γ-Irradiation Preparation of Poly(acrylic acid)–Chitosan Hydrogels for *In Vitro* Drug Release

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ABSTRACT: Biocompatible and biodegradable pH-responsive hydrogels based on poly(acrylic acid) and chitosan were prepared for controlled drug delivery. These interpolymeric hydrogels were synthesized by a γ-irradiation polymerization technique. The degree of gelation was over 96% and increased as the chitosan or acrylic acid (AAc) content increased. The equilibrium swelling studies of hydrogels prepared under various conditions were carried out in an aqueous solution, and the pH sensitivity in a range of pH 1–12 was investigated. The AAc/chitosan hydrogels showed the highest water content when 30 vol % AAc and 0.1 wt % chitosan were irradiated with a 30 kGy dose of radiation. In addition, an increase of the degree of swelling with an increase in the pH was noticed and it had 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 intestinal pH and the rest of the drug was released slowly. © 2003 Wiley Periodicals, Inc. J Appl Polym Sci 90: 3270–3277, 2003

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

INTRODUCTION

One of the most attractive applications of a 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. One of these is that the bioavailability of these drugs following oral administration is usually very low (less than 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]

Stimulus-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 3-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 of these hydrogels by grafting synthetic polymers onto the most abundant naturally occurring polysaccharides such as cellulose, starch, and alginates. Especially chitosan has been largely evaluated as a potential vehicle for oral drug administration 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 remains an unexplored area of study.

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

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

This study we report on the preparation of another polymer composed of chitosan and AAc and investigate its properties and effects by γ irradiation. In addition, the drug release characteristics of the AAc/chitosan hydrogel were studied with the 5-fluoroura-

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cil (5-FU), which is an antimetabolic drug used extensively in cancer chemotherapy. The effect of the hydrogel composition and radiation dose on the release rate of 5-FU was examined.

EXPERIMENTAL

Materials

The AAc (MW = 450,000) purchased from Junsei Chemical Co. (Tokyo) was purified by a column packed with aluminum peroxide to eliminate the hydroquinone inhibitor. Chitosan (degree of deacetylation = 83%) was procured from Tokyo Kasei Co. All other chemicals were extra pure reagent grade and were used as received.

Preparation of hydrogels

The AAc/chitosan hydrogels were synthesized by polymerization of AAc 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 AAc/chitosan solutions were prepared. Chitosan was dissolved in a mixture of water/AAc (70/30, 50/50, and 30/70% v/v). These solution mixtures were poured into a 10×3 cm (3-mm thickness) container. These samples were sealed and irradiated with a ^{60}Co source at doses of 30, 50, and 70 kGy (dose rate = 9.14×10^5 rad/h). Polymerized samples were washed with acetone and distilled water to remove unreacted monomer and vacuum dried in air for 24 h.

Hydrogel characterization

The IR absorption spectra of AAc/chitosan hydrogels and chitosan were studied by Fourier transform IR (FTIR, M series, Midac Corp.). Samples were thoroughly ground with dried KBr and disks were prepared by compression under a vacuum. Scanning electron microscopy (SEM) was performed on dried and swollen gels to analyze their morphology. The freeze- or air-dried samples were gold coated for conductance and their surfaces were examined with an SEM microscope. Because excessively high magnification might cause the collapse of the hydrogel films because of the highly focused electron beam, examinations at higher magnification were not performed. The detachment force between the gel film and the small intestine mucosa, which was washed with methanol for 24 h and polished in order to increase the interfacial area, was measured by a modified precision tensile tester (Rheometric Scientific). The AAc/chitosan hydrogels cut to the appropriate size were prewetted with distilled water and placed on a plastic plate. The plate with the 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 and 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. The results are the means of five to seven experiments.

Equilibrium swelling studies

The AAc/chitosan gels were cut with an area of 1×1 cm (10-mm thickness). The weighed dry gels were immersed in a 100-mL vial with appropriate 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 percent 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 the dried gel and W_s is the final 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 different solutions with 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 hydrogel

The 5-Fu was loaded in the hydrogel by immersing it in the drug dissolved solution. In order to trap the 5-FU in the hydrogels, an aqueous solution of 5-FU and a neutralized equivalent to NaOH were used, which was to increase the solubility of the drug. Dried hydrogels (~0.4 g) were placed in the 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–visible spectroscopy.

in vitro drug release studies

The *in vitro* release of the entrapped 5-FU was carried out by placing air-dried hydrogels loaded with the drug into 50 mL of simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) at ambient temperature. The 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. The 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 solutions were withdrawn and tested at a wavelength of maximum absorption of 267 nm for 5-FU using a UV–visible

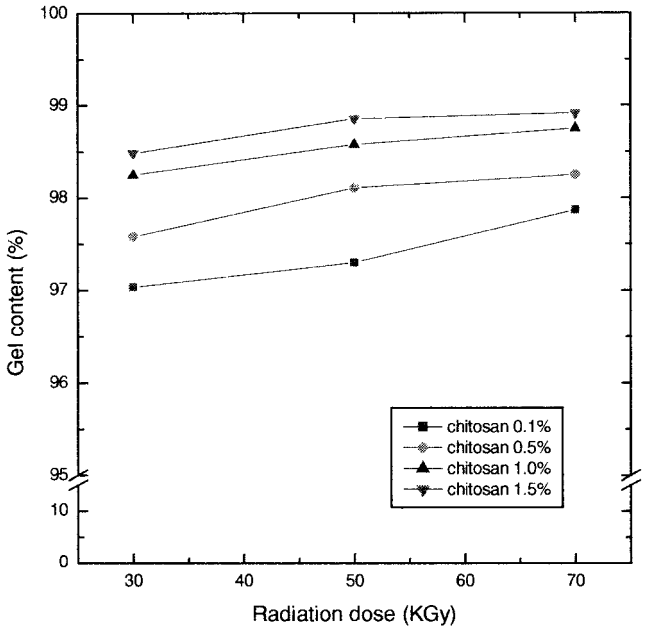

Figure 1 The effects of the dose of radiation and chitosan concentration on the gel content (AAc/water = 70:30 vol %).

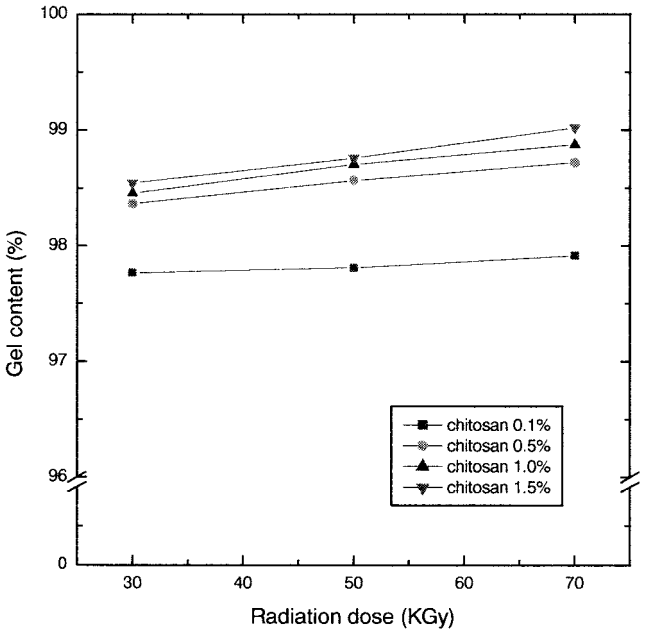

Figure 2 The effects of the radiation dose and chitosan concentration on the gel content (AAc/water = 50:50 vol %).

spectrophotometer (S-1100, SCINCO Co.). 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 hydrogels with various compositions of AAc/chitosan and various radiation doses. The degree of gelation of PAA/chitosan hydrogels was over 96% and increased as the chitosan or AAc content was increased. In addition, with the same composition of AAc/chitosan, the larger the radiation dose was, the higher the degree of gelation of the AAc/chitosan polymer complex. The results indicated that the gelation due to the crosslinking of the AAc/chitosan polymer complex was more strongly influenced by the AAc ratio than that of the chitosan with the same radiation dose. Chitosan is considered to be 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 PAAc, a weak polyacid, through an electrostatic attraction. Chitosan itself is degraded and does not form grafts, as reported by other study.[15] It was believed that the gelation of the AAc/chitosan polymer complex was mainly from the degree of breaking and crosslinking of the double bonding located in the AAc by irradiation.

Table I summarizes the composition of the AAc/chitosan hydrogels containing different chitosan parts as determined by elemental analysis. Structurally, chitosan is a linear polysaccharide consisting of $\beta(1 \rightarrow 4)$ linked D-glucosamine residues with a variable number of randomly located *N*-deacetyl-glucosamine groups. Chitosan also has a high percentage of nitrogen compared to synthetically substituted cellulose,[16] so the

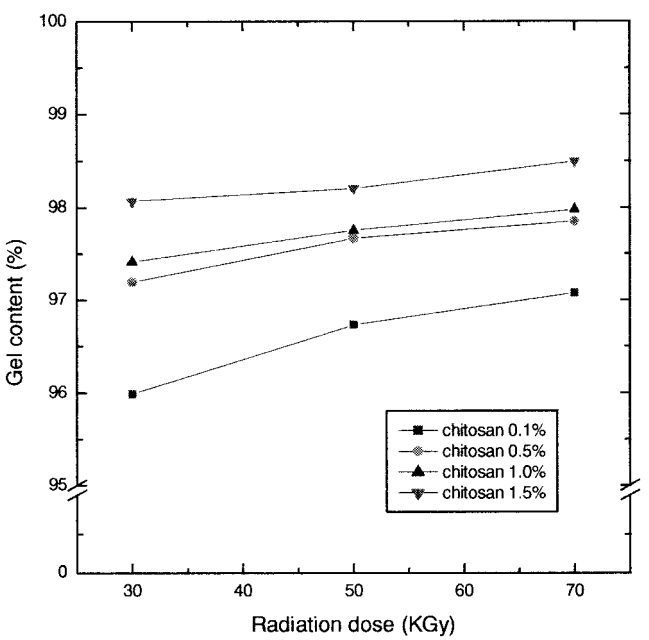

Figure 3 The effects of the radiation dose and chitosan concentration on the gel centent (AAc/water = 30:70 vol %).

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

	0.1 wt %	0.5 wt %	1 wt %	1.5 wt %
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

nitrogen content can be an indicator of its distribution 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. We thought that the formation of a polyelectrolyte complex of AAc and chitosan occurred by transfer reactions of the growing free radicals of AAc to the —OH or —NH_2 groups of the chitosan rings.

Swelling properties of gel

The equilibrium water content (EWC) of the AAc/chitosan hydrogel was measured against time at pH 6, and the results are 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 of 400–1000%. 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 the radiation dose. Figure 4 shows that, for a 70/30 vol % ratio of AAc/water 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

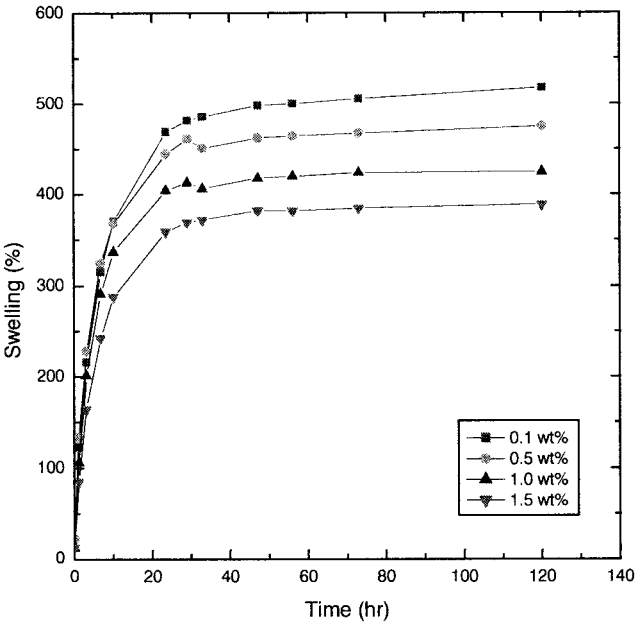

Figure 5 The degree of swelling of the AAc/chitosan hydrogel as a function of the chitosan concentration (AAc/water = 70:30 vol %, pH 6, 25°C, 50-kGy irradiation dose).

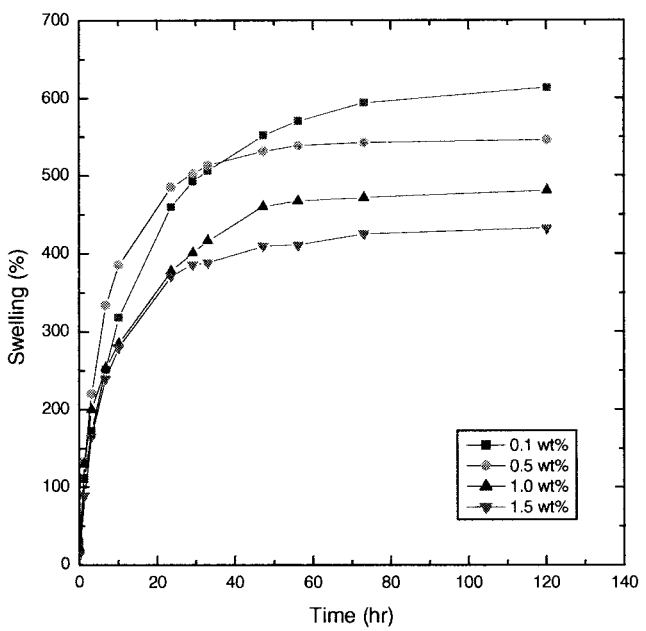

Figure 4 The swelling degree of the AAc/chitosan hydrogel as a function of the chitosan concentration (AAc/water = 70:30 vol %, pH 6, 25°C, 30-kGy irradiation dose).

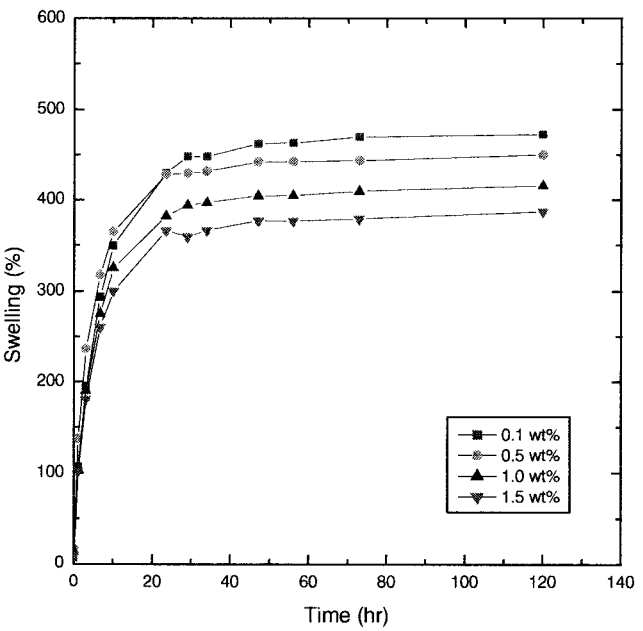

Figure 6 The swelling degree of the AAc/chitosan hydrogel as a function of the chitosan concentration (AAc/water = 70:30 vol %, pH 6, 25°C, 70-kGy radiation dose).

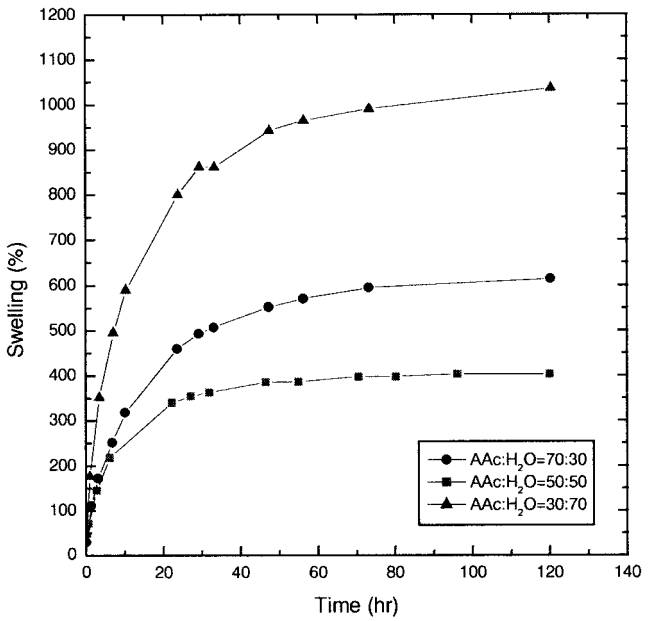

Figure 7 The degree of swelling of the AAc/chitosan hydrogel as a function of the AAc concentration (0.1 wt % chitosan, pH 6, 25°C, 30-kGy radiation dose).

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 because of the lower gel flexibility. In this study, 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 the radiation dose (50 and 70 kGy) on the degree of swelling of the AAc/chitosan hydrogel. The gel fraction was greater at the higher radiation dose; therefore, theoretically, the corresponding swelling ratio should be lower. This was consistent with the effect of the radiation dose on the gel fraction in Figures 1–3, which was that the higher the radiation dose was, the greater the gel fraction and the lower the swelling degree were. In general, the radiation dose is calculated by multiplying the dose rate by the time. Thus, the increase of the radiation dose can lead to an increase of free radicals and a longer reaction time to form complete polymer networks, which provides a perfect frame for interpolymeric networks to show their special characteristics such as changes in the 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 decreased flexibility of the gel.

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

In general, hydrogels for oral drug delivery must have an ability to hydrate and swell at relatively high pH in order 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. A slight increase of the degree of swelling with increased pH is noted. However, the EWC values of all the AAc/chitosan hydrogels at pH 3–9 are relatively small compared with the one at pH 12. We deduced that the difference in the

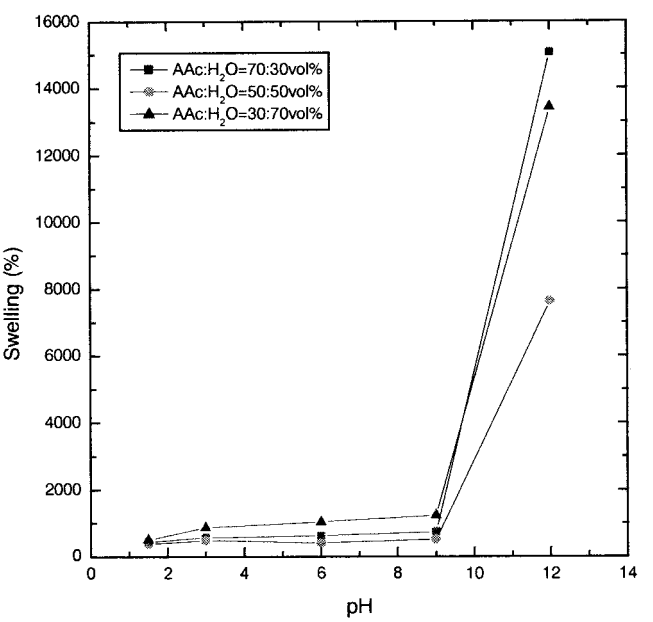

Figure 8 The effect of the AAc concentration on the swelling of the AAc/chitosan hydrogel as a function of the pH (0.1 wt % chitosan, 25°C, 30-kGy radiation dose).

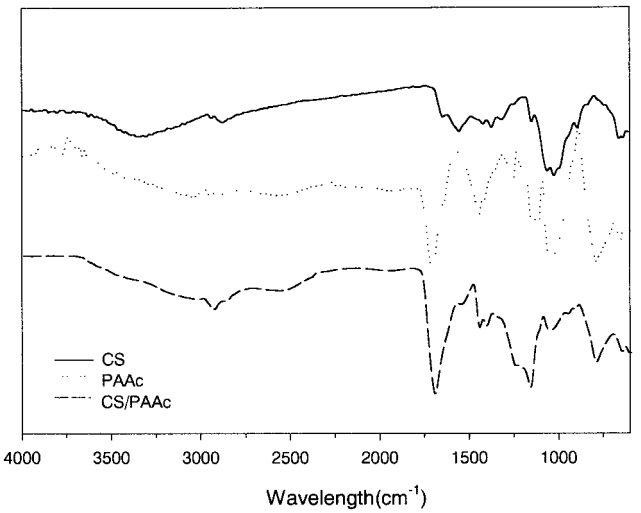

Figure 9 FTIR spectra of chitosan (CS) and CS/PAAc. The AAc/chitosan film is prepared from a hydrogel with 70:30 vol % AAc/water and 1 wt % chitosan.

swelling degree in various pH mediums was due to the extent of hydrogen bonding between AAc and chitosan in the polymer networks. As presented in other reports,[19–22] the hydrogels containing chitosan may be formed by two different mechanisms: the amidation between the free carboxylic group of the AAc and the amine groups of the chitosan, which provide for the grafting of the polyacrylic chains onto the chitosan macromolecules, and the transfer reactions of the polymeric growing radicals to functional groups of the pyran cycles of chitosan, including —NH_2 and —OH groups. Therefore, at low pH, the gel forms a compact structure composed of the ammonium ion in chitosan and the carboxylate ion in AAc, which results in a decrease in the EWC. At high pH, however, chitosan is in the form of —NH_2 but AAc exists as COO^-, resulting in an even higher EWC than that at low pH.

IR spectra

Figure 9 shows the FTIR spectra of chitosan, PAAc, and PAAc/chitosan films prepared from AAc/chitosan hydrogel. For the PAAc/chitosan film prepared from the AAc/chitosan hydrogels, the intensities of the amide I band at 1662 cm^{-1} and the amide II band at 1586 cm^{-1}, which are observed in pure chitosan, decreased dramatically, and 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 appears at 1740 cm^{-1}) and the NH_3^+ absorption of chitosan, respectively, are absorbed.[23] 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, respectively, of COO^- anion groups. These results indicate that the carboxylic groups of PAAc are dissociated into COO^-, which complexes with the protonated amino groups of chitosan through electrostatic interaction to form the polyelectrolyte complex during the polymerization procedure of the AAc in the presence of chitosan.[24,25]

SEM observation

Figure 10 shows SEM photos of freeze-dried AAc/chitosan hydrogels after swelling in an aqueous solution for long periods. The cross-sectional photos 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 the longer swollen AAc/chitosan gel, its surface pattern was like

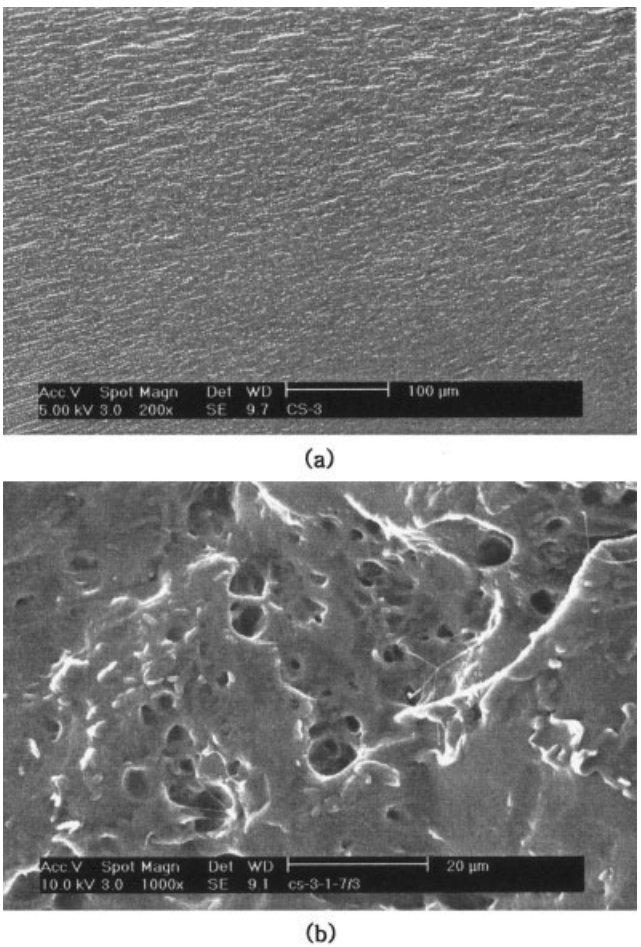

Figure 10 Cross-sectional SEM photos of AAc/chitosan hydrogels after swelling for (a) 0 or (b) 12 h in aqueous solution (AAc/water = 70:30 vol %).

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

Chitosan Concn (%)	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

a volcano surface with the increase of micro- or mesopores within the gel as observed from the photos of the cross-sectional area. Although we found no evidence that this structure of AAc/chitosan hydrogel was due to polyelectrolyte complex formation between the amino groups of the linear chitosan chain and the carboxyl groups of AAc, 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 (adherence to the mucosa 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 mucosa layer. The higher the content of chitosan in the AAc/chitosan hydrogels, the lower the adhesive force. When using acrylic hydrogels, although chitosan had a mucoadhesive force, it was indicated that the density of carboxylic groups on the polymer chain was important for mucoadhesion. An AAc/chitosan hydrogel has functional groups that form hydrogen bonds with the mucosa 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 SGF and SIF at a constant temperature of 25°C. Figures 11 and 12 show the release profiles of 5-FU from AAc/chitosan hydrogels. The amount of 5-FU released 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 AAc 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 that resulted in the decrease of the flexibility and hydration ability of the hydrogel. Therefore, the release of 5-FU by diffusion could be interfered with by the decrease of pore the size distributed in the gel fraction, which is the path for loading or release of the drug. Especially, the release

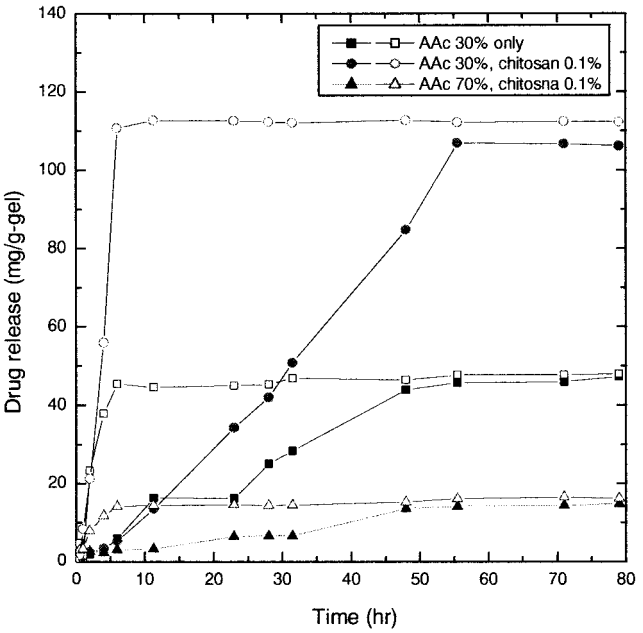

Figure 11 *In vitro* 5-FU release profiles of AAc/chitosan hydrogels in (■, ●, ▲) SGF and (□, ○, △) SIF (30-kGy radiation dose).

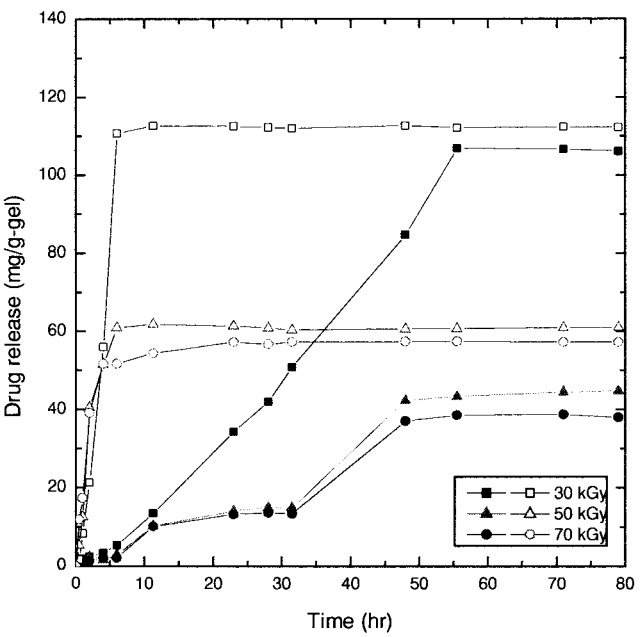

Figure 12 *In vitro* 5-FU release profiles of AAc/chitosan hydrogels in (■, ▲, ●) SGF and (□, △, ○) SIF (AAc/water = 30:70 vol %, 0.1 wt % chitosan).

of 5-FU from a hydrogel progressed more slowly in the SGF than in the SIF. This pH dependence 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 degree of swelling. In other words, the slower release of the drug in the SGF than in the SIF was due 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 of more than 80% and thereafter the release reached equilibrium in the SIF region. About 90% of the entrapped 5-FU was released in the first hour 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 due to 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 gel entrapped drug in SIF than in 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 γ irradiation for the purpose of enhancing the drug releasing ability. The AAc/chitosan hydrogels represent different degrees of gelation and crosslinking, depending on the composition of chitosan or AAc 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 hastened. In addition, the release behavior of the 5-FU from the hydrogel was different according to the pH of the release medium, the 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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