X-ray Diffraction Analysis of Poly(vinyl alcohol) Hydrogels, Obtained by Freezing and Thawing Techniques

Rosa Ricciardi,[†,‡] Finizia Auriemma,[†,*] Claudio De Rosa,[†] and Françoise Lauprêtre[‡]

Dipartimento di Chimica, Università di Napoli "Federico II", Complesso Monte S.Angelo,
Via Cintia, 80126 Napoli, Italy, and Laboratoire de Recherche sur les Polymères, UMR 7581-CNRS,
2 à 8 rue Henri Dunant, 94320 Thiais, France

Received November 5, 2003; Revised Manuscript Received January 5, 2004

ABSTRACT: The structure of physical poly(vinyl alcohol) (PVA) hydrogels prepared by subjecting a PVA/D_2O solution (11% w/w PVA) to freeze (−22 °C)/thaw (+25 °C) cycles has been systematically investigated by X-ray powder diffraction technique as a function of the number of cycles and aging time. The structural analysis has been extended to PVA "dried gels" and PVA hydrogels obtained by rehydrating the dried samples. The results of the present analysis confirm that highly stable PVA hydrogels, with a water uptake higher than 80%, may be obtained upon freeze/thaw cycles. The X-ray diffraction profiles of PVA hydrogels have been interpreted in terms of three components: "free water", crystalline PVA aggregates, and swollen amorphous PVA. The degree of crystallinity and the size of the crystals increase with increasing the number of freeze/thaw cycles and the aging time. Our results support the hypothesis that PVA hydrogels have a porous structure, with pores mainly occupied by water. The porous walls consist of swollen amorphous PVA while the crystalline domains act as knots of the gel network. The presence of crystalline knots ensures a high dimensional stability of the gel and induces elastic properties. Long time aging in sealed vials at room temperature induces large variations in the structure of freeze/thaw PVA hydrogels. The porous structure formed during freeze/thaw cycles in PVA hydrogels, instead, is not greatly altered upon drying and during the successive rehydration step; rehydrated gels, indeed, recover almost completely volume, shape, and physical properties of the as-formed freeze/thaw PVA hydrogels. Thus, the outstanding physical and mechanical properties of freeze/thaw PVA/hydrogels in the as-prepared state, may be preserved even for a long time, drying the samples immediately after the preparation and then restored when needed, upon rehydration of the dried samples.

Introduction

Poly(vinyl alcohol) (PVA), is a semicrystalline synthetic polymer able to form physically cross-linked hydrogels by different methods.[1−4] PVA hydrogels prepared by repeatedly freezing and thawing PVA aqueous solutions[5−8] have attracted much attention in the last years for their several potential applications[4] as for instance as biomedical materials,[9,10] for drug delivery,[4,11] in biotechnology for bioseparation, and as a carrier for cell immobilization.[12]

The preparation of PVA hydrogel using freezing and thawing techniques was first reported by Peppas.[5] The freeze/thaw method offers several advantages with respect to other methods, such as chemical cross-linking and radiation-induced cross-linking:[4] it is simple, it does not require any additional chemicals, and it does not need high temperature.

PVA hydrogels obtained by repeated freezing and thawing steps are thermoreversible and show many useful properties like high mechanical strength, rubberlike elasticity, stability at room temperature, ability to retain their original shape, high water content (80−90wt %), biocompatibility, and lack of toxicity.[4,8,13−15] Moreover, these PVA hydrogels are essentially insoluble in water but undergo a significant swelling if kept in water for a long time.[4]

Several parameters influence the final properties of PVA hydrogels, as, for instance, the number of freeze/thaw cycles and the polymer concentration. Therefore, for these hydrogels in which water tends to evaporate, aging is a serious problem which modifies the properties of the PVA gels and limits their applications.

As shown by previous studies, the freeze/thaw PVA gelation process results in the formation of a porous network in which polymer crystallites act as junction points.[3−5,8,16−21] The outstanding physical properties of freeze/thaw PVA hydrogels, indeed, strongly depend on the hierarchical structure extending in a very wide spatial scale.[4,8,17] Kanaya et al.[20,21] provide direct information concerning the structural organization of PVA gels formed in mixtures of DMSO and water on various length scales, through wide- and small-angle neutron-scattering and light-scattering experiments. In this continued work it was confirmed that cross-linking in these gels were small crystallites. It was shown that the crystallites had a sharp surface; the average size of the crystallites (≈70 Å) and the average distance between crystallites (on the order of 150−200 Å) in their gels were also esteemed.

The relative amount of crystal phase and the spatial arrangement of the crystals in the network play an important role in the physical properties of PVA hydrogels.[4] The microcrystalline domains are characterized by a X-ray diffraction maximum at $d \approx 4.55$ Å,[17] which was identified, on the basis of the monoclinic unit cell of PVA crystal established by Bunn,[22] as due to the superposition of equatorial $10\bar{1}$ and 101 reflections. This characteristic pattern can be clearly observed only for PVA hydrogels with a high PVA content.[16,17] However, for hydrogels with a PVA content as low as 10 to

---

* Corresponding author. Telephone: +39 081674341. Fax: +39 081674090. E-mail: auriemma@chemistry.unina.it.
[†] Università di Napoli "Federico II".
[‡] Laboratoire de Recherche sur les Polymères, CNRS.

10.1021/ma035663q CCC: $27.50

15%, ^{1}H and ^{13}C NMR have been used to derive information on the gel organization.[18,19]

The very small degree of crystallinity together with the large amount of diffuse scattering, typical of these hydrogels,[3,17,20] necessitates a careful analysis of the wide angle diffraction patterns which has not been performed until now.

This paper reports preliminary studies of the structure of PVA hydrogels obtained from freeze/thaw cycles with a low PVA content. We will examine in detail the wide-angle X-ray diffraction patterns in order to be able to determine their degree of crystallinity and apparent crystalline dimensions as a function of the number of freeze/thaw cycles. The structural characteristics of as-prepared and aged PVA hydrogels will be compared. In addition, “dried gels”, obtained by drying fresh hydrogels at room temperature under air atmosphere, will be investigated by using X-ray diffraction measurements. This structural analysis will then be extended to PVA hydrogels obtained by rehydrating the dried samples. Drying of the gel samples and rehydration of the dried gel allow one to preserve the outstanding properties of freeze/thaw PVA hydrogels for an unlimited time, decreasing the negative effects of aging.

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. To compare data obtained in the present paper to previous results derived from NMR experiments,[19] D_2O was preferred to H_2O. An aqueous solution of PVA (11% w/w) was prepared by dissolving the PVA polymer in deuterated water at 96 °C, under reflux, for about 3 h. The polymer was entirely dissolved, and the obtained homogeneous solution was slowly cooled to room temperature and kept at this temperature for one night, to eliminate air bubbles.

The aqueous PVA solution was then poured between glass slides with 1 mm spacers, at room temperature. PVA hydrogels were obtained by subjecting the polymer aqueous solution to several repeated freeze/thaw cycles, consisting of a freezing step (20 h at −22 °C) followed by a thawing step (4 h at 25 °C). The as-formed PVA hydrogels obtained by one to nine freeze/thaw cycles are denoted as GEL-1 to GEL-9 samples.

Freeze/thaw PVA hydrogels were aged by storing the as-prepared samples 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* immediately after the last *n*th freeze/thaw cycle. The drying procedure was performed until achieving a constant weight for the PVA hydrogel samples. Rehydrated PVA hydrogel samples were obtained by dipping the so obtained “dried gels” in deuterated water for 2 weeks, until achieving constant weight. The rehydrated specimens were examined by gravimetric and X-ray diffraction analyses.

Crystalline, 1 mm-thick, PVA films were obtained by melting a PVA powder in a hot press at 230 °C and slowly cooling to room temperature.

Gravimetric Measurements. Polymer weight concentrations of as-formed, aged, and rehydrated PVA hydrogels were determined by weighing each sample in the swollen state and in the corresponding dried state.

X-ray Measurements. Wide-angle X-ray powder diffraction profiles were collected at room temperature, with a Philips diffractometer using Ni-filtered Cu Kα radiation ($\lambda = 1.5418$ Å) and scans at 0.005 deg(2θ)/s in the 2θ range 10−60°. To prevent the drying of the sample during the experiment, the recording of the diffraction data was performed using a homemade brass sample holder placed in a special brass chamber covered with an out of focus Mylar film, in an atmosphere saturated with the vapors of the mother solution. During the time needed for recording the diffraction patterns (≈ 3 h), the weight loss of the sample was less than 2 wt %. Apparent crystalline dimensions along the [$10\bar{1}$] lattice direction were calculated by measuring the half-width at midheight of the corresponding Bragg reflection and applying the Scherrer formula.[23] Due to the low intensity of the Bragg peak at $2\theta = 19.4°$ in the crystalline PVA hydrogels, the standard deviation associated with the so determined apparent crystalline dimensions is on the order of ±3 Å.

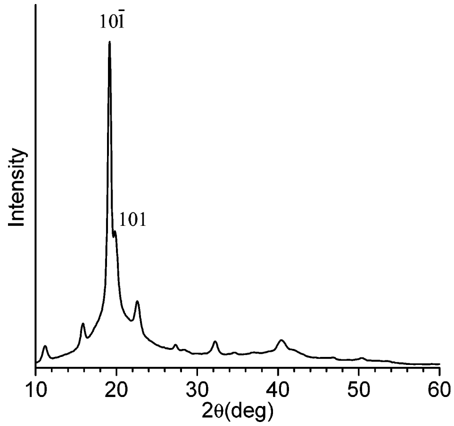

Figure 1. X-ray powder diffraction profile of a compression molded PVA film obtained by slowly cooling the melt to room temperature. The $10\bar{1}$ and 101 reflections at $2\theta = 19.4$ and 20° respectively are indicated.

Results and Discussion

Crystalline PVA. The X-ray powder diffraction profile of the melt-crystallized PVA film is reported in Figure 1. Sharp crystalline reflections, with a strong maximum at $d = 4.68$ Å ($2\theta = 19.4°$) and a shoulder at $d = 4.43$ Å ($2\theta = 20°$), typical of the crystalline atactic PVA,[24] are present. The diffraction profile indicates that, according to Bunn,[22] the sample is crystallized in the PVA structure characterized by chains in a trans-planar conformation, packed in a monoclinic unit cell with $a = 7.81$ Å, $b = 2.52$ Å (chain axis), $c = 5.51$ Å and $\beta = 91.42°$. The two strong maxima at $d = 4.68$ and 4.43 Å, correspond to the $10\bar{1}$ and 101 reflections, respectively (Figure 1). The degree of crystallinity is about 64%, whereas apparent crystalline dimensions (along the [$10\bar{1}$] lattice direction) are on the order of 156 Å.

As-Formed PVA Hydrogels. The polymer content of PVA hydrogels freshly prepared from a 11% w/w PVA solution is reported in Table 1. It slightly increases with increasing the number of freeze/thaw cycles ranging from 11.9% for GEL-1 to 14.9%(w/w) for GEL-9. This indicates that the first marked effect of the increase of the number of cycles is a slight increase of the polymer concentration in the freshly prepared gels. This result is in agreement with observation that as the number of the freeze/thaw cycles increases, PVA hydrogels expel D_2O to the surface.

The X-ray diffraction profiles of as-prepared hydrogel samples are reported in Figure 2 after subtraction of a straight baseline, approximating the background contribution. For comparison, the X-ray diffraction patterns of pure deuterated water, which is the major component of these gels, are also indicated in Figure 2. The diffraction profiles of the gels exhibit two halos centered at $2\theta \approx 28$ and 41°, as in the diffraction of pure water,

Table 1. Polymer Concentration, Total D_2O Content, Fractional Amount of Free D_2O, Degree of Crystallinity (x_c), Fraction of Swollen Amorphous PVA, Fraction of Crystalline PVA with Respect to the Sum of the Crystalline Fraction and the Swollen Amorphous Component (f_c), and Apparent Crystallite Dimensions (along the [$10\bar{1}$] Lattice Direction) of Freshly Prepared PVA/D_2O Gels Obtained by Different Numbers of Freeze/Thaw Cycles (Samples GEL-*n*, with *n* the number of freeze/thaw cycles)

gel sample	no. of freeze/thaw cycles	polymer[a] concn (% w/w)	tot. D_2O[a] (% w/w)	free D_2O[b] (%)	x_c (%)[b]	swollen[b] amorphous (%)	f_c (%)[b]	apparent[b] dimens of crystallite (Å)
GEL-1	1	11.98	88.02	86.2	0.4	13.4	2.5	28
GEL-3	3	12.69	87.31	84.2	0.8	15.0	4.8	34
GEL-5	5	13.40	86.60	80.9	1.1	18.0	5.6	35
GEL-7	7	14.72	85.28	80.1	1.1	18.8	5.7	38
GEL-8	8	13.70	86.30	78.5	1.4	20.2	6.3	40
GEL-9	9	14.94	85.06	78.1	1.4	20.6	6.3	39

[a] Determined by gravimetric measurements. [b] Determined by X-ray powder diffraction experiments.

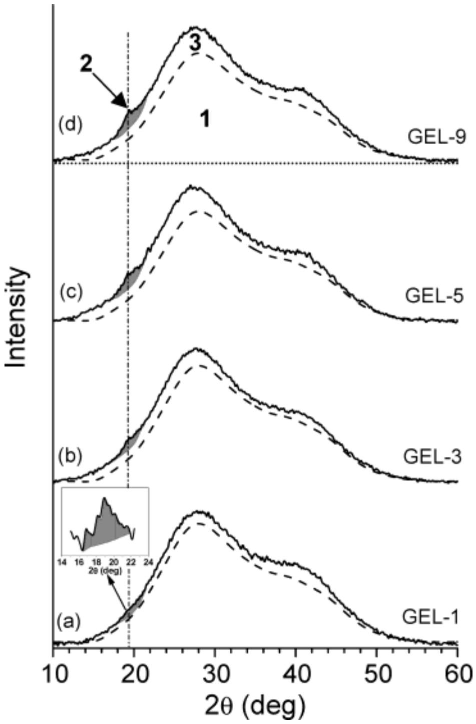

Figure 2. X-ray powder diffraction profiles of freshly prepared PVA/D_2O gels (samples GEL-*n*) obtained by different numbers *n* of freeze/thaw cycles (continuous line). The X-ray diffraction profile of liquid D_2O is also reported (dashed lines). The crystalline reflection in 2θ range 18−21° is evidenced in gray. The inset in part a shows the X-ray diffraction intensity in the 2θ range 14−24°, on enlarged scales for GEL-1.

and a weak peak in the 2θ range 18−21° which demonstrates the presence of a low amount of small crystalline PVA aggregates. Data plotted in Figure 2 show that the intensity of this reflection slightly increases with increasing the number of freeze/thaw cycles, indicating the increase of the amount of crystalline phase.

As a first approximation, the X-ray diffraction profiles of Figure 2 may be considered as arising from the sum of three contributions (Figure 2d): a large contribution (area A1) due to the scattering of the pure D_2O (dashed curves), a small diffraction component in the range 18−21° due to the crystalline aggregates (area A2), and a third component, (area A3) obtained by subtracting from the whole diffraction profiles of the gel, the diffraction profiles of the pure D_2O and of the crystalline fraction of the material. This third contribution may be attributed to the presence of an intermediate phase, containing PVA chains and water molecules intimately mixed in the network structure of the gel. Parts a−d of Figure 2 clearly show that the diffuse scattering due to this third component increases with increasing the number of freeze/thaw cycles; this effect is more particularly observed in the low 2θ region (10−30°). It is consistent with the presence of PVA chains in a disordered array closely bound to D_2O molecules, forming a swollen amorphous PVA phase.

Therefore, the relative amount of “free D_2O” molecules in the gel may be evaluated from the X-ray profiles of Figure 2 as the ratio between the area subtending the dashed curve (A1) and the area subtending the whole diffraction pattern of the gels A1/(A1 + A2 + A3). The values of the fraction of “free D_2O” in the various gel samples are reported in Table 1. These values are slightly lower than the values of the effective weight fraction of D_2O determined by gravimetric measurements.

The degrees of crystallinity (x_c) of the PVA hydrogel samples were approximately evaluated from Figure 2 as the ratio between the area of the crystalline reflection in the 2θ range 18−21° (A2) and the area subtending the whole diffraction profile of the gel sample: $x_c = A2/(A1 + A2 + A3)$. As shown in Table 1, in all cases, x_c is very low, in the range 0.4−1.4%. It increases with increasing the number of freeze/thaw cycles. The fraction of the swollen amorphous PVA phase, evaluated from the ratio A3/(A1 + A2 + A3) is also reported in Table 1.

The relative amount of crystalline PVA with respect to the sum of the crystalline and swollen amorphous portions, $f_c = A2/(A2 + A3)$, for the various PVA gel samples is reported in Figure 3 as a function of the number of freeze/thaw cycles (*n*). It is compared with the percentage of rigid protons derived from ^1H NMR free induction decay experiments on similar PVA hydrogel samples.[19] According to ref 19, as a first approximation, the free induction decay of a-PVA in the hydrogels exhibits two components characterized by a fast Gaussian-like decay with a relaxation time on the order of 20 μs and a much longer exponential decay. The former component, which corresponds to a small number of a-PVA protons, is characteristic of a rigid-lattice behavior whereas the latter component involves protons with different mobilities. In ref 19 it was assumed that the rigid a-PVA component is due to the a-PVA hydrogel crystallinity.

It must be noticed that the gel samples were here examined by X-ray diffraction analysis immediately after the end of the last imposed freeze/thaw cycle (which corresponds to zero aging time) whereas the NMR measurements were performed after a rather short delay which is specified in Figure 3 for each gel

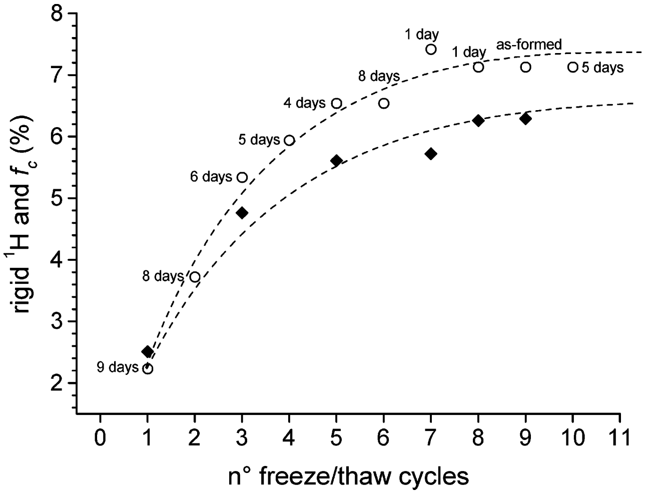

Figure 3. Fraction of crystalline PVA with respect to the total amount of PVA in the crystalline and the swollen amorphous phases, f_c, obtained by the X-ray powder diffraction profiles (◆) and fraction of rigid ^{1}H calculated by free induction decay ^{1}H NMR experiments (○) (redrawn from ref 19), as a function of the number of freeze/thaw cycles for the as-formed PVA hydrogels. The aging time imposed at each samples before ^{1}H NMR measurement is also indicated.[19]

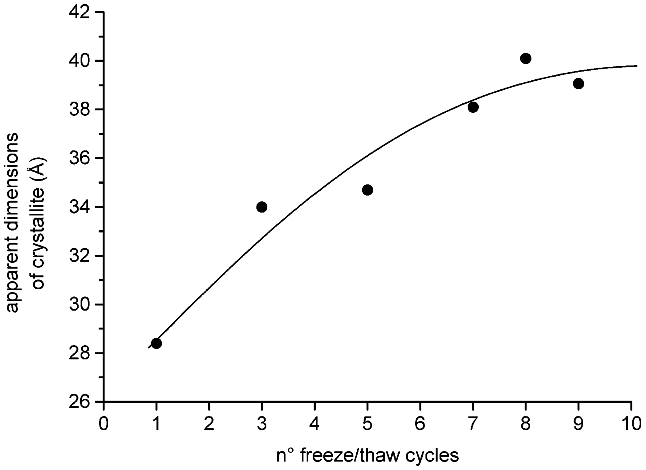

Figure 4. Apparent dimensions of crystallites along the [10$\bar{1}$] lattice direction, for as-formed PVA hydrogel samples as a function of the number of freeze/thaw cycles.

sample of ref 19. The percentage of rigid PVA protons and the values of f_c are in good agreement: they both increase with increasing the number of freeze/thaw cycles, tending to a limiting plateau after about 5 cycles. The f_c values range from 2.5 for the GEL-1 to about 6.3% for the GEL-9.

The apparent crystalline dimensions (along the [10$\bar{1}$] lattice direction) are reported in Figure 4 as a function of the number of freeze/thaw cycles for the as-formed PVA hydrogels. The crystallite dimensions increase from 28 Å for GEL-1 to 39 for the GEL-9.

The results of the present analysis are consistent with the model of the structure of PVA hydrogels obtained through freeze/thaw cycles, proposed in ref 3 and 18, based on small-angle X-ray diffraction and electron microscopy data. According to this model, freeze/thaw PVA hydrogels exhibit a porous structure, with pores mainly occupied by water. The porous walls consist of swollen amorphous PVA while the crystalline domains act as knots of the network. The formation of the crystalline knots ensures high dimensional stability and elastic properties of the gel. The quenching at low temperature, during the freeze step, induces the formation of ice crystals, which, in turn, expel amorphous polymer segments, increasing the polymer concentration in the surrounding environment. The size of ice crystals formed in the polymer-depleted pockets increases through the repeated freeze/thaw cycles. Upon thawing, the ice crystals melt and leave the porous structure of the hydrogel unaltered. Practically, water works as a porosigen in PVA solution whereas the polymer network increases its stability during the freeze step.[4,8,25,26] According to this model, our results show that both the degree of crystallinity of gels and the size of polymer crystallites increase by increasing the number of freeze/thaw cycles. The increase of degree of crystallinity is mostly due to the increase of the crystallite size, although formation of new crystalline aggregates may not be excluded. Our data also show that, whereas the overall gel concentration increases, a small amount of solution, mainly water, is expelled from the gel during each cycle and the volume of the gel decreases which, probably, also induces an increase of the concentration of the polymer in the swollen amorphous phase.

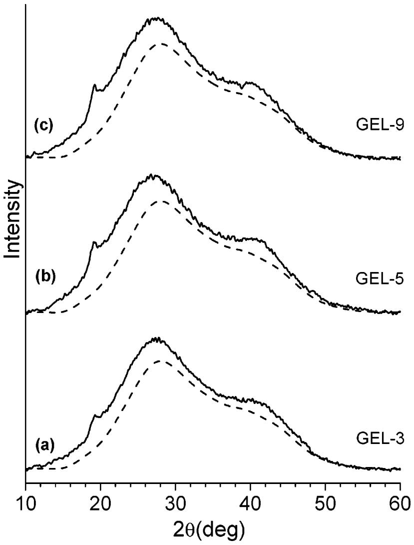

Figure 5. X-ray powder diffraction profiles of freeze/thaw PVA/D_2O gels aged for 2 months (continuous line): (a) GEL-3, (b) GEL-5, and (c) GEL-9. The X-ray powder diffraction profile of liquid D_2O is also reported as a dashed line.

Aged PVA Hydrogels. The effect of aging on the degree of crystallinity and the apparent dimensions of the crystallites for the as-formed PVA hydrogels obtained by 3, 5, and 9 freeze/thaw cycles has been also investigated. As an example, the X-ray diffraction profiles of the GEL-3, GEL-5 and GEL-9 samples, aged for 2 months, are reported in Figure 5. The polymer concentration, the total D_2O content, the relative amount of "free water", the crystallinities x_c, and f_c, the fraction of the swollen amorphous PVA and the crystal size for the as-formed GEL-3, GEL-5, and GEL-9 and for the same samples after a 2 months aging, are reported in Table 2. As shown by these data, aging induces a slight increase of polymer concentration and a significant increase of the degree of crystallinity. Moreover, the apparent crystalline dimensions, which were around 35 Å for the fresh GEL-3 and GEL-5 and 39 Å for GEL-9 samples, increase, after 2 months, to about 50 Å for the first two samples and 55 Å for the GEL-9. This increase in the crystalline dimensions is sufficient to account for

Table 2. Polymer Concentration, Total D_2O Content, Fractional Amount of Free D_2O, Degree of Crystallinity (x_c), Fraction of Swollen Amorphous PVA, Fraction of Crystalline PVA with Respect to the Sum of the Crystalline Fraction and the Swollen Amorphous Component (f_c), and Apparent Crystallite Dimensions (along the [101] Lattice Direction) of Freshly Prepared Freeze/Thaw PVA/D_2O Gels (Samples GEL-3, GEL-5 and GEL-9) and of the Same Gels 2 Months Aged at Room Temperature in Sealed Vials

		GEL-3	GEL-5	GEL-9
polymer concn[a] (% w/w)	as-formed	12.69	13.40	14.94
	2 months	18.72	21.30	20.00
total D_2O (%w/w)[a]	as-formed	87.31	86.60	85.06
	2 months	81.28	78.70	80.00
free D_2O (%)[b]	as-formed	84.2	80.9	78.1
	2 months	78.6	74.5	76.0
x_c (%)[b]	as-formed	0.8	1.1	1.4
	2 months	1.3	1.9	1.8
swollen amorphous (%)[b]	as-formed	15.0	18.0	20.6
	2 months	20.1	23.6	22.2
f_c (%)[b]	as-formed	4.8	5.6	6.3
	2 months	6.2	7.5	7.6
apparent dimens[b] of crystallite (Å)	as-formed	34	35	39
	2 months	49	50	55

[a] Determined by gravimetric measurements. [b] Determined by X-ray powder diffraction experiments.

the entirety of the increase of the PVA gel crystallinity reported in Table 2.

Dried PVA Gels. The X-ray diffraction patterns of as-formed, dried and rehydrated freeze/thaw PVA hydrogels obtained after 1 and 9 freeze/thaw cycles, are reported in Figure 6, parts A and B, respectively.

The polymer concentration, the total D_2O content, the relative amount of "free water", the crystallinities x_c and f_c, the fraction of the swollen amorphous PVA and the crystal size for the as-formed, dried and rehydrated PVA GEL-1 and GEL-9, are reported in Table 3. The X-ray diffraction profiles of dried PVA hydrogels (Figure 6) exhibit the strong diffraction maximum centered around $2\theta = 19.4°$, corresponding to the $10\bar{1}$ reflection of PVA crystals (Figure 1). As shown in Figure 6, the degree of crystallinity and the size of crystallites of dried gels increase with increasing the number of freeze/thaw cycles of the starting gels. They are quite smaller than the degree of crystallinity and the average crystallite dimensions of crystalline PVA given in Figure 1.

Rehydrated PVA Hydrogels. As shown in Figure 6, dried PVA gels, dipped in water, are able to swell and lead to rehydrated PVA hydrogels with a water content of about 77−83 wt %, depending on the number of freeze/thaw cycles of the starting PVA hydrogel sample. After swelling, all dried PVA hydrogels recover their initial shape and, apparently, their mechanical properties. Moreover, rehydrated samples of GEL-1 appear less sticky than the corresponding as prepared samples. It is worth noting that "dried gels" absorb a much higher amount of water than the melt-crystallized PVA film which can absorb 33 wt % of water only, under the same condition. During the swelling step of the dried PVA hydrogels, the amount of PVA that is dissolved in D_2O after 2 weeks is about 20wt % of the starting polymer mass for GEL-1, while it does not exceed 4 wt % for GEL-9. This could be due to the fact that an increase of the number of freeze/thaw cycles improves the stability of the whole gel structure and consequently induce a minor solubility in water of the PVA chains embedded in the network. For all the samples investigated, the amount of dissolved PVA is smaller than corresponding amounts reported in a previous work.[27] Such differences may be due to differences in the freezing duration, which was only 8 h for data reported in ref 27.

Table 3. Polymer Concentration and Total D_2O Content, Fractional Amount of Free D_2O, Degree of Crystallinity (x_c), Fraction of Swollen Amorphous PVA, Fraction of Crystalline PVA with Respect to the Sum of the Crystalline Fraction and the Swollen Amorphous Component (f_c), and Apparent Crystallite Dimensions (along the [101] Lattice Direction) of Freshly Prepared Freeze/Thaw PVA/D_2O Gels (Samples GEL-1 and GEL-9), of the Same Gels after Drying the As-Prepared Gel Samples in Air at Room Temperature ("Dried"), and of the Dried Gels after Rehydration in Water for 2 Weeks

		GEL-1	GEL-9
polymer concn[a] (% w/w)	as-formed	11.98	14.94
	dried	100.00	100.00
	rehydrated	17.20	23.32
total D_2O (% w/w)[a]	as-formed	88.02	85.06
	dried	0.00	0.00
	rehydrated	82.80	76.68
free D_2O (%)[b]	as-formed	86.2	78.1
	dried		
	rehydrated	69.6	72.6
x_c (%)[b]	as-formed	0.4	1.4
	dried	44.0	49.9
	rehydrated	1.1	2.9
swollen amorphous (%)[b]	as-formed	13.4	20.6
	dried		
	rehydrated	29.0	24.5
f_c (%)[b]	as-formed	2.5	6.3
	dried		
	rehydrated	3.7	10.6
apparent dimens[b] of crystallite (Å)	as-formed	28	39
	dried	39	42
	rehydrated	39	50

[a] Determined by gravimetric measurements. [b] Determined by X-ray powder diffraction experiments.

The X-ray diffraction patterns of rehydrated PVA hydrogels (Figure 6) show the small crystalline reflection centered around $2\theta = 19.4°$. However, the comparison of the X-ray diffraction patterns of as-formed and rehydrated PVA hydrogels in Figure 6 clearly shows a strong increase of the diffuse scattering in the low 2θ region in the case of the rehydrated gels. This phenomenon seems to be more important in the case for the GEL-1 sample than for the GEL-9 sample. This could indicate that repeated freeze/thaw cycles induce an improved organization of the polymer network in PVA hydrogel. The degrees of crystallinity in rehydrated GEL-1 and GEL-9 are 1.1 and 2.9%, respectively; they are slightly higher than the degree of crystallinity of the corresponding as-formed gels. Moreover, the apparent dimensions of crystallites of rehydrated PVA hydrogels slightly increase during rehydration.

Concluding Remarks

The results of the present analysis confirm that highly stable PVA hydrogels, with a water uptake higher than 80%, may be obtained upon freeze/thaw cycles. The X-ray diffraction profiles of PVA hydrogels have been interpreted in terms of three components: "free water", crystalline PVA aggregates, and swollen amorphous PVA.

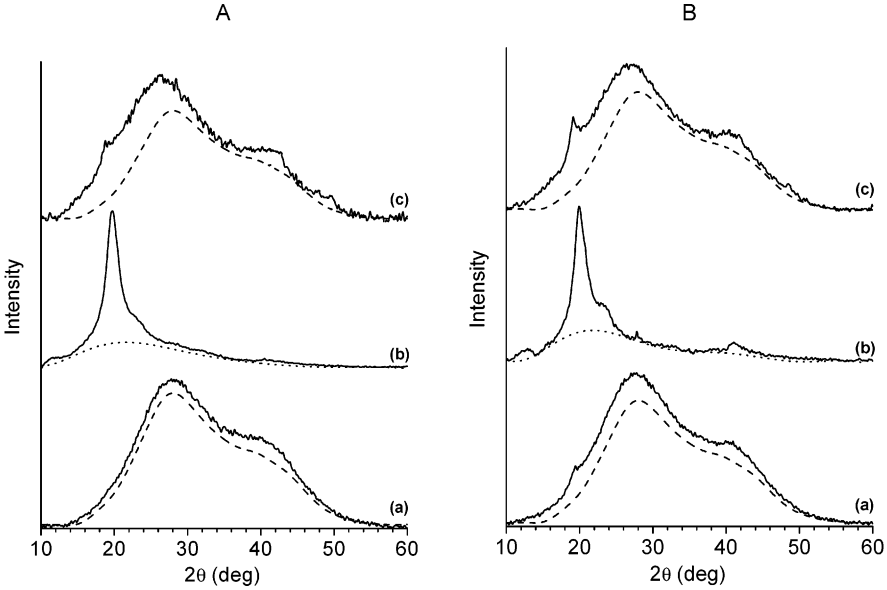

Figure 6. X-ray powder diffraction profiles of (A) GEL-1 and (B) GEL-9 in as-formed (curve a), dried (curve b), and rehydrated (curve c) states. The X-ray powder diffraction profile of liquid D_2O (dashed curves) and of amorphous PVA (dotted curves) are also shown.

The polymer concentration in as-formed PVA hydrogels slightly increases with increasing the number of freeze/thaw cycles. In freshly prepared gels, the relative amount of "free water" decreases, while the degree of crystallinity increases, with increasing the number n of freeze/thaw cycles, reaching a plateau for $n = 5$. Moreover, the apparent crystalline dimensions (along the $[10\bar{1}]$ lattice direction) increase with n. It is worth noting that, going from GEL-1 to GEL-9, the degree of crystallinity increases from 0.4 to 1.4% whereas the polymer concentration increases only from $\approx$12 to $\approx$15% w/w. Therefore, the increase of crystallinity with n is not only the result of an increase of polymer concentration in the gel, but it is also due to the significant improvement of the gel structure as a consequence of freeze/thaw cycles; in fact, each new cycle not only improves the structure of the water swollen PVA chains connecting the crystals, but it also induces an increase of the size and to a less extent of the number of the crystallites. Our analysis supports a model already proposed in the literature,[3,18] which describes the structure of freeze/thaw PVA hydrogels in terms of a porous polymer network where the crystals act as knots, the polymer segments ensure the connectivity all over the macroscopic gel sample, while free water fills the pores. Water also acts as a swelling agent in the disordered zones of polymer matrix forming hydrogen bonds with the OH groups of PVA chains. The porous structure is imprinted during the first freeze/thaw cycle by formation of ice crystals in zones placed inside the not yet well-structured gel during the freezing step. When the gel is thawed to room temperature, the ice crystals melt but the gel structure does not collapse. During successive cycles, the structure of the polymer scaffolding progressively acquires a higher stability.

Aging the samples in sealed vials at room temperature minimizes the drying process of the gel, inducing a slight increase of the polymer concentration and a significant increase of the degree of crystallinity, mainly due to an increase of the crystal size. In fact, the slow drying process occurring in gels stored for 2 months in sealed vials at room temperature, without taking any additional care to not alter the original structure of the freeze/thaw gels, leads to gel samples with apparent dimensions of the crystals higher than the crystalline dimensions of the as-prepared gel samples, and also higher than the crystalline dimensions of the "dried gels", obtained by leaving in air, at room temperature, freshly prepared freeze/thaw PVA hydrogels.

Dried PVA gels exhibit the crystalline PVA form. The degree of crystallinity and the size of crystallites of dried gels increase with increasing the number of freeze/thaw cycles of the starting gels. Their degree of crystallinity and average crystallite dimensions are smaller than those observed on PVA films crystallized from the melt.

Dipping dried gel samples in D_2O for a few minutes leads to rehydrated PVA hydrogels with a water content comparable to the water content of the as-prepared gels. Rehydration is also accompanied by the recovery of the initial shape and dimension of the freeze/thaw gel in the as-prepared state. The higher the number of cycles, the lower the amount of polymer that dissolves in water during rehydration. This result is the indication of a better organization of the polymer network and, consequently, a higher resistance to dissolution in water, for high values of n. Moreover, when n is small ($n = 1$), application of the drying procedure to freeze/thaw PVA hydrogels may lead to better physical properties in the resulting gels after rehydration, as compared to those of the gels in as-prepared state. When the structure of the freeze/thaw gels is already stabilized by imposing a larger number of freeze/thaw cycles, rehydration of dried gels induces the formation of PVA hydrogels having physical properties as good as in the original freshly prepared state. This may be due to the fact that, in the case of 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 other hand, the structure of GEL-9, which is already highly stable, is not greatly altered by rehydration.

The results of the present analysis indicates that the porous structure formed during long freeze/thaw cycles in PVA hydrogels is not greatly altered upon drying and during the successive rehydration step. However, in the case of not yet well-stabilized freeze/thaw PVA hydrogels, the drying/rehydration protocol may be quite useful, to improve the physical properties of the as-prepared freeze/thaw hydrogels. In addition, while long aging time in sealed vials at room temperature may induce large variations in the structure of freeze/thaw PVA hydrogels, the gels, once dried, can be stored for a time long at will. Rehydration of dried gels may be performed only when needed by the particular application or usage; rehydration, indeed, restores the physical properties of the freshly prepared freeze/thaw PVA hydrogel used as starting material.

Further work is in progress in order to extend the structural analysis of PVA hydrogels obtained by freeze/thaw technique to larger spatial scales and to ultimately relate the structural features to the physical properties of these systems.

Acknowledgment. The Centro di Competenza “Nuove Tecnologie per le Attività Produttive”Regione Campania P.O.R. 2000-2006 Misura 3.16 is gratefully acknoweledged for the financial support.

References and Notes

(1) Peppas, N. A.; Merrile, E. W. *J. Appl. Polym. Sci.* **1977**, *21*, 1763.

(2) Guenet, J. M. *Thermoreversible Gelation of Polymers and Biopolymers*; Academic Press: San Diego, CA, 1992.

(3) Yokoyama, F.; Masada, I.; Shimamura, K.; Ikawa, T.; Monobe, K. *Colloid Polym. Sci.* **1986**, *264*, 595.

(4) Hassan, C. M.; Peppas, N. A. *Adv. Polym. Sci.* **2000**, *153*, 37.

(5) Peppas, N. A. *Makromol. Chem.* **1975**, *176*, 3433.

(6) Nanbu, M. JP58036630 1983.

(7) Nanbu, M. JP59082939 1984; JP59082940 1984.

(8) Lozinsky, V. I. *Russ. Chem. Rev.* **1998**, *67*, 573.

(9) Tamura, K.; Ike, O.; Hitomi, S.; Isobe, J.; Shimizu, Y.; Nambu, M. *Trans.-Am. Soc. Artif. Intern. Organs* **1986**, *32*, 605. Oka, M.; Noguchi, T.; Kumar, P.; Ikeuchi, K.; Yamamuro, T.; Hyon, S. H.; Ikada, Y. *Clin. Mater.* **1990**, *6*, 361. Noguchi, T.; Yamamuro, T.; Oka, M.; Kumar, P.; Kotoura, Y.; Hyon, S. H.; Ikada, Y. *J. Appl. Biomater.* **1991**, *2*, 101. Cha, W. I.; Hyon, S.-H.; Ikada, Y. *Macromol. Symp.* **1996**, *109*, 115.

(10) Hyon, S.-H.; Cha, W.-I.; Ikada, Y.; Kita, M.; Ogura, Y.; Honda, Y. *J. Biomater. Sci.—Polym. Ed.* **1994**, *5*, 397.

(11) Peppas, N. A.; Mongia, N. K. *Eur. J. Pharmacol. Biopharm.* **1997**, *43*, 51. Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharmacol. Biopharm.* **2000**, *50*, 27.

(12) Lozinsky, V. I. *Trends Biotechnol.* **2003**, *21*, 445.

(13) Urushizaki, F.; Yamaguchi, H.; Nakamura, K.; Numajiri, S.; Sugibayashi, K.; Morimoto, Y. *Int. J. Pharm.* **1990**, *58*, 135.

(14) Stauffer, S. R.; Peppas, N. A. *Polymer* **1992**, *33*, 3932.

(15) Peppas, N. A.; Scott, J. E. *J. Controlled Release* **1992**, *18*, 95.

(16) Komatsu, M.; Inoue, T.; Miyasaka, K. *J. Polym. Sci., Part B: Polym. Phys.* **1986**, *24*, 303.

(17) Watase, M.; Nishinari, K. *J. Polym. Sci., Part B: Polym. Phys. Ed.* **1985**, *23*, 1803; *Macromol. Chem.* **1989**, *190*, 155.

(18) Willcox, P. J.; Howie, D. W.; JR.; Schimdt-Rohr, K.; Hoagland, A.; Gido, S. P.; Pudjijanto, S.; Kleiner, L. W.; Venkatraman, S. *J. Polym. Sci. Part B: Polym. Phys.* **1999**, *37*, 3438.

(19) Ricciardi, R.; Gaillet, C.; Ducouret, G.; Lafuma, F.; Lauprêtre, F. *Polymer* **2003**, *44*, 3375.

(20) Kanaya, T.; Ohkura, M.; Kaji, Furusaka, M.; Misawa, M. *Macromolecules* **1994**, *27*, 5609.

(21) Takeshita, H; Kanaja, T.; Nishida, K.; M.; Kaji, K. *Macromolecules* **1999**, *32*, 7815. Kanaja, T.; Ohkura, M.; Takeshita, H.; Kaji, K.; Furusaka, M.; Yamaoka, H.; Wignall, G. D. *Macromolecules* **1995**, *26*, 3168. Kanaja, T.; Takeshita, H.; Nishikoji, Y.; Ohkura, M.; Nishida, K.; Kaji, K. *Supramol. Sci.* **1998**, *5*, 215. Takeshita, H.; Kanaja, T.; Nishida, K.; Kaji, K.; Takahashi, T.; Hashimoto, M. *Phys. Rev. E* **2000**, *61*, 2125.

(22) Bunn, C. W. *Nature (London)* **1948**, *161*, 929.

(23) Klug, H. P.; Alexander, L. E. *X-ray Diffraction Procedures*; J. W. Sons: New York, 1959.

(24) Mooney, B. C. L. *J. Am. Chem. Soc.* **1941**, *68*, 2828.

(25) Chen, J.; Park, H.; Park, K. *J. Biomed. Mater. Res.* **1999**, *44*, 53.

(26) Oxley, H. R.; Corkhill, P. H.; Fitton, J. H.; Tighe, B. J. *Biomaterials* **1993**, *14*, 1065.

(27) Hassan, C. M.; Peppas, N. A. *J. Appl. Polym. Sci.* **2000**, *76*, 2075.

MA035663Q