Structure and Morphology of Freeze/Thawed PVA Hydrogels

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ABSTRACT: The structure and morphology of poly(vinyl alcohol) (PVA) gels prepared by repeated cycles of 8 h freezing at −20 °C and 4 h thawing at +25 °C were examined. Long-term morphological changes of such gels were determined upon swelling in water at 37 °C for 6 months. The preparation conditions were examined by varying such parameters as the number of freezing and thawing cycles, the concentration of aqueous solution, and the PVA molecular weight. The overall structure and stability were examined in terms of water content, fractional PVA dissolution, degree of crystallinity, and crystal size distribution. An increase in the number of freezing and thawing cycles served to reinforce existing crystals within the structure. Increased initial concentrations of aqueous PVA solutions resulted in hydrogels that contained initially higher crystallinity and added stability upon swelling. An increase in the PVA molecular weight resulted in crystals of higher lamellar thickness and a broadening of the crystal size distribution due to an increase in PVA chain length. Secondary crystallization was more pronounced for more loosely cross-linked samples. An increase in the free volume and mobility within the network allowed for additional crystallization to proceed during swelling. Overall, freeze/thawed PVA gels of intermediate molecular weight ($\bar{M}_n = 64\,000$) and increased number of freezing and thawing cycles showed enhanced stability during swelling at 37 °C for a 6-month period of time demonstrating their appropriateness for long-term biomedical applications.

Introduction

A physical method of gelation and solidification of poly(vinyl alcohol) (PVA) has been examined to avoid component leaching associated with traditional chemical cross-linking techniques. Specifically, the method of interest involves the casting of dilute, aqueous solutions of PVA, then cooling to −20 °C and thawing back to room-temperature several times.[1] Such a technique produces stable hydrogels that are physically cross-linked by the presence of crystalline regions. In addition to their nontoxic nature, these freeze/thawed gels have demonstrated enhanced mechanical properties, particularly for biomedical applications. High mechanical strength and high elasticity are a few of the attributes to these materials that make them suitable for artificial tissue and contact lens applications.[2,3]

When considering these freeze−thawed gels for many pharmaceutical and medical applications, it is important to characterize the stability of the system over long time periods. Some of the problems that have been associated with PVA gels produced by freezing/thawing techniques include the dissolution of PVA chains, the melting out of crystallites, and additional crystallization over long time periods, or secondary crystallization. These problems can significantly alter the behavior of the gels over time and need to be addressed when considering the materials for any long-term applications.[4−6] For example, Peppas and Scott[7] examined the crystalline nature of freeze/thawed gels during swelling. An initial decrease in the degree of crystallinity was observed as smaller crystalline regions melted out. This stage was followed by an increase in the crystallinity over longer time periods which was attributed to additional crystallite formation due to aging. Murase et al.[8] and Tanigami et al.[9] also examined changes in the crystalline structure of freeze/thawed PVA gels over time. They noted the syneresis or solvent exclusion due to an increase in the crystallinity. The densification of the structure was attributed to phase separation. Some initial insight has been gained as to the instabilities of freeze/thawed PVA gels over long time periods of swelling. However, the long-term stability of such gels still remains an important issue. In particular, there is a continued need for an improved understanding of structure−property relationships on the overall morphology of freeze/thawed PVA hydrogels.

Here we examine the morphology and long-term stability of PVA gels prepared by these benign manufacturing processes in which an aqueous PVA solution is exposed to repeated cycles of freezing and thawing. In particular, the importance of the preparation conditions including the number of freezing and thawing cycles, the concentration of aqueous solution, and the PVA molecular weight was investigated.

Experimental Section

Freezing and Thawing PVA Gel Preparation. Aqueous solutions of 7, 10, or 15 wt % PVA were prepared by dissolving PVA (Elvanol grades, E. I. du Pont de Nemours, Wilmington, DE) in deionized water for 6 h at 90 °C. Three different PVA molecular weights, $\bar{M}_n = 35\,740$, $\bar{M}_n = 64\,000$, and $\bar{M}_n = 88\,880$ were used which had degrees of hydrolysis varying from 99.0 to 99.8% and respective polydispersity indices of 2.15, 2.02, and 2.10. The prepared aqueous solutions were cast between glass microscope slides with 0.7 mm thick spacers. The samples were then exposed to three to seven cycles of freezing for 8 h at −20 °C and thawing for 4 h at 25 °C.

Swelling and Dissolution Studies. The behavior and stability of PVA gels (prepared by freezing/thawing techniques) upon swelling in water was examined. Swelling and dissolution experiments yield important information concerning the stability of PVA gels in terms of the water content (degree of swelling) and the fractional dissolution of PVA. Equilibrium swelling studies were conducted in deionized water at 25 °C. The PVA films prepared by repeated cycles of freezing and thawing were cut into thin disks of 12 mm diameter using a cork borer. Each disk was initially weighed in air and heptane and then placed in a jar containing 50 mL of deionized water.

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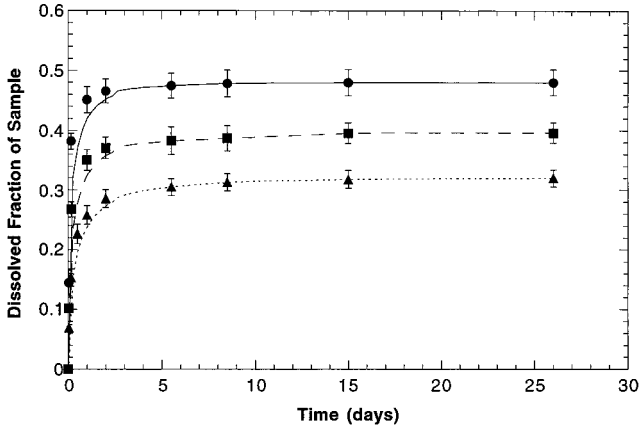

Figure 1. Initial fractional dissolution in water at 37 °C of PVA samples with three (●), five (■), and seven (▲) cycles of 8 h freezing and 4 h thawing with $\bar{M}_n$ = 35 740 and a concentration of 15 wt %.

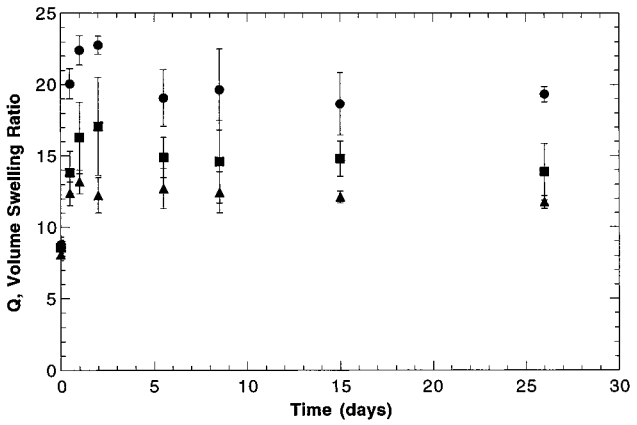

Figure 2. Initial swelling in water at 37 °C of PVA samples with three (●), five (■), and seven (▲) cycles of 8 h freezing and 4 h thawing with $\bar{M}_n$ = 35 740 and a concentration of 15 wt %.

At specific times during swelling, the samples were blotted and weighed in air and heptane, and 5 mL samples were removed from the swelling media.

The swelling media samples were analyzed for PVA dissolution by complexing each 5 mL sample of aqueous PVA with 2.5 mL of a 0.65 M boric acid solution and 0.3 mL of a 0.05 M I_2/0.15 M KI solution and then diluting to 10 mL with deionized water at 25 °C. The absorbance of visible light at 671 nm was then measured with a UV/vis spectrometer (Lambda 10 model, Perkin-Elmer, Norwalk, CT) to determine the concentration of complexed PVA in solution.

DSC Studies. The crystalline nature of PVA gels prepared by freezing and thawing processes was more closely examined to analyze the morphology of the system. Of particular utility was the use of differential scanning calorimetry (DSC, model 2910, TA Instruments, New Castle, DE) to determine the degrees of crystallinity and crystal size distributions of samples in the initial state (before swelling) and at various times during swelling in deionized water at 37 °C over 6 months. With DSC, the difference in heat flow between a sample and an inert reference is measured as a function of time and temperature as both the sample and reference are exposed to a temperature change. In a typical experiment, 5−10 mg of a partially dried sample was placed in an aluminum pan and heated at a scanning rate of 5 °C/min from 40 to 250 °C. A nitrogen purge through the sample chamber was implemented to obtain a more uniform, stable thermal environment.

Results and Discussion

Swelling and Dissolution Studies. Upon placement in water at 37 °C, all PVA samples prepared by freezing and thawing techniques swelled to a high extent. In addition, it was observed that a significant fraction of PVA chains that were not incorporated into the overall crystalline structure dissolved into solution.

The swelling and dissolution behavior typical for PVA gels prepared by freezing and thawing techniques was analyzed for samples of $\bar{M}_n$ = 35 740, 15 wt % concentration, and three to seven cycles of freezing and thawing. Using the boric acid technique, it was determined that a certain amount of amorphous PVA was dissolved at 37 °C in water over the initial period (first month) of swelling as shown in Figure 1. These results indicate that there is initial dissolution of the samples due to PVA chains that did not participate in the crystallite formation process and therefore were not incorporated in the physical structure of the gel. A lower degree of PVA dissolution was observed with samples that were prepared with increased cycles of freezing and thawing. For example, upon increasing to seven cycles of freezing and thawing, approximately 20% more of the chains did participate in the overall crystalline structure. Therefore, it is evident from these results that there is an increased degree of physical cross-linking associated with an increased number of freezing/thawing cycles.

By taking the dissolution results into consideration, it was possible to calculate a true volume swelling ratio which accounts for PVA chain dissolution effects. Figure 2 shows the volume swelling ratio, Q, as a function of time for samples prepared with three, five, and seven cycles during the initial month of swelling. It can be seen that in all cases the swelling ratio increases with time and passes through a maximum during the first few days. This maximum is followed by a reequilibration to a lower value. After the initial period of instability and overshoot during the first 5 days, all gels attained a relatively constant volume swelling ratio although some variation is observed. The samples treated for three cycles showed a much more swollen structure than those treated for five or seven cycles. Increased freezing/thawing cycles lead to further crystal formation and therefore increased physical cross-linking. The ensuing physical network seems to be relatively stable. However, the "overshoot" in the volume swelling ratio is a strong indication of some initial instability due to the loss of initial crystallinity, chain dissolution, or even possible additional crystallization.

Although swelling studies provide initial insight as to the structure and morphology of PVA gels with varying number of freezing and thawing cycles, the crystalline nature of these materials will be discussed further in terms of overall degrees of crystallinity as well as crystal size distributions.

Analysis of Degree of Crystallinity. A typical DSC thermogram for a PVA sample exposed to repeated cycles of freezing and thawing is shown in Figure 3. The broad peak at approximately 100 °C represents the evaporation of residual water present in the sample. The sharp peak at approximately 230 °C represents the melting of PVA.

Degrees of crystallinity were calculated by analyzing the DSC thermogram obtained for each PVA sample exposed to a constant rate of heating. The heat required for melting of a sample, ΔH, was determined by integrating the area under the melting peak over the range 190−240 °C. This ΔH value was corrected for the residual water present in the sample by also analyzing

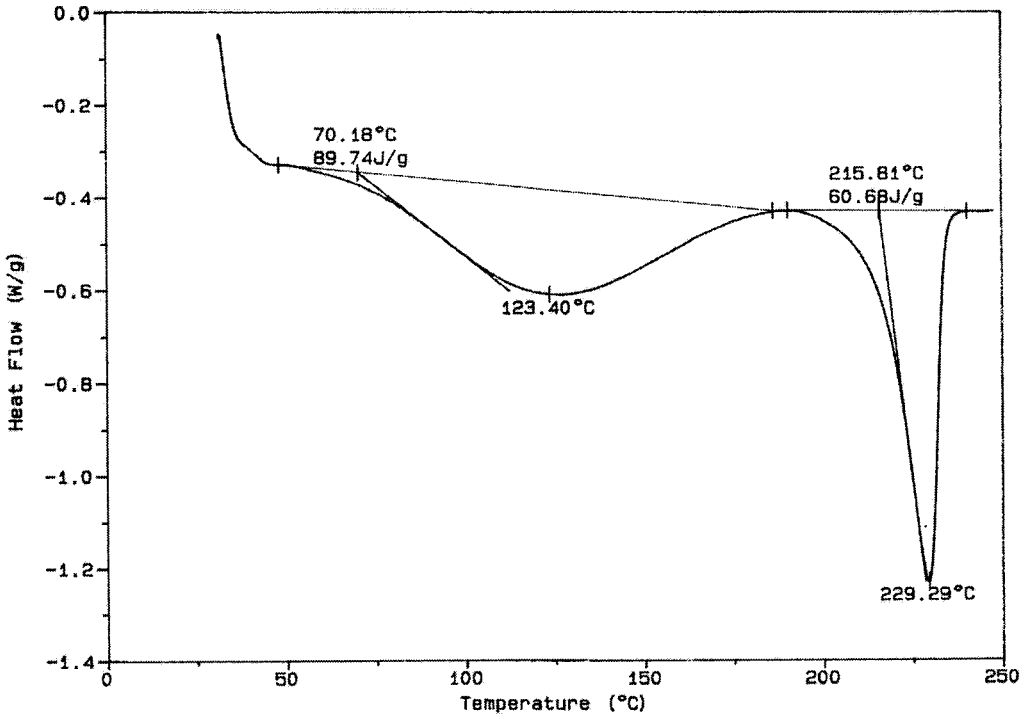

Figure 3. Typical DSC thermogram for a PVA sample prepared by freezing and thawing techniques.

the peak representing the heat required for the evaporation of water (at approximately 100 °C).

The degree of crystallinity, X_t, based on dry PVA volume, was calculated by dividing the corrected ΔH by the heat required[10] for melting a 100% crystalline PVA sample, $\Delta H_c = 138.6$ J/g. Modified degrees of crystallinity, $X_{mod,t}$, were also calculated for partially dissolved samples during swelling using the data from the dissolution analysis. Therefore, the changes in crystallinity could be examined based on the original weight of the sample. This is important for the overall analysis of the change in the structure of PVA gels prepared by freezing and thawing processes.

To analyze the dynamics of the system based on its original state, a modified value of the degree of crystallinity at time t, $X_{mod,t}$, during swelling was calculated by

$$X_{mod,t} = X_t[1 - w_{diss,t}] \quad (1)$$

Here, X_t is the original calculated degree of crystallinity at time, t, during swelling and $w_{diss,t}$ is the weight fraction of the sample dissolved at time, t.

The crystalline structure of PVA gels was examined in terms of the overall degree of crystallinity as obtained using DSC experiments. Parameters that were investigated included the number of freezing and thawing cycles, PVA molecular weight, the concentration of aqueous solution, as well as the time of swelling. The error associated with the modified degrees of crystallinity was estimated to be ±1.2%.

The modified degrees of crystallinity calculated for samples prepared with three, five, and seven cycles of freezing and thawing and then swollen in water at 37 °C over 6 months are shown in Table 1. Samples that were exposed to five and seven cycles of freezing and thawing showed a slightly more stable crystalline structure upon swelling as indicated by the modified degrees of crystallinity. Initially, samples that were exposed to three, five, or seven cycles of freezing and thawing had similar degrees of crystallinity of approximately 60%. However, upon swelling over a period of 6 months, a considerably higher percent of the crystalline regions melt out of the three-cycle samples. In fact, approximately 27% of the initial crystallinity was lost in the first day of swelling. In comparison, the five- and seven-cycle samples retained much higher fractions of the initial crystallinity. Specifically, only a 15% decrease in the degree of crystallinity was noted for the samples exposed to seven cycles of freezing and thawing.

Table 1. Modified Degrees of Crystallinity for PVA Samples of $\bar{M}_n = 35\,740$ and Concentration of 15 wt % Exposed to Three, Five, or Seven Cycles of Freezing and Thawing

time of swelling (days)	modified degree of crystallinity (%)		
	3 cycles	5 cycles	7 cycles
0	59.5	56.7	62.6
1	32.5	39.1	47.1
15	34.7	39.3	46.1
46	33.8	39.6	44.9
71	30.5	38.0	42.3
96	33.5	33.4	42.3
127	34.6	38.3	44.2
187	33.3	37.7	44.9

Thus, enhanced stability is created when exposing a PVA sample to an increased number of freezing and thawing cycles. Although an increased number of freezing and thawing cycles does not necessarily increase the overall initial degree of crystallinity in a PVA sample, it is apparent that repeated cycles reinforce crystals that already exist. When examining the three-, five-, and seven-cycle samples over long periods of swelling, no further melting out of crystals is observed.

The modified degrees of crystallinity were calculated for samples of varying molecular weight during swelling at 37 °C for 6 months. The molecular weights of $\bar{M}_n = 35\,740$ and $\bar{M}_n = 64\,000$ were compared (Table 2) with a constant concentration of 15 wt % and three cycles of freezing and thawing. Prior to swelling, the lower molecular weight sample initially had a 7.5% higher

Table 2. Modified Degrees of Crystallinity for PVA Samples of $\bar{M}_n$ = 35 740 and $\bar{M}_n$ = 64 000 and a Concentration of 15 wt % Exposed to Three Cycles of Freezing and Thawing

time of swelling (days)	modified degree of crystallinity (%)	
	$\bar{M}_n$ = 35 740	$\bar{M}_n$ = 64 000
0	59.5	52.0
1	32.5	46.6
15	34.7	45.0
46	33.8	49.3
71	30.5	47.0
96	33.5	44.8
127	34.6	45.5
187	33.3	47.4

Table 3. Modified Degrees of Crystallinity for PVA Samples of $\bar{M}_n$ = 64 000 and $\bar{M}_n$ = 88 880 and a Concentration of 10 wt % Exposed to Three Cycles of Freezing and Thawing

time of swelling (days)	modified degree of crystallinity (%)	
	$\bar{M}_n$ = 64 000	$\bar{M}_n$ = 88 880
0	49.9	57.7
1	41.8	36.1
15	41.1	40.3
46	43.5	40.2
71	40.8	37.0
96	39.6	41.1
127	39.4	40.8
187	40.2	41.8

degree of crystallinity. However, upon swelling in water for 1 day, a large fraction (27%) of the crystals melted out from the samples of $\bar{M}_n$ = 35 740. The gels of higher molecular weight ($\bar{M}_n$ = 64 000) were much more stable, retaining all but 5% of their initial degree of crystallinity.

When one examines these degrees of crystallinity over longer time periods of 6 months, it is apparent that both gels are considerably stable. The $\bar{M}_n$ = 35 740 samples retained a constant degree of crystallinity of approximately 33%. The samples of higher PVA molecular weight were capable of retaining a degree of crystallinity of approximately 46%. Although both samples were extremely stable over time, the samples of higher molecular weight showed enhanced stability with respect to retaining more of the initial crystalline structure over a long time period of swelling.

The crystalline nature of PVA gels of $\bar{M}_n$ = 64 000 and $\bar{M}_n$ = 88 880 was also examined during swelling at 37 °C for 6 months (Table 3). These gels of varying molecular weights had a constant concentration of 10 wt % and were exposed to three cycles of freezing and thawing. Initially, the samples of $\bar{M}_n$ = 88 880 had a modified degree of crystallinity of nearly 8% higher than samples of $\bar{M}_n$ = 64 000. However, upon swelling in water for 24 h, the higher molecular weight samples exhibited a nearly 22% loss in crystallinity. The $\bar{M}_n$ = 64 000 gels only showed a loss of approximately 8%. Therefore, the intermediate molecular weight of $\bar{M}_n$ = 64 000 showed the capability of retaining significantly more of its originally formed crystalline network. In fact, when examining these gels over a 6-month time period, the crystallinity is quite stable at approximately 41%. Although the gels of $\bar{M}_n$ = 88 880 were also relatively stable over long time periods, with a degree of crystallinity of approximately 40%, some increase in variation was observed. This is likely due to the fact that there is less physical cross-linking with such a system which allows for additional rearrangement of the structure over time during the swelling process.

Table 4. Modified Degrees of Crystallinity for PVA Samples of $\bar{M}_n$ = 64 000 and a concentration of 7, 10, or 15 wt % Exposed to Three Cycles of Freezing and Thawing

time of swelling (days)	modified degree of crystallinity (%)		
	7 wt %	10 wt %	15 wt %
0	43.4	49.9	52.0
1	37.8	41.8	46.6
15	31.2	41.1	45.0
46	27.3	43.5	49.3
71	33.2	40.8	47.0
96	31.6	39.6	44.8
127	31.2	39.4	45.5
187	31.5	40.2	47.4

Overall, with the comparison of the degrees of crystallinity of PVA samples of varying molecular weight, conclusions can be drawn as to the enhanced stability associated with an intermediate molecular weight of $\bar{M}_n$ = 64 000. This molecular weight appears to define a PVA chain length that allows for the formation of crystalline regions that are more stable upon swelling in water at 37 °C for long time periods of 6 months. The effect of molecular weight on the overall crystalline nature and stability of freeze−thawed PVA gels will be addressed further in terms of the size and number of crystalline regions.

The modified degrees of crystallinity of freeze−thawed gels prepared with varying PVA concentrations of 7, 10, and 15 wt % were also examined as a function of swelling time (Table 4). These gels of varying concentration were prepared with a molecular weight of $\bar{M}_n$ = 64 000 and three cycles of freezing and thawing. Initially, the degree of crystallinity increased with an increase in PVA concentration. In the region of interest, the formation of stable crystalline regions became more probable with increased concentration due to an increase in the overlap of PVA chains as well the promotion of polymer chain folding. Therefore, there was a steady increase in the degree of crystallinity as the concentration was increased from 7 to 15 wt %. The increase was more significant, however, between the 7 and 10 wt % samples (6.5%) than between the 10 and 15 wt % samples (2.1%). Therefore, as the concentration was raised further, one would expect the crystallinity to level off or to start decreasing. Under such conditions, entanglements among the polymer chains may act to inhibit the folding of polymer chains and thus the formation of crystals.

It is interesting to also examine the stability of these hydrogels of varying concentrations during swelling. Initially, the 7 wt % samples showed a loss of approximately 6% of the crystallinity upon swelling for 1 day. There was then a loss of another 6% over the next 2 weeks of swelling. During the following month, there was still some additional loss of 4%. Therefore, the samples prepared from 7 wt % PVA solutions were quite unstable upon swelling, showing continued melting out of crystalline regions over a 1.5 month time frame. After this initial instability, due to the loss of crystallinity, there was further instability introduced in the form of additional crystallization at longer times. For example, between day 46 and 71 of swelling, an additional 4% of crystals was introduced into the network. This was likely due to the increase in free volume and mobility in the loosely cross-linked sample. After this point, the gels appear to be quite stable over the remaining 4 months of swelling.

PVA gels prepared from concentrations of 10 and 15 wt % exhibited much greater stability during the swelling process. There was an initial loss of crystallinity of 8% for the 10 wt % sample and only a 5% initial loss for the 15 wt % sample. After the initial 24 h of instability, the gels were quite stable, with constant degrees of crystallinity of approximately 41% for the 10 wt % sample and 46% for the 15 wt % sample. Overall, the concentration of aqueous PVA has a significant impact on the overall degree of crystallinity as well as the stability of the crystals during swelling. In particular, the 10 and 15 wt % concentrations provided for extremely stable physical networks.

Crystal Size Distribution Analysis. The DSC endotherms representative of the melting of PVA were analyzed to obtain the distribution of melting temperatures for each sample. Melting temperatures were then converted to crystallite thicknesses using the Thomas–Gibbs equation[11]

$$T_m = T_m^\circ\left(1 - \frac{2\sigma_e}{\Delta H L}\right) \tag{2}$$

where T_m is the observed melting temperature, L is the lamellar thickness of the crystal, T_m° is the equilibrium melting temperature of an infinitely thick crystal, σ_e is the surface free energy per unit area of the chain folds, and ΔH is the heat of fusion per unit volume of the crystals. In performing such an analysis, eq 2 is valid for crystals of large lateral dimensions with respect to the thickness. In addition, it is assumed that the heat flow value at each melting temperature is proportional to the fraction crystalline lamellae of thickness L.

The value of T_m° was determined for each molecular weight sample by following the analysis of Peppas and Hansen.[12] Essentially, the melting temperature of the polymer, T_m, was plotted as a function of the crystallization temperature, T_c. The point at which this plot intersected the line $T_m = T_c$ was determined to be T_m°. The highest melting temperature was used in this analysis. The values of T_m° were 520.5 K for PVA with $\bar{M}_n$ = 35 740, 516.5 K for PVA with $\bar{M}_n$ = 64 000, and 512.6 K for PVA with $\bar{M}_n$ = 88 880. A value of 3.3×10^{-8} cm was used[13] for $\sigma_e/\Delta H$.

Crystal size distributions were normalized so that the area under each curve was proportional to modified degrees of crystallinity to account for chain dissolution effects. This analysis gave information as to the size and number of crystallites.

To examine the effect of varying cycles of freezing and thawing, the distributions of the three-, five-, and seven-cycle samples before swelling and after 15 days of swelling are shown together in Figure 4. In the initial state, there were no significant differences in the crystal size distributions between samples prepared with three, five, or seven cycles of freezing and thawing. In all cases, the lamellar thickness ranged from 50 to 300 Å with the largest fraction of crystals having thickness of approximately 170 Å. However, it is apparent from these distributions that with an increase in the number of freezing and thawing cycles, increased stability is imparted into the network. This increased stability can be described in three different ways. First, samples with increased cycles of freezing and thawing retain more of their overall crystalline structure. This is represented by an increase in the overall area under each distribution. Upon swelling of the sample for 15 days, a significantly lower fraction of PVA crystals melted out of the samples of increased freezing and thawing cycles.

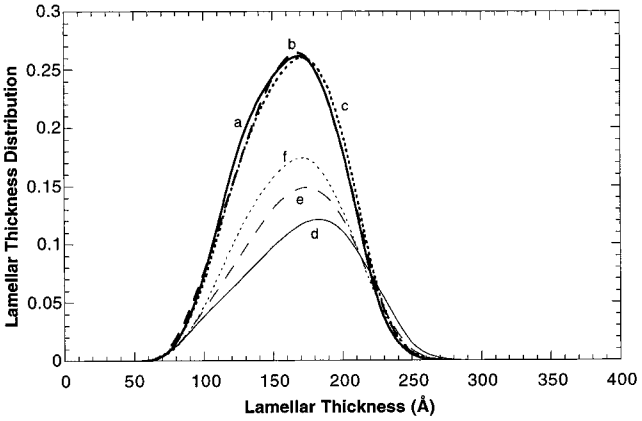

Figure 4. Crystal size distributions for PVA samples of three (a), five (b), and seven (c) cycles of freezing and thawing before swelling and for three (d), five (e), and seven (f) cycles of freezing and thawing after 15 days of swelling.

Second, the stability can be analyzed in terms of the shift in the peak lamellar thickness. The average lamellar thickness essentially remained constant for PVA samples exposed to repeated cycles of freezing and thawing. However, with the three-cycle samples, there was increased rearrangement of the size and number of crystalline regions as indicated by the overall shift in the distribution.

Finally, instability in the network can be examined in terms of the presence of additional crystallization. When comparing the distributions after 15 days of swelling, crystals of higher lamellar thickness were present in the three-cycle samples. This effect was somewhat small, but was worth being noted. Samples exposed to fewer cycles of freezing and thawing were less physically cross-linked and, thus, swelled to a higher extent. Therefore, there was an increase in the free volume and mobility present in the network which allowed for additional crystallization to proceed. Some secondary crystallization appeared to be present after 15 days of swelling in the three-cycle samples.

It can be concluded that the number of freezing and thawing cycles does not significantly impact the size and number of crystalline regions of PVA gels prepared by freezing and thawing techniques. Rather, an increase in freezing and thawing cycles serves to add stability to existing crystals. Thus, samples of higher freezing and thawing cycles exhibit greater stability as rearrangement within the structure is held to a minimum. However, with a broad range of the number of freezing and thawing cycles (three to seven), much of the instability is sustained after the initial 15 days of swelling. This stability was also analyzed over long time periods of up to 6 months to more accurately predict the impact of the number of freezing and thawing cycles on the stability and, thus, usefulness of the materials for long-term applications.

Crystal size distributions were obtained with samples of varying PVA molecular weights of $\bar{M}_n$ = 35 740, $\bar{M}_n$ = 64 000, and $\bar{M}_n$ = 88 880. First, the molecular weights of $\bar{M}_n$ = 35 740 and $\bar{M}_n$ = 64 000 were compared. During preparation, these molecular weight samples were exposed to three cycles of freezing and thawing using a PVA concentration of 15 wt %.

When these samples are compared both in the initial state and after 15 days of swelling (Figure 5), some

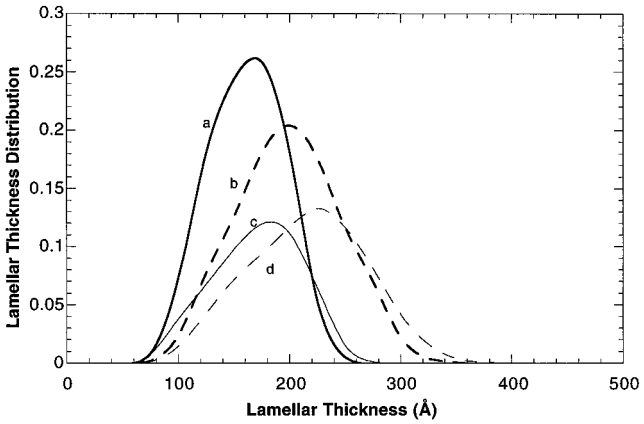

Figure 5. Crystal size distributions for PVA samples of $\bar{M}_n$ = 35 740 (a) and $\bar{M}_n$ = 64 000 (b) before swelling and $\bar{M}_n$ = 35 740 (c) and $\bar{M}_n$ = 64 000 (d) after 15 days of swelling.

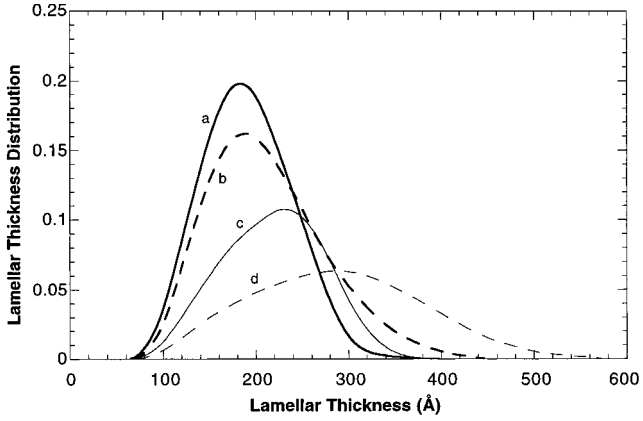

Figure 6. Crystal size distributions for PVA samples of $\bar{M}_n$ = 64 000 (a) and $\bar{M}_n$ = 88 880 (b) before swelling and $\bar{M}_n$ = 64 000 (c) and $\bar{M}_n$ = 88 880 (d) after 15 days of swelling.

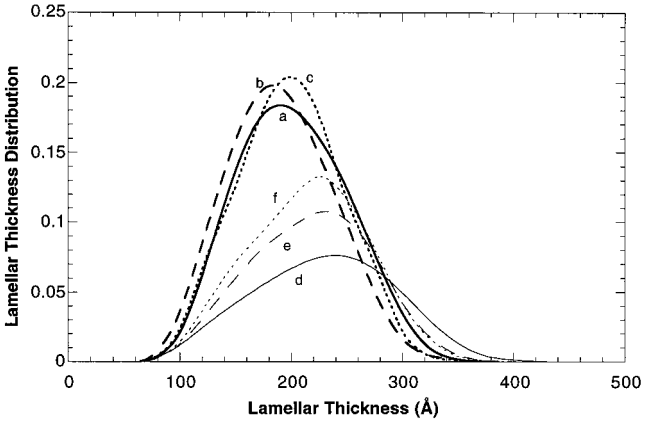

Figure 7. Crystal size distributions for PVA samples of 7 (a), 10 (b), and 15 (c) wt % concentration before swelling and for 7 (d), 10 (e), and 15 (f) wt % concentration after 15 days of swelling.

interesting trends can be observed. Upon examination of the initial distributions, it can be noted that the higher molecular weight PVA allowed for the formation of crystals of higher lamellar thickness due to the increase in PVA chain length. In addition, the average value for crystals within the sample increased significantly from 170 to 200 Å with a higher molecular weight PVA. Overall, however, the sample is less uniform in terms of the size and number of crystals as indicated by the presence of a broader distribution.

Both samples exhibited quite similar overall stability upon swelling. Similar changes in the distributions were present between the initial state and after 15 days of swelling for each sample. In particular, shifts of approximately 15−25 Å were observed for both the $\bar{M}_n$ = 35 740 and $\bar{M}_n$ = 64 000 samples. In addition, similar degrees of secondary crystallization were present in both samples after 15 days of swelling. The higher molecular weight appeared to allow for a slightly higher degree of additional crystallization during swelling. This was likely due to the fact that this sample had additional PVA chain length to allow for further crystallization to proceed. However, the difference was not very significant because the higher molecular weight sample actually had decreased free volume and mobility, due to increased physical cross-linking, that may have prevented a very high degree of secondary crystallization from occurring.

It is also interesting to examine higher molecular weights of $\bar{M}_n$ = 64 000 and $\bar{M}_n$ = 88 880. During preparation, these molecular weight samples were exposed to three cycles of freezing and thawing using a PVA concentration of 10 wt %. The samples were compared both in the initial state and after 15 days of swelling as shown in Figure 6. The higher molecular weight PVA ($\bar{M}_n$ = 88 880) did allow for the formation of crystals of higher lamellar thickness due to the increase in PVA chain length. The average lamellar thickness of the crystals was not significantly impacted by an increase in the molecular weight of PVA. Overall, the presence of a broader distribution is representative of a less uniform sample in terms of the size and number of crystals. It is apparent that, upon swelling, the higher molecular weight sample exhibited more instability in terms of the overall degree of crystallinity and crystal uniformity. The $\bar{M}_n$ = 88 880 sample exhibited significant rearrangement upon swelling which resulted in considerably more instability. There are two characteristics of this molecular weight material that appear to contribute to increased instability. First, there is an increase in the PVA chain length which could contribute to additional crystallization upon swelling. Second, through a swelling analysis, the $\bar{M}_n$ = 88 880 sample actually demonstrated higher overall volume swelling ratios. This indicates that the sample is less physically cross-linked which creates increased free volume and mobility. The combination of these two characteristics lead to a significant amount of secondary crystallization and overall instability.

Overall, it can be concluded that the molecular weight significantly impacts the size and number of crystalline regions with PVA gels prepared by freezing and thawing techniques. In particular, a combination of an increase in PVA chain length and an increase in free volume lead to structures that are potentially the most unstable. However, in all cases, much of the instability appears to be sustained after the initial 15 days of swelling.

Crystal size distributions were obtained for samples of varying initial PVA solution concentrations of 7, 10, and 15 wt %. During preparation, these samples were exposed to three cycles of freezing and thawing using a PVA molecular weight of $\bar{M}_n$ = 64 000. To examine the differences between the 7, 10, and 15 wt % samples, the initial crystal size distributions before swelling and the distributions after 15 days of swelling are shown in Figure 7. It is apparent that, upon swelling for 15 days, all samples exhibited similar changes of decreased crystallinity, shifting of the peak lamellar thickness, and

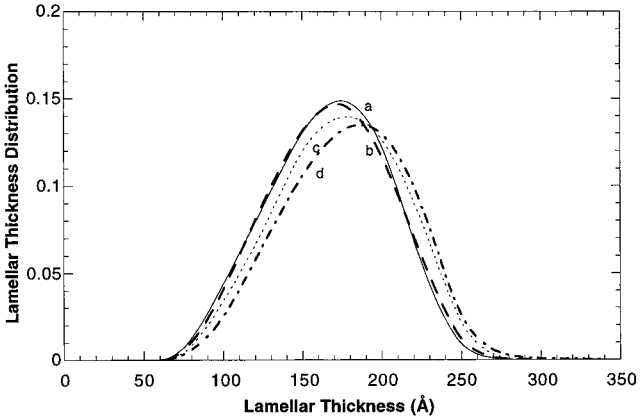

Figure 8. Crystal size distributions after 15 days (a), 1.5 months (b), 3 months (c), and 6 months (d) of swelling for PVA samples prepared with five cycles of freezing and thawing, concentration of 15 wt %, and $\bar{M}_n = 35\,740$.

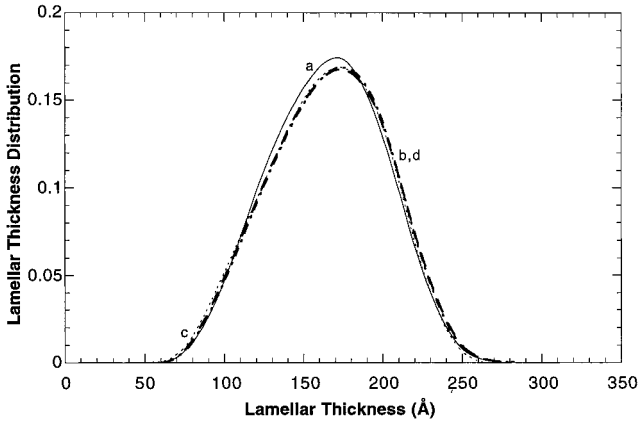

Figure 9. Crystal size distributions after 15 days (a), 1.5 months (b), 3 months (c), and 6 months (d) of swelling for PVA samples prepared with seven cycles of freezing and thawing, concentration of 15 wt %, and $\bar{M}_n = 35\,740$.

additional crystallization. However, the increased overall stability of the 15 wt % sample is worth noting. A PVA solution of initially higher concentration that has been exposed to repeated cycles of freezing and thawing shows enhanced stability with respect to both retaining a higher degree of crystallinity as well as exhibiting lower secondary crystallization. Lower PVA solution concentrations result in gels of lower cross-linking and, thus, higher degrees of swelling. However, along with this effect, there is increased free volume to permit the rearrangement and resulting instability of the structure.

Analysis of Crystallinity at Long Times. A considerable degree of instability has been demonstrated in the initial 1−15 days of swelling for PVA gels prepared by freezing and thawing techniques. However, such materials could be washed for an initial period of time to remove excess PVA chains. Such a washing technique would allow for the melting out of less stable crystalline regions as well as the possible rearrangement of the network in terms of secondary crystallization. Therefore, PVA were analyzed in terms of crystal size distributions at time intervals of 15 days, 1.5 months, 3 months, and 6 months during swelling to better ascertain information as to the appropriateness of such materials for long-term biomedical or pharmaceutical applications.

The first hydrogels that will be further discussed in terms of long-term network stability are the gels prepared from 15 wt % PVA solutions of molecular weight $\bar{M}_n = 35\,740$. These gels were prepared with five and seven cycles of freezing and thawing. The distributions over 6 months of swelling for the five-cycle samples are shown in Figure 8. Initially, these materials were very stable between 15 days and 1.5 months of swelling with nearly identical crystal size distributions. After this point, there was a trend of continued shifting of the distribution to a higher lamellar thickness. Although this effect was small, there is an indication that additional crystallization occurred within the structure and overall long-term instability.

Some interesting results are found when examining the seven-cycle samples over this 6-month period of swelling (Figure 9). Between 15 days and 1.5 months of swelling, a very slight loss of crystallinity occurred. After this point, however, the gels were extremely stable with overlapping distributions representing 1.5, 3, and 6 months of swelling. These results emphasize that increasing the number of freezing and thawing cycles

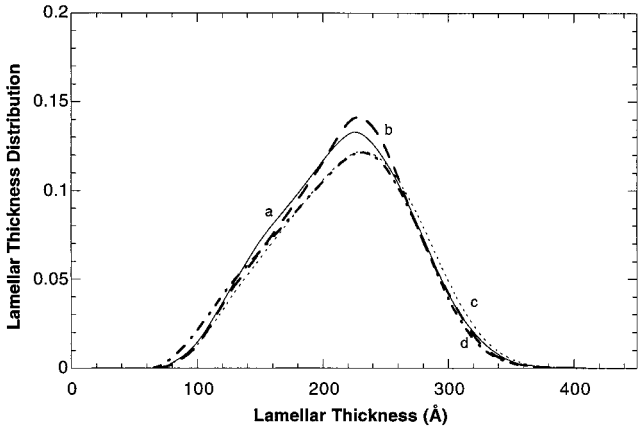

Figure 10. Crystal size distributions after 15 days (a), 1.5 months (b), 3 months (c), and 6 months (d) of swelling for PVA samples prepared with $\bar{M}_n = 64\,000$, concentration of 15 wt %, and three cycles of freezing and thawing.

can significantly increase the initial stability as well as long-term stability of PVA gels prepared by freezing and thawing techniques. Increased freezing and thawing cycles act to reinforce existing crystals within the structure. In addition, samples exposed to increased freezing and thawing cycles are less physically cross-linked resulting in decreased free volume and mobility within the network. Therefore, additional crystallization over long time periods is not observed. Such materials show great promise for use in biomedical and pharmaceutical applications because of their enhanced overall stability.

PVA gels of higher molecular weights were also examined in terms of their crystal size distributions at long times during swelling. The distributions for the gels prepared from 15 wt % solutions of PVA of $\bar{M}_n = 64\,000$ with three cycles of freezing and thawing are shown in Figure 10. Through experimental analysis, these gels have demonstrated enhanced stability in terms of fractional PVA dissolution, swelling dynamics, and overall degree of crystallinity. However, some rearrangement of the structure occurred during long-term swelling. In particular, between 15 days and 3 months of swelling there was a slight increase followed by a more significant decrease in the degree of crystallinity as indicated by the change in the area under the peak. However, after this time, the materials were very stable between 3 and 6 months of swelling with nearly identi-

cal distributions at these time intervals. Even though some instability was observed in terms of the degree of crystallinity, no shift in the overall distribution was noted. We would also expect that, with an increase in the number of freezing and thawing cycles, this sample would exhibit further enhancement of network stability.

Conclusions

The overall structure, morphology, and stability of PVA gels prepared by freezing and thawing techniques have been investigated. Prepared gels were characterized in terms of their swelling and dissolution behavior, degrees of crystallinity, and crystal size distributions. In addition, the long-term stability was addressed in order to consider the appropriateness of such materials for long-term biomedical or pharmaceutical applications. Overall conclusions can be drawn as to the effect of several parameters on the structure and stability of PVA gels prepared by freezing and thawing processes. The parameters of particular interest are the number of freezing and thawing cycles, PVA molecular weight, and the concentration of aqueous solution. Variation of these parameters significantly impacts the resulting behavior of the ensuing materials.

When considering stability issues of such materials for long-term biomedical or pharmaceutical applications, there are some key points to consider. The stability can be significantly enhanced by increasing the number of freezing and thawing cycles. In addition, it is actually desirable to use a low to intermediate PVA molecular weight to prevent the possible rearrangement of the structure over long time periods. Results indicate that an increase in PVA chain length and an increase in the free volume within the network together allow for secondary crystallization to proceed as the material swells. This phenomenon was more pronounced in the $\bar{M}_n = 88\,880$ sample, which had the longest polymer chain length and swelled to a very high extent. Obviously, such additional crystallization that occurred over a 6-month period of swelling is extremely undesirable for most applications because the performance characteristics of the gel can be significantly altered. In particular, the mechanical strength, diffusive profile, and adhesive characteristics may be impacted in a negative way. In addition, the increased water fraction in loosely cross-linked systems provides for greater potential chain dissolution and overall rearrangement of the structure.

PVA gels prepared by freezing and thawing techniques can be described as a very unique and complicated system. We are capable of varying many parameters to change or even optimize overall properties of the material for a specific application. It is, however, interesting to observe the effect that there can be considerable overlap in the structure, behavior, and stability by the careful manipulation of preparation conditions.

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