Investigation of the Crystallinity of Freeze/Thaw Poly(vinyl alcohol) Hydrogels by Different Techniques

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ABSTRACT: The crystallinity of freeze/thaw poly(vinyl alcohol) (PVA) hydrogels, either fresh or aged or obtained by dipping dried freeze/thaw gel samples in water immediately after their preparation, was investigated by using different techniques. Free induction decays obtained from ^{1}H NMR experiments provide the most accurate measurement of the degree of crystallinity of these systems. Values thus obtained are in a good agreement with data obtained by X-ray diffraction for all the samples under study. The degrees of crystallinity, determined by using differential scanning calorimetry (DSC), instead, are lower than those obtained by the other two methods, for all the gel samples, but the aged gels. This result is due to the occurrence of the gel−sol transition during the heating scan which is characterized by the endothermic melting of the crystallites and the exothermic solubilization and solvation of PVA chains in water. In as-prepared and rehydrated gels, the endothermic and exothermic effects overlap, which leads to an underestimated value of the degree of crystallinity. For aged samples, the crystallites are larger and more perfect; the corresponding melting endotherms are narrower and shifted toward higher temperatures, which permits the separation of the endothermic and exothermic effects and leads to a more accurate measurement of the degree of crystallinity. Thus, the comparative analysis of the degree of crystallinity in PVA hydrogels measured by different techniques provides indirect information concerning their complex structure.

Introduction

Application of freeze−thaw cycles to aqueous solutions of poly(vinyl alcohol) (PVA) permits to obtain gels with improved physical properties with respect to PVA hydrogels obtained with other techniques.[1] Typically, freeze/thaw PVA hydrogels are elastic, they manifest a long time dimensional stability, they can be extended up to 5−6 times their initial length, and they do not lose elasticity even after immersion in water for a long time.[2,3] The tested biocompatibility of freeze/thaw PVA hydrogels and their ability to incorporate and release large amounts of host molecules of different size in their structure make these systems particularly attractive for biomedical and biotechnological applications.[1,4,5]

The outstanding physical properties of PVA hydrogels derive from their complex structure, where PVA chains and solvent molecules are organized at different hierarchical scales. PVA hydrogels exhibit a porous structure, with pores filled by a polymer-poor phase. The network scaffolding is ensured by highly interconnected regions of a polymer-rich phase. The latter phase is itself organized and consists of small micellar crystalline aggregates of PVA chains and amorphous domains. The PVA chains in the amorphous domains are swollen by the solvent and act as tie chains which connect the fringed micelle-like crystals.

The size and amount of crystalline aggregates in PVA hydrogels play an important role on their performances in applications since they determine the dimensional stability, the toughness, and strength to external stresses of the samples. A too high crystallinity is deleterious for the elasticity and makes the gels more fragile, whereas if crystallinity is too low, gels are poorly coherent and rather sticky. Therefore, it is very important to control the crystallinity of PVA hydrogels obtained by freeze/thaw techniques.

The degree of crystallinity of PVA hydrogels depends on various parameters such as the number of freeze/thaw cycles and the time/temperature history of the sample. It is in all cases very low. Because of the low amount of crystallinity, obtaining quantitative information requires accurate measurements and special care in order not to alter the state of the sample during the measurements.

The presence of crystals in freeze/thaw hydrogels has been indicated by different authors employing several techniques as, for instance, solid-state ^{13}C NMR,[6,7] DSC,[2,3,7−10] and diffraction techniques.[7,11−15] All these studies indicated the existence of a low crystallinity. However, most of these studies did not report any quantitative analysis of the amount of crystals in these systems.

We have recently performed a systematic quantitative analysis of the structure of PVA hydrogels, prepared by subjecting PVA/D_2O solutions (11−15% w/w PVA) to freeze (−22 °C)/thaw (25 °C) cycles, as a function of the number of cycles[16,17] and concentration of mother solution.[16] The techniques used were X-ray diffraction and ^{1}H NMR determinations of the free induction decays. Results obtained from the two techniques were in good agreement. They indicated that the degree of crystallinity is of the order of 2−6% for freshly prepared gels. It increases with increasing the PVA concentration and/or number of freeze/thaw cycles. Besides, the degree of crystallinity and crystallite size depend on the state of

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Table 1. Comparison between the Degree of Crystallinity of PVA GEL-*n* Samples in As-Formed, Aged, and Rehydrated State Derived from Different Techniques: ^{1}H NMR, f_c(NMR); DSC, f_c(DSC); X-ray, f_c(XR), and Weight Fraction of Polymer in the Gels As Determined by Gravimetric Measurements

PVA GEL-*n* state	no. of freeze/thaw cycles	f_c(NMR)[a] (%)	f_c(DSC) (%)	f_c(XR)[b] (%)	polymer concn[c] (% w/w)
as-prepared	1	2.2	1.6	2.5	12.0
	2	3.7			12.3[d]
	3	5.3	3.9	4.8	12.7
	4	5.9			13.1[d]
	5	6.5	4.6	5.6	13.4
	6	6.5			13.8[d]
	7	7.4		5.7	14.2[d]
	8	7.1		6.3	14.5
	9	7.1	4.7	6.3	14.9
	10	7.1			15.4[d]
aged	1	4.5	4.2		14.4
	3	6.6	7.1	6.2	18.7
	5	7.3	7.8	7.5	21.3
	9	7.7	8.0	7.6	20.0
24 h rehydrated	1	6.1	5.5		16.6
	2	8.6			
	3	9.9	6.6		22.1
	5	10.1	6.8		22.3
	9	10.6	8.2		23.0
14 days rehydrated	1	7.0	5.7	3.7	17.2
	2	9.3			
	3	9.9	6.8	9.6	23.6
	5	10.9	6.5		23.3
	9	11.9	7.3	10.6	23.3

[a] f_c(NMR) data of as-prepared GEL-*n* samples are taken from ref 16. [b] Determined in ref 17. [c] Determined by gravimetric measurements. [d] Interpolated values.

the samples, whether they are fresh, aged, or prepared by rehydrating dried samples. It must be noticed that, for this small PVA crystallinity range, results obtained from ^{1}H NMR were shown to be more accurate than X-ray diffraction data.

It is worth noting that a widely used technique for the quantitative detection of the crystalline fraction in polymer systems is the differential scanning calorimetry (DSC).[18] Freeze/thaw PVA hydrogels have been extensively studied by DSC by Watase and Nishinari.[2,3,8−10,19] The endothermic peak, observed on heating the samples, was related to the melting of crystallites. However, any quantitative estimation of the degree of crystallinity was not attempted from measurements of the enthalpy of melting, probably because the presence of the solvent complicated the analysis.

The present paper reports the comparison of quantitative determinations of the degree of crystallinity of freeze/thaw PVA hydrogels by free induction ^{1}H NMR experiments and DSC. The samples under study are fresh PVA hydrogels, aged PVA hydrogels, and PVA hydrogels prepared by rehydrating dried samples.

Experimental Section

PVA hydrogels were prepared by using 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* triads are 22.1, 50.1, and 27.8%, respectively.

Aqueous solutions of PVA with an 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 transparent solutions were slowly cooled to room temperature and kept at this temperature for one night to eliminate air bubbles.

The aqueous PVA solutions were then poured between glass slides with 1 mm spacers at room temperature. 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. The as-formed PVA hydrogels obtained by 1−10 freeze/thaw cycles are identified as GEL-1 to GEL-10 samples.

Aged freeze/thaw PVA hydrogel samples were obtained by storing the as-prepared samples at room temperature in sealed vials 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* samples 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 films were obtained by dipping the so-obtained "dried gels" in deuterated water for 24 h and 14 days, respectively.

Polymer weight concentrations of as-formed, aged, and rehydrated PVA hydrogels were determined by weighing each sample in the swollen and in the corresponding dried state by using a Gibertini analytical balance. The values thus determined are reported in Table 1.

Solid-state ^{1}H NMR experiments were performed 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 $\pi/2$ pulse duration of 3 μs and delay time of 60 s. Since the hydrogels were made from D_2O solutions, the observed FID is the signal of the PVA protons only. The fraction of rigid protons in PVA gel samples, $(\text{rigid } ^1\text{H})_{PVA}/(\text{total } ^1\text{H})_{PVA}$, was determined by measuring the fraction of protons that relax during the first 20 μs. The degree of crystallinity, f_c(NMR), was calculated with respect to the PVA content:

$$f_c(\text{NMR}) = \frac{(\text{rigid } ^1\text{H})_{PVA}}{(\text{total } ^1\text{H})_{PVA}} \times 100$$

The error on each measurement was estimated to be of the order of ±0.5%.

The DSC measurements were performed by using a Perkin-Elmer DSC-7 differential scanning calorimeter, calibrated against an indium standard (T_m = 156.6 °C), with scans at a 10 °C/min heating rate under a flowing nitrogen atmosphere. Specimens weighing between 3 and 7 mg were cut from the center of the PVA hydrogel films. The specimens were hermetically sealed inside stainless steal pans provided with a

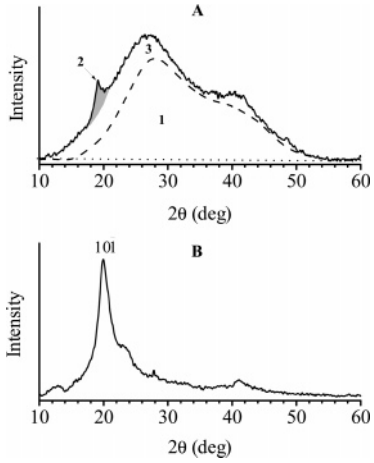

Figure 1. X-ray powder diffraction profiles of rehydrated PVA hydrogel sample (A) and of dried gel (B), obtained after nine freeze/thaw cycles (continuous line). The X-ray diffraction profile of liquid D_2O is also reported (dashed line). The crystalline reflection 2 in the 2θ range 18°−21° is evidenced in gray, whereas the $10\bar{1}$ reflection of the crystalline PVA is indicated in (B).

(Viton rubber) O-ring (Perkin-Elmer, large volume capsules) to prevent water loss from gels during scans from 10 to 110 °C. This kind of capsule eliminates the interfering effects of vaporization by suppressing the vaporization of water; well-sealed capsules are able to withstand the internal pressure generated upon heating the sample. The correct sealing procedure was ensured by checking that the weight of the sealed capsules before and after the DSC scans remained constant. For all the measurements, the reference was an empty pan. This procedure provided reproducible thermograms.

The degree of crystallinity, f_c(DSC), of a PVA GEL-n was determined as the ratio between the heat of fusion, ΔH_m, of the PVA hydrogel sample (normalized for the mass of the polymer in the gel) and the thermodynamic enthalpy of melting of a 100% crystalline PVA, ΔH_m^0:[7,20,21]

$$f_c(\mathrm{DSC}) = \frac{\Delta H_m}{\Delta H_m^0} \times 100$$

with $\Delta H_m^0 = 150$ J/g.[7,20,21]

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.[22] Because of 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 of the order of ±3 Å.

Results and Discussion

X-ray Diffraction Characterization. The X-ray diffraction profile of rehydrated GEL-9 hydrogel sample along with the X-ray diffraction profile of the dried GEL-9 sample is reported in Figure 1 as an example, after subtraction of a straight baseline which approximates the background contribution. For comparison, the X-ray diffraction pattern of pure deuterated water, which is the major component of these gels, is also indicated in Figure 1A (dashed line). The diffraction profile of our gels in the different states (Figure 1A) always exhibits (independent of the fresh, aged, or rehydrated state of the gels) two halos centered at $2\theta \approx 28°$ and 41°, as in the diffraction profile of pure water, and a weak peak in the 2θ range 18°−21°, which corresponds to the diffraction reflection of crystalline PVA (Figure 1B).[17] This result demonstrates the presence of a low amount of small crystalline PVA aggregates in the as-formed and rehydrated gel samples.

With reference to Figure 1A and consistent with the analysis performed in a previous paper,[17] the X-ray diffraction profile of as-formed and rehydrated GEL-n samples is considered as the sum of three contributions: a large contribution (area A1) due to the scattering of pure D_2O (dashed curves), a small diffraction component in the range from 18° to 21° due to the crystalline aggregates (area A2), and a third component (area A3) due to the presence of amorphous PVA swollen by water molecules.

The relative amount of crystalline PVA with respect to the sum of the crystalline and swollen amorphous portions, f_c(XR), has been determined in ref 17 by measuring the areas A1, A2, and A3 through the ratios A2/(A2 + A3). The f_c(XR) values thus obtained for GEL-n samples in different states, reported in Table 1, are compared, in the following sections, to the degrees of crystallinity obtained by ^{1}H NMR, f_c(NMR), and DSC, f_c(DSC).

^{1}H NMR Characterization. Aged PVA hydrogels. The percentage of rigid protons, f_c(NMR), measured for fresh GEL-n samples, was reported in a previous paper.[16] The effect of 2 months aging, in sealed vials, on the degree of crystallinity of PVA hydrogels obtained by submitting a 11% PVA/D_2O solution to freeze/thaw cycles is investigated here.

As explained in ref 16, as a first approximation, the ^{1}H free induction decay of PVA in the hydrogels exhibits at least two components characterized by a very 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 PVA protons, is characteristic of a rigid-lattice behavior whereas the latter component involves protons with different mobilities. The rigid PVA component is very likely due to the PVA hydrogel crystallinity.

The percentage of rigid protons, f_c(NMR), is reported in Figure 2 as a function of the number of freeze/thaw cycles, n, for aged PVA GEL-n samples with n ranging from 1 to 9. It is compared with the percent of rigid protons, f_c(NMR), of fresh GEL-n samples determined in ref 16.

As shown in Figure 2, as the number of freeze/thaw cycles increases, the fraction of rigid ^{1}H, f_c(NMR), increases. The degree of crystallinity, f_c(NMR), for aged GEL-n samples is systematically higher than the degree of crystallinity of as-prepared GEL-n hydrogels, ranging from approximately 4.5 to 7.5%. This result can be explained by a growth of primary crystallites, even though the formation of a new class of crystallites upon aging cannot be excluded. It is worth noting that the increase of the degree of crystallinity, f_c(NMR), in PVA GEL-n, on aging, is more pronounced for low n values.

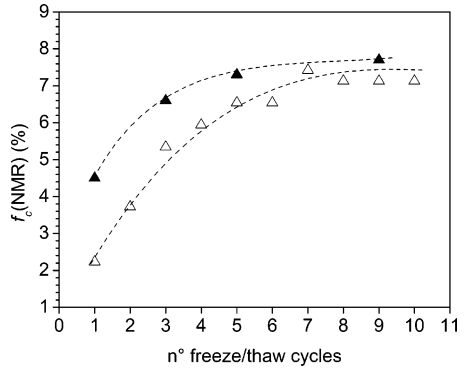

Figure 2. Percentage of rigid protons, f_c(NMR), in 11% w/w PVA hydrogels as a function of the number of freeze/thaw cycles: (△) as-formed PVA hydrogels;[16] (▲) 2 months aged PVA hydrogels.

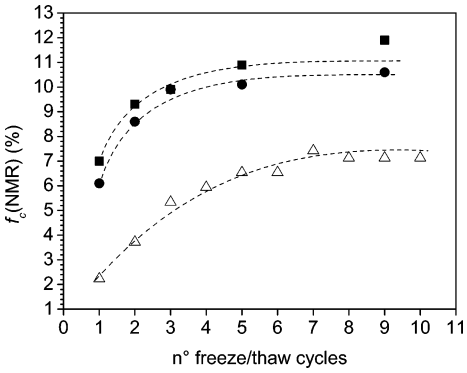

Figure 3. Percentage of rigid protons in PVA hydrogels as a function of the number of freeze/thaw cycles: (△) 11% w/w as-formed PVA hydrogels;[16] (●) 24 h rehydrated 11% w/w PVA hydrogels; (■) 14 days rehydrated 11% w/w PVA hydrogels.

For n higher than 5, aging only slightly alters the degree of crystallinity of GEL-n samples.

The different behaviors of GEL-n samples with aging, as n increases, may be explained by the fact that consecutive freeze/thaw cycles progressively strengthen the structure of the network scaffolding imprinted by the first cycle, making the whole structure more stable and less susceptible to effects of aging.

Rehydrated PVA Hydrogels. The fraction of rigid protons, f_c(NMR), in 24 h and 14 days rehydrated PVA GEL-n samples is reported in Figure 3 as a function of n. In both cases, f_c(NMR) first increases with increasing the number of freeze/thaw cycles and then reaches a plateau after the first four cycles. The degree of crystallinity achieved after 14 days rehydration is only slightly higher that the value achieved after only 24 h of permanence in water.

The f_c(NMR) values for rehydrated samples are compared to those of fresh PVA GEL-n samples in Figure 3. As shown in our previous work,[17] drying and successive rehydration of freshly prepared GEL-n samples result in a neat increase of polymer concentration, and therefore, rehydrated PVA hydrogels exhibit a higher crystallinity than as-prepared gels.

Differential Scanning Calorimetry. As-Formed PVA Hydrogels. Figure 4 (curves a) shows the DSC thermograms of the as-formed PVA hydrogels. The thermograms exhibit an endothermic peak due to the melting of crystallites. The melting endotherm is rather broad for GEL-1 and GEL-3 and becomes narrower for GEL-5 and GEL-9. The melting peak is in the range from 46 to 62 °C for GEL-1 and from 46 to 68 °C for GEL-3 while it appears at approximately 56−57 °C for GEL-5 and GEL-9.

The sharpening of the endothermic peak in GEL-5 and GEL-9 sample with respect to GEL-1 and GEL-3 could indicate the presence of better-defined crystals as n increases. It reflects the fact that, on increasing the number of freeze/thaw cycles, the width of the distribution of the crystallite sizes decreases.

In the DSC thermograms of Figure 4 (curves a), an exothermic peak appears immediately after the melting of the crystals in all the as-formed PVA hydrogels. The phase diagram of PVA−water system determined by Komatsu and co-workers in ref 23 indicates that at temperatures higher than the melting temperatures of our hydrogels (50−70 °C), and for the PVA concentrations typical of our systems (12−24% w/w PVA; see Table 1), a homogeneous solution is stable. Since the polymer concentration does not greatly change during the DSC scans and the pressure increases only slightly, the exothermic peak appearing after the endothermic peak may be assigned to solubilization and solvation of PVA chains, although the occurrence of recrystallization of PVA chains into more stable crystals may not be excluded. The melting of crystals and solubilization of the polymer chains in water correspond to the gel−sol phase transition in PVA hydrogels.

On increasing the number of freeze/thaw cycles, the exothermic peak shifts toward higher temperatures which are 66 and 73 °C for GEL-1 and GEL-9, respectively.

For the as-prepared GEL-n samples, as the number of freeze/thaw cycles increases from 1 to 9, the enthalpy of melting (normalized for the PVA content in the gel), ΔH_m, increases from 2.4 to 7.0 J/g, indicating an increase of the degree of crystallinity, f_c(DSC). The degree of crystallinity of freshly prepared PVA GEL-n samples determined by DSC measurements, f_c(DSC), is compared in Figure 5 with f_c(XR) and f_c(NMR) values determined, in our previous works, by using wide-angle X-ray diffraction[17] and ^{1}H NMR,[16,17] respectively.

As shown in Figure 5, the f_c(DSC) values increase from 1.7 to 4.7%, as n increases, reaching a plateau after the first 3−5 cycles. The f_c(DSC) values are systematically lower than the f_c(XR) and fractions of rigid protons values, f_c(NMR), obtained by X-ray and ^{1}H NMR, respectively. This is likely due to the fact that, as a result of both the small size of crystallites in PVA hydrogels and the presence of large amounts of water, for temperatures higher than 50 °C there is an overlap between endothermic and exothermic phenomena corresponding to the melting of PVA crystals in the gel and to polymer solubilization and solvation (or even recrystallization phenomena) in the presence of water, respectively.

At temperatures lower than 65−70 °C, endothermic phenomena prevail, whereas at temperatures higher than 65−70 °C, exothermic phenomena are dominant. Overlap of endo- and exothermic phenomena leads to an underestimation of the degree of crystallinity obtained from the measurement of the area of endothermic peaks in the DSC scans.

Aged PVA Hydrogels. The DSC thermograms recorded on PVA hydrogels aged for 2 months in sealed vials are compared in Figure 4 (curves b) with DSC thermograms of fresh GEL-n samples (curves a). As shown in Figure 4, on aging, the endothermic peaks grow and shift toward higher temperatures up to 66−

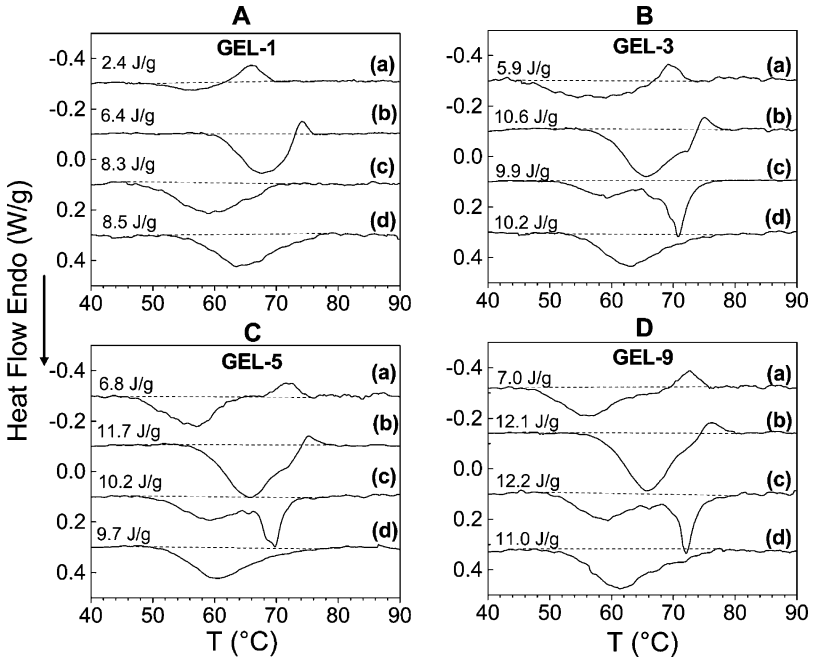

Figure 4. DSC heating scans of freeze/thaw (A) GEL-1, (B) GEL-3, (C) GEL-5, and (D) GEL-9 in as-formed (curve a), 2 months aged (curve b), 24 h rehydrated (curve c), and 14 days rehydrated (curve d) samples. The ΔH_m values of endothermic peak (normalized for the weight of PVA in the gel) are indicated.

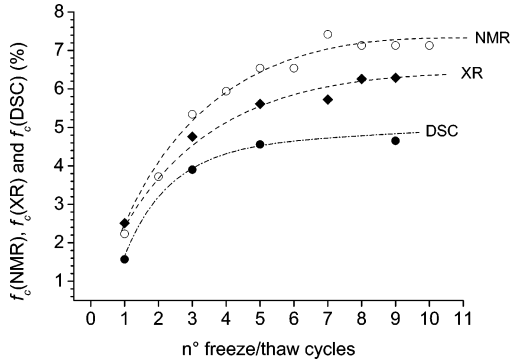

Figure 5. Fractions of crystalline PVA with respect to the total amount of PVA in the crystalline and swollen amorphous phases, obtained by the X-ray powder diffraction profiles, f_c(XR) (◆),[17] fractions of crystalline PVA with respect to the total amount of PVA in hydrogels, determined by DSC, f_c(DSC) (●), and fractions of rigid ^{1}H, calculated from the ^{1}H free induction decay experiments, f_c(NMR) (○),[16] as a function of the number of freeze/thaw cycles for the as-formed PVA hydrogels.

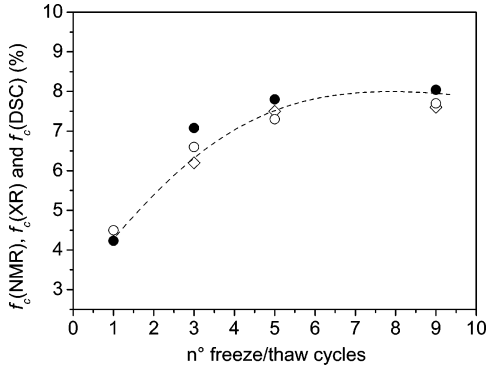

Figure 6. Fractions of crystalline PVA with respect to the total amount of PVA in the crystalline and the swollen amorphous phases, obtained by the X-ray powder diffraction profiles, f_c(XR) (◇),[17] fractions of crystalline PVA with respect to the total amount of PVA in hydrogel, determined by DSC, f_c(DSC) (●), and fractions of rigid ^{1}H, calculated from the free induction decay experiments, f_c(NMR) (○), as a function of the number of freeze/thaw cycles, for the 2 months aged PVA hydrogels.

68 °C. The increase of the melting temperature in aged PVA hydrogels, with respect to the melting temperature of as-prepared samples, can be interpreted in terms of morphological changes mainly involving a growth of the dimensions of crystallites rather than an increase of the number of crystallites. Accordingly, the endothermic peaks, shown in Figure 4 (curves b) for 2 months aged PVA GEL-*n* samples, are narrower than those of fresh GEL-*n* samples (curves a).

The crystallinity degrees of aged GEL-*n* samples evaluated from the DSC scans of Figure 4 are reported in Figure 6 as a function of *n* and compared to crystallinity degrees derived from X-ray diffraction and NMR data.

As the number of freeze/thaw cycles increases, the enthalpy of melting, ΔH_m, of aged GEL-*n* samples increases from 6.4 to 12.1 J/g, and as a consequence, the degree of crystallinity, f_c(DSC), increases from approximately 4.2 to 8.0%, in good agreement with results from ^{1}H NMR and X-ray diffraction. Moreover, the growth of the crystallite dimensions on aging is in agreement with the results of X-ray measurements that have shown that the apparent crystallite dimensions, which are around 35 Å for the fresh GEL-3 and GEL-5 samples and 39 Å for GEL-9, increase, after 2 months, to about 50 Å for the first two samples and to 55 Å for the GEL-9 sample.[17]

As shown in Figure 4 (curves b), after 2 months, the exothermic peak shifts to temperatures (74−76 °C) higher than the temperatures observed for the freshly prepared gels.

Rehydrated PVA Hydrogels. Figure 4 (curves c and d) shows the DSC thermograms of dried GEL-*n* (n = 1, 3, 5, 9) after rehydration in D_2O for 24 h and 14 days. They are compared with DSC thermograms of the corresponding fresh GEL-*n* samples (curves a). The DSC thermogram of GEL-1, after 24 h rehydration, exhibits

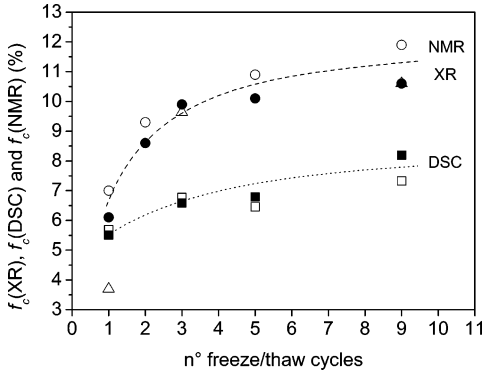

Figure 7. Fractions of crystalline PVA with respect to the total amount of PVA in the crystalline and the swollen amorphous phases, obtained by the X-ray powder diffraction profiles, f_c(XR) (△), and DSC curves, f_c(DSC) (■, □), and fractions of rigid ^{1}H, calculated from the free induction decay experiments, f_c(NMR) (●, ○), as a function of the number of freeze/thaw cycles for PVA hydrogels rehydrated during 24 h (full symbols) and 14 days (open symbols), respectively.

a wide peak at about 59 °C, whereas the thermograms of PVA gels with a higher number of freeze/thaw cycles, submitted to the same drying/rehydrating procedure, show a relatively sharp peak at 70−72 °C with a shoulder at lower temperatures (58−59 °C).

The presence of a sharp peak at 70−72 °C in 24 h rehydrated GEL-*n* samples (Figure 4, curves c) for n = 3, 5, and 9 indicates a better resistance to dissolution in water of these gels with respect to gels obtained from dried GEL-1 samples.

For 24 h rehydrated GEL-*n* samples, the enthalpy of melting, ΔH_m, increases slightly from 8.3 (for $n = 1$) to 12.2 J/g (for $n = 9$), with increasing the number of freeze/thaw cycles, and as a consequence, the corresponding degree of crystallinity, f_c(DSC), increases from approximately 5.5 to 8.2% (see Figure 7).

For 14 days rehydrated GEL-*n* samples, the DSC curves show only one broad endothermic peak (Figure 4, curves d). As n increases, this peak shifts slightly from 64 to 61 °C, whereas the enthalpy of melting increases from 8.6 (for $n = 1$) to 11.0 J/g (for $n = 9$). The sharp endothermic peak at $T \approx 72$ °C exhibited by 24 h rehydrated GEL-*n* samples for $n > 1$ (Figure 4, curves c) disappears in the DSC thermograms of 14 days rehydrated gels (Figure 4 curves d) probably because upon effect of prolonged swelling crystallites become less perfect and water molecules enter the crystalline lattice.

The degree of crystallinity of rehydrated gels, f_c(DSC), increases from approximately 5.7 for GEL-1 to approximately 7.3% for GEL-9 (see Figure 7). As shown in Figure 4 (curves c and d), for all rehydrated PVA hydrogel samples (24 h and 14 days), the exothermic peaks are not apparent in the DSC curves.

The comparison of the degree of crystallinity of rehydrated samples determined by DSC, f_c(DSC), with f_c(XR) obtained by X-ray diffraction, and with the fraction of rigid protons, determined by ^{1}H NMR experiments, f_c(NMR) (Figure 7) shows that DSC underestimates the crystallinity of rehydrated PVA hydrogels.

It must be noted that the gel−sol transition of rehydrated PVA hydrogels may be considered as the result of simultaneous endothermic and exothermic phenomena. In rehydrated PVA hydrogels, crystals, during the prolonged swelling in water, become highly hydrated and include a large amount of water and structural defects. By heating the samples at temperatures higher than 45 °C, crystals easily melt. Owing to large imperfections of crystals, endothermic peaks of rehydrated PVA hydrogels are broader than those of freshly prepared samples. They overlap with the exothermic peak due to solubilization and solvation of polymer chains in the solvent. Thus, the simultaneous occurrence of endothermic (melting) and exothermic (solubilization and solvation) phenomena reduces the area of endothermic peak, resulting into an apparent degree of crystallinity, f_c(DSC), lower than the value evaluated by using other techniques (Figure 7).

It is worth noting that the f_c(XR) value of the 14 days rehydrated GEL-1 sample is lower than the ones determined with the other techniques. Because of the small degree of crystallinity of GEL-1, the degree of crystallinity is affected by a large error, and this error is larger in the case of X-ray diffraction measurements.

The formation of largely hydrated and imperfect crystals in rehydrated PVA hydrogels is also supported by the fact that, for 14 days rehydrated GEL-*n* with n higher than 1, the melting temperature is lower and the endothermic peak is broader than in the case of the 24 h rehydrated gels. In fact, a large rehydration time in water necessarily induces a larger solvation and higher amount of structural defects in the crystals.

The degrees of crystallinity as determined by using the different (X-ray, ^{1}H NMR, and DSC) techniques are summarized in Table 1 for all the gel samples under study.

Conclusions

Comparison of the degrees of crystallinity of freeze/thaw PVA hydrogels measured by using DSC, X-ray diffraction, and ^{1}H NMR techniques shows that the degrees of crystallinity determined from ^{1}H free induction experiments can be considered as the most accurate ones. They are in good agreement with results obtained by X-ray diffraction technique, for all samples, whereas degrees of crystallinity deduced from DSC are lower than those obtained by the other two methods, for all the gel samples, but aged samples.

A detailed analysis of DSC data indicates that the gel−sol phase transition occurring during heating scans involves endothermic and exothermic phenomena. The first phenomenon corresponds to the melting of crystals whereas the second one is due to solubilization and solvation of PVA chains in the solvent and probably also to the occurrence of recrystallization phenomena.

For freshly prepared gels, the degrees of crystallinity calculated from DSC thermograms are systematically lower than those calculated by X-ray or ^{1}H NMR because, in these systems, the crystals are small and highly hydrated, so that endothermic peaks are broad and overlap with exothermic peaks.

Aging GEL-*n* samples mainly induces crystallite growth and formation of less hydrated crystals, probably containing a less amount of imperfections. In this case, endothermic peaks due to the melting of crystallites are narrower than those of fresh samples. Therefore, the gel−sol transition in these systems is better solved into two separate phenomena due to melting of crystals and chain solubilization and solvation. As a consequence, the degrees of crystallinity determined by DSC, f_c(DSC), for aged samples agree quite well with results obtained by using other techniques.

In rehydrated PVA hydrogels, crystals are highly hydrated and include large amounts of water and structural defects, as a result of prolonged swelling of dried samples in water. The melting of these crystals results in a broad endotherm concealing the exothermic peak. In this case, the gel−sol transition is not resolved into two separate steps. For this reason, for all rehydrated gels, the degree of crystallinity calculated from DSC thermograms, f_c(DSC), is systematically lower than results obtained by using X-ray and ^{1}H NMR.

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