Rehydrated GEL-1 samples, indeed, loose elasticity at smaller strain than GEL-1 in the as-prepared state, indicating that the drying/rehydration procedure significantly alters the viscoelastic properties of native PVA gel, obtained by imposing only one freeze/thaw cycle. It is worth noting that the G' value for 24 h rehydrated GEL-1 sample is similar to that of 14 days rehydrated GEL-1. These results indicate that, for GEL-1, the drying/rehydration procedure gives rise to a gel more fragile than the fresh gel and that the new properties are achieved in the first 24 h and do not change greatly under further rehydration in D_2O. In the case of GEL-*n* samples with $n \neq 1$, instead, rehydration does not greatly alter the viscoelastic properties of the samples in the as-prepared state. The values of the storage modulus, G', determined at 1 Hz for the as-formed PVA gel samples[22] are compared in Figure 8 to those of the corresponding rehydrated PVA GEL-*n* as a function of the number of freeze/thaw cycles, n. The storage modulus, G', for all rehydrated GEL-*n* samples, increases with increasing the number of freeze/thaw cycles, n, tending to a plateau value after the first 3−5 freeze/thaw cycles. The storage modulus, G', for 24 h rehydrated GEL-*n* samples is lower than that of freshly prepared samples, except for the GEL-1, for which the storage modulus in the freshly prepared state and after 24 h rehydration of dried samples are similar. The dried GEL-*n* samples, at a higher number of freeze/thaw cycles, need a 14 days rehydration to give rise to G' values comparable to those observed in the as-prepared state. In the case of GEL-1 sample, a 14 days rehydration, instead, results in G' values higher than the G' values determined in fresh samples.

In summary, whereas the analysis of the tensile properties of our gels indicates that rehydrated samples show higher toughness than GEL-*n* samples in the fresh state (Figure 1), the viscoelastic measurements indicate the rehydrated gels are less stiff (Figure 8).

Shear Storage Modulus and Crystallinity of PVA Hydrogels. In the preceding section, we have shown that, for a given number of freeze/thaw cycles, the shear storage modulus at low frequency and low strain amplitude are lower in the aged and rehydrated GEL-*n* samples than in the fresh GEL-*n* samples, for all n, except in the case when $n = 1$. The increase of dynamic viscoelastic modulus on increasing the number of freeze/thaw cycles in PVA hydrogels has been previously related to the density of cross-links and, therefore, of microcrystals.[1,22,27]

As shown by data reported in Table 1, aging and drying/rehydration protocols induce an increase in the polymer concentration and degree of crystallinity in these systems. These changes, in turn, strongly affect their viscoelastic behavior.

To investigate this aspect, the G' values of the GEL-*n* samples in the as-prepared, aged, and rehydrated states are plotted in Figure 9 as a function of the weight fraction of polymer, Q_w, in the gels (Figure 9A), determined by gravimetric analysis (Table 1), and of degree of crystallinity (Figure 9B), determined as the fraction of rigid protons in PVA hydrogels using ^{1}H free induction experiments, f_c(NMR). The G' values for each set of GEL-*n* samples may be roughly fitted with a straight line. Extrapolation of these lines to $G' = 0$ leads to values for the polymer content and crystallinity in the samples, around 10 and 1%, respectively, that are independent of the sample preparation. This result indicates that a minimum concentration of PVA and a minimum of crystallinity is required for PVA/D_2O systems to form gels. It is worth noting that the value of 1% crystallinity deduced from extrapolation in Figure 9B is coincident with the extrapolated value of minimum crystallinity required for gel formation obtained in ref 22, for independent sets of gel samples. In the latter case, indeed, the samples used for the measurements of G' and the percentage of rigid protons were GEL-*n* samples in the freshly prepared state, obtained by using PVA/D_2O solutions with different PVA concentration.

Moreover, in ref 22, it was observed that, for a given crystallinity, the storage modulus increases as the polymer concentration of mother solution increases. In our case, inspection of Figure 9B shows that, for a given crystallinity, the storage modulus decreases with aging time and exhibits the lowest value for rehydrated gels, independently of the rehydration duration. As a result

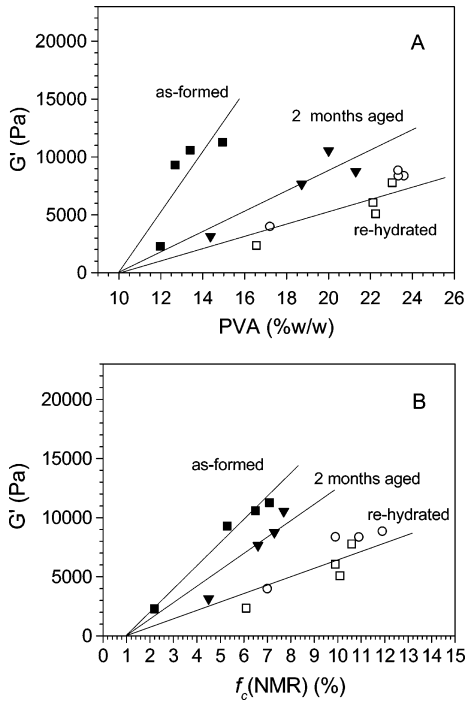

Figure 9. Storage modulus, G', at 1 Hz, as a function of the polymer weight fraction (A) and as a function of the percent of protons on rigid regions of the polymer (B) for 11% w/w PVA hydrogels in the as-formed state (■), 2 months aged state (▼), 24 h rehydrated state (□), and 14 days rehydrated state (○).

of these two opposite tendencies, although the polymer concentration and the degree of crystallinity in aged and rehydrated gels increase with respect to the degree of crystallinity of as-prepared gels, the storage modulus decreases. These observations indicate, in agreement with conclusions of ref 22, that although hydrogel crystallinity is the main factor that controls the G' values at low strain amplitude, G' values are also strongly affected by other structural factors, as discussed in the next section. Aging and drying/rehydration protocol, indeed, not only induce strong changes in the polymer content and degree of crystallinity in these systems but also affect other relevant structural parameters, which determine changes in their viscoelastic properties.

Correlation between the Storage Modulus (G') and the Mesh Size. As a first approximation, the structure of the gels under study has been related to their mechanical properties by using the theory of elasticity of polymer gels. This theory is based on a model which stipulates that the gel contains a collection of randomly coiled and cross-linked macromolecules that undergo affine deformation.[28] The main assumption of the affine network model is that, given a network consisting of strands connecting randomly positioned junctions, the relative deformation of each "network strand" is the same as the macroscopic relative deformation imposed to the whole network. In other terms, we have made the hypothesis that our gels may be regarded as a classic network, although they present a complex hierarchical structure. The simple assumption that the structure of our gels lacks complexity and may be modeled as a classic network permits to relate the storage modulus determined at low strain amplitude (0.1−0.6%) and low frequency (1 Hz) of our gels (i.e., in the fully elastic, reversible regime), G', to the average number of equivalent units in a "network strand", N, connecting two "ideal" junctions by use of the following formula:[28]

$$G' = \frac{RT}{N_{Av}a^3N}\phi_0^{2/3}\phi^{1/3} \tag{2}$$

where N_{Av} is the Avogadro number, G' is the shear modulus of PVA gel samples, R is the gas constant, T is the absolute temperature, ϕ_0 and ϕ are the polymer volume fractions of gel in the relaxed and in the swollen state, $N_{Av}a^3$ is the molar volume of the solvent, and N is the average number of equivalent units with volume equal to the solvent volume (a^3), comprised between two junctions (in a "network strand").[28] For deuterated water $N_{Av}a^3$ corresponds to 18 cm^3 mol^{-1}.

In the assumption that the relaxed state corresponds to the dry state, ϕ_0 is set equal to 1 for all the samples. This assumption relies on the fact that in the dried state the tie chains in the amorphous phase, connecting the physical cross-links, are in the unstretched state and correspond to the unperturbed dimensions. The values of ϕ ($= V_{dry}/V_{sw}$, the ratio between the volume of the gel in the dry state, V_{dry}, and the swollen, V_{sw}, state) are reported in Table 2.

The values of the network mesh size $L_c \propto \sqrt{N}a$ in as-formed, rehydrated, and aged PVA GEL-n samples have been approximately estimated by using the following equation:[30]

$$L_c = \phi^{-1/3}(C_\infty N)^{1/2}a \tag{3}$$

where C_∞ is the characteristic ratio of PVA, equal to 8.3.[31]

Values thus obtained are reported in Table 2. They indicate that the network mesh size of the gels is of order of hundred nanometers and thus ~10 times higher than the average correlation distance between the fringed micelle-like crystallites, determined in previous studies.[20,32] Such large values of L_c, indeed, reflect the microscopic heterogeneity of freeze/thaw PVA hydrogels originating from their phase-separated nature, including pores of various dimensions (from nanometers to micrometers[9,18,33]), highly interconnected through the network scaffolding ensured by the PVA chains.

The L_c values of GEL-n samples in as-prepared, aged, and rehydrated state decrease with increasing the number of freeze/thaw cycles. GEL-1 samples show always the largest values of L_c, within a given set of samples.

In all cases, except for GEL-1, L_c values are larger in 24 h rehydrated samples than in the as-prepared state. Therefore, the first marked effect of rehydration is a neat increase of the pore size with consequent formation of less stiff gels. As the time of permanence of dried samples increases, the pore size decreases. This rearrangement of the network structure leads to gels only slightly softer than the gels in as formed state. In the case of GEL-1 samples, instead, the L_c values of 2 months aged and rehydrated samples are lower than in the as-prepared state and the resulting gels become more stiff. This result is in agreement with a previous observation that the structure of GEL-1 sample is not yet well formed and, therefore, highly susceptible to significant structural changes upon rehydration and/or aging.[16,21]

^{1}H Pulsed Gradient Spin-Echo Nuclear Magnetic Resonance. In the present study, we have

Table 2. Polymer Volume Fraction of Gel in the Swollen State, ϕ, Storage Modulus, G' at 1 Hz, Average Number of Equivalent Units in a "Network Strand", N, and Average Length of "Network Strands", L_c, in As-Formed, 24 h Rehydrated, 14 Days Rehydrated, and 2 Months PVA GEL-*n* Samples

gel sample	no. of freeze/thaw cycles	gel state	ϕ^a	G' (at 1 Hz) (Pa)	N	$L_c{}^b$ (nm)
GEL-1	1	as-formed	0.107	2288	30803	318
		2 months aged	0.125	3146	23591	264
		24 h rehydrated	0.148	2351	33324	297
		14 days rehydrated	0.153	4001	19790	226
GEL-3	3	as-formed	0.113	9293	7728	156
		2 months aged	0.159	7659	10468	163
		24 h rehydrated	0.199	6066	14187	176
		14 days rehydrated	0.211	8383	10449	148
GEL-5	5	as-formed	0.119	10584	6908	145
		2 months aged	0.154	8758	9065	153
		24 h rehydrated	0.200	5095	16923	192
		14 days rehydrated	0.211	8371	10459	148
GEL-9	9	as-formed	0.133	11271	6721	138
		2 months aged	0.159	10542	7594	139
		24 h rehydrated	0.208	7771	11221	154
		14 days rehydrated	0.218	8853	10002	143

[a] Determined using the equation $\phi = \rho_s/[\rho_s + \rho_p(Q_w^{-1} - 1)]$, where ρ_p is the density of amorphous PVA (1.269 g/cm^3),[29] ρ_s the density of solvent (1.112 g/cm^3 for D_2O, 99.9% pure), and Q_w the polymer weight fraction of PVA in the gels. [b] Determined by eq 3.

analyzed the effect of the number of freeze/thaw cycles and of a drying/rehydration procedure on the water diffusion within the gel samples. For comparison the water intradiffusion coefficient of water in the mother solution has also been determined.

The X-ray diffraction study of our gels supports a model of PVA hydrogel samples containing two kinds of water: water molecules swelling the macropores (>80% of total water) and water molecules in the polymer amorphous phase.[16] The relaxation time of protons of the water molecules entrapped in the polymer-rich amorphous phase is expected to be much lower than that of "free" water molecules in the macropores. Therefore, by choosing an appropriate value of the duration of the spin-echo sequence, T_E, it should be possible to selectively monitor one kind of diffusing water molecules. In particular, by using high T_E values, water protons in the amorphous phase relax before echo detection, and only the diffusion of water molecules in the macropores is detected.

For all the examined samples, a monoexponential decay of the ^{1}H signal of water was observed, corresponding to just one detectable diffusing species, whatever the T_E values used in the spin-echo sequence. Measurements were also performed on a swollen PVA sample (characterized by 33 wt % water content) obtained by dipping, for 1 week, a melt-crystallized PVA film in a D_2O/H_2O (80/20 vol %) mixture. In this case, a broad and weak ^{1}H NMR peak was observed in the echo spectrum, whose intensity did not vary under the strongest gradient available by the instrument. This result indicates a D value lower than 10^{-12} m^2 s^{-1} for the water molecules swelling the polymer amorphous phase of the melt-crystallized film. It confirms the assumption that, under the chosen experimental conditions, only the mobility of the water inside the pores of the gel samples is observed.

The size of pores may be qualitatively derived from the measured intradiffusion coefficient by writing the root-mean-square diffusion displacement (X_{rms}) of a solvent molecule over the duration of the experiment as

$$X_{rms} = (2DT_E)^{1/2}$$

In the case of unrestricted diffusion (e.g., isotropic liquid solutions), D does not depend on the T_E value; in contrast, in the case of restricted diffusion (e.g., solvent inside a microemulsion droplet or a vesicle) D varies by changing T_E since X_{rms} cannot exceed the dimension of the microenvironment. Measurements performed on several PVA hydrogel systems at T_E values ranging between 0.1 and 0.4 s led to quite the same D value. This experimental evidence indicates that the motion of water molecules inside the hydrogel sample is unrestricted. This may be due either to the fact that the pore size is higher than the maximum X_{rms} experimentally detectable value (~20 μm) or to the fact that the pores are interconnected so that water molecules can easily move from one pore to another.

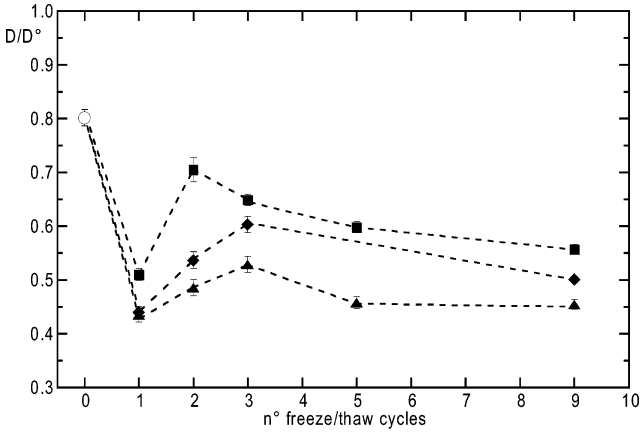

Figure 10. Water intradiffusion coefficients in PVA hydrogel samples as a function of the number of freeze/thaw cycles: (○) starting liquid mother solution (11% w/w PVA); (■) as-formed GEL-*n* samples; (◆) GEL-*n* samples dried and rehydrated for 24 h; (▲) GEL-*n* samples dried and rehydrated for 14 days.

In the following, to perform a detailed comparison of the water mobility in the different investigated samples, all the measurements were performed at $T_E = 0.16$ s.

The water intradiffusion coefficients, normalized with respect to the diffusion coefficient of neat water, $D/D°$, are reported in Figure 10 as a function of the number of freeze/thaw cycles for as-formed gels, samples dried and rehydrated for 24 h, and samples dried and rehydrated for 14 days. The water diffusion coefficient determined for the mother solution is also reported in Figure 10. For a 20/80 vol % mixture of H_2O and D_2O, the $D°$ value is 1.96×10^{-9} m^2 s^{-1}.

Inspection of Figure 10 shows that the translational mobility of water is appreciably reduced by the presence of dissolved PVA in the 11 wt % PVA solution. When the PVA solution is transformed into a hydrogel by the freeze/thaw procedure, a further decrease of $D/D°$ is observed, whose extent depends on the number of cycles performed. The data relative to the three kinds of samples show the same trend; particularly, the samples on which just one cycle has been performed (GEL-1) exhibit the lower solvent mobility. With increasing the number of cycles, $D/D°$ increases and reaches a maximum for two−three cycles; it then slightly decreases again, tending to a plateau.

By comparing the $D/D°$ data relative to the different kinds of samples, it can be observed that the values measured for the as-formed hydrogels are higher than those measured for rehydrated samples. Furthermore, samples rehydrated for 24 h exhibit a higher solvent mobility with respect to samples rehydrated for 14 days probably because a prolonged swelling reduces the pore size and results in a better swelling of PVA chains in the amorphous regions.

Solvent mobility in solution can be interpreted in terms of obstruction and solvation effects. In polymer solutions, both effects are important: the polymer chains obstruct the diffusion of small molecules, and particularly for good solvents, a large number of solvent molecules are involved in the polymer chain solvation. However, it has been shown that, over a wide range of concentration, the solvent intradiffusion in polymer solutions is generally dominated by obstruction and nonspecific kinetic effects rather than any chemical binding such as hydrogen bonding.[34] In this framework, the $D/D°$ decrease in hydrogels with respect to the PVA solution corresponds to an enhancement of the obstruction, which could be explained in terms of the formation of a network scaffolding, i.e., the formation of polymer-rich regions, highly interconnected, consisting of crystalline and amorphous PVA, rather than to the obstruction effect of the single polymer chains.

The variation of $D/D°$ with increasing the number of freeze/thaw cycles, and, particularly, the increase after the first cycle, has never been observed before and is not of straightforward understanding. The first freeze/thaw cycle induces a microscopic phase separation in the system, accompanied by the formation of crystallites in the polymer-rich phase. As discussed above, the polymer-poor phase is confined in regions embedded in the network scaffolding and constitutes a sort of interconnected macro- and micropores.[1,2,9] Since the freezing procedure is quite fast, the phase separation occurring during the first cycle may be not complete; i.e., it may not correspond to the thermodynamic stability. This means that the polymer-poor phase contains more polymer than expected. On the other side, the polymer-rich phase contains only few small crystallites[16] so that, when the sample is thawed, the amorphous fraction of the polymer-rich phase can be partially redissolved in the pores. In other words, GEL-1 results from an only partial segregation phenomenon. On repeating the freeze−thaw cycles, the gel structure improves, and a further phase separation of the polymer-poor phase within the pores occurs, forming a new, more dilute, polymer-poor phase and a more concentrated polymer-rich phase. Therefore, the increase of the $D/D°$ value from 1 to 2 cycles may be due to two factors: the decrease of the polymer concentration in the water solution swelling the pores and/or to the increase of the pore size during the second freeze/thaw cycle. The subsequent slight decrease of the $D/D°$ value passing from 3 to 9 cycles could be related to either the reduction of the pore size or the sample syneresis.

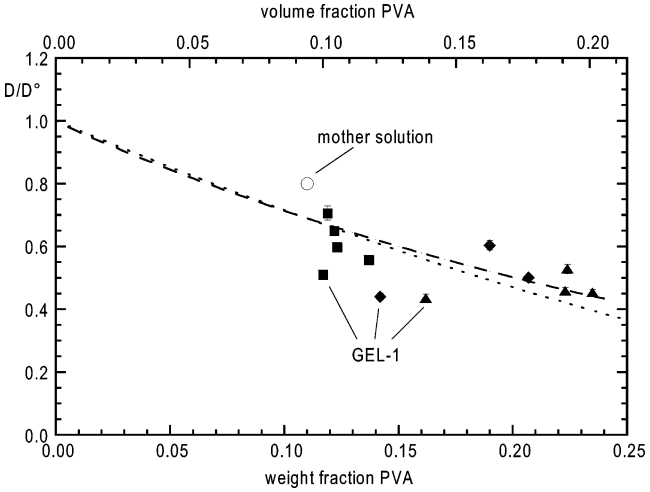

Figure 11. Water intradiffusion coefficients in PVA hydrogel samples as a function of the polymer weight fraction: (○) starting liquid mother solution (11% w/w PVA); (■) as-formed GEL-*n* samples; (◆) GEL-*n* samples dried and rehydrated for 24 h; (▲) GEL-*n* samples dried and rehydrated for 14 days. Dashed line: prediction of the Mackie−Meares model;[35−37] dotted line: prediction of the free volume theory.[34,38,39]

The $D/D°$ values for GEL-*n* samples obtained from different preparation protocols, for the same value of *n*, depend on the water content. As shown in Table 1, during the rehydration procedure, the gel samples does not acquire the full amount of water that it has lost during the drying step.

$D/D°$ values obtained for the whole set of gel samples are reported in Figure 11 as a function of the polymer weight fraction and polymer volume fraction. The data relative to GEL-*n* samples with $n > 1$ may be described by a single line, the $D/D°$ values for the mother solution and for GEL-1 samples lying above and below that trend, respectively, in agreement with differences observed between the behavior of GEL-1 and GEL-*n* (with $n > 1$) samples.

The trends of $D/D°$ predicted by two different hydrodynamic models are also shown in Figure 11. The Mackie−Meares model[35−37] (dashed line) is based on tortuosity (the increased path length between two points due to obstruction) and the simple cubic lattice model. The dependence of $D/D°$ as a function of the polymer volume fraction, ϕ, can be written as

$$\frac{D}{D^0} = \frac{(1-\phi)^2}{(1+\phi)^2} \tag{4}$$

Equation 4 is completely predictive, without any adjustable fitting parameters.

The Vrentas−Duda free volume theory[34,38,39] takes into account specific solute−solvent interactions; for solutions with concentration lower than 50 wt %, the concentration dependence of the diffusion coefficient is written as

$$\log\left(\frac{D}{D°}\right) = \frac{AQ_w}{1-Q_w} \tag{5}$$

where A is a system-dependent parameter and Q_w is the polymer weight fraction in GEL-n samples. The fit of eq 5 to experimental data is shown in Figure 11 as a dotted line. Although this method is unable to be completely predictive, the experimental results indicate that A is close to -1 for many polymer−solvent systems.[34] In the present case, an A value equal to -1.31 ± 0.11 is obtained. Both models adequately fit the experimental data for GEL-n with n higher than 1, indicating that, in these samples, which are different from GEL-1, obstruction plays a major role in determining the solvent mobility.

Conclusions

The mechanical behavior of PVA hydrogels, obtained by the freeze/thaw technique in as-prepared, 2 months aged, and rehydrated states, has been investigated by determining the stress−strain curves and storage and loss shear moduli at low strain amplitude.

The stress−strain curves of PVA hydrogels show that the toughness of gels increases with increasing the number of freeze/thaw cycles. Moreover, the gel samples, after a drying/rehydrating procedure, exhibit a further drastic increase of toughness, probably due to the increase of polymer concentration.

In all cases, the values of the storage modulus increase with increasing the number of freeze/thaw cycles and reach a plateau for n equal to 4 or 5.

For a given number of freeze/thaw cycles, aging induces only a slight decrease of the storage modulus (G') values with respect to the storage modulus of as-formed samples, while the drying/24 h rehydrating procedure induces a dramatic decrease in G' values. This G' decrease is an evidence for the chain reorganization that occurs in the crystalline and amorphous phase during the rehydration step. After 14 days of rehydration, the G' values, and, as a consequence, the PVA chains organization in amorphous and crystalline regions, are quite close to those observed in the as-formed samples.

When n is equal to 1, the application of the drying procedure improves the physical properties of the hydrogels. When the structure of the freeze/thaw gels has been stabilized by a larger number of freeze/thaw cycles, rehydration of dried PVA hydrogels leads to systems having physical properties as good as in the original freshly prepared state. These results may be due to the fact that, in GEL-1 whose structure is not yet well-fixed, rehydration improves the structure by dissolving the polymer fraction not included in the gel network. On the contrary, the structure of GEL-9, which is already highly stable, is not significantly altered by rehydration.

The ^{1}H PGSE-NMR technique has been used to investigate the water mobility inside the pores. The intradiffusion coefficient of water in the pores, for all the gel samples, is lower than in PVA solution. The water mobility in GEL-1 sample in as-formed, aged, and rehydrated states is always lower than the water mobility in GEL-n with $n > 1$. The water mobility inside the pores is governed by the pore size and/or the PVA concentration in the polymer-poor phase filling the pores.

^{1}H PGSE-NMR experiments also indicate that the polymer-poor regions embedded in the network scaffolding (due to interconnected polymer-rich regions) are in turn interconnected. Therefore, the network structure of PVA gels may be described in terms of the presence of two bicontinuous phases.

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