Preparation of Poly(acrylic acid)–Chitosan Hydrogels by Gamma Irradiation and *In Vitro* Drug Release

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Received 23 April 2002; accepted 28 May 2003

ABSTRACT: Biocompatible and biodegradable pH-responsive hydrogels based on poly(acrylic acid) (AAc) and chitosan were prepared for controlled drug delivery. These interpolymeric hydrogels were synthesized by a gamma irradiation polymerization technique. The degree of gelation was over 96% and increased as the chitosan or acrylic acid content increased. The equilibrium swelling studies of hydrogels prepared in various conditions were carried out in an aqueous solution, and the pH sensitivity in the range of pH 1–12 was investigated. The AAc/chitosan hydrogels showed the highest water content when the 30 vol % AAc and 0.1 wt % chitosan were irradiated with a 30-kGy radiation dose. Also, an increase of swelling degree with an increase in the pH was noticed and showed the highest value at pH 12. The drug, 5-fluorouracil, was loaded into these hydrogels and the release studies were carried out in simulated gastric and intestinal fluids. The *in vitro* release profiles of the drugs showed that more than 90% of the loaded drugs were released in the first 1 h at the intestinal pH and the rest of the drug had been released slowly. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 3660–3667, 2003

Key words: hydrogels; drug delivery systems; irradiation; radiation; gelation

INTRODUCTION

One of the most attractive applications of the drug delivery system (DDS) is the delivery of bioactive agents from polymeric materials at a specific site. However, there are many problems to be overcome in that the bioavailability of these drugs after oral administration is usually very low (<1%) because the molecules undergo degradation in the gastrointestinal tract and a substantial portion of the amount absorbed is removed and metabolized by the liver.[1]

For several years, stimuli-responsive polymeric hydrogels that swell or shrink in response to changes in environmental conditions have been extensively studied and used as smart materials for various biomedical applications.[2–4] Hydrogels are three-dimensional, hydrophilic, polymeric networks capable of imbibing large amounts of water or biological fluids. These polymeric hydrogels are prepared from a limited number of synthetic polymers and their derivatives such as copolymers of methacrylic acid, acrylamide, and *N*-isopropylacrylamide.[5–7]

In recent years, considerable interest has been focused on modification by grafting synthetic polymers onto the most abundant naturally occurring polysaccharides such as cellulose, starch, and alginates. In particular, chitosan has been largely evaluated as a potential vehicle for drugs administered orally because of its cationic nature and high charge density in a solution. Moreover, it is acknowledged that chitosan possesses good mucoadhesive properties[8]; thus sustained release and improved bioavailability of drugs can be achieved by prolonging the residence time of drug carriers at the absorption site. However, the development of hydrogel matrices incorporated with chitosan for oral drug delivery is still a virgin area of study.

Recent reports on chitosan/poly(acrylic acid) (PAA) complexes deal with the work of thermal or photoinitiated polymerization and the blend method to obtain self-curing chitosan/PAA polymer complexes with an interpenetrating network structure.[9–11] However, polymerization of acrylic acid monomer in an aqueous chitosan solution by radiation has not been investigated.

Because no catalyst or heat is required in radiation polymerization, there is no possibility for toxicity in the system and decomposition of the drugs. Moreover, by using a radiation technique, the hydrophilicity as well as porosity can be controlled at a low temperature,[12–14] and it is a very convenient method for preparing a drug delivery system.

In this study, we report on the preparation of another polymer composed of chitosan and acrylic acid (AAc), and investigate its properties and effects by gamma irradiation. Also, the drug-release characteristics of the AAc/chitosan hydrogel were studied with 5-fluorouracil (5-FU), which is an antimetabolic drug

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Journal of Applied Polymer Science, Vol. 90, 3660–3667 (2003)

used extensively in cancer chemotherapy. The effect of hydrogel composition and radiation dose on the release rate of 5-FU was examined.

EXPERIMENTAL

Materials

Acrylic acid (MW 72; Junsei Chemical Co., Tokyo, Japan) was purified by a column packed with aluminum peroxide to eliminate the hydroquinone inhibitor. Chitosan (degree of deacetylation: 83%) was purchased from Tokyo Kasei Co. All other chemicals were extrapure reagent grade and were used as received.

Preparation of hydrogels

AAc/chitosan hydrogels were synthesized by polymerization of acrylic acid in the presence of chitosan. Various compositions (99.9/0.1, 99.5/0.5, 99/1.0, and 98.5/1.5 w/w %) of aqueous acrylic acid/chitosan solutions were prepared. Chitosan was dissolved in a mixture of water/acrylic acid (70/30, 50/50, 30/70 vol/vol %). These solution mixtures were poured into a 10 × 3 cm (thickness: 3 mm) container. These samples were sealed and irradiated by ^{60}Co source at 30, 50, and 70 kGy dose (dose rate: 9.1410^5 rad/h). Polymerized samples were washed with acetone and distilled water to remove unreacted monomer, after which they were vacuum dried for 24 h.

Characterization of hydrogels

To measure the gel content, the irradiated hydrogel samples were placed in a 200-mesh stainless steel net and washed with distilled water three times after extraction in distilled water at 80°C for 24 h in an autoclave. The remaining gel was freeze-dried to constant weight. Gel content was measured gravimetrically.

$$\text{Gelation (\%)} = \frac{W_d}{W_0} \times 100$$

where W_d is the weight of dry gel after extraction and W_0 is the initial weight of dry gel. Infrared absorption spectra of AAc/chitosan hydrogels and chitosan were studied by FTIR (M series, Midac Corp., CA). Samples were thoroughly ground with dried KBr and discs were prepared by compression under a vacuum. Scanning electron microscopy (SEM) of dried and swollen gels was performed to analyze their morphology. The freeze- or air-dried samples were gold-coated for conductance and their surfaces were examined by SEM. Because magnifications in excess might cause the collapse of the hydrogel films as a result of the highly focused electron beam, examinations at higher magnification were not performed. The detachment force between the gel film and the mucosal layer of the small intestines, which was washed with methanol for 24 h and polished to increase the interfacial area, was measured by a modified precision tensile tester (Rheometric Scientific, Poole, UK). AAc/chitosan hydrogels cut to the appropriate size were prewetted with distilled water and placed on the plastic plate. A plastic plate with hydrated gel was mounted on the upper clamp of the apparatus. The mucosal layer of the small intestine was mounted on the lower support of the apparatus, then put in contact with the hydrated gel. The detachment force needed for the separation of the two surfaces was determined 2 min after the contact. Results were obtained by means of five to seven experiments.

Equilibrium swelling studies

AAc/chitosan gels were cut having an area of 10 × 10 × 2 mm and the weighed dry gels were immersed in a 100-mL vial with appropriate amounts of distilled water at room temperature until equilibrium was attained. After the excess surface water was removed, the weight of the swollen samples was measured at various time intervals. The procedure was repeated until there was no further weight increase. The percentage swelling was calculated by the following equation:

$$\text{\% Swelling} = \frac{W_s - W_d}{W_d} \times 100$$

where W_d represents the initial weight of dried gel and W_s is the weight of the swollen gel at time t. To investigate the influence of pH on the swelling behavior, the water content of the hydrogel was determined by placing the hydrogel in solutions of different pH values, ranging from 2 to 12. The pH of the medium solution was adjusted with 0.1*N* HCl and 0.1*N* NaOH.

Drug inclusion in the hydrogel

The 5-FU was loaded in the hydrogel by immersing it in the drug-dissolved solution. To trap the 5-FU in the hydrogels, an aqueous solution of 5-FU was used and neutralized equivalent to NaOH, with the purpose of increasing the solubility of the drug at room temperature. Dried hydrogels (~ 0.4 g, 10 × 10 × 2 mm) were placed in 50 mL 5-FU solution (1 mg/mL) for 24 h at room temperature. The amount of drug included in the AAc/chitosan hydrogel was determined by UV–vis spectroscopy.

In vitro drug release studies

The *in vitro* release of the entrapped drug 5-FU was carried out by placing air-dried hydrogels loaded with

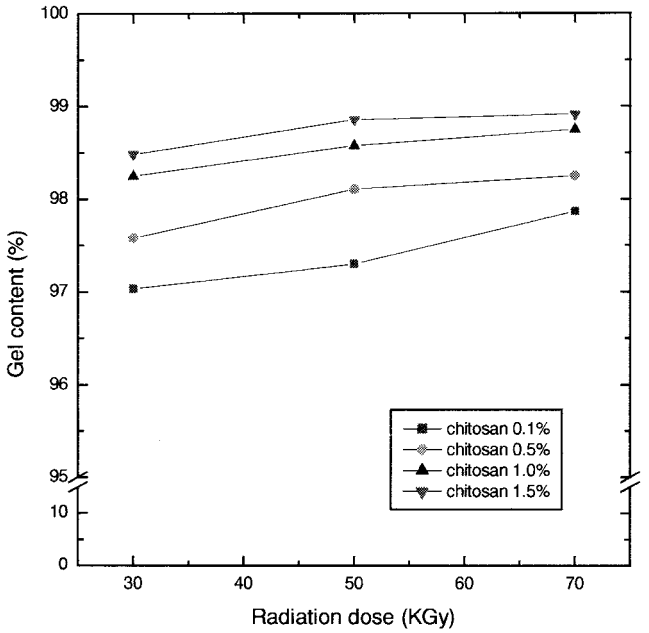

Figure 1 Effect of radiation dose and chitosan concentration on gel content. AAc : water = 70 : 30 vol %.

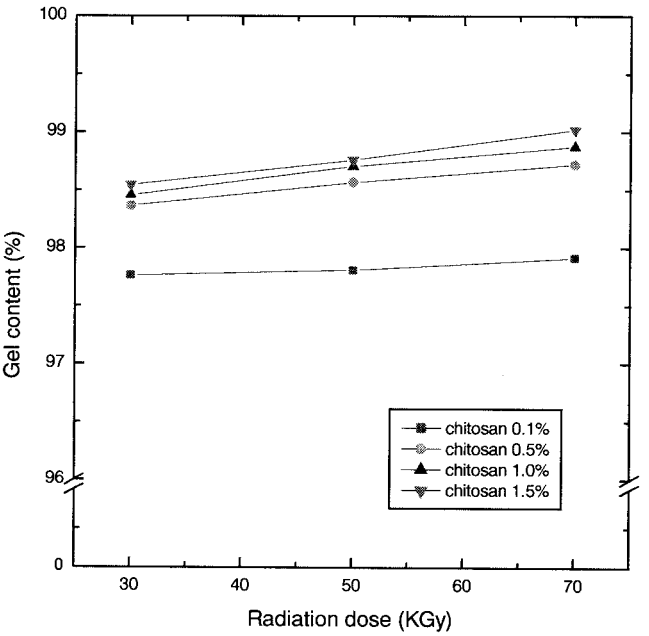

Figure 2 Effect of radiation dose and chitosan concentration on gel content. AAc : water = 50 : 50 vol %.

the drug into 50 mL of the simulated gastric fluid (SGF) and the simulated intestinal fluid (SIF) at an ambient temperature. SGF (pH 1.2) was prepared by dissolving 2 g of sodium chloride and 7 mL of concentrated HCl in 1 L of distilled water. SIF (pH 6.8) was prepared by mixing 250 mL of 0.2*M* KH_2PO_4 and 118 mL of 0.2*N* NaOH. At periodic intervals, 100 μL of solution was withdrawn and tested at λ_{max} 267 nm for 5-FU using a UV–vis spectrophotometer (S-1100, Scinco Co., Seoul, Korea). The release media were replaced periodically with an equal volume of fresh solution to create infinite sink conditions. The data represent mean values from three independent experiments.

RESULTS AND DISCUSSION

Degree of gelation

Figures 1–3 show the degree of gelation of AAc/chitosan hydrogel from various compositions of AAc/chitosan at various radiation doses. The degree of gelation of PAA/chitosan hydrogels was over 96% and increased as the chitosan or acrylic acid content was increased. Also with the same composition of AAc/chitosan, the greater the radiation dose, the higher the degree of gelation of the AAc/chitosan polymer complex. The results indicated that the gelation attributed to the crosslinking of the AAc/chitosan polymer complex was also influenced by concentration of the AAc/chitosan with the same radiation dose. It is considered that chitosan is a weak polybase because of the large quantities of amino groups in its chains; thus chitosan has the possibility of forming a polyelectrolyte complex with poly(acrylic acid), a weak polyacid, through an electrostatic attraction. Chitosan itself is degraded, not subject to grafting in complexation, as reported in another study.[15] It was thought that the gelation of the AAc/chitosan polymer complex was mainly attributed to the degree of

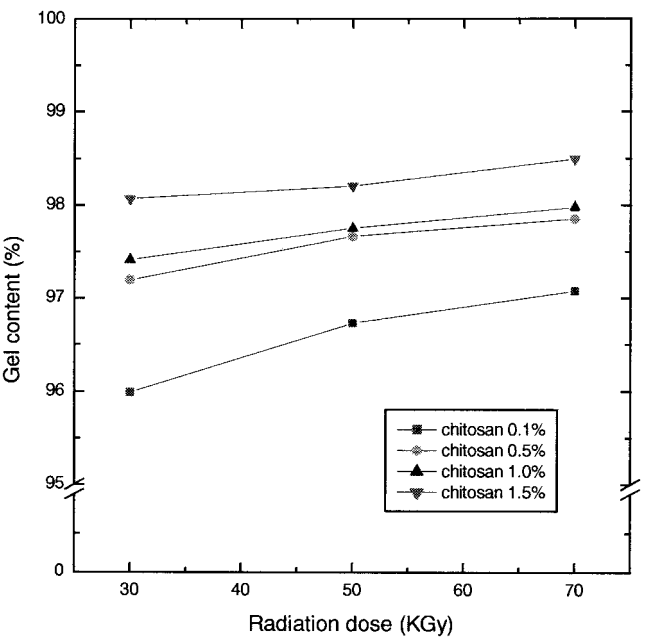

Figure 3 Effect of radiation dose and chitosan concentration on gel content. AAc : water = 30 : 70 vol %.

TABLE I
Elemental Analysis of AAc Chitosan Hydrogels Containing Different Chitosan Concentrations (AAc : H_2O = 70 : 30 vol %, 30 kGy)

	Chitosan concentration (wt %)			
Element	0.1	0.5	1.0	1.5
N	0.17	0.21	0.28	0.4
C	45.76	45.67	45.9	45.61
H	5.99	6.14	6.05	6.14
S	0	0	0	0
O	48.08	47.98	47.77	47.85

breaking and crosslinking of the double bond located in the acrylic acid by irradiation.

Table I summarizes the elemental analysis of the AAc/chitosan hydrogel containing different proportions of chitosan. Structurally, chitosan is a linear polysaccharide consisting of $\beta(1\rightarrow4)$ linked D-glucosamine residues with a variable number of randomly located *N*-deacetyl-glucosamine groups. Also, chitosan has an amine side group that is responsible for its polycationic character,[16] so the nitrogen content can be an indicator as to how it is distributed in the AAc/chitosan hydrogel. The results indicated that the nitrogen content of the AAc/chitosan hydrogel increases in proportion to an increase of the chitosan content in the feed polymer complex.

Swelling properties of the gel

The equilibrium water content (EWC) of the AAc/chitosan hydrogel was measured against time at pH 6 and is shown in Figures 4–7. All the samples revealed a fast increase in water content and reached equilibrium after 120 h, having high equilibrium water contents ranging from 400 to 1000%. From the results of Figures 1–3, the swelling degree of AAc/chitosan hydrogel might be related to the gel content, which was different depending on the weight ratios of AAc/chitosan and radiation dose. Figure 4 shows that, for the ratio of AAc/water (70/30 vol %) and 30 kGy, the swelling degree of AAc/chitosan hydrogels prepared in various chitosan contents (0.1–1.5 wt %) increased as the chitosan content in the gel decreased. In general, the swelling is strongly affected by the crosslinking ratio defined by the molar ratio of the repeating unit of the polymer to the crosslinking agent.[17] Therefore, a higher crosslinking ratio may lead to a decrease of the swelling degree as a result of the lower gel flexibility. In this study, it is thought that the decrease in swelling with the addition of chitosan is attributed to the loose networks of hydrogels because chitosan hinders the crosslinking of AAc.

Figures 5 and 6 also show the effect of radiation dose (50, 70 kGy) on the swelling degree of the AAc/chitosan hydrogel. The gel fraction was greater at the higher radiation dose, and theoretically the corresponding swelling ratio should be lower. It was consistent with the effect of radiation dose on gel fraction in Figures 1–3 that the higher the radiation dose, the greater the gel fraction and the lower the swelling

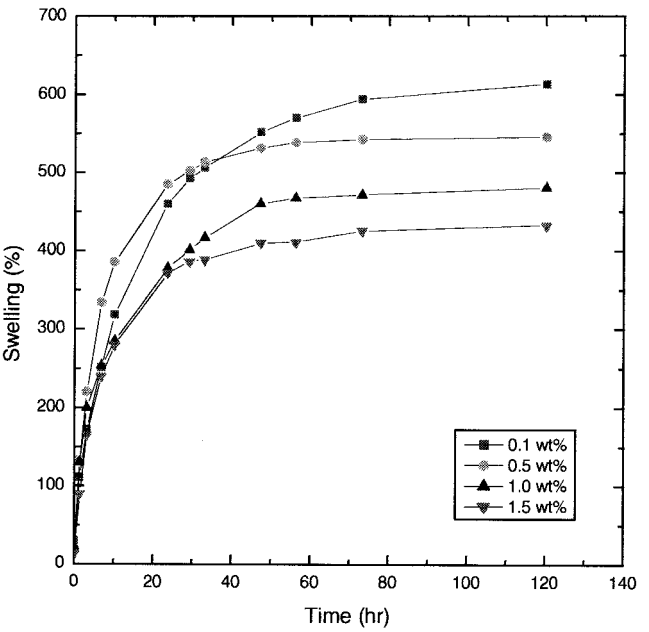

Figure 4 Swelling degree of AAc/chitosan hydrogel as a function of chitosan concentration. AAc : water = 70 : 30 vol %; pH 6; 25°C; radiation dose, 30 kGy.

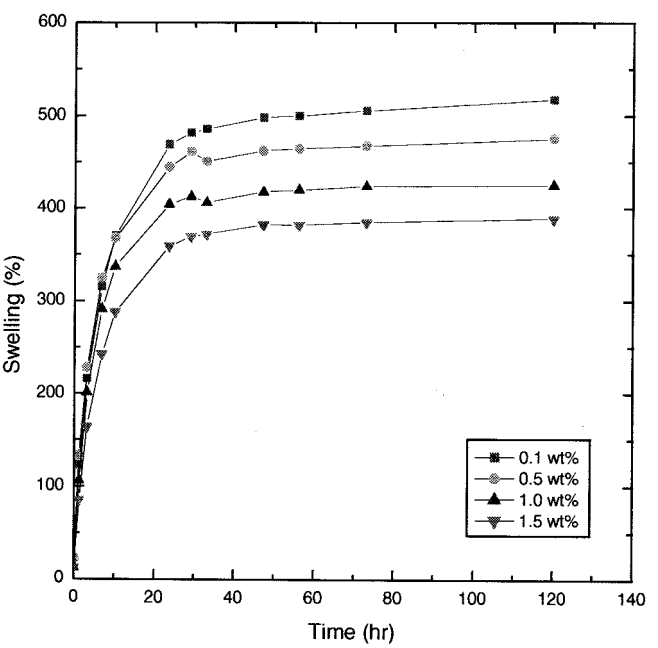

Figure 5 Swelling degree of AAc/chitosan hydrogel as a function of chitosan concentration. AAc : water = 70 : 30 vol %; pH 6; 25°C; radiation dose, 50 kGy.

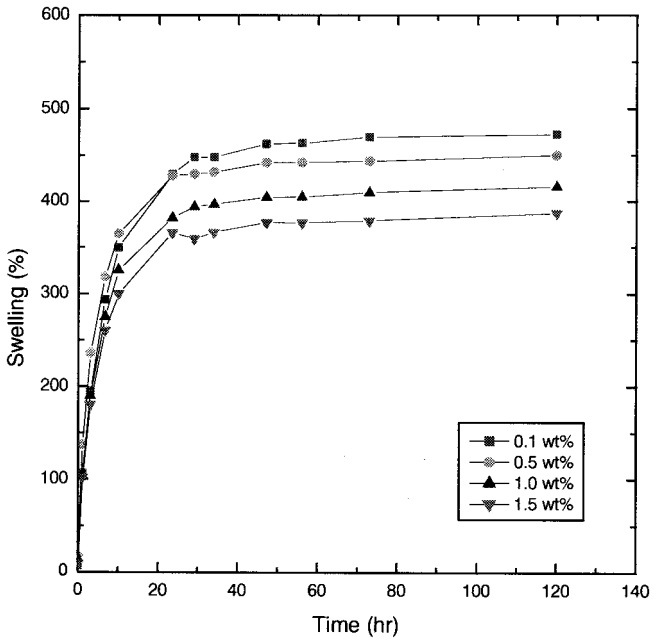

Figure 6 Swelling degree of AAc/chitosan hydrogel as a function of chitosan concentration. AAc : water = 70 : 30 vol %; pH 6; 25°C; radiation dose, 70 kGy.

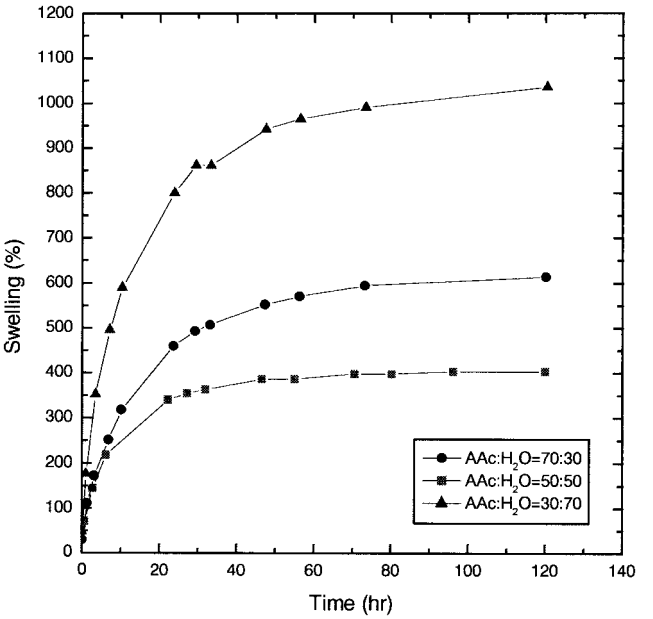

Figure 7 Swelling degree of AAc/chitosan hydrogel as a function of AAc concentration. Chitosan, 0.1 wt %; pH 6; 25°C; radiation dose, 30 kGy.

degree. In general, radiation dose was calculated by multiplying the dose rate by time, so the increase of radiation dose can lead to an increase of free radicals and longer reaction time to form complete polymer networks that provide a perfect frame for interpolymeric networks to show their special characteristics such as change of mechanical strength, swelling capacity, and gel flexibility. In this study, the decreased swelling degree of the AAc/chitosan hydrogel at a high radiation dose might be attributable to a high crosslinking ratio of polymeric gel networks, resulting in the decrease of the flexibility of the gel.

Figure 7 shows the swelling degree of AAc/chitosan hydrogels prepared at various ratios of 70, 50, and 30 vol % acrylic acid. The results indicated that the swelling degrees of hydrogels decreased as the ratio of the acrylic acid increased. The hydrogels having AAc/water (30/70 vol %) and 0.1 wt % chitosan content represented the highest water content, although there were slight differences depending on the pH of the solution and radiation dose. Comparing the results of Figures 4–6, in which the difference of swelling degree was 150–200%, the difference reveals about 700% according to the acrylic acid content. This result indicates that the network structure and swelling degree are mainly dependent on the acrylic acid content in the gel. However, for the hydrogels having AAc/water (50/50 vol %), the swelling degree was the lowest among the three samples. It was contrary to the results in Figures 1–3 that the higher the content of AAc and chitosan, the greater the gel fraction and the lower the corresponding swelling ratio. Although it could not explain the reason as presented in other studies,[18,19] the 50/50 volume fraction of AAc/water might be the most appropriate ratio to become a comparatively tight and complete network structure resulting in a decrease of the hydration ability and swelling of the gel.

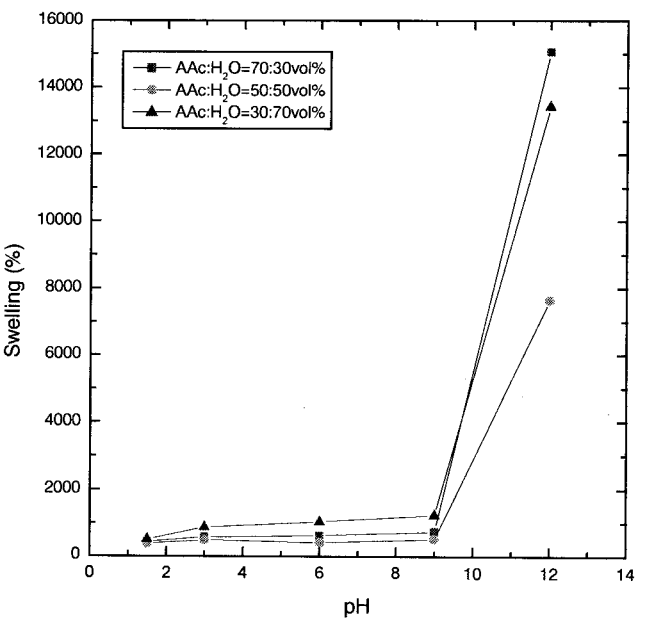

Figure 8 Effect of AAc concentration on swelling of AAc/chitosan hydrogel as a function of pH. Chitosan, 0.1 wt %; 25°C; radiation dose, 30 kGy.

In general, a hydrogel for oral drug delivery must have the ability to hydrate and swell at relatively high pH to release a drug in the intestinal region. Figure 8 shows the swelling degree of AAc/chitosan hydrogels having 0.1 wt % chitosan content measured at various pH values at 25°C. The swelling degrees of all the AAc/chitosan hydrogels in the range of pH 3–9 are relatively small compared with that at pH 12. It was deduced that the difference in the swelling degree in various pH media was attributed to the extent of hydrogen bonding between AAc and chitosan in the polymer networks. At low pH, the gel forms a compact structure composed of ammonium ion in chitosan and carboxylate ion in AAc, resulting in a decrease in EWC. At high pH, however, chitosan is in the form of $-NH_2$; however, AAc exists as COO^-, resulting in even higher EWC than that at low pH.

IR spectra

Figure 9 shows the FTIR spectra of chitosan, PAAc, and PAAc/chitosan film prepared from AAc/chitosan hydrogel. For the PAAc/chitosan film prepared with AAc/chitosan hydrogels, the intensities of amide band I at 1662 cm^{-1} and amide II at 1586 cm^{-1}, which are observed in pure chitosan, decreased dramatically. Two new absorption bands at 1731 and 1628 cm^{-1}, which can be assigned to the absorption peaks of the carboxyl groups of PAA (the absorption peaks of the carboxyl groups in pure PAAc appear at 1740 cm^{-1}) and the NH_3^+ absorption of chitosan, respectively, are absorbed.[20] The broad peaks appearing at 2500 and 1900 cm^{-1} confirmed the presence of NH_3^+ in AAc/chitosan hydrogels. Furthermore, the absorption peaks at 1532 and 1414 cm^{-1} could be assigned to asymmetric and symmetric stretching vibrations of COO^- anion groups. These results indicate that the carboxylic groups of PAAc are dissociated into COO^-, which complex with protonated amino groups of chitosan through electrostatic interaction to form the polyelectrolyte complex during the polymerization procedure of the acrylic acid in the presence of chitosan.[21,22]

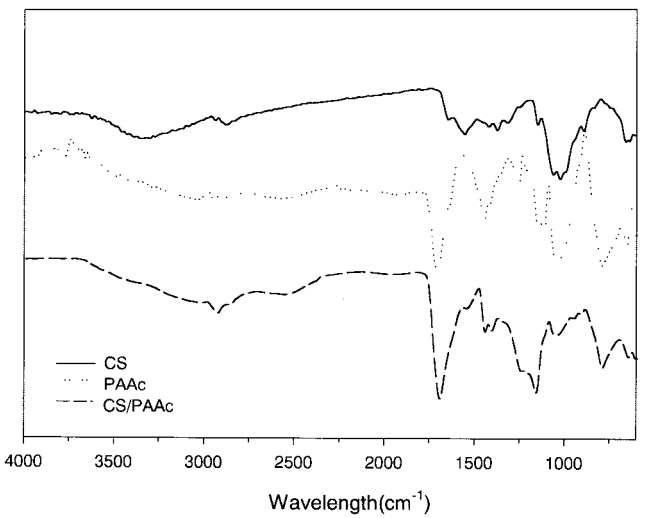

Figure 9 FTIR spectra of CS (chitosan), PAAc (polyacrylic acid), and CS/PAAc (AAc/chitosan film prepared from hydrogel involving AAc : water = 70 : 30 vol %, chitosan 1 wt %).

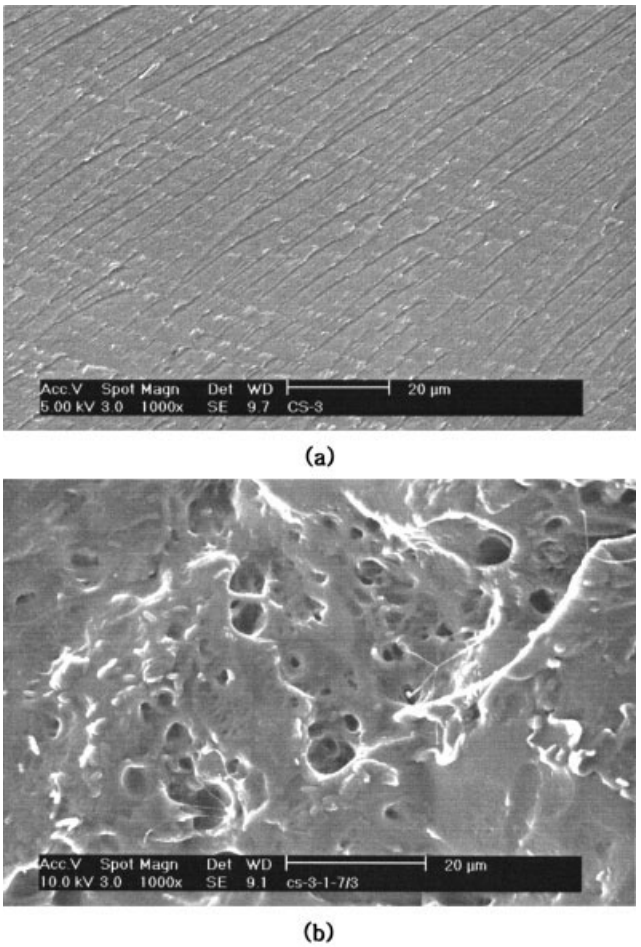

Figure 10 Cross-sectional SEM photos of AAc/chitosan hydrogels after swelling for (a) 0 h and (b) 12 h in aqueous solution. AAc : water = 70 : 30 vol %.

SEM observation

Figure 10 shows SEM micrographs of freeze-dried AAc/chitosan hydrogels after swelling for long periods in an aqueous solution. All the cross-sectional micrographs represent the irregular surface pattern, which was a typical surface pattern of N-acetylated chitosan prepared from chitin. The surface morphology of dried AAc/chitosan gel without swelling was more dense and compact than the sample swollen for 12 h and there were no pores or cracks on the surface. For AAc/chitosan gel swollen for longer periods, its

TABLE II
Adhesive Force of the AAc/Chitosan Hydrogels with Respect to Concentration of Chitosan (AAc : H_2O = 30 : 70 vol %, 30 kGy)

Chitosan concentration (%)	Adhesive force (kg_f/cm^2)
0	0.93
0.1	0.80
0.5	0.77
1.0	0.68
1.5	0.62

surface pattern had a volcano-like surface with the increase of micro- or mesopores within the gel, as observed from the micrographs of the cross-sectional area. Although we found no evidence that this structure of AAc/chitosan hydrogel was attributable to polyelectrolyte complex formation between the amino groups of the linear chitosan chain and the carboxyl groups of acrylic acid, these microscopic findings suggest that micro- or mesopores should be the path for loading or release of a drug from a hydrogel.

Adhesive force

Table II shows the adhesive force of AAc/chitosan hydrogels. It has been proposed that mucoadhesion, which adheres to the mucosal layer, may be used to prolong the retention time of oral dosage forms. In this study, the adhesive force was obtained by measuring the force required to break the contact between the AAc/chitosan hydrogel and the mucosal layer. The higher the content of chitosan in the AAc/chitosan hydrogels, the lower the adhesive force. It was found that through the use of acrylic hydrogels, although chitosan had a mucoadhesive force, the density of carboxylic groups on the polymer chain was important for mucoadhesion. AAc/chitosan hydrogel has functional groups to form hydrogen bonds with the mucosal layer and their chains are flexible enough to form as many hydrogen bonds as possible.

In vitro drug release studies

The *in vitro* release experiments of the 5-FU from AAc/chitosan hydrogels prepared with various formulations were carried out in simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at a constant temperature of 25°C. Figures 11 and 12 show the release profiles of 5-FU from AAc/chitosan hydrogels. The release amount of 5-FU from a hydrogel with 0.1% chitosan prepared by 30-kGy irradiation was the largest of all the samples. For the hydrogels having the same chitosan content, the release of 5-FU increased as the acrylic acid and radiation dose decreased. These results indicated that the release of 5-FU was related to the swelling degree of the hydrogel. Too large a monomer content and radiation dose led to a dense and tight polymeric gel network, resulting in the decrease of flexibility and hydration ability of the hydrogel. Therefore, the release of 5-FU by diffusion could be hindered by the decrease of pore size distributed in the gel fraction, which is the path for loading or re-

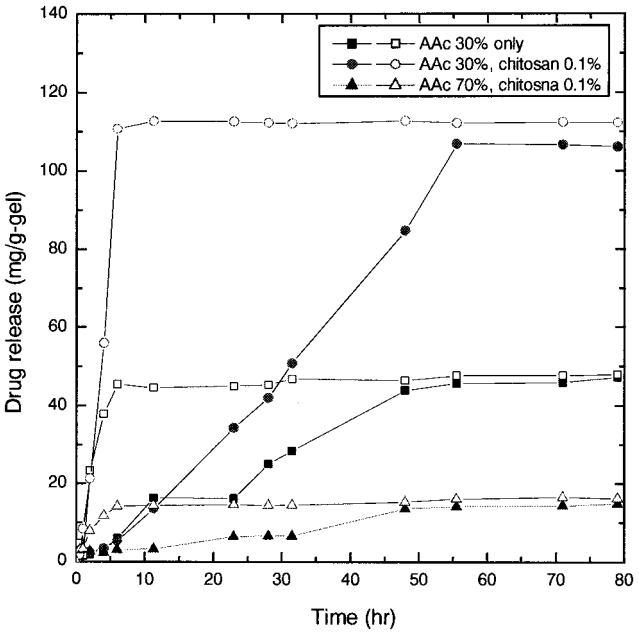

Figure 11 *In vitro* 5-FU release profiles of AAc/chitosan hydrogels in SGF (solid) and SIF (hollow). Radiation dose: 30 kGy.

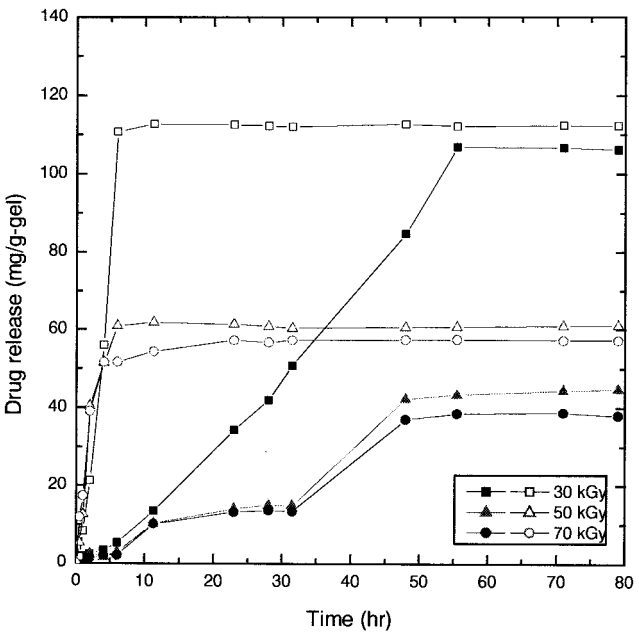

Figure 12 *In vitro* 5-FU release profiles of AAc/chitosan hydrogels in SGF (solid) and SIF (hollow). AAc : water = 30 : 70 vol %, chitosan 0.1 wt %.

lease of the drug. In particular, the release of 5-FU from a hydrogel progressed more slowly in the SGF than in the SIF. This pH dependency of 5-FU release was caused by the extent of hydrogen and chemical bonding between AAc and chitosan, which underwent a reversible change according to the pH of the medium, causing the difference in the swelling degree. In other words, a slow release of the drug in the SGF than in the SIF was attributed to the compact polyion complex formed between the ammonium ion in the chitosan and the carboxylate ion in the AAc. However, there was an initial burst release of the drug to an extent of more than 80% and thereafter the release reached equilibrium in the SIF region. An amount of about 90% entrapped 5-FU was released in the first 1 h in the SIF. This initial burst effect may be attributed to the diffusion of the drug caused by rapid gel swelling and the release of the drug adsorbed toward the surface of the gel matrix. After 10 h, close to 100% of the loaded drug had been released. This may be because of the diffusion of the drug entrapped in the core of the gel. The remaining drug in the gel may be released in a very slow fashion because of the slow rate of degradation of the gel matrix. This result corroborates well with the greater release of the drug entrapped in the gel in SIF than in the SGF.

CONCLUSIONS

A new pH-responsive hydrogel based on AAc/chitosan polymerization was developed for oral drug delivery. The preparation of these copolymeric hydrogels was carried out using the radical polymerization technique by gamma irradiation for the purpose of enhancing the drug-release ability. The AAc/chitosan hydrogels represent different gelation and crosslinking degrees depending on the composition of chitosan or acrylic acid and radiation dose. The equilibrium swelling measurements clearly showed the mucoadhesive and pH-responsive nature of these hydrogels. The *in vitro* release profiles of 5-FU were established in SGF and SIF. The hydrogel with the lower content of chitosan and AAc was quickly swollen in the medium solution and the release rate of the 5-FU from it was expedited. Also, the release behavior of the 5-FU from the hydrogel was different according to the pH of the release medium, content of the monomer, and the radiation dose. This investigation of chitosan-based interpolymeric pH-responsive hydrogels indicates that the rate of drug release can be modulated by the appropriate chemical modification of the crosslinking densities of these gels and further modification of these hydrogels can lead to a successful application for localized drug delivery to the intestinal environment.

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