Preparation and Swelling Properties of Glycoside-Bearing Hydrogels by Gamma-Ray Radiation to Glycoside-Bearing Polymer Aqueous Solutions

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ABSTRACT: γ-Ray radiation on poly(glucosyloxyethyl methacrylate) [poly(GEMA)] and poly(glucosyloxyethyl acrylate) [poly(GEA)] aqueous solutions without any crosslinkers gives glycoside-bearing hydrogels in a high yield. The degree of the swelling ratios of each obtained hydrogel in water was decreased with an increase in the total radiation dose to each polymer solution. In order to clarify the formation mechanism of obtained poly(GEMA) hydrogel by γ-ray radiation, the swelling properties of each hydrogel were also compared with those of poly(GEA) under various conditions. Poly(GEMA) in an organic solvent, *N,N*-dimethylformamide and dimethylsulfoxide, was not gelled by γ-ray radiation, although poly(GEA) was gelled under these conditions. These results suggest that the radiation formation mechanism of the poly(GEMA) hydrogels is different from that of the poly(GEA) hydrogels. In addition, the radiolysis of water is necessary in order to form the hydrogels for poly(GEMA). Next, we predicted the radiation formation mechanism of the poly(GEMA) hydrogels by the crosslinking between the glucoside moieties in poly(GEMA). © 1998 John Wiley & Sons, Inc. J Appl Polym Sci 70: 965–972, 1998

Key words: poly(glucosyloxyethyl methacrylate); hydrogel; γ-ray radiation; glycopolymer; crosslinking polymer

INTRODUCTION

Hydrogels have been of great interest to researchers in various areas of polymer chemistry and polymer physics. One of the most promising classes of materials for biomedical applications seems to be the hydrogels,[1] which usually have high water holding substances and have the original rheological properties. Hence, they have been researched thoroughly and are often used as contact lenses,[2–4] wound dressings,[5,6] artificial articular cartilage[7,8] or drug delivery system (DDS) devices.[9–11] To synthesize hydrogels, water-soluble vinyl monomers, such as acrylamide,[12] 2-hydroxyethyl methacrylate,[13] vinylpyrrolidone,[14] and methacrylic acid,[15] are generally copolymerized with divinyl compound to give crosslinked polymers. Using a radical initiator and a crosslinker in the synthesis of hydrogels for the purpose of using them as biomaterials, however, has several drawbacks, such as residual unreaction monomers, crosslinkers, or initiators in obtained hydrogel. These residuals may cause inflammation, or toxicity. Electron beams[16] or γ-ray[17,18] radiation on purified polymers were used in order to avoid these problems and obtain the hydrogels without any crosslinkers. The ad-

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vantage of this method for hydrogel synthesis is that it may form hydrogels that have homogeneous crosslinking points.

In recent years, the rising interest in glycobiology has led to a similar interest in carbohydrates that are conjugated into a variety of materials, including proteins, lipids, and synthetic polymers. These carbohydrate derivatives and their matrices will be useful tools in areas such as clinical medicine. For example, Kobayashi and Akaike tried to create a novel hybrid artificial liver by utilizing the hepatocyte cells, with the attachment of lactose-containing polymers.[19] We also synthesized novel functional glycoside-bearing polymers, poly(glucosyloxyethyl methacrylate) sulfate [poly(GEMA) sulfate], as a heparinoid and found that poly(GEMA) sulfate had anticoagulant activity.[20–22] Glycoside-bearing and sulfated glycoside-bearing hydrogels also appear to be useful a new clinical medicine material in synthetic matrices.

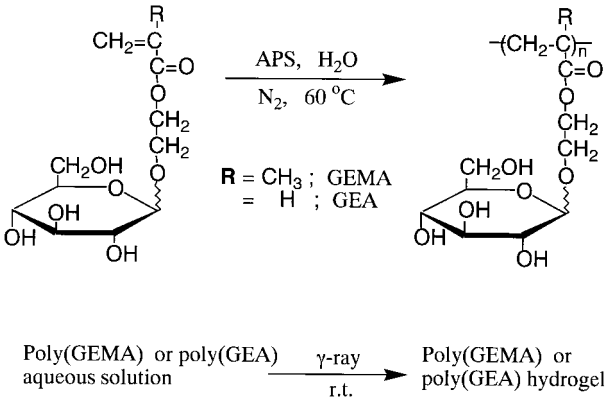
In our previous study, by using glucosyloxyethyl methacrylate (GEMA) (which has very low toxicity[23,24]) and *N,N,N′,N′*-tetramethylethylenediamine as a divinyl crosslinker, we prepared GEMA hydrogels, and we evaluated their swelling properties.[24] In order to prepare more highly biocompatible hydrogels, water dissolved GEMA homopolymers, (which was purified by reprecipitation to remove unreacted monomers and initiators), was irradiated by γ-rays without any initiators or crosslinkers.[25]

It is important to clarify the hydrogel formation mechanism of the glycoside-bearing polymers. It is known that methacrylate type polymers generally cannot be gelled in water by γ-ray irradiation,[26] although poly(GEMA) has been gelled. In this study, in order to evaluate the swelling properties and to clarify the mechanism of poly(GEMA) hydrogel formation by γ-ray radiation, we first prepared poly(glucosyloxyethyl acrylate) [poly(GEA)], containing many α-hydrogens (α-hydrogens containing polymers are easily crosslinked by γ-ray radiation); next, the crosslinking formation ability and swelling properties of poly(GEMA) and poly(GEA) aqueous solutions were compared under various conditions. Finally, we predicted the radiation formation mechanism of the poly(GEMA) hydrogels.

MATERIALS AND METHODS

Materials

GEMA and GEA were provided by Nippon Fine Chemical Co. (Takasago, Japan).[19] Dextran and Pullulan were purchased from Nacalai Tesque (Kyoto, Japan). Solvents were purified in the usual way prior to use and the other reagents were used without further purification.



Scheme 1 Synthesis of poly(GEMA) or poly(GEA) polymer hydrogel.

Synthesis of Poly(GEMA) or Poly(GEA)

Poly(GEMA) and poly(GEA) were synthesized as follows (Scheme I). Each polymer was prepared by the free-radical polymerization of GEMA or GEA using ammonium peroxodisulfate (APS) as an initiator at 60°C for 5 or 3 h in distilled water under a nitrogen atmosphere, respectively. The contents were cooled at 0°C to stop the reaction, and the reaction product was poured into a large quantity of isopropyl alcohol. The precipitates that were formed were collected and dried at room temperature in vacuo. These polymers were purified by reprecipitation 3 times and were dialyzed for 3 days with distilled water to remove the isopropyl alcohol. These purified polymers were collected as white powder by lyophilizing the dialyzed polymer solution. These products were characterized by the ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectrum with a JNM-GSX-400 Fourier transform NMR spectrometer (Japan Electro Optics Co., Akishima, Japan). The number-average molecular weight of poly(GEMA) and poly(GEA) was determined by gel permeation chromatography (GPC) as 10.9×10^4 (373 monomer units) and 9.9×10^4 (356 monomer units), respectively. GPC analysis was done on a Shimadzu LC-6A HPLC system equipped with RI (Shodex RI SE-51) detector using a guaranteed class *N,N*-dimethylformamide as an eluent at a flow rate of 1.0 mL min^{-1}. The Shodex GPC column (AD-80M/S; 28 × 0.8 cm i.d.) was connected

in a series. The molecular weight calibration curves were obtained with Tosoh PEG and PEO standards.

Preparation of Poly(GEMA) or Poly(GEA) Hydrogels

A γ-ray radiation apparatus was used (GAMMACELL 220, Atomic Energy of Canada Ltd., Ottawa, Canada) with ^{60}Co-γ-rays as the radiation source. Purified poly(GEMA) and poly(GEA) were dissolved at 20 wt % in distilled water. These samples were deaerated in glass ampoules by purging with high purity nitrogen to remove the oxygen that causes the main chain scission of the polymers. They were irradiated at a dose rate in a range from 141–160 krad h^{-1} at room temperature. After irradiation on each polymer solution, the obtained gels were slowly rinsed and swollen in water for 3 days in order to remove noncrosslinked polymers. The swelling ratio of each hydrogel was then estimated as follows: swelling ratio = (weight of swollen gel (25°C) − weight of lyophilized gel)/(weight of lyophilized gel).

RESULTS AND DISCUSSION

Synthesis of GEMA or GEA Homopolymers

GEMA or GEA was easily polymerized with ammonium peroxodisulfate as an initiator at 60°C in water. The resulting poly(GEMA) and poly(GEA) were purified by reprecipitation and dialysis and were obtained as a powder. The ^{13}C-NMR spectrum of poly(GEMA) and poly(GEA) is shown in Figure 1. ^{13}C-NMR measurement can readily obtain the data concerning the structure and functional group distribution of polysaccharides and glycoside-bearing polymers because there are many carbons and many hydroxyl groups in these polymers, and this method was effectively used for the structure determination of polysaccharide derivatives by Uryu and his fellow researchers.[27] We found the peaks of the main chain in poly(GEMA) and poly(GEA), which were assigned at 48–57 and 37–45 ppm, respectively; then we could not find the peaks of the vinyl group in GEMA and GEA, which were assigned at 130–139 and 131–135 ppm. The branching in obtained polymers, which is caused by a chain transfer reaction, was not observed in these spectra.

Poly(GEMA) and poly(GEA) were soluble in polar solvents such as *N,N*-dimethylformamide (DMF) and dimethylsulfoxide (DMSO) and were insoluble in methanol, ethanol, and acetone. The obtained polymers did not have an effect on L929 fibroblast cells proliferation and adhered cells formation in a range of 0.1–10 μg mL^{-1}.[28] Similar results for poly(GEA) have also been obtained, although these data have not yet been published. Therefore, poly(GEMA) and poly(GEA) will be useful materials for biomedical uses because of the low toxicity to cell line in the presence of these polymers.

Synthesis and Properties of Poly(GEMA) and Poly(GEA) Hydrogels

γ-Ray irradiation of the poly(GEMA) solution gave a transparent glycoside-bearing hydrogel. The molecular weight dependence of the swelling ratio on poly(GEMA) in a constant irradiation dose, and the same concentration is shown in Figure 2. The swelling ratio is the adsorbed water weight in the obtained hydrogels per lyophilized hydrogel weight. The swelling ratio of the poly(GEMA) decreased with an increase in the number-average molecular weight of poly(GEMA) in the constant total radiation doses and polymer concentrations. The pseudocrosslinking points in the hydrogels may have increased with an increase in the number-average molecular weight of the poly(GEMA). This tendency may also be observed in poly(GEA). On the basis of the above data, the number of monomer units between poly(GEMA) and poly(GEA) were equal in subsequent experiments.

Figure 3 shows the effects of γ-ray radiation on an aqueous solution of poly(GEMA) or poly(GEA). The viscosity of the poly(GEMA) aqueous solution increased with an increase in the total radiation dose, and its polymer solution ultimately gelled to more than 500 krad γ-ray radiation. On the other hand, the poly(GEA) aqueous solution gelled to more than 10 krad radiation. The hydrogel yield was measured from the lyophilized hydrogel weight to the fed polymer weight. The yield of each polymer hydrogel increased, and the swelling ratio of the obtained hydrogels decreased, depending on the total irradiation dose, indicating that irradiation caused some crosslinking between each polymers and that the crosslinking point increased with an increase in the radiation dose with the poly(GEMA) solution. The swelling ratio of the poly(GEMA) hydrogels was larger than that of the poly(GEA) hydrogels. Poly-

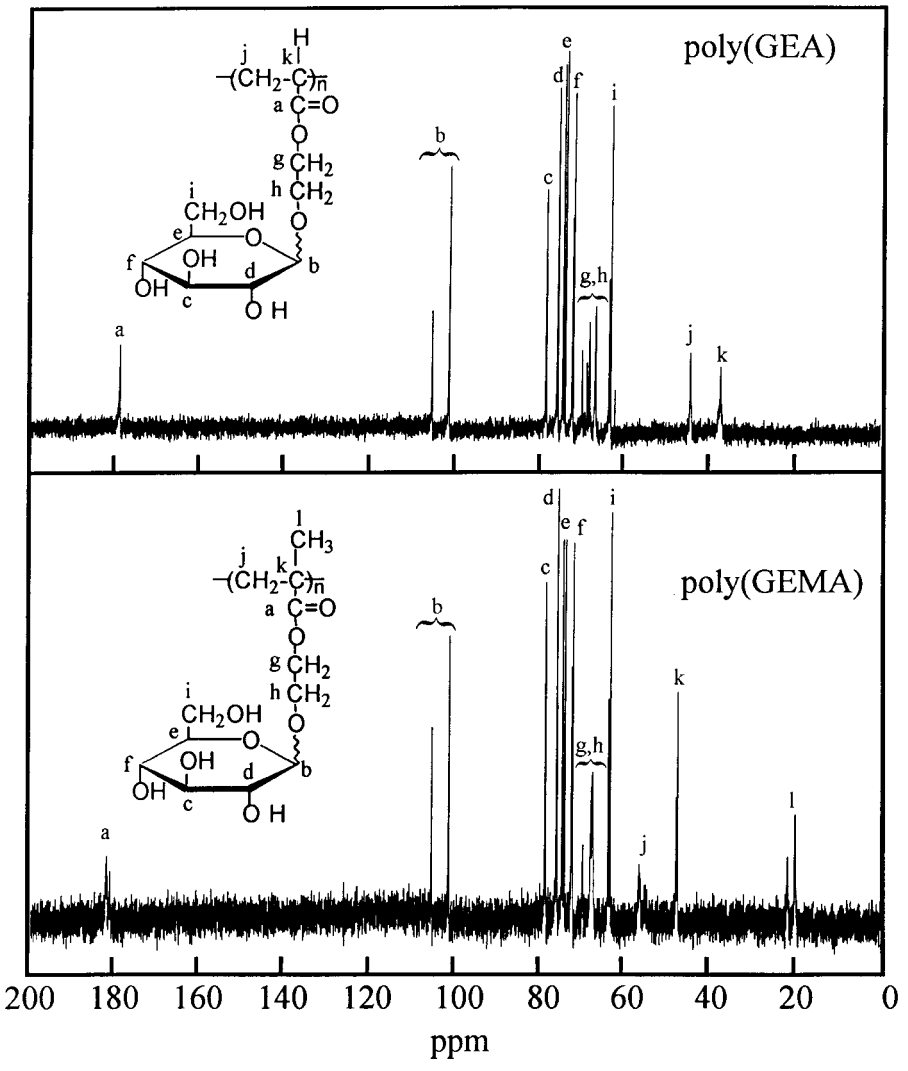


Figure 1 ^{13}C-NMR spectra of poly(GEMA) and poly(GEA) at 50°C in D_2O. Each polymer was dissolved at 20 wt %.

(GEA) hydrogels were formed with a lower radiation dose than the poly(GEMA) hydrogels. These results suggest that the crosslinking point in poly(GEMA) was less than that in poly(GEA) at the same dose, and the mechanism of the formed poly(GEMA) hydrogel differed from that of poly(GEA).

γ-Ray irradiation of a poly(GEMA) and poly(GEA) mixture aqueous solution also gave a transparent hydrogel (Fig. 4). The swelling ratio of these hydrogels decreased with an increase in the amount of poly(GEA). Therefore, it is possible that the swelling ratio of the poly(GEMA)–poly(GEA) mixed hydrogels was controlled by the poly(GEA) content in the hydrogels in the constant total radiation dose and the polymer concentration. Although Figure 4 shows some scattering, the trends are clear. This appeared to be due to the heterogeneous distribution of mixed polymers in the formed hydrogels.

These polymers were irradiated under various conditions (Fig. 5 and Table I) in order to understand why the radiation formation of the hydrogels of poly(GEMA) and poly(GEA) occurred. In the absence of water, the irradiation of the poly(GEMA) powders caused a molecular weight decrease in these polymers, suggesting that the only main chain scission of poly(GEMA) was caused by γ-ray radiation. On the other hand, in the case of poly(GEA), the molecular weight of its polymer increased with an increase in the total irradiation dose, and its

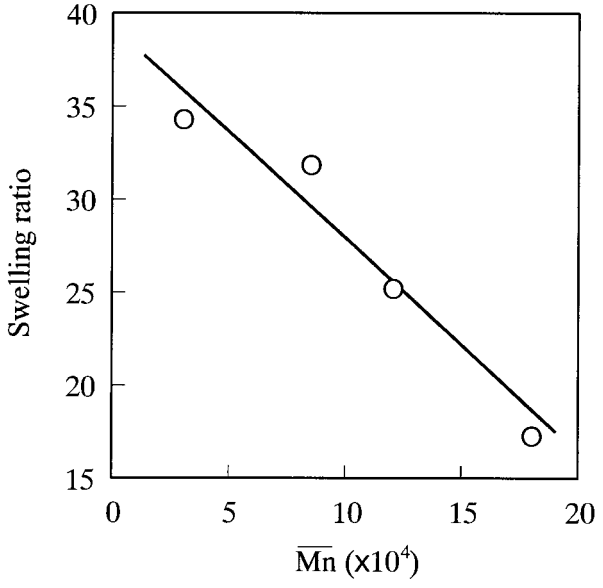


Figure 2 Molecular weight dependence of swelling ratio of poly(GEMA) hydrogel. Polymer concentration was 16 wt %. Each point is the mean of triplicate experiments.

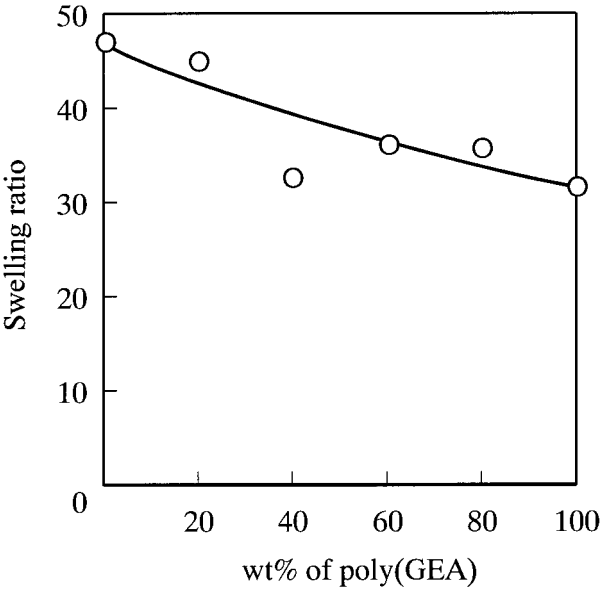


Figure 4 Effect of poly(GEA) content in poly(GEMA)–poly(GEA) mixed solution on swelling ratio of obtained mixed hydrogel. Polymer concentration was 20 wt %. Each point is the mean of duplicate experiments.

polymer ultimately gelled by over 500 krad γ-ray radiation.

The gelation behavior of the γ-ray irradiated polymers is shown in Table I. First, poly(methacrylic acid) [Poly(MA)] and poly(acrylic acid) [Poly(AA)] were prepared by the free-radical polymerization of methacrylic and acrylic acid in distilled water using APS as an initiator, and then their aqueous solutions were irradiated by γ-ray radiation. A decrease in the viscosity of the PMA aqueous solution by γ-ray radiation, which

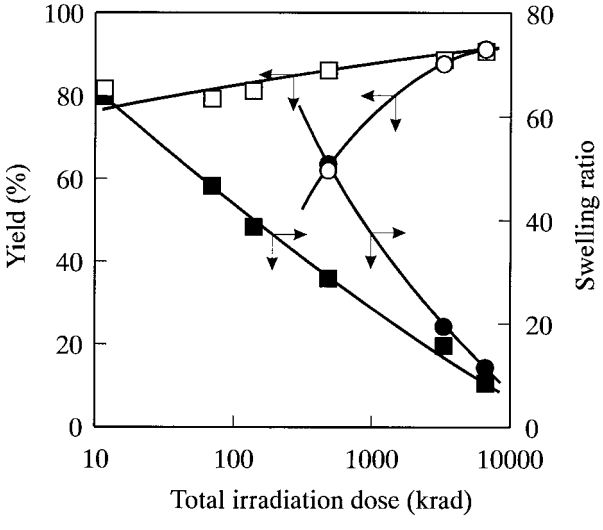


Figure 3 The yield of poly(GEMA) (○) and poly(GEA) (□) hydrogels and the swelling ratio of these hydrogels (●, ■) with γ-ray radiation. Each polymer concentration was 20 wt %. Each point is the mean of duplicate experiments.

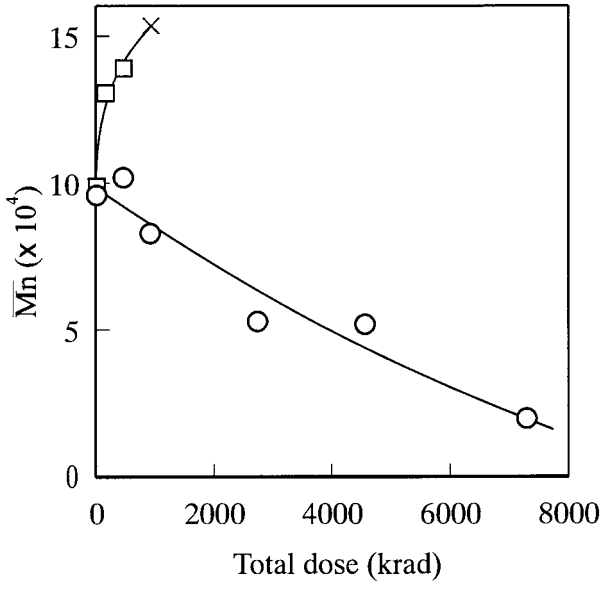


Figure 5 Total irradiation dose dependence of the number-average molecular weight of poly(GEMA) (○) or poly(GEA) (□) powder.

Table I Result of Gamma-Ray Radiation on Various Polymer Solutions.

Run No.	Polymer	Solvent	Concentration (wt %)	Gelation
1	Poly(GEMA)[a]	Water	20	○
2	Poly(GEMA)[a]	DMF	20	×
3	Poly(GEMA)[a]	DMSO	20	×
4	Poly(GEA)[a]	Water	20	○
5	Poly(GEA)[a]	DMF	20	○
6	Poly(GEA)[a]	DMSO	20	○
7	Poly(MA)[b]	Water	5	×
8	Poly(AA)[b]	Water	5	○
9	Dextran	Water	20	○
10	Pullulan	Water	20	×

Total irradiation dose is 3.3 Mrad. Reaction temperature is room temperature.
[a] Poly(GEMA); $M_n = 10.9 \times 10^4$, poly(GEA); $M_n = 9.9 \times 10^4$.
[b] Poly(MA); $M_n = 29.2 \times 10^4$, poly(AA); $M_n = 23.7 \times 10^4$.

caused the main chain scission in the methacrylate-type polymers,[29] was observed. The poly(GEMA) aqueous solution, however, was gelled by γ-ray radiation, although poly(GEMA) is a methacrylate-type polymer. These results suggest that the saccharide moieties in poly(GEMA) were necessary in order to crosslink the polymers. However, it is obvious that all types of saccharide units cannot be gelled by γ-ray radiation because of the no-gelation of pullulan. Then, poly(GEA) and poly(AA) were easily gelled, and poly(GEA) was also gelled in DMF or DMSO by γ-ray radiation. In these experiments, we did not use poly(2-hydroxyethyl methacrylate) (HEMA) as a sample for γ-ray radiation because it is insoluble in water.

In general, the —[CH_2—CHR]— repeat units have polymers that are preferentially crosslinked by irradiation without water, but the —[CH_2—C(CH_3)R]— repeat units have polymers that degrade due to irradiation. The radiolysis of these polymers in a large number of solvents has been researched by A. Henglein.[30] In spite of the above data, this polymer was crosslinked, and the swelling ratio was decreased by γ-ray radiation. The mechanism of poly(vinylalcohol) hydrogel formation is known due to the dehydrogenation on its main chain. Therefore, these results suggest that these hydrogel formation processes are the intermediate products of the radiolysis of water like the crosslinking of poly(vinylalcohol).[17] If the hydrogel formation mechanism of poly(GEMA) was similar to that of poly(vinylalcohol), it is possible that the open rings of the glucoside moieties in poly(GEMA) did not occur, and the formation mechanism of the poly(GEMA) or poly(GEA) hydrogels is shown in Figure 6.

In conclusion, poly(GEMA) hydrogels were easily obtained by γ-ray radiation on a poly(GEMA) aqueous solution. We found that the radiation formation mechanism of the poly(GEMA) hydrogels was different from that of the poly(GEA) hydrogels and that the radiolysis of water is necessary in order to form the hydrogels for poly(GEMA). We also predicted the radiation formation mechanism of poly(GEMA) hydrogel by crosslinking between the glucoside moieties in poly(GEMA).

In another study, we evaluated the biological activity of glycoside-bearing and sulfated glycoside-bearing hydrogels. We found that poly(GEMA) hydrogels had very low toxicity on cell proliferation and poly(GEMA) sulfate hydrogels had a cell adhesive property. Therefore, the glycoside-bearing hydrogels (which were obtained by γ-radiation) will be suitable materials for biomedical uses. In this study, we were not able to confirm the crosslinking point in glycoside-bearing hydrogel by using ^{13}C-NMR. In order to accomplish this, it is necessary to use various analyzers, such as solid-state high-resolution NMR and electron spin resonance (ESR).

Recently, glycoside-bearing polymers have been also extensively studied as an analogy to polysaccharide[23] and cellular surfaces.[31–34] Therefore, it is predicted that these glycoside-bearing polymer can be easily gelled and that the obtained hydrogels have specific bioactivity. Then, we also synthesized poly(GEMA-*co*-HEMA) hydrogels by γ-ray radiation on a poly(GEMA-*co*-HEMA) polymer aqueous solution. These results will be analyzed in a future article.

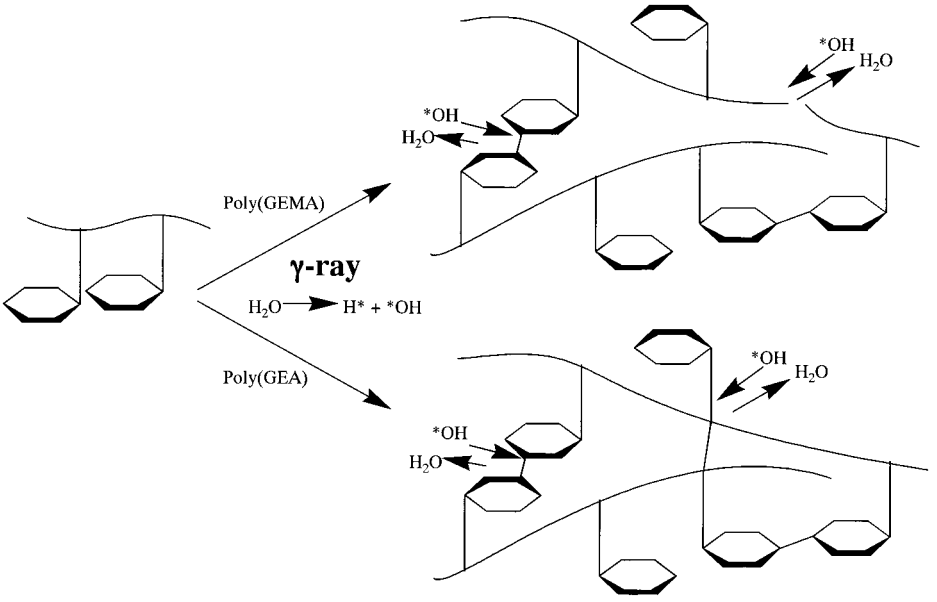


Figure 6 The schematic mechanism of hydrogel formation of poly(GEMA) and poly-(GEA) in water by γ-ray radiation.

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