Swelling Behavior and Drug Release of Amphiphilic *N*-Isopropylacrylamide Terpolymer Xerogels Depending on Polymerization Methods: γ-Irradiation Polymerization and Redox Initiated Polymerization

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Amphiphilic terpolymer xerogels were prepared from *N*-isopropyl acrylamide, acrylic acid, and *n*-dodecyl acrylamide by both chemical redox initiated polymerization and γ-irradiation polymerization. The swelling equilibrium and swelling kinetics of amphiphilic xerogels prepared by these two polymerization methods were compared. Drug release behavior was also compared using 5-fluorouracil (5-FU) as a model drug, in order to elucidate the effect of polymerization method. γ-Irradiation polymerization provided amphiphilic terpolymer xerogels which exhibited slower swelling and drug release than chemical redox initiated polymerization.

Key words Hydrogel; amphiphilic; γ-irradiation; drug release; swelling

Polymeric networks containing ionic and hydrophobic moieties that exhibit phase transition in response to external environmental changes, such as solvent composition,[2] buffer composition,[3] pH,[4] temperature,[4,5] pressure,[6] electronic field,[7] electromagnetic radiation[8] and photoelectric stimuli[9] have recently received increasing attention. These hydrogels are generally composed of a thermosensitive component such as *N*-isopropylacrylamide (NIPAAm) and a hydrophilic comonomer such as acrylic acid.[10—14] In particular, amphiphilic terpolymer hydrogels prepared by incorporating hydrophobic components in the gel networks have received much attention since they possess a hydrophilic/hydrophobic heterophase structure in aqueous media.[15,16] The controlled hydrophilic/hydrophobic balance of these amphiphilic hydrogels can provide versatile controlled delivery features for hydrophilic, hydrophobic and amphiphilic drugs.

These amphiphilic terpolymer hydrogels have been synthesized by polymerization using chemical redox initiators such as ammonium persulfate (APS), and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED), or by γ-irradiation polymerization.[17,18] Hydrogels prepared by chemical redox initiated polymerization require a process to remove the initiators before drug loading, in contrast to γ-irradiation polymerization. γ-Irradiation polymerization appears to be more useful if controlled drug release is obtained without any unfavorable effects on drug substances.

In the present paper, amphiphilic terpolymer xerogels were prepared from *N*-isopropyl acrylamide, acrylic acid, and *n*-dodecyl acrylamide by both chemical redox initiated polymerization and γ-irradiation polymerization. The swelling equilibrium and swelling kinetics of amphiphilic xerogels prepared by γ-irradiation polymerization were compared with those prepared by chemical redox initiated polymerization. Drug release behavior from these xerogels was also compared using 5-fluorouracil (5-FU) as a model drug, in order to elucidate the effect of different polymerization methods.

Experimental

Materials 5-FU, NIPAAm, acrylic acid (AAc), acryloyl chloride, *n*-dodecylamine, APS, and TEMED were purchased from Wako Pure Chemical Industries, Ltd. NIPAAm was recrystallized from petroleum ether (60—70 °C). AAc was distilled at a reduced pressure (56—57 °C/24 mmHg) under N_2. Acryloyl chloride was distilled under N_2 before use. *N*,*N*′-methylene-bis-acrylamide (MBAAm) (Aldrich) was used as received, without further purification. *N*-*n*-dodecyl acrylamide (DA) was prepared from acryloyl chloride and *n*-dodecylamine at a low temperature.[16] Other chemical regents were all of analytical grade.

Preparation of Xerogels Xerogels were prepared from NIPAAm (91.5 mol%), AAc (5 mol%), DA (2 mol%) and crosslinker MBAAm (1.5 mol%). Monomers and crosslinker (total weight: 2 g) were dissolved in 5 ml of an ethanol/water (v/v=9 : 1) mixture containing 5-FU (5 mg/ml). Nitrogen was bubbled for 15 min to remove oxygen.

Redox initiated polymerization was carried out by adding 12.5 mg of APS and 0.125 ml of TEMED with even stirring. The solution was immediately injected into the space between two silanized glass plates separated by 2 mm-thick silicone rubber with extreme care to avoid the introduction of air bubbles into the solution. Polymerization was performed at room temperature for 4 h. The resulting gels were cut into disks (9 mm diameter) and dried under a vacuum at under −20 °C for 1 d and at room temperature for 3 d.

For γ-irradiation polymerization, 5-FU ethanol/water (v/v=9 : 1) solution containing three monomers (NIPAAm, AAc and DA) and crosslinker MBAAm was injected into the space between two silanized glass plates. Polymerization was carried out using γ-irradiation of 5 kGy dose (^{60}Co). After γ-irradiation, gels obtained were cut into disks (9 mm diameter) and dried under a vacuum, as described above.

Swelling Measurements. Swelling was measured with both the gels prepared by redox initiated polymerization and γ-irradiation polymerization. Dried gel disks were initially immersed and equilibrated in 0.05 M buffer solutions of different pH values ranging from pH 1.4 to pH 7.4 in glass vials at 10 °C for 3 d. Citrate buffer (0.05 M) was used for pH 1.4, 4.4 and 5.4, and phosphate buffer (0.05 M) for pH 7.4. These vials were in turn immersed in a water bath (Yamato-Komatsu Coolinics Circulator CTE42A) at a fixed temperature (15 °C to 60 °C). After equilibrium at each temperature for 24 h, each sample was removed from the buffer solution and weighed directly by an electrobalance (Mettler AE100) after being tapped with filter paper to remove excess water on the surface. The swelling ratio (SW), that is, the weight of absorbed water per weight of dried disk, was determined: $SW=(W_{H_2O}-W_d)/W_d$, where W_d and W_{H_2O} are the dry and wet weight of the gel, respectively.

Swelling from the dry state was also measured by immersing the dried gel disks directly in 0.05 M of the buffer solution (pH 1.4) at a fixed temperature (10 °C to 60 °C). The swelling ratio was determined after 24 h, as described above. Furthermore, swelling kinetics of the dried gel disks were measured in 0.05 M buffer solutions of pH 1.4 and pH 7.4 at 37 °C, by determining the swelling ratio at appropriate intervals.

Release of 5-FU from Xerogels The dried gels were put in a phosphate buffer (pH 7.4), and the drug release rate from the gel disks was determined at 37 °C in a water bath with a shaker. Samples were removed at appropriate intervals with volume replacement of the removed sample. "Sink" condition[19] was maintained such that the amount of 5-FU released did not exceed 10% of its solubility in water. An assay of 5-FU was carried out by high performance liquid chromatography (HPLC). The stationary phase was ODS-C_{18}, 5 μm 4.6 mm×150 mm column (GL Sciences Inc., Japan). The column

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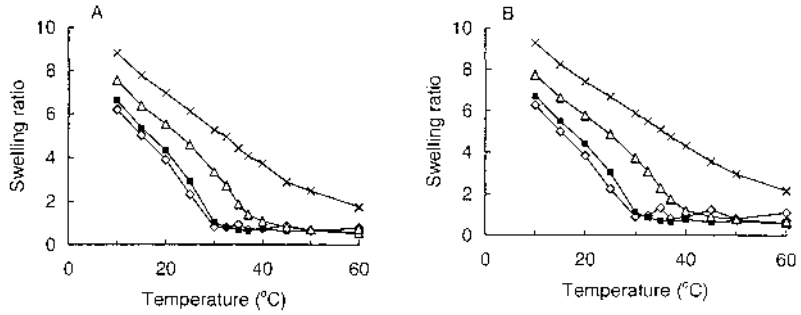

Fig. 1. Effect of Temperature and pH on the Equilibrium Swelling Ratio of NIPAAm-AAc-DA Gels Prepared by Redox Initiated Polymerization (A) and by γ-Irradiation (B)

Gels were prehydrated at 10 °C. pH: ◇, 1.4; ■, 4.4; △, 5.4;, ×,7.4.

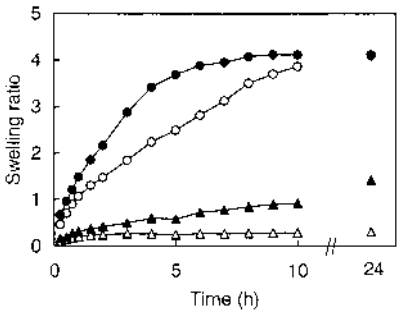

Fig. 2. Swelling Kinetics of NIPAAm-AAc-DA Xerogels Prepared by Redox Initiated Polymerization (●▲) and by γ-Irradiation (○△)

pH: ▲△, 1.4; ●○,7.4.

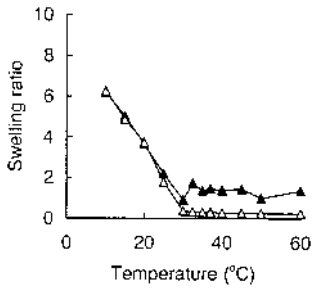

Fig. 3. Temperature Dependence of Swelling of Xerogels Prepared by Redox Initiated Polymerization (▲) and by γ-Irradiation (△), at pH 1.4

Swelling was measured from the dry state.

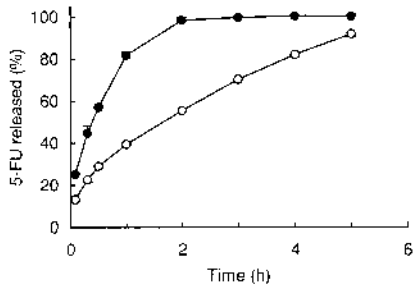

Fig. 4. 5-FU Release from NIPAAm-AAc-DA Xerogels Prepared by Redox Initiated Polymerization (●) and by γ-Irradiation (○), at pH 7.4 and 37 °C

was kept as 35 °C. The eluent was 1/75 M phosphate buffer solution with pH 7.0. The flow rate was 1 ml/min and the detector wavelength was 270 nm. Cytosine-1-β-D-arabinofuranoside was used as an internal standard.

Results

Swelling Equilibrium of Amphiphilic Xerogels The swelling equilibrium of amphiphilic terpolymer xerogels prepared by γ-irradiation polymerization was compared with that of xerogels prepared by redox initiated polymerization (Fig. 1). No significant difference in swelling behavior was observed between gels prepared by these two polymerization methods. The swelling ratio was strongly dependent on both pH and temperature, such that swelling increased with increasing pH due to the ionic repulsion of the ionized carboxylic groups (pK_a of 4.3) and with decreasing temperature due to a reduced hydrophobic interaction. These gels demonstrated thermosensitive swelling behavior with a lower critical solution temperature (LCST) between 30 °C and 35 °C at pH 1.4 and pH 4.4. The LCST of the gels increased to 45 °C at pH 5.4, and the thermosensitivity was obscure at pH 7.4.

Swelling Kinetics and Drug Release of Amphiphilic Xerogels The swelling kinetics of the dried amphiphilic gel prepared by γ-irradiation polymerization was compared with that prepared by redox initiated polymerization. The former xerogel exhibited slower swelling than the latter xerogel at pH 7.4 and 37 °C, as shown in Fig. 2. At pH 1.4, the swelling ratio of the gel prepared by γ-irradiation polymerization reached a plateau within 3 h, whereas that of the gel prepared by redox initiated polymerization continued to increase after 3 h. The swelling ratio of these gels after 24 h depended on temperature, as shown in Fig. 3. Xerogel prepared by γ-irradiation polymerization exhibited a small degree of swelling at 30 °C and higher, whereas xerogel prepared by redox initiated polymerization showed a significant degree of swelling, even at temperatures above 30 °C.

Figure 4 shows the drug release profiles from the dried amphiphilic gel prepared by γ-irradiation polymerization and by redox initiated polymerization at pH 7.4. Drug release from the former xerogel was slower than that from the latter xerogel.

Discussion

Amphiphilic xerogels prepared by γ-irradiation polymerization exhibited a slower drug release than xerogels prepared by redox initiated polymerization at pH 7.4 (Fig. 4). This may be ascribed to the slower swelling of γ-irradiation xerogels than that of redox initiated polymerization xerogels at pH 7.4 (Fig. 2). The swelling kinetics of xerogels appears to govern the drug release rate from these xerogels.

The swelling behavior of γ-irradiation xerogels was simi-

lar to that of the redox initiated polymerization xerogels when xerogels were equilibrated at various temperatures after swelling at 10 °C (Fig. 1). This suggests that crosslinking caused by γ-irradiation[20] is negligible compared to that caused by the crosslinker. On the other hand, swelling behavior from the dry state differed between these two xerogels. γ-Irradiation xerogels exhibited clear thermosensitive swelling behavior without swelling at pH 1.4 and at temperatures of 30 °C and higher, while redox initiated xerogels showed a substantial extent of swelling at these temperatures (Fig. 3). This may be explained by assuming that the swelling of γ-irradiation xerogels may be inhibited at temperatures of 30 °C and higher by increased hydrophobicity at the surface region, while the swelling of redox initiated polymerization xerogels may be brought about due to hydrophilic portions distributed at the surface region, even at temperatures of 30 °C and higher. This may result in the differing swelling rate of xerogels as shown in Fig. 2.

Conclusion

γ-Irradiation polymerization of NIPAAm, AAc and DA provided amphiphilic terpolymer xerogels which exhibited slower drug release and sharper thermo-responsiveness than redox initiated polymerization.

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References and Notes

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