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Facile Preparation of Biodegradable Glycol Chitosan Hydrogels Using Divinyladipate as a Crosslinker

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Abstract: Biodegradable, pH-sensitive, glycol chitosan (GC) hydrogels were prepared using divinyl adipate (DVA) as a crosslinker and acetic acid as a catalyst. DVA has highly reactive double vinyl ester groups and GC contains a high density of hydroxyl groups, with two in every glucosamine unit. The transesterification reaction between vinyl esters and hydroxyl groups produced crosslinked GC hydrogels. The initial crosslinking reaction was monitored by measuring the viscosity of the reaction mixture. When DVA was added to the GC solution and heated to 50 °C, the viscosity of the GC solution gradually increased, implying a crosslinking reaction and hydrogel formation. A new peak from the ester group was observed in the FTIR spectra of the GC hydrogels, confirming the crosslinking reaction. The synthesized GC hydrogel showed pH-dependent water absorbency, mainly due to the presence of amine groups ($-NH_2$) at the C-2 position of the glucosamine unit of GC. The water absorbency greatly increased at acidic pH and slightly decreased at alkaline pH. The GC hydrogel gradually degraded in 37 °C water due to hydrolysis of the ester bonds, which were intermolecular crosslinking sites. A red dye, 5-carboxyltetramethyl-rhodamine (CTMR), was entrapped in the GC hydrogels as a model compound. CTMR was released from GC hydrogels in two steps: an initial burst release mainly due to desorption and diffusion, and a second sustained release possibly due to gradual degradation.

Keywords: chitosan, hydrogels, divinyladipate, biodegradable, pH-sensitive.

Introduction

Hydrogels are generally defined as three-dimensional macromolecular networks that swell significantly in water or biological fluids. Polymer hydrogels are of interest in various biomedical applications such as drug delivery systems and tissue engineering, due to excellent biocompatibility in human tissues.[1,2] Hydrogels can have controlled drug release by altering the gel structures in response to environmental stimuli, such as pH or temperature.[3-5]

Chitosan, a partially deacetylated derivative of chitin, is one of the most abundant natural polysaccharides. Chitosan has received increasing attention in a broad range of industrial areas such as biomedical, agricultural, pharmaceutical, and environmental fields. Chitosan has many appealing intrinsic properties, such as biocompatibility, biodegradability, antimicrobial activity, and wound-healing properties.[6-8] In particular, because of the excellent biocompatibility, chitosan is considered one of the most promising biomaterials. Glycol chitosan (GC) is a chitosan derivative containing glycol groups at C-6. GC is soluble in water at any pH while unmodified chitosan is only soluble in acidic solutions below pH 6. Chitosan hydrogels can be obtained by physical or chemical crosslinking.[9-15] Polyelectrolyte complex hydrogels are formed by the electrostatic interaction between two oppositely charged polyelectrolytes such as chitosan and alginate.[9] Covalent crosslinking between chitosan chains leads to formation of chemical chitosan hydrogels with a permanent network structure. The most common crosslinkers used with chitosan are dialdehydes such as glyoxal, in particular glutaraldehyde.[12] γ-Irradiation has been frequently used for the synthesis of chitosan-based chemical hydrogel.[15]

Herein, a facile preparation method of biodegradable GC hydrogels using divinyladipate (DVA) as a crosslinker is reported. In contrast to common chemical crosslinking with glutaraldehyde or γ-irradiation, the ester linkage formed by reaction between vinyl esters of DVA and hydroxyl groups of polymers is slowly hydrolyzed in an aqueous medium.[16] Hydrogel formation by crosslinking was investigated using a FTIR spectrometer and viscometer. The pH-dependent swelling behavior of GC hydrogels was investigated and the release behavior from the hydrogels was studied using 5-

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carboxytetramethyl-rhodamine (CTMR) as a model compound.

Experimental

Materials. Glycol chitosan (GC, degree of polymerization: 400) was purchased from Wako, Japan. Divinyladpipate (DVA) was purchased from TCI, Japan. 5-Carboxyltetramethyl-rhodamine (CTMR) was purchased from Fluka, UK. All other chemicals were of analytical grade and were used as received.

Preparation and Characterization of GC Hydrogels. GC (600 mg) was dissolved in 15 mL of dimethylsulfoxide (DMSO) and 3 mL of dilute acetic acid (10%). DVA (0.082 M, 300 mg) was added drop-wise to the GC solution. The mixture of GC and DVA were allowed to react at 30 °C for 6 h under gentle stirring. The gel-like, viscous solution was transferred into a petri dish, covered, and sealed. The dish was incubated in an oven at 50 °C for 72 h. The resulting hydrogels were removed from the dishes and cut into disks with a diameter of 10 mm and a thickness of 4 mm. The hydrogels were immersed in distilled water at 4 °C for seven days, and the media was changed daily to remove DMSO, acetic acid, and unreacted DVA.

The gelation of the reaction mixture containing GC and DVA was monitored using a viscometer. The viscosity of the reaction mixture was measured as a function of time using a digital viscometer (HVDV-II+Pro CP, Brookfield Engineering Laboratories, MA, USA) at 50 °C under a 0.1 s^{-1} shear rate. For FTIR spectroscopy, GC hydrogels were freeze-dried and ground. KBr pellets of GC and GC hydrogels were prepared. The FTIR spectra of the GC hydrogel were recorded on a Nicolet 6700 FTIR spectrometer (Thermo Fisher Scientific Inc., MA, USA).

pH-Dependent Swelling of GC Hydrogels. The water absorbency of GC hydrogels was investigated in different pH buffer solutions (pH 4.0, 7.4, and 10.0). GC hydrogel specimens of a known weight (W_o) were immersed in buffer solutions at 37 °C. The swollen hydrogels were taken out from the buffer solution at predetermined intervals and weighed (W_t) after removing excess solution from the surface with a wet filter paper. The swelling ratio was recorded as $Q=W_t/W_o$, where W_o=the weight of the hydrogel at time zero and W_t=the weight of the hydrated hydrogel at time t. Experiments were performed in triplicate.

Degradation of GC Hydrogels. A disk shaped GC hydrogel was placed in 20 mL of 37 °C distilled water. At predetermined time intervals, the hydrogel was weighed after removing excess water with a wet filter paper.

Dye-Loading into Hydrogels. A red dye, 5-carboxytetramethyl-rhodamine (CTMR), was used as a model compound. CTMR was added to the GC solution under gentle stirring. The reaction mixture containing GC, DVA, and CTMR was allowed to form into a hydrogel. The resulting CTMR-loaded GC hydrogels were cut into disks with a diameter of 10 mm and a thickness of 4 mm.

Release of CTMR from GC Hydrogels. CTMR-loaded GC hydrogels were placed in 20 mL of PBS (pH 7.4) and incubated in a shaking water bath at 37 °C. At predetermined time intervals, the release medium was replaced by the same volume of fresh PBS (pH 7.4). The CTMR released from the GC hydrogels was measured by absorption at 550 nm on a UV-VIS spectrophotometer (Ultrospec 2100 pro, General Electric, NY, USA). All of the experiments were performed in triplicate.

Results and Discussion

Synthesis of GC Hydrogels. GC hydrogels were synthesized according to the reaction scheme shown in Figure 1. DVA has double vinyl ester groups and GC contains a high density of hydroxyl groups; two in every glucosamine unit. All of the hydroxyl groups could be reaction sites with vinyl ester groups of DVA. The transesterification reaction between vinyl esters and hydroxyl groups has been used for the synthesis of water-soluble paclitaxel derivatives,[17] glucose derivatives,[18] polyesters,[19] and polymer hydrogels.[16,20] Polyesters were synthesized from polytransesterification of DVA and diols.[19] Dextran-based hydrogels were prepared from a biocatalytic transesterification reaction between dextran and DVA.[16] Polytransesterification of inulin with DVA produced inulin polyesters and crosslinked inulin hydrogels.[20]

In the transesterification reaction of DVA with hydroxyl groups, an anhydrous condition in the reaction medium is a requisite because of a competing reaction of the hydrolysis of DVA in the presence of water. However, in the present study, water (15%) was added to the reaction media because of the limited solubility of GC in neat DMSO. Without the addition of water, homogeneous GC hydrogels were not prepared. Instead, excess DVA was added to the reaction mixture. Figure 2(c) shows transparent GC hydrogels synthesized by the transesterification reaction between hydroxyl groups in GC and vinyl esters in DVA. Acetic acid catalyzes the transesterification of GC with DVA.

The crosslinking reaction between GC and DVA was monitored by a viscometer, as shown in Figure 3. The reaction mixture containing GC and DVA in a mixture of DMSO/water/acetic acid (50/9/1 v/v/v) flowed freely before crosslinking, as shown in Figure 3(a). As the reaction proceeded at 50 °C, the viscosity correspondingly increased up to approximately 5,000 cps in 180 min, clearly indicating crosslinking between GC and DVA. After further incubation for three days at 50 °C, the reaction mixture formed a hydrogel, as shown in Figure 2(b) and (c).

The crosslinking reaction by DVA was confirmed by FTIR spectroscopy.[21,22] The FTIR spectra of the original and crosslinked GC hydrogel are shown in Figure 4. The FTIR

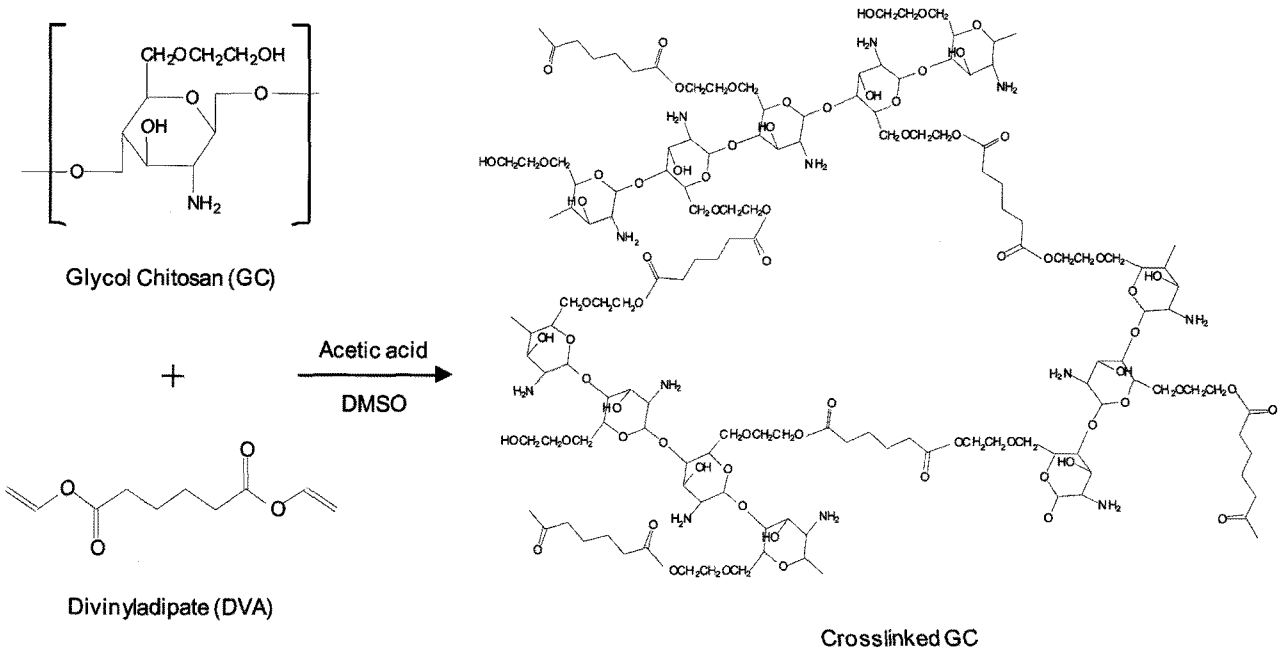

Figure 1. Schematic representation of gelation of GC using DVA as a crosslinker.

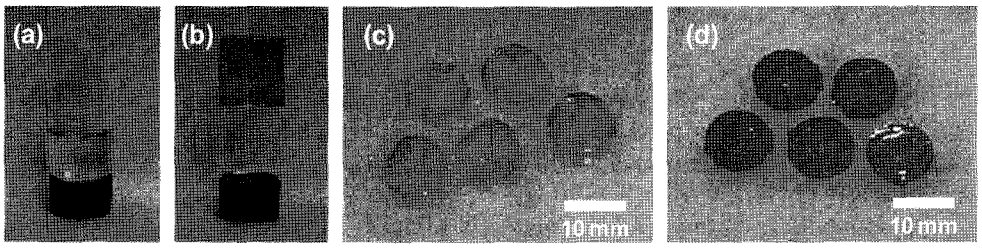

Figure 2. Images of (a) a reaction mixture containing GC and DVA in DMSO/water/acetic acid (50/9/1 v/v/v) before crosslinking, (b) GC hydrogel after crosslinking reaction, (c) GC hydrogel disks, and (d) CTMR (red dye used as a model drug) loaded GC hydrogel disks.

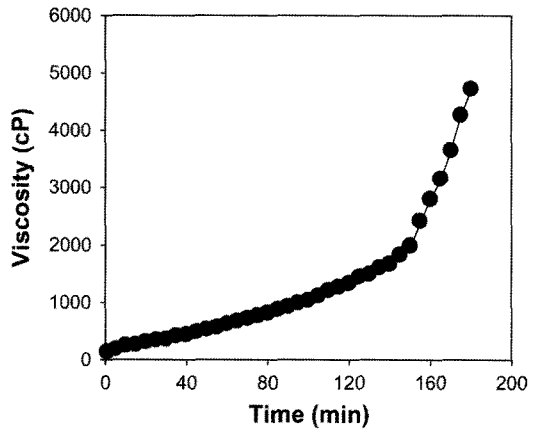

Figure 3. Viscosity of a reaction mixture containing GC and DVA in DMSO/water/acetic acid (50/9/1 v/v/v) as a function of reaction time at 50 °C.

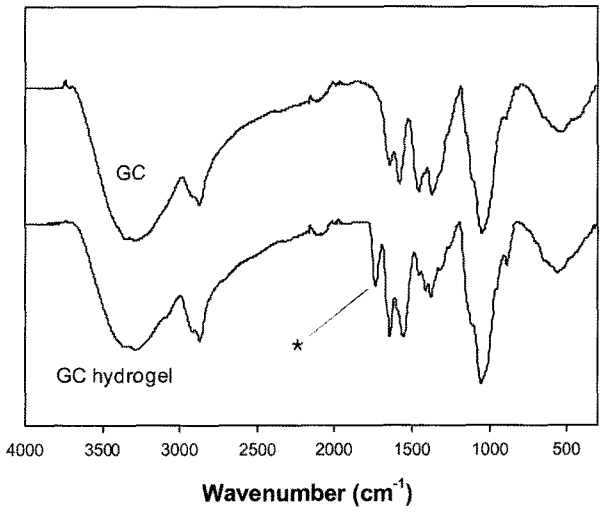

Figure 4. FTIR spectra of GC and a GC hydrogel.

spectrum of the original GC showed broad O-H and N-H stretching vibration bands at 3000-3500 cm^{-1}. The C-H symmetric and asymmetric stretching vibrations of CH_2 groups were observed at 2917 and 2872 cm^{-1}, respectively. The absorptions at 1644 cm^{-1} was due to the amide C=O of

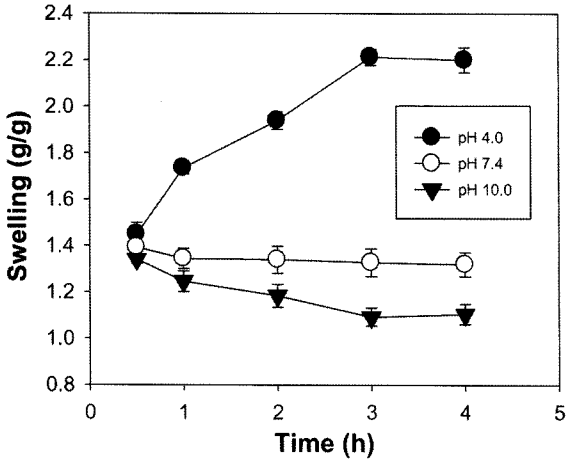

Figure 5. pH-Dependent swelling behavior of GC hydrogels.

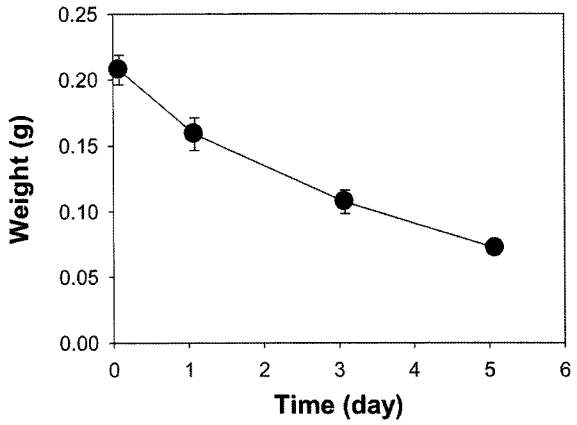

Figure 6. Weight loss of GC hydrogels in distilled water at 37 °C.

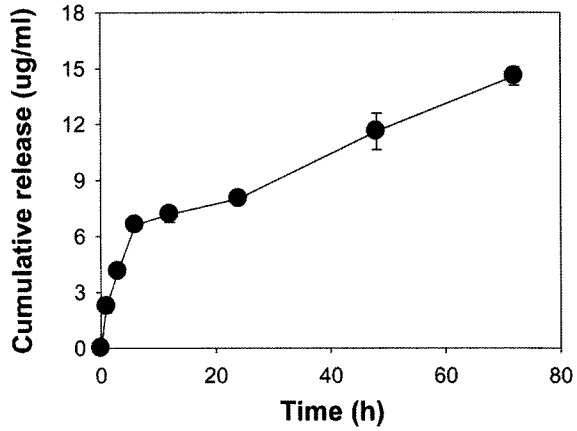

Figure 7. A release profile of CTMR from GC hydrogels in PBS at 37 °C.

the *N*-acetylglucosamine unit in GC. The degree of deacetylation (DD) of GC measured by ^{1}H NMR spectroscopy was 0.85, indicating that GC has 15% *N*-acetylglucosamine unit. The FTIR spectra of the crosslinked GC hydrogels showed a strong new absorption peak at 1737 cm^{-1} (*), which was due to the C=O of the ester bonds which formed between GC and DVA.

pH-Dependent Swelling of GC Hydrogels. The swelling behavior of GC hydrogels was investigated at 37 °C in three buffer solutions of pH 4.0, 7.4 and 10.0, as shown in Figure 5. The synthesized GC hydrogel showed pH-dependent water absorbency, mainly due to amine groups ($-NH_2$) at C-2 of the glucosamine unit of GC. At pH 7.4, the water absorbency roughly maintained a constant value at 1.4 g/g. At pH 4.0, the GC hydrogel swelled to 2.2 g/g. The pK_a of the amine group of chitosan was reported to be approximately 6.5.[23] Therefore, at pH 4.0, the amine group is protonated to form $-NH_3^+$, which causes electrostatic repulsion between GC chains. That is, GC hydrogel networks could be in an expansion state. By contrast, at pH 10.0, the deprotonation of $-NH_3^+$ occurs, which forces GC chains to aggregate, resulting in the slight shrinkage of the GC network. The pH-dependent swelling behavior of the hydrogels could render them suitable candidates for environment-sensitive controlled drug delivery systems.

Degradation of GC Hydrogels. It was reported that the ester linkage formed by reaction between DVA and hydroxyl groups was hydrolyzed in aqueous media.[16] The hydrolysis is facilitated at elevated temperatures. The degradation behavior of GC hydrogels in distilled water was investigated at 37 °C, as shown in Figure 6. The swollen GC hydrogel gradually lost weight due to the hydrolysis of the ester bonds, which were intermolecular crosslinking sites of GC chains. As mentioned previously, GC is water-soluble. Therefore, degraded GC chains were released from the GC hydrogel network, which resulted in weight loss. On the 5th day, the GC hydrogel lost approximately 60% of its original weight.

Release of CTMR from GC Hydrogels. CTMR was simply added to the crosslinking reaction mixture, which produced a CTMR-incorporated GC hydrogel, as shown in Figure 2 (d). Figure 7 shows the *in vitro* CTMR release profile from GC hydrogels in PBS at 37 °C. CTMR seemed to be released from GC hydrogels in two steps; the initial burst release and a second sustained release. The initial burst release may be due to diffusion and desorption of CTMR from the surface of GC hydrogels. The second sustained release may be attributed to gradual degradation of the GC hydrogels. This result suggested that these biodegradable GC hydrogels could potentially entrap a wide variety of drugs or biomolecules, with a controlled delivery rate.

Conclusions

Biodegradable GC hydrogels were synthesized under mild conditions using DVA as a crosslinker and acetic acid as a catalyst. A crosslinking reaction at 50 °C for three days produced a transparent and homogeneous GC hydrogel. The synthesized GC hydrogel showed pH-dependent swelling behavior due to the protonation of the amine groups ($-NH_2$)

of chitosan under acidic milieu. The GC hydrogels were gradually degraded at 37 °C in water due to the hydrolysis of the ester bonds at the crosslinking sites. A model compound, CTMR, was incorporated into the GC hydrogels. CTMR was slowly released due to both simple diffusion and gradual hydrogel degradation. Overall, the results imply that this newly synthesized biodegradable GC hydrogel is a promising candidate for a controlled drug release system.

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