Effect of Gelatin on the Swelling Behavior of Organic Hybrid Gels Based on *N*-Isopropylacrylamide and Gelatin

Wen-Fu Lee, Sung-Chuan Lee

Department of Chemical Engineering, Tatung University, Taipei 10451, Taiwan, Republic of China

Received 1 May 2004; accepted 19 July 2004
DOI 10.1002/app.21287
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: Organic hybrid gels based on poly(*N*-isopropylacrylamide) and a natural polymer, gelatin, were prepared through two-step crosslinking with genipin or glutaraldehyde. The effects of the gelatin content on the swelling behaviors and physical properties of these hybrid gels were investigated. The results indicated that the swelling ratio decreased with an increase in the content of gelatin in these hybrid gels. The swelling ratio for the gel crosslinked by genipin was significantly smaller than that for the gel crosslinked by glutaraldehyde. The results also showed that the gel crosslinked with genipin had a higher crosslinking density and a higher gel strength. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 98: 1092–1099, 2005

Key words: hydrogels; swelling; stimuli-sensitive polymer

INTRODUCTION

Hydrogels are three-dimensional hydrophilic polymers that swell but do not dissolve when brought into contact with water, and they sometimes undergo a volume phase change in response to a change in the surrounding conditions, including the temperature,[1,2] pH,[3] ionic strength,[4] and electric field.[5,6]

A thermosensitive hydrogel, one of the environmental stimuli-response hydrogels, collapses at elevated temperature through the critical gel-transition temperature (CGTT). The volume change occurs within a quite narrow temperature range. The permeability of water through the gel can be changed by an on–off switch according to the environmental temperature. Therefore, such materials can be used in many applications, such as drug delivery systems and enzyme activity control.[7–14]

A poly(*N*-isopropylacrylamide) (PNIPAAm) hydrogel demonstrates a nearly continuous volume transition and an associated phase transition (a highly swollen gel network from a low temperature to a collapsed phase at a high temperature, near its critical point between 31and 35°C).[15] Hirotsu[16] investigated the phase behavior of the PNIPAAm gel/water/alcohol system and explained its thermoshrinkage by the destruction of hydrogen bonds between the water molecules and amide group of *N*-isopropylacrylamide (NIPAAm).

Gelatin obtained by thermal denaturation or physical and chemical denaturation of collagen is the most widespread protein in the body, occurring in most connective tissues, such as skin, tendon, and bone.[17–19] The food, pharmaceutical, and photographic industries are the main users of gelatin, which has several other technical applications. Its most frequent uses in the biomedical field include hard and soft capsules, microspheres, wound dressings, and adsorbent pads for surgical use; furthermore, it is much cheaper and easier to obtain in concentrated solutions.[17,18,20]

At a temperature of about 40°C, gelatin solutions are in the sol state, and they change into gels when they are cooled at room temperature, as long as their concentration is high enough.[21] The sol–gel transformation is due to a conformational disorder–order transition of the gelatin chains, which form thermoreversible networks by associating helices in junction zones stabilized by hydrogen bonds. The mechanism of gelation and the properties of gelatin gels have been extensively investigated.[21–23] On the other hand; gelatin exhibits poor mechanical properties, which limit its possible applications as a biomaterial. Because gelatin is soluble in aqueous solutions, gelatin materials for applications must be submitted to crosslinking. Physical crosslinking methods include dehydrothermal treatments and ultraviolet and γ irradiation. Furthermore, because of the large number of functional side groups it contains, gelatin readily undergoes chemical crosslinking. This is usually performed with bifunctional reagents, such as glutaraldehyde (GA)

Correspondence to: W.-F. Lee (wflee@che.ttu.edu.tw).
Contract grant sponsor: Tatung University (Taipei, Taiwan); contract grant number: B91-1413-01.

and diisocyanates, as well as carbodiimides, polyepoxy compounds, and acyl azide.[24–26]

GA is by far the most widely used agent because of its high efficiency of collagenous material stabilization. The crosslinking of collagenous samples with GA involves the reaction of free amino groups of lysine or hydroxylysine amino acid residues of the polypeptide chains with the aldehyde groups of GA.[26] The polymerization of GA molecules in aqueous solutions with observable reductions in free aldehyde has been reported previously.[27,28] In the polymerization, the aldehyde functional groups of two GA molecules may undergo aldol condensation.[27] With GA polymerization, after crosslinking, a network crosslinking structure can be created intramolecularly and intermolecularly within collagen-based biomaterials. The mechanism of crosslinking of collagen-based biomaterials with GA can be found in the literature.[27,29] GA is widely available and inexpensive, and its aqueous solutions can effectively crosslink collagenous tissues in a relatively short period.[25] However, if released into the host because of biodegradation, GA is toxic.[26]

Genipin (GP) is a naturally occurring crosslinking agent with promising characteristics. GP can be obtained from an iridoid glucoside, geniposide, which is abundant in gardenia fruits. GP has been widely used in herbal medicine,[30] and the dark blue pigments obtained by its spontaneous reaction with amino acids or proteins have been used in the fabrication of food dyes.[31] The reaction mechanism of amino-group-containing compounds with GP has been discussed in the literature.[31,32] It has been proposed that a GP-amino-group monomer is formed though a nucleophilic attack by amino-group-containing compounds such as gelatin on the third carbon of GP. This is followed by the opening of the GP ring, and an aldehyde group is formed. Subsequently, the resulting aldehyde group is attacked by the attached secondary amino group. Dimerization occurs during the second stage, perhaps by a radical reaction. Therefore, GP may form intramolecularly and intermolecularly crosslinked products with a heterocyclic structure.

The main propose of this study was to prepare a series of organic hybrid hydrogels based on NIPAAm and gelatin and to investigate systemically their swelling behavior and physical properties in deionized water at different temperatures. In addition, the solvent effect on the swelling behavior for the NIPAAm/gelatin hybrid hydrogels was studied.

EXPERIMENTAL

Materials

NIPAAm (Wako Pure Chemical Co., Ltd., Osaka, Japan) was recrystallized in *n*-hexane before use for the removal of an inhibitor. *N,N′*-Methylene bisacrylamide (NMBA; Sigma Chemical Co., St. Louis, MO), used as a crosslinking agent, and *N,N,N′,N′*-tetramethylethylenediamine (TEMED; Fluka Chemical Co., Buchs, Switzerland), used as an accelerator, were used as received. Ammonium persulfate (APS; Wako Pure Chemical), used as an initiator, was further purified by recrystallization. Gelatin from porcine skin (type A, 300 Bloom), corresponding to a molecular weight range of 50–100 kDa (Sigma Chemical), used as a natural polymer, was used as received. GA (Wako Pure Chemical) and GP (Challenge Bioproducts, Taichung, Taiwan), used as gelatin crosslinking agents, were used as received.

Preparation of the hydrogels

The hydrogels were prepared by free-radical polymerization in deionized water (18.3 MΩ cm). NIPAAm and gelatin in various ratios and 4 mol % NMBA (based on the total monomer content) were dissolved in 10 mL of deionized water. To this solution, 1 mol % APS and 1 mol % TEMED as a redox initiator were added, and the mixture was immediately injected into the space between two glass plates. The gel membrane thickness was adjusted with a silicone spacer between the two glass plates. Polymerization was carried out at 25°C for 1 day. After the gelation was completed, the gel membrane was further crosslinked with 20 mL of 1 wt % GA or GP solutions in a phosphate buffer solution of pH 7.4 for 24 h at room temperature. After this crosslinking reaction was completed, the gel membrane was cut into disks 10 mm in diameter and then immersed in an excess of deionized water for 3 days to remove the residual unreacted monomer. The hybrid gels were dried at 40°C for 3 days and then further dried in a 25°C vacuum oven for 1 day.

Measurement of the swelling ratio (SR)

The preweighed dried gels were immersed in an excess amount of deionized water [or various volume ratios of dimethyl sulfoxide (DMSO) to H_2O] at 25°C until swelling equilibrium was attained. Each gel was then removed from the water bath, tapped with filter paper to remove excess surface water, and weighed as the wet weight (W_w). SR and the equilibrium solvent content (ESC) were calculated as follows:

$$\mathrm{SR} = \left(\frac{W_w - W_d}{W_d}\right) \tag{1}$$

$$\mathrm{ESC} = \left(\frac{W_w - W_d}{W_w}\right) \times 100\% \tag{2}$$

where W_d is the weight of the dried gel.

Measurement of the dynamic swelling

The dried gels were immersed in an excess amount of deionized water (or various volume ratios of DMSO to H_2O) at different temperatures. SR was obtained by the weighing of the initial and swollen samples at various time intervals. The amount of water absorbed (W_t) was reported as a function of time, and the equilibrium absorption at an infinitely long time was designated W_∞. Equation (3) was used to calculate the diffusion coefficient (D) for $W_t/W_\infty \leqq 0.8$:[33]

$$\frac{W_t}{W_\infty} = \left(\frac{4}{\pi^{0.5}}\right)\left(\frac{Dt}{L^2}\right)^{0.5} \tag{3}$$

where t is the time and L is the initial thickness of the dried gel. To investigate the diffusion model of the gel, we fitted the initial swelling data to the exponential heuristic equation for $W_t/W_\infty \leqq 0.6$:[34,35]

$$\frac{W_t}{W_\infty} = Kt^n \tag{4}$$

where K is a characteristic constant of the gel and n is a characteristic exponent of the mode transport of the penetrant.

Measurement of the equilibrium SR at various temperatures

Two preweighed dried gels were immersed in 10 mL of deionized water, and the water temperature was kept at 25, 28, 30, 32, 33, 36, or 40°C for 1 day. After the swelling equilibrium was attained, the equilibrium SR for the gels at every temperature was calculated.

Measurement of the thermoreversibility

The dried gels were equilibrated in 10 mL of deionized water at 25°C, and the wet gels were weighed. The gels were then transferred to 10 mL of deionized water at 37°C and were weighed at each fixed time interval. When the weight of the gels was kept constant, the gels were transferred into deionized water at 25°C again and were weighed at each fixed time interval. This operation was conducted for several cycles.

Measurement of the swell–deswell reversibility

The dried gels were equilibrated in 10 mL of a 10% (v/v) DMSO/H_2O aqueous solution, and the wet gels were weighed. The gels were then transferred into 10 mL of a 50% (v/v) DMSO/H_2O aqueous solution and were weighed at each fixed time interval. When the weight of the gels was kept constant, the gels were transferred into a 10% (v/v) DMSO/H_2O aqueous solution again and were weighed at each fixed time interval. This operation was conducted for several cycles, and the SRs were calculated.

Measurement of the physical properties

The gel strengths of these samples were measured with a uniaxial compression experiment with a Lloyd LRX universal tester (RX; J. J. Lloyd, Poole, UK). Equation (5) was used to calculate the shear modulus (G):[36,37]

$$\tau = F/A = G(\lambda - \lambda^{-2}) \tag{5}$$

where τ is the compression stress, F is the compression load, A is the cross-sectional area of swollen gels, and λ is the compression strain (L/L_0). At low strains, a plot of the shear stress versus $-(\lambda - \lambda^{-2})$ yielded a straight line, the slope of which was G. The effective crosslinking density (ρ_x) could then be calculated from G and the polymer volume fraction (ν_2) as follows:

$$\rho_x = G/\nu_2^{1/3}RT \tag{6}$$

where R is the gas constant (8.48×10^4 g cm/mol K) and T is the absolute temperature.

RESULTS AND DISCUSSION

Characterization of the PNIPAAm/gelatin hybrid gels

The characterization of the PNIPAAm/gelatin hydrogels with various feed compositions is shown in Table I. N represents the PNIPAAm hydrogel; GP5, GP10, GP20, GP30, and GP40 represent 5, 10, 20, 30, and 40 wt % gelatin dispersed in the network of N and crosslinked with 1 wt % GP, respectively. GA5, GA10, GA20, GA30, and GA40 represent 5, 10, 20, 30, and 40 wt % gelatin dispersed in the network of N and crosslinked with 1 wt % GA, respectively.

The equilibrium SRs for these gels are shown in Table I. The results shown in Table I indicate that the equilibrium SRs for these gels in deionized water were in the order of N > GP5 > GP10 > GP20 > GP30 > GP40 and N > GA5 > GA10 > GA20 > GA30 > GA40. In other words, the greater the gelatin content was, the lower the SR was for these gels. This was because the incorporation of gelatin into the N matrix and further crosslinking with GP or GA made the gel network denser. Besides, the gels crosslinked with GP had lower SRs than those gels crosslinked with GA, and this was due to the heterocyclic structure of GP. A comparison of the heterocyclic structure of GP and the linear structure of GA showed that the gels crosslinked with the heterocyclic structure of the GP appeared to be relatively more difficult in relaxation than the structure of those gels crosslinked with GA.

TABLE I
Characterization of the NIPAAm/Gelatin Hybrid Gels

Sample codes	NIPAAm (g)	Gelatin (g)	Gelatin (wt %)	NMBA (mol %)	Equilibrium SR at 25°C (g/g)
N	1.1316	0	0	4	12.0
GP5	1.1316	0.056	5	4	6.27
GP10	1.1316	0.113	10	4	4.55
GP20	1.1316	0.0226	20	4	3.32
GP30	1.1316	0.0339	30	4	3.01
GP40	1.1316	0.452	40	4	2.81
GA5	1.1316	0.056	5	4	6.41
GA10	1.1316	0.113	10	4	4.90
GA20	1.1316	0.0226	20	4	4.02
GA30	1.1316	0.0339	30	4	3.40
GA40	1.1316	0.452	40	4	3.13

Swelling Kinetics of the PNIPAAm/gelatin hybrid gels in deionized water

The SRs as a function of time for PNIPAAm/gelatin crosslinked with GP or GA hybrid hydrogels at 25°C in deionized water are shown in Figures 1 and 2, respectively. The results indicated that SR decreased with an increase in the content of gelatin in the hybrid gels. The n, K, and D values, calculated with eqs. (3) and (4), are listed in Table II. The results show that the values of D for these hybrid gels in deionized water were in the order of N < GP5 < GP10 < GP20 < GP30 < GP40 and N < GA5 < GA10 < GA20 < GA30 < GA40. These results showed that the greater the gelatin content was in the hybrid gels, the higher the rate was of water penetration into the gel during the swelling process. At the same time, the results shown in Table II also indicate that the transport mechanisms for the hybrid gels with a low SR belonged to Fickian transport according to the classification of diffusion types presented by Alfrey et al.[38]

Effect of the temperature on SR

The CGTT was around 32°C for the NIPAAm hydrogels. For the NIPAAm gel, the hydrophilic group (amido —NHCO—) in the polymer structure formed an intermolecular hydrogen bond with surrounding water at low temperature (below the gel-transition temperature); however, this trend changed when the temperature was greater than the gel-transition temperature. The hydrogen bond between the hydrophilic group and the surrounding water broke, and the hydrogels became more hydrophobic; this resulted in a collapsed state for the gels. This phenomenon made the SR of the gel rapidly decrease at the gel-transition temperature. The effect of the temperature on the equilibrium SR for the NIPAAm/gelatin hybrid gels, shown in Figure 3, indicated that the equilibrium SRs dramatically decreased around 32°C. The results showed that the CGTTs of the gels were not affected by the contents and crosslinking agent of the gelatin. The results in Figure 3 also show that the more gelatin

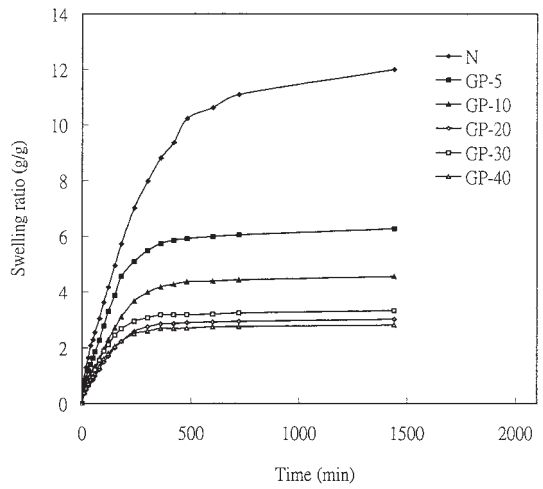

Figure 1 SR as a function of time for NIPAAm/gelatin crosslinked with GP hybrid hydrogels at 25°C in deionized water.

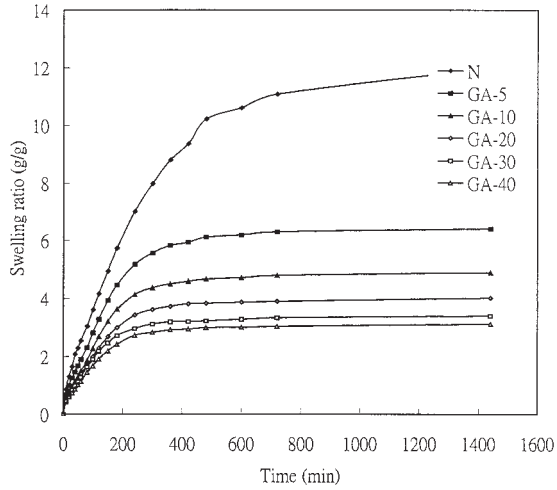

Figure 2 SR as a function of time for NIPAAm/gelatin crosslinked with GA hybrid hydrogels at 25°C in deionized water.

TABLE II
Fundamental Properties of the NIPAAm/Gelatin Hybrid Gels

Sample code	$D \times 10^7$ (cm^2/s)	n	$K \times 10^2$
N	0.60	0.44	0.75
GP5	1.08	0.38	1.76
GP10	1.10	0.29	3.69
GP20	1.12	0.27	6.05
GP30	1.14	0.25	6.83
GP40	1.42	0.24	7.36
GA5	1.02	0.37	1.93
GA10	1.04	0.31	3.22
GA20	1.37	0.29	4.39
GA30	1.41	0.28	5.03
GA40	1.45	0.23	7.37

was incorporated into the gels, the smoother the SR profile was.

Thermoreversibilities of the NIPAAm/gelatin hybrid gels in deionized water

A thermoreversible gel exhibits a swell–deswell transition. This behavior depends on the weak hydrogen bonding of amide groups, which are transferred from a swelling state to a deswelling state within a certain temperature range. Figure 4 shows the change in SR for the GP and GA gels when they were immersed in deionized water at 25 and 37°C. All the gels could swell and deswell for a period of time when the temperature was cycled through their gel-transition temperatures. As shown in Figure 4, the deswelling equilibrium time was faster than that of swelling between 37 and 25°C. The water inside the gels was squeezed out quickly by an elastic retractive force of networks with a violent volume phase change at a higher temperature (37°C). This driving force was larger than water infiltrating into the gels during the swelling process of the gel from a high temperature to a low temperature (25°C). These results indicated that the equilibrium times for the GP and GA series gels were shorter than that of the NIPAAm gel during the swelling process. However, the deswelling equilibrium times observed for the GP, GA, and N gels were short and not affected by the gelatin content and crosslinking agent. Additionally, those gels still showed good thermoreversible behavior.

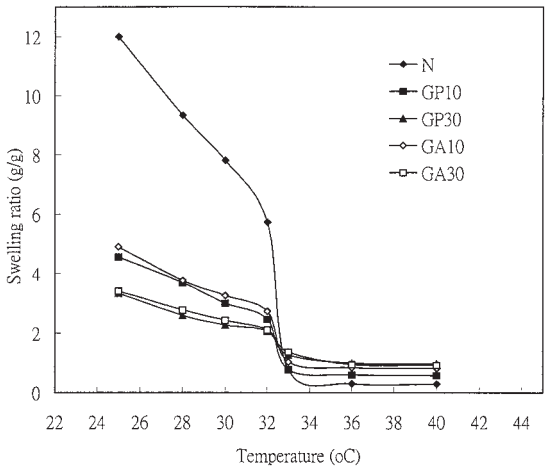

Figure 3 SR as a function of temperature for NIPAAm/gelatin hybrid hydrogels (N, GP10, GP30, GA10, and GA30) in deionized water.

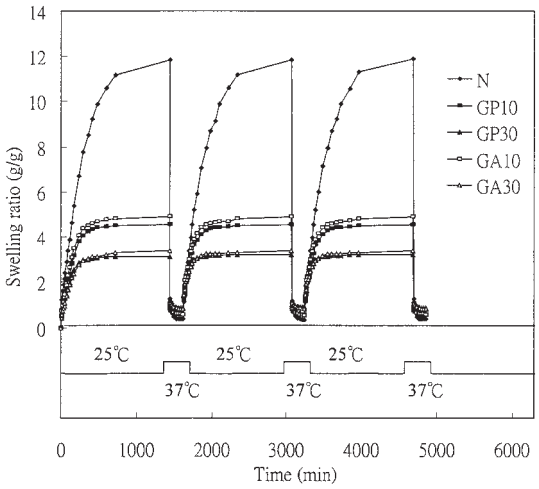

Figure 4 NIPAAm/gelatin hybrid hydrogels (N, GP10, GP30, GA10, and GA30) swelling and deswelling between 25 and 37°C in deionized water.

Swelling kinetics of the PNIPAAm/gelatin hybrid gels in various ratios

The ESCs for the hybrid gels obtained from eq. (2) are shown in Figure 5. The results indicated that the profiles of ESC for the gels exhibited a re-entrant type of behavior in various DMSO aqueous solutions. The addition of DMSO gradually reduced the solubility of the polymer from 20% (v/v) onward rather sharply; however, with the continuous addition of DMSO, its solubility increased gradually. Increasing the content of DMSO made the medium less compatible with the gelatin hydrogels. This could be attributed to more polar DMSO; the water molecules were rearranged such that hydrogen-bonding interactions with organic and polar DMSO were better optimized; this left the polymer stabilized by intramolecular hydrogen bonds. At this juncture, its solubility decreased. This was interpreted under the assumption that the mixing free energy between the polymer segments was not a linear function of the solvent composition. This behavior was also investigated by Tanaka et al.,[39] Kim et al.,[40] Schild et al.,[41] and Winnik et al.[42]

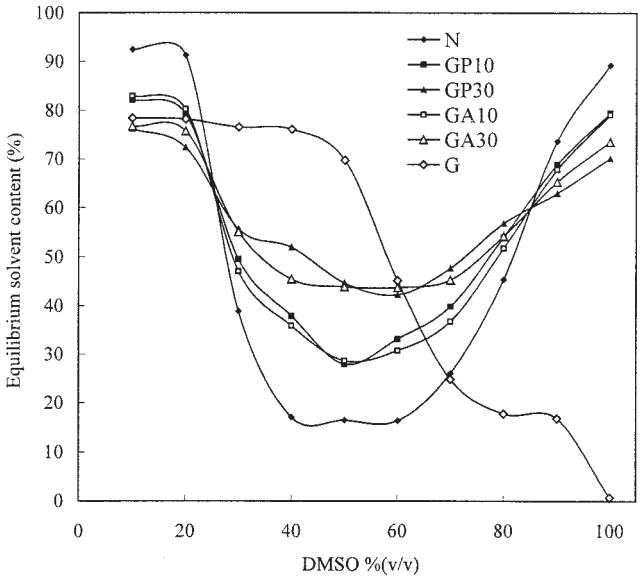

Figure 5 ESC as a function of the various volume ratios of DMSO to H_2O for NIPAAm/gelatin hybrid hydrogels [N, GP10, GP30, GA10, GA30, and the pure gelatin hydrogel (G)] at 25°C.

The SRs as a function of time for PNIPAAm/gelatin crosslinked with GP or GA interpenetrating network gels at 25°C in various DMSO aqueous solutions are shown in Figures 6–8. N-10, N-30, N-50, N-80, and N-100 represent the gels immersed in 10, 30, 50, 80, and 100% (v/v) DMSO aqueous solutions, respectively; similar symbols in Figures 7 and 8 stand for the same conditions used for the N gel. The results indicated that the SRs for all the gels decreased with an increase in the DMSO concentrations until 50% (v/v); with the continuous addition of DMSO, SR increased gradually. Their n, K, and D values, calculated with eqs. (3) and (4), are listed in Table III. The results showed that the values of D for the these gels in different concentrations of DMSO were in the order of N-10 > N-100 > N-30 > N-80 > N-50; GP30-10 > GP30-100 > GP30-30 > GP30-80 > GP30-50 and GA30-10 > GA30-100 > GA30-30 > GA30-80 > GA30-50. These results showed that the gels had preferential adsorption of the water and then pure DMSO. As described previously, when the DMSO concentration was 50%, the water–DMSO interactions were stronger than the water–gel interactions. The results also indicated that the transport mechanism for these gels in

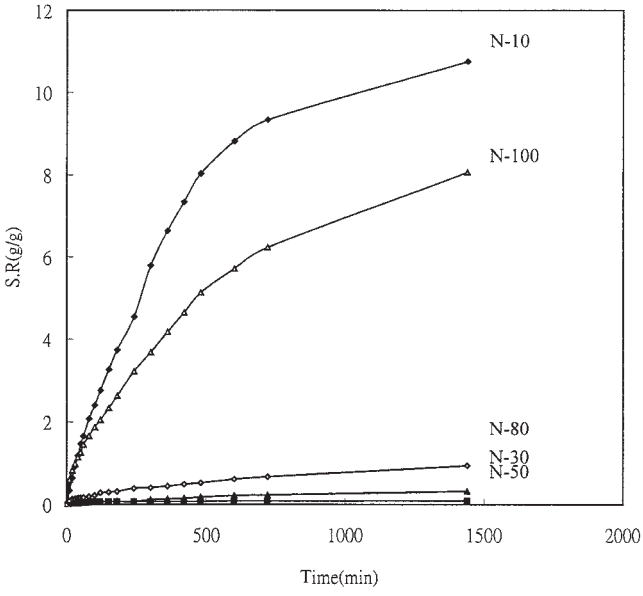

Figure 6 SR as a function of time for the NIPAAm hydrogels in various volume ratios of DMSO aqueous solutions at 25°C.

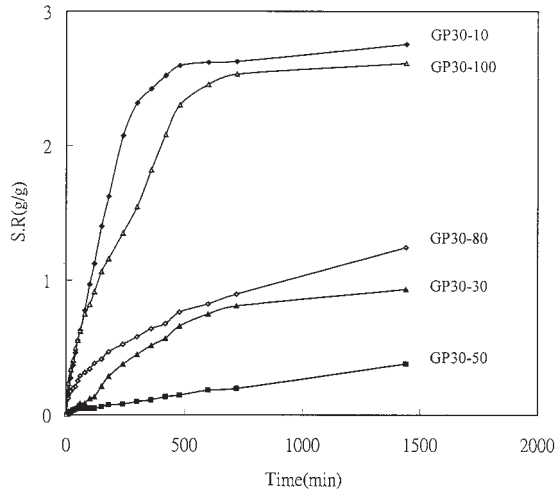

Figure 7 SR as a function of time for the GP30 gels in various volume ratios of DMSO aqueous solutions at 25°C.

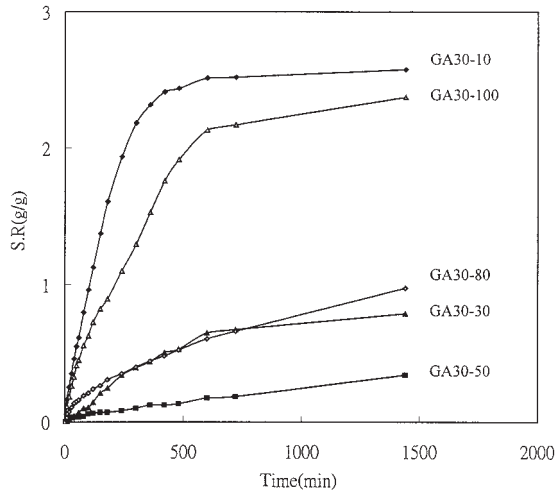

Figure 8 SR as a function of time for the GA30 gels in various volume ratios of DMSO aqueous solutions at 25°C.

TABLE III
Fundamental Properties of the NIPAAm/Gelatin Hybrid Gels in Various DMSO Aqueous Solutions

Sample code	$D \times 10^8$ (cm^2/s)	n	$K \times 10^2$
N-0	6.00	0.44	0.75
N-10	5.12	0.48	0.46
N-30	2.35	—	—
N-50	—	—	—
N-80	2.31	0.04	41.76
N-100	3.47	0.33	1.80
GP30-0	11.40	0.25	6.83
GP30-10	7.61	0.24	5.93
GP30-30	3.14	0.04	39.58
GP30-50	1.02	—	—
GP30-80	2.28	0.07	30.00
GP30-100	3.92	0.19	8.99
GA30-0	14.10	0.28	5.03
GA30-10	8.28	0.24	6.25
GA30-30	3.30	0.03	45.78
GA30-50	1.30	—	—
GA30-80	2.04	0.06	33.70
GA30-100	6.63	0.19	8.87

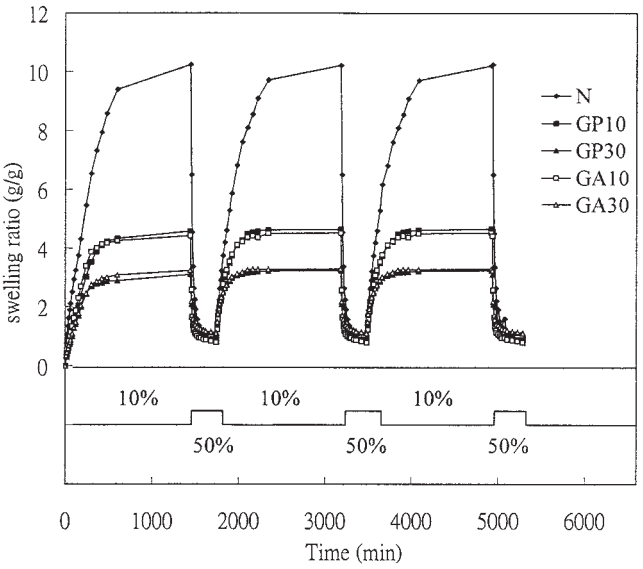

Figure 9 NIPAAm/gelatin hybrid hydrogels (N, GP10, GP30, GA10, and GA30) swelling and deswelling between 10 and 50% (v/v) at 25°C.

various DMSO aqueous solutions belonged to Fickian transport.

Reversibility of the NIPAAm/gelatin hybrid gels between various DMSO aqueous solutions

Because these hybrid gels showed different swelling behaviors in various DMSO aqueous solutions, the swelling–deswelling reversibility for these gels between 10 and 50% (v/v) DMSO aqueous solutions were investigated. The results shown in Figure 9 indicated that the same differences in the SR for these gels at every swelling–deswelling cycles were observed. This behavior showed a good reversibility for the gels between 10 DMSO and 50% (v/v) DMSO aqueous solutions.

Effect of the crosslinked gelatin on the gel strength (G) and ρ_x

The G and ρ_x values for these gels, calculated with eqs. (5) and (6), are listed in Table IV. The results showed that the GP and GA series gels had higher values of G than the PNIPAAm gel (N). This was because the hybrid gel had a larger polymer density and made the gel stronger. Furthermore, the hybrid gels showed the highest polymer density and made G steeply increase. This was because the hybrid gel, which contained gelatin and was crosslinked with GP or GA, had the highest ρ_x and G values. Besides, the gels crosslinked with GP had higher ρ_x values than those crosslinked with GA because the gels crosslinked with the heterocyclic structure of GP possessed higher rigidity than those gels crosslinked with a linear structure of GA.

CONCLUSIONS

The experimental results showed that the addition of gelatin to N by two-step crosslinking with GP or GA made the network structure of the gel denser. The SRs in deionized water for the gels decreased with the addition of gelatin crosslinked with GP or GA. In the diffusion transport mechanism, the results indicated that the swelling exponents (n) for all the hybrid gels at 25°C were 0.23–0.44. This implied that the swelling transport mechanism was Fickian transport. The D values for the gels increased with an increase in the gelatin content, so water molecule easily infiltrated the hydrogels for gels containing more gelatin. The CGTT of the gels was not significantly affected by the extent of gelatin in the interpenetrating network gels. The ESC for these gels exhibited a re-entrant type of behavior in different concentrations of DMSO. The com-

TABLE IV
G and ρ_x of the NIPAAm/Gelatin Hybrid Gels

Sample codes	G (N/cm^2)	ρ_x (10^{-5} mol/cm^3)
N	272.3 ± 6.50%	2.08 ± 6.58%
GP5	543.0 ± 7.80%	4.21 ± 7.60%
GP10	585.3 ± 7.77%	4.49 ± 7.79%
GP20	1180.4 ± 3.49%	9.05 ± 3.45%
GP30	1517.0 ± 4.09%	11.6 ± 3.44%
GP40	2077.9 ± 3.07%	15.9 ± 3.14%
GA5	520.7 ± 0.86%	3.99 ± 0.08%
GA10	772.8 ± 3.62%	5.92 ± 3.54%
GA20	931.9 ± 6.13%	7.15 ± 6.01%
GA30	1217.5 ± 6.50%	9.34 ± 6.50%
GA40	1452.4 ± 6.04%	11.12 ± 6.08%

pression moduli for the gels increased with the addition of gelatin with crosslinked GP or GA.

References

1. Hoffman, A. S. J Controlled Release 1987, 6, 297.
2. Bae, Y. H.; Okano, T.; Kim, S. W. J Polym Sci Part B: Polym Phys 1990, 28, 923.
3. Hirokawa, E.; Tanaka, T. J Phys Chem 1984, 81, 6379.
4. Ricka, J.; Tanaka, T. Macromolecules 1984, 17, 2916.
5. Eisenberg, S. R.; Grodzinski, A. J. J Membr Sci 1984, 19, 173.
6. Kwon, I. C.; Bae, Y. H.; Okano, T.; Kim, S. W. J Controlled Release 1991, 17, 149.
7. Hoffman, A. S.; Afrassiabi, A.; Dong, L. C. J Controlled Release 1986, 4, 213.
8. Dong, L. C.; Hoffman, A. S. J Controlled Release 1991, 5, 141.
9. Wu, X. S.; Hoffman, A. S.; Yager, P. J Intell Mater Syst Struct 1993, 4, 202
10. Yoshida, R.; Sakai, K.; Okano, T.; Sakurai, Y. Polym J 1991, 23, 1111.
11. Okayama, Y.; Yoshida, R.; Sakai, K.; Okano, T.; Sakurai, Y. J Biometry Sci Polym Ed 1993, 4, 545.
12. Bea, Y. H.; Okano, T.; Kim, S. W. Makromol Chem Rapid Commun 1987, 8, 481.
13. Freltas, R. F. S.; Cussler, E. L. Sep Sci Technol 1987, 22, 911.
14. Dong, L. C.; Hoffman, A. S. J Controlled Release 1986, 4, 223.
15. Heskins, M.; Guillet, J. E. J Macromol Sci Chem 1968, 2, 1441.
16. Hirotsu, S. J Phys Soc Jpn 1987, 56, 233.
17. Ustaa, M.; Piechb, D. L.; MacCronec, R. K.; Hillig, W. B. Biomaterials 2003, 24, 165.
18. Rose, P. J.; Mark, H. F.; Bikales, N. M.; Overberger, C. G.; Menges, G.; Kroschwitz, J. I. Encyclopedia of Polymer Science and Engineering, 2nd ed., Wiley-Interscience: New York, 1987; vol. 7, p 7.
19. Nimni, M. E.; Cheung, D. T.; Strates, B.; Kodama, M.; Sheikh, K. Collagen 1998, 3, 38.
20. Tanioka, A.; Miyasaka, K.; Ishikawa, K. Biopolymers 1976, 15, 1505.
21. Ross-Murphy, S. B. Polymer 1992, 33, 2622.
22. Bigia, A.; Cojazzib, G.; Panzavoltaa, S.; Roveria, N.; Rubini, K. Biomaterials 2002, 23, 4827.
23. Michon, C.; Cuvelier, G.; Relkin, P.; Launay, B. J Biol Macromol 1997, 20, 259.
24. Rault, I.; Frei, V.; Herbage, D.; Abdul-Malak, N.; Huc, A. J Mater Sci: Mater Med 1996, 7, 215.
25. Khor, E. Biomaterials 1997, 18, 95.
26. Damink, L. H. H.; Dijkstra, P. J.; Luyn, M. J. A.; Wachem, P. B.; Nieuwenhuis, P.; Feijen, J. J Mater Sci: Mater Med 1995, 6, 460.
27. Nimni, M. E.; Cheung, D.; Strates, B.; Kodama, M.; Sheikh, K. Collagen; CRC: Boca Raton, FL, 1988; Vol. 3, p 1.
28. Bigia, A.; Cojazzib, G.; Panzavoltaa, S.; Rubini, K.; Roveria, N. Biomaterials 2001, 22, 763.
29. Jayakrishnan, A.; Jameela, S. R. Biomaterials 1996, 17, 471.
30. Akao, T.; Kobashi, K.; Aburada, M. Biol Pharm Bull 1994, 17, 1573.
31. Touyama, R.; Takeda, Y.; Inoue, K.; Kawamura, I.; Yatsuzuka, M.; Ikumoto, T.; Shingu, T.; Yokoi, T.; Inouye, H. Chem Pharm Bull 1994, 42, 668.
32. Touyama, R.; Inoue, K.; Takeda, Y.; Yatsuzuka, M.; Ikumoto, T.; Moritome, N.; Shingu, T.; Yokoi, T.; Inouye, H. Chem Pharm Bull 1994, 42, 1571.
33. Kabra, G.; Gehrke, S. H.; Hwang, S. T. J Appl Polym Sci 1991, 2409, 42.
34. Franson, M.; Peppas, N. A. J Appl Polym Sci 1983, 1299, 28.
35. Korsemeyer, M.; Merrwall, E. W.; Peppas, N. A. J Polym Sci Part B: Polym Phys 1986, 409, 24.
36. Peppas, N. A.; Barr-Howell, B. D. Hydrogels in Medicine and Pharmacy; CRC: Boca Raton, FL, 1986; Vol. 1, p 27.
37. Treloar, L. R. G. The Physics of Rubber Elasticity; Clarendon: Oxford, 1975.
38. Alfrey, T.; Gurnee, E. F.; Lloyd, W. G. J Polym Sci 1966, 12, 249.
39. Katayama, S.; Hirokawa, V.; Tanaka, Y. Macromolecules 1984, 17, 2641.
40. Mukae, K.; Sawamura, S.; Makino, K.; Kim, S. W.; Ueda, I.; Shirahama, K. J Phys Chem 1993, 97, 737.
41. Schild, H. G.; Muthukumar, M.; Tirrell, D. A. Macromolecules 1991, 24, 948.
42. Winnik, F. M.; Ringsdrof, H.; Venzmer, J. Macromolecules 1990, 23, 2415.