

Investigation on the Formation Mechanisms of Hydrogels Made by Combination of γ -Ray Irradiation and Freeze-Thawing

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ABSTRACT: Hydrogels based on poly(vinyl alcohol) (PVA)/water-soluble chitosan (ws-chitosan)/glycerol were prepared by γ -ray irradiation, freeze-thawing, and combination of γ -ray irradiation and freeze-thawing, respectively. The influence of freeze-thawing cycles, the irradiation doses, and the sequence of freeze-thawing and irradiation processes on the rheological, swelling, and thermal properties of these hydrogels was investigated to evaluate the formation mechanisms of hydrogels made by combination of irradiation and freeze-thawing. For hydrogels made by freeze-thawing followed by irradiation, the physical crosslinking is destroyed partially while chemical crosslinking is formed by irradiation. However, the chemical crosslinking

density reduces with the increase of freeze-thawing cycles. Hydrogels made by irradiation followed by freeze-thawing bear less degree of physical crosslinking with the increase of irradiation dose for the increased chemical crosslinking density. It is found that these hydrogels own larger swelling capacity and better transparent appearance than those made by freeze-thawing followed by irradiation. Moreover, the former hydrogels have larger mechanical strength than the latter at low freeze-thawing cycles. © 2008 Wiley Periodicals, Inc. *J Appl Polym Sci* 108: 1365–1372, 2008

Key words: hydrogels; irradiation; freeze-thawing; water-soluble chitosan; rheology

INTRODUCTION

Hydrogels are three-dimensional polymer networks in which hydrophilic polymer chains are connected by physical or chemical bonds. These bonds give rise to the integrity and physical stability of the networks, whereas the thermodynamic compatibility of the polymer chains with water allows these materials to swell in aqueous solvents.¹ There are numerous applications of hydrogels, particularly in the medical and pharmaceutical sectors. Because of their biocompatibility and high water contents, hydrogels can be used for tissue engineering,² drug delivery devices,³ contact lenses,⁴ materials for artificial skin,^{5,6} etc.

Irradiation has long been used to make hydrogels for medical applications.⁷ It is easy to control the process and harvest the product without adding any initiators or crosslinkers, which may be harmful and difficult to remove. In addition, the radiation process can join hydrogel formation and sterilization in one technological step. Hydrogels prepared by freeze-thawing without any initiators or crosslinkers, too,

from PVA aqueous solutions have better mechanical strength than the irradiated products. However, this method yields opaque hydrogels with limited swelling capacity and thermal stability. PVA, a water-soluble polyhydroxy polymer, has been used widely in practical applications for its excellent chemical and physical properties. Chitosan has been well known to be able to accelerate the reepithelialization and normal skin regeneration,⁸ and to confer considerable antibacterial activity against a broad spectrum of bacteria.^{9,10} Protonated chitosan, a kind of water-soluble chitosan, can simplify the hydrogel-making process and improve the antibacterial activity. Glycerol is found to be able to improve the swelling capacity of the hydrogels.

In a former study, it was found that hydrogels made by combination of γ -ray irradiation and freeze-thawing, namely irradiation followed by freeze-thawing and freeze-thawing followed by irradiation, showed very different properties at a given irradiation dose and fixed freeze-thawing cycles. However, there is little information about the difference in the formation mechanisms of these two kinds of hydrogels. Herein, PVA/ws-chitosan/glycerol blend hydrogels were prepared by irradiation, irradiation followed by freeze-thawing, freeze-thawing, and freeze-thawing followed by irradiation, respectively. The effects of irradiation dose and freeze-thawing

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cycles on the rheological, swelling, and thermal properties were investigated. The formation mechanisms for hydrogels made by combination of irradiation and freeze-thawing were discussed.

EXPERIMENTAL

Materials

Poly(vinyl alcohol) (PVA) and glycerol were provided by Sinopharm Chemical Reagent Co., China. The degrees of polymerization and hydrolysis of PVA were 1750 ± 50 and 98%, respectively. Water-soluble chitosan (ws-chitosan), manufactured by protonating chitosan in HCl/CH₃CH₂OH solution, was obtained from Jinhu Chitin Co., China. The molecular weight and deacetylation degree of the chitosan before protonation were about 200,000 and 91.7%, respectively.

Preparation of the hydrogels

PVA was dissolved in distilled water at 96°C for 3 h under refluxing. The homogeneous solution, containing 7 wt % PVA, 2 wt % ws-chitosan, and 1 wt % glycerol, was obtained by stirring the mixture solutions of PVA, ws-chitosan, and glycerol at 40°C with a physical stirrer for 30 min before placing them in an ultrasonic water bath at 40°C for 15 min to remove bubbles. The aqueous solution was poured into petri dishes for preparing hydrogels by γ -ray irradiation (Irra.), irradiation followed by freeze-thawing (Irra. + FT), freeze-thawing (FT), and freeze-thawing followed by irradiation (FT + Irra.), respectively. Irradiations were performed in N₂ atmosphere by ⁶⁰Co γ -ray to the doses of 30 and 70 kGy, respectively, at a dose rate of 0.75 kGy/h. The freezing and thawing, repeated from one to six times, were performed at -20°C for 1.5 h and then at 25°C for 1 h. The hydrogel samples were stored at 4°C for further study.

Rheological measurements

The rheological measurements were conducted on a strain-controlled ARES rheometer (TA Instruments, Twin Lakes, WI) using parallel plates of 25 mm in diameter with plate-to-plate distance of 1–2 mm. Frequency scanning was performed in the frequency range of 0.4–10 rad/s at 25°C and with a strain of 0.2%. Temperature dependence of the rheological properties was investigated from 25 to 100°C at 1 rad/s with a strain of 0.2% and a ramp rate of 5°C/min. The strain sweeping experiments were conducted for strains ranging from 0.05 to 200% at 1 rad/s and at 25°C. Some of the hydrogels were extracted in distilled water for 72 h and were further

immersed in 80°C water for 20 min before the rheological experiment. To prevent dehydration during rheological measurements, a thin layer of vaseline was placed on the periphery surface of the hydrogel held between the plates. Each test was performed at least twice, on two different disc specimens from the same hydrogel sample.

Swelling behavior

Hydrogels were extracted in distilled water at 42°C for 72 h with water being changed every 8 h. The hydrogels were then immersed in distilled water at room temperature until the equilibrium state of swelling was achieved (with a weight of W_s). The water-absorbed gels were dried at 60°C for 48 h to a constant weight (W_d). The degree of swelling (DS) of the gels was calculated according to the equation $DS = W_s/W_d$.

Differential scanning calorimetry analysis

The differential scanning calorimetry (DSC) experiment was performed with Mettler Toledo DSC822e. The physical blend and the extracted dry hydrogels of about 9 mg were heated from 30 to 250°C under 50 mL/min N₂ atmosphere with a scanning rate of 10°C/min.

RESULTS AND DISCUSSION

Appearance of the hydrogels

Hydrogels prepared by irradiation to 30 kGy were too adhesive to be removed without damaging the shape whereas other hydrogels could be easily removed from the petri dishes. Hydrogels made by Irra. are transparent because of the homogeneous network structure. The opaque appearance of hydrogels made by FT is attributed to the formation of crystallites and the microphase separation that occurs in the early stage of the gelation process.^{11–13} As a result, the crystallites can be characterized by the turbidity of the PVA hydrogel.^{14,15} Hydrogels made by Irra. + FT are more transparent than those made by FT + Irra. as shown in Figure 1. Moreover, the larger irradiation dose results in a better transparent appearance for the former hydrogels [Fig. 1(b,d)]. The transparent property of the higher dose prepared hydrogel indicates that larger chemical crosslinking density inhibits the formation of crystallites and the phase separation during the freeze-thawing process.

Rheological properties of the hydrogels

Figure 2 presents the temperature dependence of both storage (G') and loss (G'') modulus for hydrogel

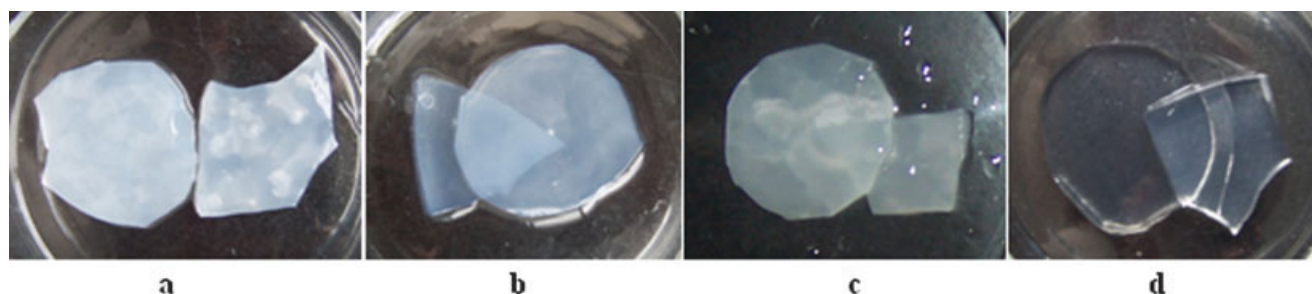


Figure 1 The morphology of hydrogels made under various conditions. (a–d) Hydrogels made by two cycles of freeze-thawing followed by irradiation to 30 kGy (FT-2 + 30 kGy), irradiation to 30 kGy followed by two cycles of freeze-thawing (30 kGy + FT-2), two cycles of freeze-thawing followed by irradiation to 70 kGy (FT-2 + 70 kGy), and by irradiation to 70 kGy followed by two cycles of freeze-thawing (70 kGy + FT-2), respectively. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

made by three cycles of freeze-thawing followed by irradiation to 30 kGy. The hydrogel possesses high G' values which show no temperature dependence at low temperatures, but above 55°C, G' and G'' decrease and come to the lowest values at about 80°C. This is due to the breakdown of the PVA physical network, as proposed by Hatakeyema et al.¹⁶ However, G' is considerably higher than G'' , even at high temperatures, indicating that the elastic network is still present. This network is due to the chemical crosslinking induced in the irradiation process. Other hydrogels made by FT + Irra. show similar property. Thereby, it is proposed that the extent of chemical crosslinking can be evaluated from the storage modulus of hydrogels after being immersed in distilled water at high temperatures.

The effects of freeze-thawing cycles and irradiation doses on the storage modulus for hydrogels made by FT + Irra. are demonstrated in Figure 3. Before the measurement, these hydrogels were immersed in

distilled water at 80°C for 20 min, in which process the physical network could be destroyed. As shown, a decrease in storage modulus is observed with the increase of freeze-thawing cycles at a given irradiation dose, indicating a decreased chemical crosslinking density. As the physical crosslinking increases with the increase of freeze-thawing cycles (see Figs. 4 and 6), it is therefore concluded that the formation of chemical crosslinking induced by irradiation is hindered by the physical crosslinking formed in the freeze-thawing process. Furthermore, hydrogels made by Irra. + FT must own larger chemical crosslinking density than those made by FT + Irra., for the chemical crosslinking formed by irradiation cannot be affected by freeze-thawing. Figure 3 also shows that hydrogels made at 70 kGy show much larger storage modulus than the corresponding hydrogels made at 30 kGy. This implies that hydrogels made at high doses possess larger chemical crosslinking density.

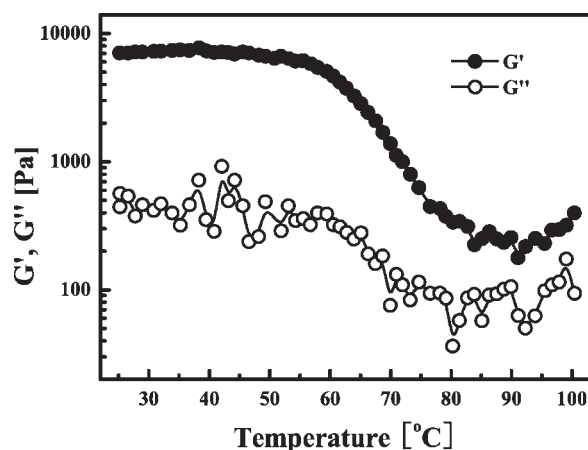


Figure 2 Storage and loss modulus (G' and G'') at 1 rad/s as a function of temperature for hydrogel made by three cycles of freeze-thawing followed by irradiation to 30 kGy (FT-3 + 30 kGy).

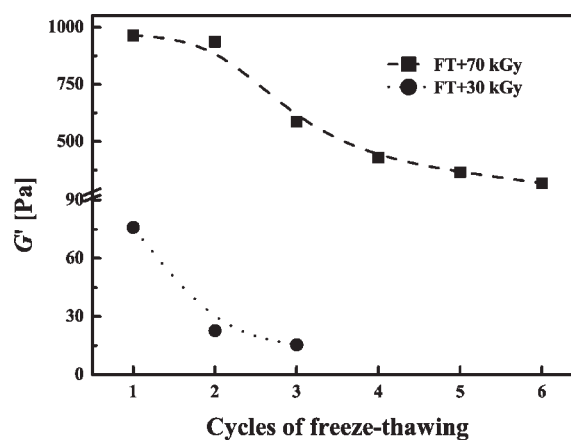


Figure 3 Storage modulus (G') at 1 rad/s as a function of the number of freeze-thawing cycles for hydrogels made by freeze-thawing followed by irradiation to 30 and 70 kGy (i.e., FT + 30 kGy, FT + 70 kGy), respectively. The hydrogels were immersed in 80°C water for 20 min before the rheological measurement.

The storage modulus as a function of freeze-thawing cycles for hydrogels made under different conditions is compared in Figure 4. G' increases with the increase of freeze-thawing cycles, indicating an increased degree of physical crosslinking. Hydrogels made by FT + Irra. show larger storage modulus than those made by FT when the number of freeze-

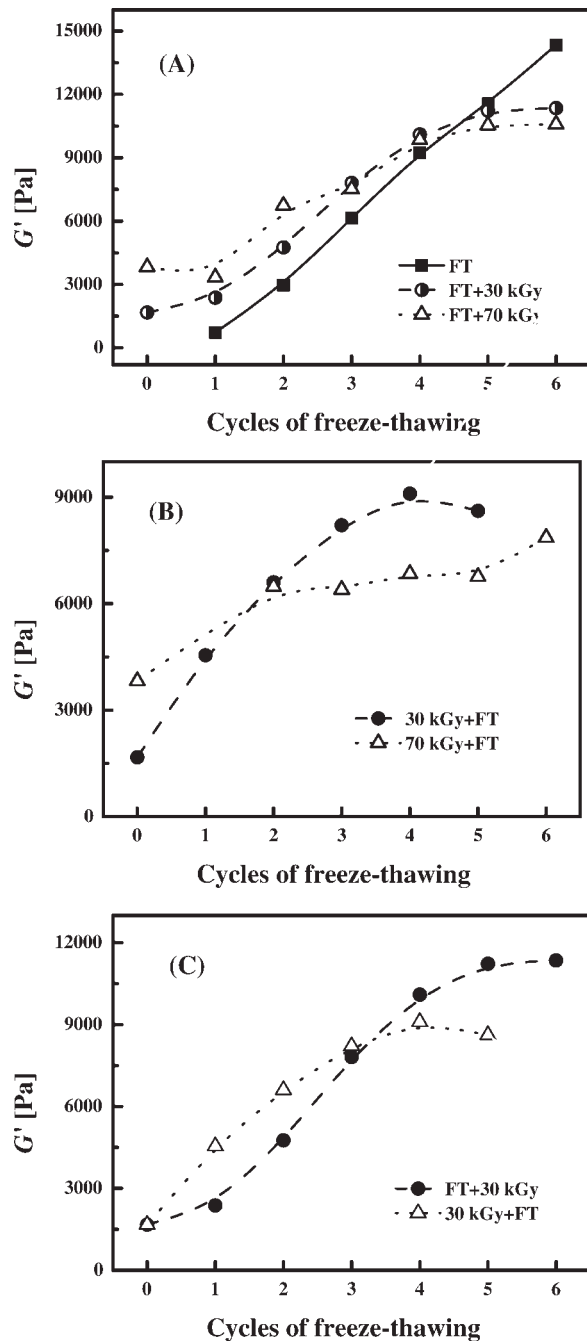


Figure 4 Storage modulus (G') at 1 rad/s as a function of freeze-thawing cycles for hydrogels made by freeze-thawing (FT), freeze-thawing followed by irradiation to 30 or 70 kGy (i.e., FT + 30 kGy, FT + 70 kGy), and by irradiation to 30 or 70 kGy followed by freeze-thawing (i.e., 30 kGy + FT, 70 kGy + FT), respectively.

thawing cycles is less than three, and the higher the irradiation dose, the larger the storage modulus is [Fig. 4(A)]. This implies that the following irradiation can effectively improve the mechanical strength at lower number of freeze-thawing cycles because of the chemical crosslinking. However, the relationship is just the opposite when the number of freeze-thawing cycles is larger than four, which indicates that irradiation can damage the physical crosslinking network to a certain extent, accompanying with the formation of chemical crosslinking network. Figure 4(B) shows that the storage modulus of hydrogels made by Irra. + FT increases with increasing the number of freeze-thawing cycles and levels off at high freeze-thawing cycles. However, the enhancement is not significant for hydrogels irradiated to 70 kGy. This implies that less physical crosslinking can be formed by freeze-thawing at higher irradiation dose for the strong chemical crosslinking network. From Figure 4(C), it is found that at low freeze-thawing cycles, hydrogels made by Irra. + FT have larger storage modulus than those made by FT + Irra.

Figure 5 shows the strain dependence of the storage modulus at 1 rad/s for hydrogels made under different conditions. The value of G' has been normalized by its maximum value, G'_0 . At low strain amplitudes, G' is independent of the strain amplitude, indicating that the deformation imposed on the network structure is entirely reversible. At higher strain amplitudes, G' is a decreasing function of the strain amplitude and the deformation is no longer reversible. A lower strain amplitude at the end of the constant regime means less flexibility of the hydrogel.¹² Thus, Figure 5(A) shows that the flexibility of hydrogels made by FT decreases with the increase of freeze-thawing cycles and comes to a constant value at large number of freeze-thawing cycles. Hydrogels made by combination of irradiation and freeze-thawing show similar property. Figure 5(B) reveals that the flexibility reduces for hydrogels made by one cycle of freeze-thawing followed by irradiation (FT-1 + 30 kGy, FT-1 + 70 kGy) comparing with those made by pure freeze-thawing (FT-1), and this effect is more significant at high irradiation dose (FT-1 + 70 kGy). However, when the number of freeze-thawing cycles is sufficiently high (e.g., six cycles), the flexibility is almost the same. The reduction of flexibility is likely ascribed to the chemical crosslinking network formed by irradiation, and a larger irradiation dose introduces more chemical crosslinking points. However, the chemical crosslinking network is hard to form when the number of freeze-thawing cycles is large (Fig. 3), which results in little change in flexibility. As seen from Figure 5(C), little difference in flexibility is observed for hydrogels made by irradiation alone to different doses. Hydrogel made by irradiation to 70 kGy

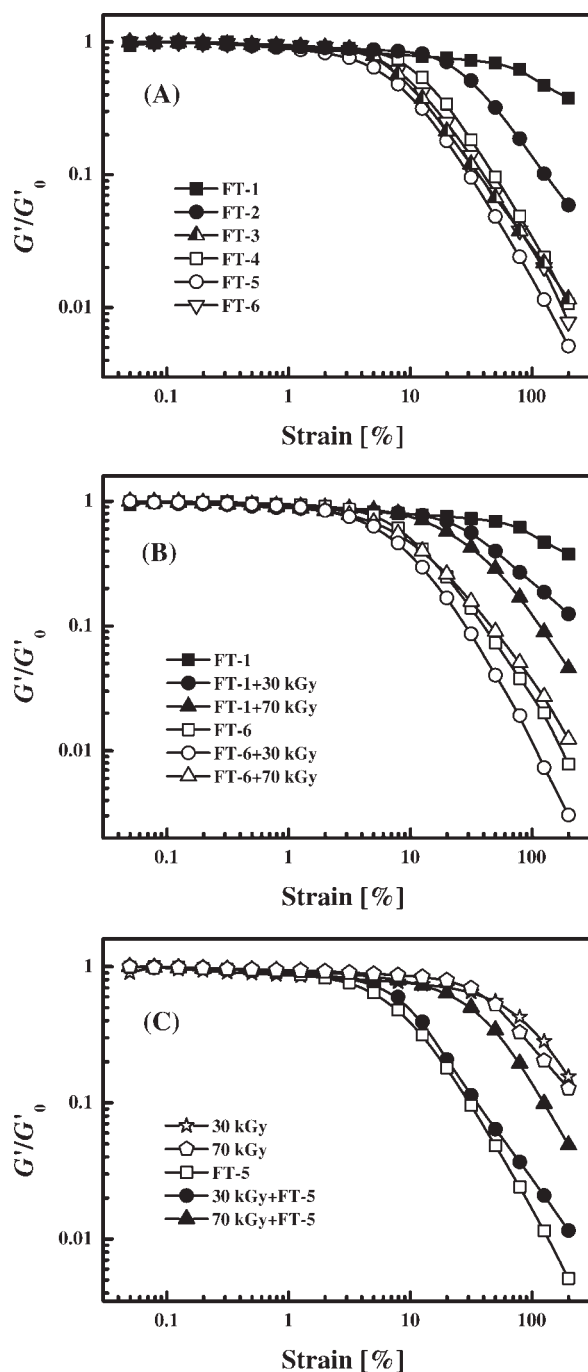


Figure 5 Storage modulus (G') at 1 rad/s as a function of strain values for hydrogels obtained under different conditions. The storage modulus (G') had been normalized with respect to its maximum value (G'_0). The number following "FT" indicates the number of freeze-thawing cycles.

followed by five cycles of freeze-thawing (70 kGy + FT-5) is much more flexible than that made by five cycles of freeze-thawing (FT-5), and it shows similar flexibility to that made by irradiation to 70 kGy. However, the hydrogel made by irradiation to 30 kGy followed by five cycles of freeze-thawing (30 kGy + FT-5) shows almost the same flexibility as

that prepared by five cycles of freeze-thawing alone (FT-5), and they are more fragile than that made by irradiation to 30 kGy alone. These phenomena indicate that the flexibility of hydrogels made by Irra. + FT can be significantly reduced at low dose for the formation of large physical crosslinking network. However, little physical crosslinking network can be formed at high irradiation dose [Fig. 4(B)], resulting in little influence on flexibility.

Swelling behavior of the hydrogels

The water absorption capacity of hydrogels is closely related to the crosslinking density, the presence of hydrophilic groups, and the percentage of amorphous regions in the polymeric structure.¹⁷ The increase of the crosslinking density causes reduction of the distances between the crosslinking points, demanding larger force to deform the polymeric chain. Consequently, systems with high crosslinking density possess low DS.

Figure 6 shows the DS with respect to the freeze-thawing cycles for hydrogels made under various conditions. The results reveal that the swelling capacity decreases with increasing the number of freeze-thawing cycles for all the hydrogels, because an increased degree of physical crosslinking is associated with an increased number of freeze-thawing cycles. This is similar to the result described by Peppas and Hassan.¹⁸ Hydrogels made by Irra. show the largest swelling capacity because of the special chemical crosslinking networks. With the dose increasing from 30 to 70 kGy, the swelling capacity decreases because of the increased degree of chemical crosslinking.

At low freeze-thawing cycles, the swelling capacity for hydrogels made by FT + Irra. is greatly improved comparing with the corresponding hydrogels made by FT, especially at low irradiation dose.

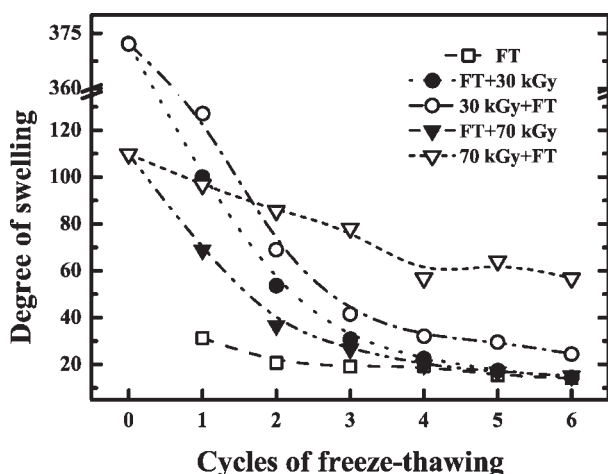


Figure 6 Degree of swelling as a function of freeze-thawing cycles for hydrogels made under various conditions.

However, the swelling capacity is almost the same when the number of freeze-thawing cycles is larger than four. As known, there are physical and chemical crosslinkings in these hydrogels, and hydrogels with chemical crosslinking have larger swelling capacity than those with physical crosslinking as mentioned earlier. So the enhanced swelling capacity at low freeze-thawing cycles indicates that the physical crosslinking density must be reduced partially by irradiation, and the DS is mainly determined by chemical crosslinking. When the number of freeze-thawing cycles is larger than four, the physical crosslinking is in dominant position.

The swelling capacity is greatly decreased for hydrogels made by Irra. + FT compared with the corresponding hydrogels made by Irra. However, this effect is not pronounced at large irradiation dose, and hydrogels made by irradiation to 70 kGy followed by freeze-thawing (70 kGy + FT) have even larger swelling capacity than those made at 30 kGy (30 kGy + FT) when the number of freeze-thawing cycles is larger than one. The decrease of swelling capacity is due to the physical crosslinking formed in the freeze-thawing process, and the less degree of physical crosslinking of hydrogels made at higher irradiation dose [Fig. 4(B)] results in the larger swelling capacity.

It is worthy of noting that hydrogels made by Irra. + FT exhibit larger water absorption capacity than those made by FT + Irra. As the chemical crosslinking is larger in the former hydrogels than in the latter (see Fig. 3), it can be proposed that the former hydrogels possess less degree of physical crosslinking than the latter hydrogels. Meanwhile, the former hydrogels own larger storage modulus than the latter at low number of freeze-thawing cycles as found in Figure 4(C). So it can be concluded that the storage modulus of hydrogels made by combination of irradiation and freeze-thawing is mainly determined by chemical crosslinking at low cycles of freeze-thawing, and when the number of freeze-thawing cycles is large enough, it is mainly determined by physical crosslinking.

DSC analysis

PVA has been reported to exhibit an endothermic peak around 230°C in DSC curve that corresponds to the melting temperature.¹⁹ ws-chitosan and glycerol do not show significant transition in the temperature range of 200–235°C. The melting temperatures of the physical blend and extracted hydrogels are shown in Table I. The physical blend exhibits much lower melting temperature than pure PVA. This indicates that the ordered association of the PVA molecules is decreased by the presence of ws-chitosan and glycerol, as found by some researchers.^{19,20} A significant

TABLE I
Melting Temperatures of PVA/ws-Chitosan/Glycerol Physical Blend and Hydrogels Made Under Various Conditions

Irradiation (kGy)	Melting temperature (°C)						
	Irra.	FT + Irra.			Irra. + FT		
		FT-1 ^a	FT-3	FT-5	FT-1	FT-3	FT-5
0	211.7	233.4	233.3	232.2	233.4	233.3	232.2
30	214.1	221.7	224.5	227.1	219.8	219.9	220.0
70	205.4	217.1	220.5	221.9	206.6	205.6	205.5

^a The number following "FT" indicates the number of freeze-thawing cycles.

decrease in the melting temperature is observed with increasing irradiation dose for all the hydrogels. However, hydrogels made by FT show much larger melting temperatures than the physical blend. The melting temperature variations are caused by morphological and chemical modifications. The morphological changes involve the thickness of the crystallites and the degree of crystallinity. The chemical changes involve the crosslinking and grafting reactions, and they also affect the morphological ones.^{19,21} Thereby, in this work, the chemical crosslinking induced by irradiation reduces the melting temperature, whereas the crystallites introduced by freeze-thawing increases the melting temperature. The increase of freeze-thawing cycles shows little influence on the melting temperatures for hydrogels made by FT and by Irra. + FT, indicating that the changes of morphological and chemical state of these hydrogels are not significant with the increase of freeze-thawing cycles. However, hydrogels made by FT + Irra. demonstrate higher melting temperatures with the increase of freeze-thawing cycles. This is because that the chemical crosslinking decreases with the increase of freeze-thawing cycles (Fig. 3). Hydrogels made by Irra. + FT show increased melting temperatures comparing with those made by Irra., but the increase is not pronounced for hydrogels prepared at high dose. This is due to that crystallites are hard to form for hydrogels made by Irra. + FT at high irradiation dose (Fig. 1).

Formation mechanisms for hydrogels made by combination of γ -ray irradiation and freeze-thawing

Hydrogel formation under irradiation is a result of formation of chemical crosslinking networks. The chemistry underlying radiation-induced crosslinking of water-soluble polymers has been studied in detail.^{22–26} In brief, highly reactive water-derived species and polymer macromolecule radicals are formed in the aqueous solution by irradiation. Fast reactions

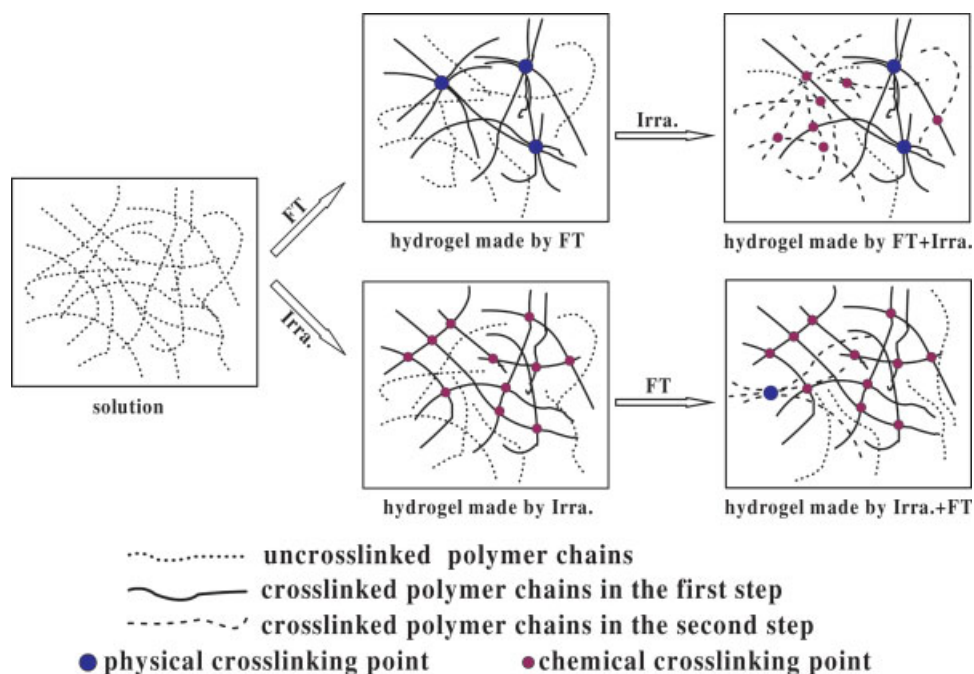


Figure 7 Schematic illustrations of hydrogel formations by combination of irradiation and freeze-thawing. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

of some of the intermediates with macromolecules (mainly through H-atom abstraction from the polymer by OH radicals) lead to the formation of polymer radicals in most cases. Covalent bond linking and therefore the crosslinking networks are formed through the recombination of the polymer radicals.²⁷

The formation of PVA hydrogels prepared via freeze-thawing is ascribed to the formation of PVA crystallites which act as physical crosslinking sites in the network.^{28–30} PVA molecules are aggregated during ice formation, and hydrogen bond is established between molecular chains, resulting in the formation of crystallites. Loose crosslinking networks are formed in the first freezing and thawing cycle, and with increasing freeze-thawing cycles, the number of crosslinking points increases and molecular chains aggregate.¹⁶

The schematic illustrations of hydrogel formations by combination of irradiation and freeze-thawing are presented in Figure 7. Polymer radicals can be generated when hydrogels formed by freeze-thawing are subjected to irradiation. However, the combination opportunity of these radicals is greatly reduced because of the less mobility of the polymer radicals due to the physical crosslinking networks. Thus, the chemical crosslinking density is smaller than that of hydrogels made by irradiation from the aqueous solution. Meanwhile, the physical crosslinking points can be destroyed partially by irradiation. On the other hand, the physical crosslinking is hard to form for hydrogels made by Irra. + FT. This is because

the mobility of the polymer chains is much reduced because of the chemical crosslinking networks formed under irradiation, which hinders the phase separation and the formation of crystallites. The degree of physical and chemical crosslinkings is much different for these two kinds of hydrogels, which results in different properties. It is thereby concluded that not only the irradiation dose and freeze-thawing cycles but also the sequence of irradiation and freeze-thawing processes determine the properties of the hydrogels prepared by combination of irradiation and freeze-thawing.

CONCLUSIONS

Blend hydrogels based on PVA/ws-chitosan/glycerol were prepared by irradiation, freeze-thawing, and combination of irradiation and freeze-thawing, respectively. The difference in the sequence of irradiation and freeze-thawing endows the hydrogels made by combination of irradiation and freeze-thawing with much different degree of physical and chemical crosslinkings which affects the physical properties. The mechanical strength increases while the swelling capacity and flexibility decrease when the freeze-thawing cycles increases from one to six due to the increase of physical crosslinking density. For hydrogels made by freeze-thawing followed by irradiation, the mechanical strength and swelling capacity are enhanced at low cycles of freeze-

thawing whereas they are reduced by irradiation at high cycles of freeze-thawing. For hydrogels made by irradiation followed by freeze-thawing, the already formed chemical crosslinking hinders the formation of physical crosslinking induced by freeze-thawing, especially at large irradiation dose. Moreover, hydrogels made by irradiation followed by freeze-thawing have larger swelling capacity and better transparent appearance than those made by freeze-thawing and freeze-thawing followed by irradiation. And at low freeze-thawing cycles, the former has larger mechanical strength than the corresponding hydrogels prepared via other methods.

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