Environmental Responses of poly(*N*-isopropylacrylamide-*co*-glycidyl methacrylate derivatized dextran) Hydrogels

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ABSTRACT: A series of intelligent hydrogels (poly(NIPA-*co*-GMA-Dex)) were synthesized by copolymerization of *N*-isopropylacrylamide (NIPA) and glycidyl methacrylate derivatized dextran (GMA-Dex) in aqueous solution with different ratios. Their swelling behaviors at different temperatures and in different pH and ionic strengths, and their mechanical properties were studied. It has found that poly(NIPA-*co*-GMA-Dex) hydrogels are temperature-, pH-, and ionic strength-sensitive associated with the roles of the component PNIPA and GMA-Dex, respectively. Most significantly, poly (NIPA-*co*-GMA-Dex) hydrogels exhibit simultaneously good swelling properties and mechanical properties. © 2005 Wiley Periodicals, Inc. J Appl Polym Sci 96: 2435–2439, 2005

Key words: hydrogels; copolymerization; *N*-isopropylacrylamide; dextran; temperature-sensitive; mechanical properties

INTRODUCTION

In recent years, the unique swelling properties of stimuli-responsive gels have led to proposals for gel-based separation processes. In particular, temperature-sensitive hydrogels have been widely studied in view of the application in biological separation.[1] Using temperature-sensitive hydrogels to concentrate or separate enzyme and protein has many merits, such as simple operation, lower operating temperature, and economy.

The best-known temperature-sensitive gel is poly-*N*-isopropylacrylamide (PNIPA) hydrogel. PNIPA hydrogel exhibits a lower critical solution temperature (LCST) at 32°C. Below the LCST, it absorbs water from the environment and swells. In contrast, it shrinks dramatically and forms a compact structure above the LCST. Therefore, it is expected to be useful in the separation processes of protein. Kawaguchi and coworkers[2] used the monodisperse hydrogel microspheres of PNIPA as an adsorbent of human gamma globulin (HGG) at 25 and 40°C. The result showed that the amount of HGG absorbed by PNIPA microspheres at 40°C is nearly five times that absorbed at 25°C. Thus the separation of protein can be achieved by changing the environmental temperature above and below the LCST. However, the insufficient mechanical properties of PNIPA hydrogel have severely limited its use.

Dextran is a bacterial polysaccharide. In recent years, dextran and its derivative are becoming increasingly important in biomedical, pharmaceutical, and biotechnological fields due to their good biocompatibility, innocuity, and degradability. Van Dijk-Wolthuis et al.[3] prepared glycidyl methacrylate derivatized dextran (GMA-Dex) by transesterification of glycidyl methacrylate (GMA) with dextran molecules at room temperature. Hydrogels were successfully obtained by free radical polymerization of GMA-Dex in aqueous solution using ammonium peroxydisulfate and *N*,*N*,*N*′,*N*′-tetramethylenediamine as an initiator system. In addition, Chiu et al.[4–6] prepared pH-sensitive dextran hydrogel by free radical polymerization of GMA-Dex, acrylic acid, and *N*-*t*-butylacrylamide.

In this study, dextran was introduced into PNIPA gel and a series of temperature-sensitive hydrogels were synthesized by copolymeration of GMA-Dex with NIPA in aqueous solutions. The mechanical properties and the influence of weight ratio r (r = GMA-Dex/(GMA-Dex + NIPA)) in the feed on the swelling ratio (SR), deswelling kinetics have been studied. The effects of the ionic strength and pH of the environment on swelling properties were also investigated.

EXPERIMENTAL

Materials

NIPA was purchased from Kohjin Co. Ltd., Japan. 4-Dimethylaminopyridine (DMAP) and dextran (T40) were obtained from Shanghai Chemical Reagents Co., China. GMA was obtained from Suzhou Anli Chemi-

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cal Plant, China. All other reagents, including dimethyl sulfoxide (DMSO), ammonium peroxydisulfate (APS), *N*,*N*,*N*′,*N*′-tetramethylenediamine (TEMED), *N*,*N*′-methylenebis (acrylamide) (MBAA), ethanol, sodium chloride, etc. were analytical grade made in China, and used as received without further purification.

Synthesis of GMA-Dex

To obtain a dextran-based reactive monomer that can be copolymerized with NIPA to form a hydrogel, GMA-Dex was synthesized and characterized according to Van Dijk-Wolthuis et al.[3,7,8] In brief, dextran (10 wt %) and DMAP (2 wt %) were dissolved in DMSO, and GMA was added corresponding with a ratio of 1 mol of GMA per 20 mol of glucopyranosyl units afterward. Under the protection of nitrogen gas, the reaction mixture was stirred for 48 h at room temperature. After the neutralization of DMAP with an equimolar amount of concentrated hydrochloric acid, the solution was added dropwise into ethanol while stirring. The precipitate was collected by filtration and washed with ethanol twice. The GMA-Dex was dried under vacuum at room temperature. The product yield ratio was 85%.

Synthesis of poly(NIPA-*co*-GMA-Dex) hydrogels

Poly(NIPA-*co*-GMA-Dex) hydrogel was prepared by free radical copolymerization of NIPA and GMA-Dex in aqueous solution using APS and TEMED as an initial system. Specifically, GMA-Dex and NIPA (total 20 wt %) were dissolved in distilled water according to *r* 0.2, 0.4, 0.5, 0.6, and 0.8, respectively, and then APS (0.2 wt %) was added. After bubbling with nitrogen gas to remove oxygen, TEMED (0.5 wt %) was added with stirring and the copolymerization was processed at room temperature for 24 h. In the same way, the poly-GMA-Dex hydrogel was prepared with only GMA-Dex and the PNIPA hydrogel was prepared with only NIPA monomer in the presence of MBAA (2 wt %).

The obtained hydrogels were cut into thin disks of 24 mm in diameter and then immersed in distilled water for 1 week to remove the unreacted monomer. They were dried in a vacuum at 60°C for 1 day.

Measurements

GMA-Dex monomer and dried poly(NIPA-*co*-GMA-Dex) hydrogels were powdered with KBr, pressed into pellets under reduced pressure, and infrared spectroscopy measurements were carried out (Vector 22 FTIR, BRUKER Co., Germany).

^{1}H-NMR measurements were conducted at room temperature using D_2O as solvent (Varian UNITY Plus-400 NMR, USA).

The compressive stress–strain measurements were performed using a tensile-compressive tester (Tensilon RTC-1310A, Oricntec Co., Japan) at 20°C. The swelling hydrogels were cut into a 9-mm-dia. piece. The shear modulus was calculated according to the following equation:[9] $\tau = F/A = -G(\lambda - \lambda^{-2})$, where τ is the compressive stress, F is the applied force, A is the surface area of the deformed gel, G is the shear modulus, and $\lambda = h/h_0$ (where h and h_0 are the equilibrium heights of deformed and original gels, respectively).

Swelling characterization of hydrogels

Dried hydrogels were immersed in distilled water for 48 h over a range of temperatures between 20 and 55°C. After the swelling equilibrium was reached, hydrogels were removed from water and weighed. The equilibrated swelling ratio (SR) is defined as W_s/W_d, where W_s and W_d are the weights of swollen and dry gels, respectively.

The deswelling experiments were performed at 50°C. Hydrogels after equilibrium swelling at 20°C were immersed into the distilled water of 50°C quickly. The hydrogels were weighed at different times and then the water retaining (WR) was calculated using the equation as follows. $WR = 100\ (W_t - W_d)/W_s$, where W_t is the weight of swollen gel at different times and W_s and W_d are defined as above.

The dried hydrogels were immersed in buffer solutions in a range of pH 2.6–10.0 with an ionic strength of 0.2 mol L^{-1} or in the solution of different ionic strength at 20°C for 1 week. The sized changes of gels were followed by measuring the SR of them.

RESULTS AND DISCUSSION

Preparation and characterization of GMA-Dex and poly(NIPA-*co*-GMA-Dex) hydrogel

The structures of GMA-Dex and poly(NIPA-*co*-GMA-Dex) hydrogels were determined by IR and ^{1}H-NMR. For GMA-Dex, IR measurements (KBr, cm^{-1}): 1706 and 950 cm^{-1} are indicative of the carbonyl group and double bond of the methacrylate group, respectively. ^{1}H-NMR measurement (D_2O): the peaks at 5.8, 6.2, and 1.95 ppm can be assigned to CH_2=C and methyl protons from GMA, respectively. The integrated signals of 4.95 ppm and between 3.3 and 4.05 ppm were assigned to anomeric and remaining protons of dextran molecules. Accordingly, the degree of substitution (DS, the molar ratio of GMA per glucopyranosyl unit), determined by ^{1}H-NMR[3] was 4 in this study. For poly(NIPA-*co*-GMA-Dex) hydrogels, IR spectra show

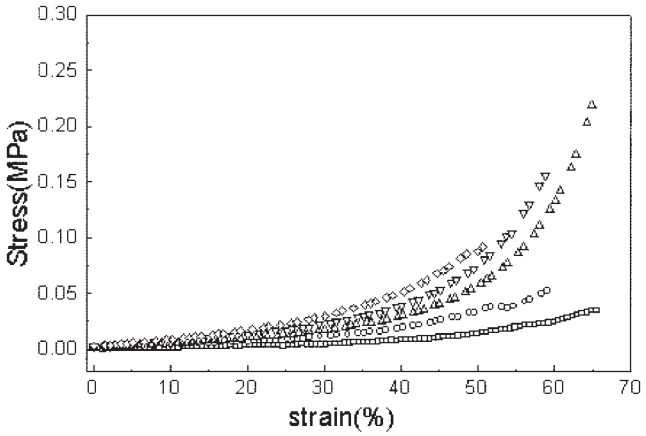

Figure 1 Stress–strain curves of poly(NIPA-*co*-GMA-Dex) hydrogels (r = 0.2 (□), 0.4 (○), 0.5 (△), 0.6 (▽), 0.8 (◇)) (20°C).

a C=O stretching vibration around 1706 cm^{-1} from GMA-Dex segments. A C=O stretching vibration around 1648 cm^{-1} and a N–H deformation vibration around 1547 cm^{-1} from PNIPA segments can also be observed. The absorption at 950 cm^{-1} disappeared in comparison with the spectrum of GMA-Dex.

Effect of temperature on SR

As to any hydrogel, swelling and mechanical properties are the two major aspects in determining its practical application. In this study, we found that poly(NIPA-*co*-GMA-Dex) hydrogels show excellent mechanical properties. Figure 1 shows the stress–strain curves of poly(NIPA-*co*-GMA-Dex) hydrogels. The fracture stresses are in the range of 10^4–10^5 Pa, which are obviously strong enough to conduct further studies when compared with PNIPA hydrogel, which is too soft to measure. Figure 2 illustrates the relationship between r and the shear modulus of the hydrogel calculated according to the method mentioned above. The shear modulus increases with the increase of r, which indicates that the shear modulus of the hydrogel can be adjusted by changing the preparation ratio of it as desired. The swelling behaviors of poly(NIPA-*co*-GMA-Dex) hydrogels with different r values were investigated at various temperatures, and the results are shown in Figure 3. We can see that all of these hydrogels exhibit LCST and the volume changes at LCST are sharper in comparison with PNIPA gel. In a general way, when the SR of gel increases, its mechanical property becomes weaker. So, it is very significant for poly(NIPA-*co*-GMA-Dex) hydrogels to show both high SR and good mechanical properties.

The value of the LCST of the hydrogels was defined as the temperature where the volume of it shrunk most dramatically. Then the relationship between LCST and r is shown in the iconograph of Figure 3. It is found that the LCST of poly(NIPA-*co*-GMA-Dex) hydrogel is higher than that of PNIPA hydrogel (32°C) and increases with the increase of r (Fig. 3, inset). This result also indicates that poly(NIPA-*co*-GMA-Dex) hydrogels maintain the temperature-sensitive characteristics of PNIPA hydrogel, suggesting the temperature-sensitive component PNIPA played a dominant role for the volume phase transition of hydrogels in response to the temperature. Incorporating the hydrophilic group (–OH) from glucopyranosyl units of dextran to the hydrogel results in the increase of the ratio of hydrophile to hydrophobe and the number of hydrogen bonds as well. Therefore, it needs more energy to destroy these hydrogen bonds and the LCST increased correspondingly. In addition, the increase of the LCST might be associated with the weakening of the intermolecular hydrophobic effect since the amount of PNIPA decreased with the increase of r.

The deswelling kinetics of poly(NIPA-*co*-GMA-Dex) hydrogels was studied. Figure 4 shows the dynamic shrinking behaviors of gels at 50°C. As seen here, the abrupt shrinkage was observed with all these hydrogels, and then saturated. The deswelling speed decreased and the WR increased with the increase of r; it might be associated with the increase of the amount of GMA-Dex and thus the force that forces the PNIPA chain into globule conformation was weakened with the increase of r.

Effect of pH and ionic strength on SR

Figure 5 shows the influence of pH values and ionic strength on the SR of poly(NIPA-*co*-GMA-Dex) hydrogel. The SR increased slightly with the increase of pH. The similar pH dependence of SR of poly-GMA-Dex hydrogel was found in our study; it might be due to the different ionization of –OH groups in various pH solutions. At the low pH region, –OH groups can not

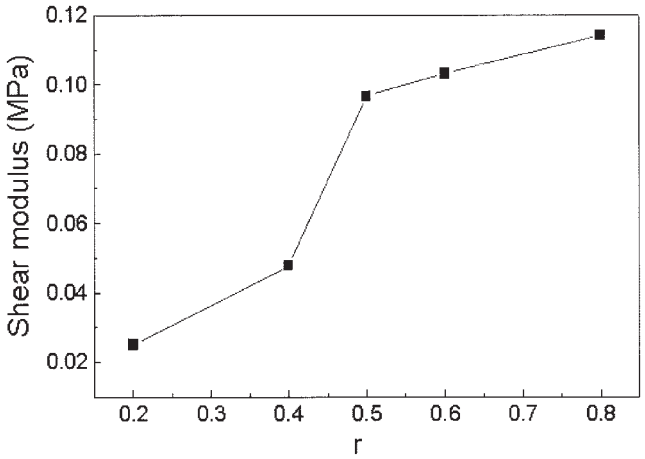

Figure 2 Relationship between r and the shear moduli of poly(NIPA-*co*-GMA-Dex) hydrogels.

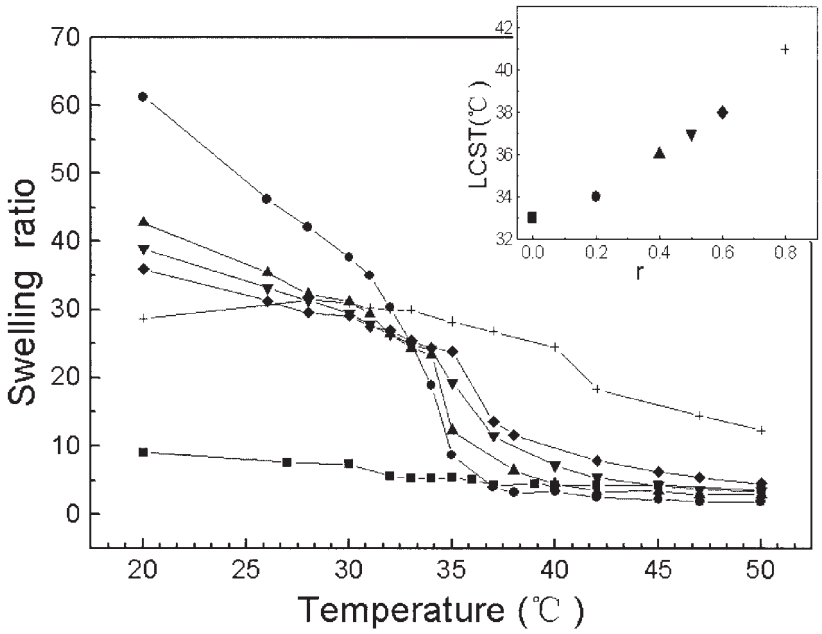

Figure 3 Temperature dependence of the SR of PNIPA hydrogel (■) and poly(NIPA-*co*-GMA-Dex) hydrogels (r = 0.2 (●), 0.4 (▲), 0.5 (▼), 0.6 (♦), 0.8 (+)).

be easily ionized, consequently, it may initiate of the formation of hydrogen bonding between –OH in GMA-Dex and –CONH–in the NIPA part and enhance the interaction between the macromolecular chain. As a result, the SR decreased. With the increase of pH values, the –OH group begins to ionize and the hydrogel swells due to the electrostatic repulsive force. Additionally, we can see that the SR remained high when ionic strength was < 0.5 mol L^{-1}, but drop significantly after that. This result is due to the shielding effect from counter ions in solution and the existence in the Donnan potential.[10] As seen from Figure 5, we also found that the changing range of SR as a function of pH is smaller than that of ionic strength, showing that the effect of pH on SR is weak comparatively.

Effect of reversibility on SR

The oscillatory swelling experiments were further investigated to examine the reversibility and the responses of poly(NIPA-*co*-GMA-Dex) hydrogels to the temperature, and the results are shown in Figure 6. It was found that poly(NIPA-*co*-GMA-Dex) (r = 0.2, 0.5, 0.8) hydrogels could swell and deswell well over a

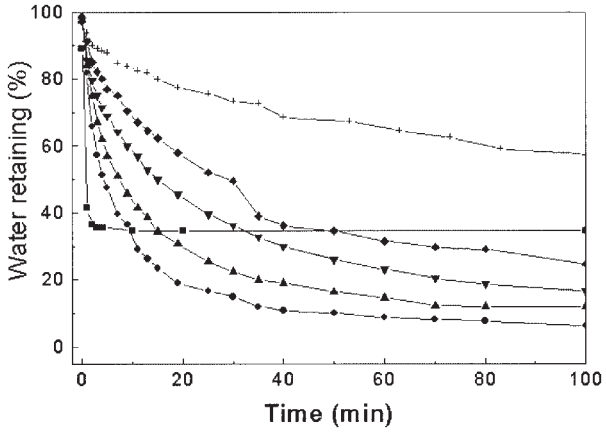

Figure 4 Deswelling kinetics of PNIPA hydrogel (■) and poly(NIPA-*co*-GMA-Dex) hydrogels with different r values (r = 0.2 (●), 0.4 (▲), 0.5 (▼), 0.6 (♦), 0.8 (+)).

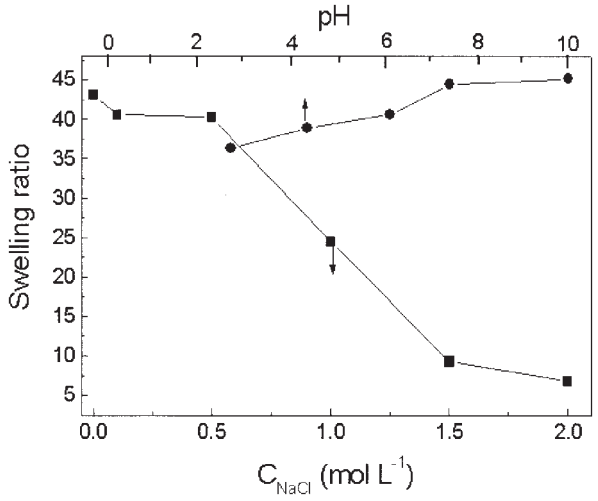

Figure 5 SR as a function of pH (●) and ionic strength (■) at 20°C for poly(NIPA-*co*-GMA-Dex) hydrogels (r = 0.4).

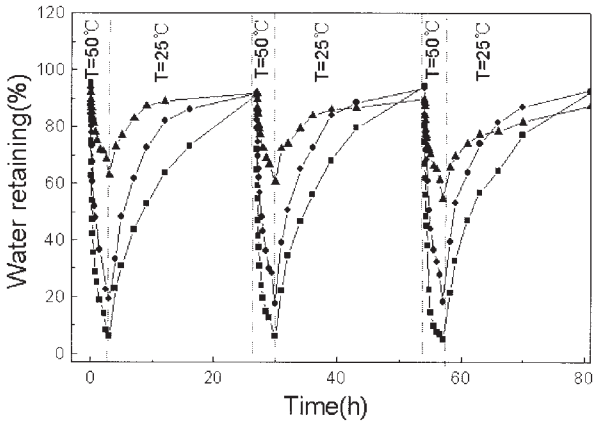

Figure 6 Swelling reversibility of poly(NIPA-*co*-GMA-Dex) hydrogels (r = 0.2 (■), 0.5 (●), 0.8 (▲)) at 25 and 50°C.

period of time when temperature was varied periodically between the higher temperature and the lower temperature, and the more PNIPA in the hydrogel, the more obvious the thermosensitive effect is, and the greater the range of reversibility is.

CONCLUSIONS

1. Glycidyl methacrylate derivatized dextran (GMA-Dex) and a series of poly(NIPA-*co*-GMA-Dex) hydrogels were successfully synthesized. Both GMA-Dex monomer and poly(NIPA-*co*-GMA-Dex) gels were characterized by using IR spectra and ^{1}H-NMR.
2. The swelling behaviors of hydrogels responding to external stimuli were studied, and it was found that poly(NIPA-*co*-GMA-Dex) hydrogels are temperature-, pH-, and ionic strength-sensitive associated with the roles of the component PNIPA and GMA-Dex, respectively.
3. Compared with PNIPA hydrogel, poly(NIPA-*co*-GMA-Dex) hydrogel exhibits not only simultaneously better swelling property and mechanical property, but also a better reversibility depending on temperature.

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