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Design and characterization of chitosan nanoparticles as delivery systems for paclitaxel

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ABSTRACT

The main objective of this research is to design a new extended release multiparticulate delivery system by incorporation into nanoparticles made of chitosan-polymethacrylic acid copolymers. As the first part of a continued research on conversion of chitosan to useful biopolymer-based materials, by grafting polymethacrylic acid (PMAA), free radical graft copolymerization was carried out at 70 °C, with bis-acrylamide as a cross-linking agent and persulfate as an initiator. Equilibrium swelling studies were carried out in enzyme-free simulated gastric and intestinal fluids. Also, the paclitaxel as a model drug was entrapped in these nano-gels and in vitro release profiles were established separately in both enzyme-free SGF and SIF. The drug release was found to be faster in SIF.

1. Introduction

Natural polymers have potential pharmaceutical applications because of their low toxicity, biocompatibility, and excellent biodegradability. In recent years, biodegradable polymeric systems have gained importance for design of surgical devices, artificial organs, drug delivery systems with different routes of administration, carriers of immobilized enzymes and cells, biosensors, ocular inserts, and materials for orthopedic applications (BrØndsted & Kopeček, 1990). These polymers are classified as either synthetic (polyesters, polyamides, polyanhydrides) or natural (polyamino acids, polysaccharides) (Giammona, Pitarresi, Cavallora, & Spadaro, 1999; Krogars et al., 2000). Polysaccharide-based polymers represent a major class of biomaterials, which includes agarose, alginate, carageenan, dextran, and chitosan. Chitosan [β(1,4)2-amino-2-D-glucose] is a cationic biopolymer produced by alkaline N-deacetylation of chitin, which is the main component of the shells of crab, shrimp, and krill (Chiu, Hsiue, Lee, & Huang, 1999; Jabbari & Nozari, 2000). Chitosan is a functional linear polymer derived from chitin, the most abundant natural polysaccharide on the earth after cellulose, and it is not digested in the upper GI tract by human digestive enzymes (Fanta & Doane, 1986; Furda, 1983). Chitosan is a copolymer consisting of 2-amino-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucose units links with β-(1-4) bonds. It should be susceptible to glycosidic hydrolysis by microbial enzymes in the colon because it possesses glycosidic linkages similar to those of other enzymatically depolymerized polysaccharides. Among diverse approaches that are possible for modifying polysaccharides, grafting of synthetic polymer is a convenient method for adding new properties to a polysaccharide with minimum loss of its initial properties (Saboktakin, Maharramov, & Ramazanov, 2007; Peppas, 1987). Graft copolymerization of vinyl monomers onto polysaccharides using free radical initiation, has attracted the interest of many scientists. Up to now, considerable works have been devoted to the grafting of vinyl monomers onto the substrates, especially starch and cellulose (Jabbari & Nozari, 2000; Xu & Li, 2005). Existence of polar functionally groups as carboxylic acid need not only for bioadhesive properties but also for pH-sensitive properties of polymer (Ratner, 1989; Thierry, Winnik, Mehri, & Tabrizian, 2003), because the increase of MAA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH. It is as a part of our research program on chitosan modification to prepare materials with pH-sensitive properties for use as drug delivery (Mahfouz, Hamm, & Taupitz, 1997; Schmitz et al., 2000; Bloembergen & Pershan, 1967).

In this study, we hypothesized that the absorption of paclitaxel could be enhanced by administration with chitosan-polymethacrylic acid nanoparticles because of their greater per-

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meability properties (Puttpipatkhachorn, Nunthanid, & Yamamato, 2001).

2. Materials and methods

2.1. Materials

Chitosan (33 kDa) was obtained from TaeHoon Bio. Co. (Korea) and used as received. The viscosity and degree of deacetylation as determined by the supplier were 2.8 cps (0.5% solution in 0.5% acetic acid at 20 °C) and 90% respectively. Sodium tripolyphosphate was purchased from Sigma–Aldrich, USA and used without further purification.

2.2. Instruments

The powder morphology chitosan/PMAA nanoparticles in the form of pellets (to measure grain size) was investigated using Philips XL-30 E SEM scanning electron microscope (SEM) at 30 kV (max.). The samples were prepared by physical vapor disposition method. The gold layer thickness at these samples was 100 Å. They were carried out in Chemistry Department of Tarbiat Modares University. The DSC curves were obtained on a TGA/SDTA 851 calorimeter at heating and cooling rates of 10 °C/min under N_2. The amount of released drug was determined on a Philips PU 8620 UV spectrophotometer at the absorption maximum of the free drug in aqueous alkali) λ_{max} = 275 nm) using a 1 cm quartz cell. Enzyme-free SGF (pH 1) or SIF (pH 7.4) was prepared according to the method described in US Pharmacopeia.

2.3. The free radical graft copolymerization of chitosan -methacrylic acid

Chitosan with 1:1 molar ratios of methacrylic acid were polymerized at 60–70 °C in a thermostatic water bath, bis-acrylamide as a cross-linking agent (CA), using persulfate as an initiator ([I] = 0.02 M) and water as the solvent (50 mL). The polymeric system was stirred by mechanical stirrer to sticky nanoparticles and it was separated from medium without solvent addition. All the experiments were carried out in Pyrex glass ampoules. After the specific time (48 h), the precipitated network polymer was collected and dried in vacuum.

2.4. Preparation and characterization of chitosan-methacrylic acid nanoparticles

Chitosan-methacrylic acid copolymer suspensions of 0.2% (w/v) were prepared in 1% acetic acid. Sodium tripolyphosphate (TPP, 1.0%) was added dropwise to 6 mL of chitosan with strirring, followed by sonication with a dismembrator for 10 s at a power setting of 3 W.The resulting chitosan particle suspension was centrifuged at 10,000 × *g* for 10 min.The pelleted particles were resuspended in deionized water with 10 s sonication and lyophilized. The mean size and zeta potential of the chitosan-methacrylic acid nanoparticles were determined by photon correlation spectroscopy using ZetaPlus particle analyzer.

2.5. Adsorption of paclitaxel by chitosan–methacrylic acid nanoparticles

The major amount of drug was adsorbed by 2 mg of chitosan nanoparticles in a certain time period. Chitosan-methacrylic acid nanoparticle suspensions (4 mg/mL) were mixed with paclitaxel solutions (0.5 and 1 mg/mL), vortexed, and incubated at 37 °C for 1, 6, 12 and 18 h. After adsorption, the suspensions were centrifuged at 10,000 × *g* for 10 min and free drug was measured in the supernatant by a colorimetric method using periodic acid/Schiff (PAS) staining. Schiff reagent was prepared by diluting pararosaniline solution (40 g/L in 2 M HCl, Sigma) with water to give a final concentration of 1.0% sodium bisulfite (80 mg) was added to 5 mL of Schiff reagent and the resultant solution was incubated at 37 °C until it became colorless or pale yellow. Periodic acid solution was freshly prepared by adding 10 μL of 50% periodic acid to 7 mL of 7% acetic acid. Supernatants were mixed with 100 μL of dilute periodic acid and incubated for 2 h at 37 °C. Then, 100 μL of Schiff reagent was added at room temperature, and after 30 min the absorbance was measured at 560 nm.

2.6. Stability of paclitaxel during nanoparticles preparation

The following procedure was used to assess the stability of paclitaxel during the bead preparation process. The prepared nanoparticles were extracted twice with a solvent mixture of 1:1 acetonitrile and ethanol (v/v), the extract was evaporated, the residue was injected onto HPLC column. Stability-indicating chromatographic method was adopted for this purpose. The method consisted of a Symmetry C18 column (254 mm × 4.6 mm; 5 μm) run using a mobile phase of composition methanol:water (70:30, v/v) at a flow rate of 0.5 mL/min, a Waters pump (600E), and eluants.

2.7. Release rate studies

A definite weight range of 10–15 mg of nanoparticles were cut and placed in a 1.5 mL capacity microcentrifuge tube containing 1 mL of release medium of the following composition at 37 °C: phosphate buffered saline (140 mM, pH 7.4) with 0.1% sodium azide and 0.1% Tween 80. At predetermined time points, 100 μL of release medium was sampled with replacement to which 3 mL of scintillation cocktail was added and vortexed before liquid scintillation counting. The cumulative amount of paclitaxel released as a function of time was calculated.

2.8. Solid-state characterization

To study the molecular properties of paclitaxel and chitosan-PMAA, the solid-state characterization was done by the application of thermal, X-ray diffraction, and microscopy techniques. During these studies, the solid characteristics of paclitaxel and chitosan-PMAA were compared with those of nanoparticles to reveal any changes occurring as a result of nanoparticle preparation.

2.8.1. Differential scanning calorimetry

Differential scanning calorimetry (DSC) studies were performed with a Mettler Toledo 821 thermal analyzer (Greifensee, Switzerland) calibrated with indium as standard. For thermogram acquisition, sample sizes of 1–5 mg were scanned with a heating rate of 5 °C/min over a temperature range of 25–300 °C. In order to check the reversibility of transition, samples were heated to a point just above the corresponding transition temperature, cooled to room temperature, and reheated up to 300 °C.

2.8.2. Scanning electron microscopy

Paclitaxel samples and chitosan-PMAA beads were viewed using a Philips XL-30 E SEM scanning electron microscope (SEM) at 30 kV (max.) for morphological examination. Powder samples of paclitaxel and beads were mounted onto aluminium stubs using double-sided adhesive tape and then sputter coated with a thin layer of gold at 10 Torr vacuum before examination. The specimens were scanned with an electron beam of 1.2 kV acceleration potential, and images were collected in collected in secondary electron mode.

2.8.3. X-ray diffraction studies

Molecular arrangement of paclitaxel and chitosan-PMAA in powder as well as in nanoparticles were compared by powder X-ray diffraction patterns acquired at room temperature on a Philips PW 1729 diffractometer (Eindhoven, Netherlands) using CuKα radiation. X-ray diffraction is a proven tool to study crystal lattice arrangements and yields very useful information on degree of sample crystallinity.

3. Results

3.1. Chemical stability of paclitaxel

In the present study, nanoparticles were prepared by the classical method, which involves spreading a uniform layer of polymer dispersion followed by a drying step for removal of solvent system. Since bead preparation methodology involved a heating step, it may have had a detrimental effect on the chemical stability of drug. Hence, stability assessment of paclitaxel impregnated in bead was done using stability-indicating method. For this purpose, paclitaxel was extracted from bead and analyzed by HPLC. A single peak at 21.2 min representing paclitaxel (with no additional peaks) was detected in the chromatogram, suggesting that the molecule was stable during preparation of beads (chromatograms not shown).

3.2. Content uniformity

Paclitaxel was extracted from different regions of chitosan-PMAA nanoparticles using acetonitrile:ETOH (1:1, v/v) solvent system. After normalization of amount of paclitaxel on weight basis of nanoparticles, the results indicated that the variation in distribution of paclitaxel in different regions of nanoparticles were <16%.

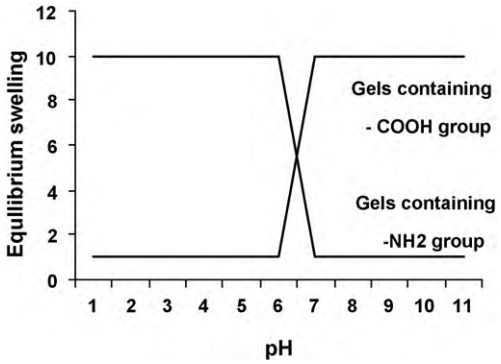

Fig. 1. Equilibrium degree of swelling in response to pH.

3.3. Nanoparticles morphology

The composition of the polymer defines its nature as a neutral or ionic network and furthermore, its hydrophilic/hydrophobic characteristics. Ionic hydrogels, which could be cationic, containing basic functional groups or anionic, containing acidic functional groups, have been reported to be very sensitive to changes in the environmental pH. The swelling properties of the ionic hydrogels are unique due to the ionization of their pendent functional groups. The equilibrium swelling behavior of ionic hydrogels containing acidic and/or basic functional groups is illustrated in Fig. 1. Hydrogels containing basic functional groups are found increased swelling activity in acidic conditions and reduced in basic conditions but pH-sensitive anionic hydrogels shows low swelling activity in acidic medium and very high activity in basic medium. As shown in Fig. 2, an increase in the content of MAA in the freed monomer mixtures resulted in less swelling in simulated gastric fluid but greater swelling in and simulated intestinal fluids. This is because the increase of MAA content in the hydrogels provides more hydrogen bonds at low pH and more electrostatic repulsion at high pH.

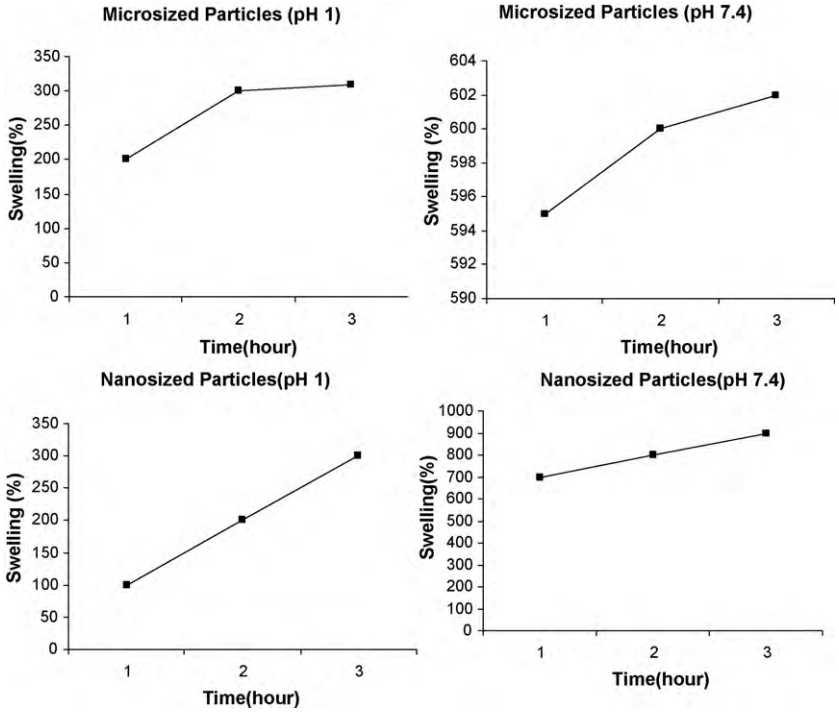

Fig. 2. Time-dependent swelling behavior of micro-and nano-carriers for paclitaxel drug model as a function of time at 37 °C.

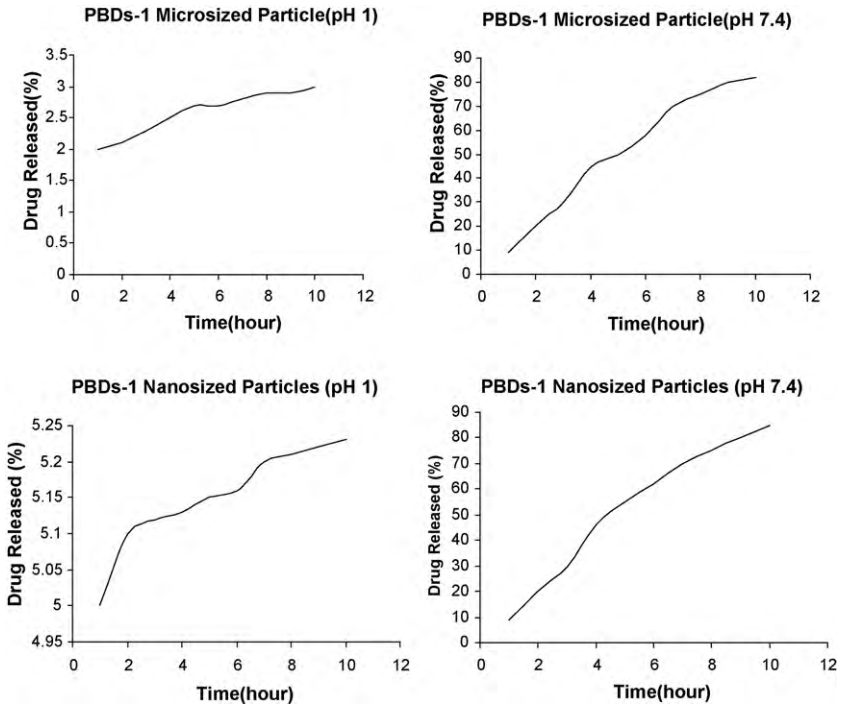

Fig. 3. Release of paclitaxel drug from micro- and nano-polymeric carriers as a function of time at 37 °C.

Fig. 4 shows scanning electron microscope (SEM) of graft chitosan copolymer with polymethacrylic acid and nano-polymer bonded drug.

3.4. In vitro release studies

Nano- and micro-polymer bonded drugs (50 mg) were poured into 3 mL of aqueous buffer solution (SGF: pH 1 or SIF: pH 7.4). The mixture was introduced into a cellophane membrane dialysis bag. The bag was closed and transferred to a flask containing 20 mL of the same solution maintained at 37 °C. The external solution was continuously stirred, and 3 mL samples were removed at selected intervals. The removed volume was replaced with SGF or SIF. Triplicate samples were used. The sample of hydrolyzate was analyzed by UV spectrophotometer, and the quantity of paclitaxel was determined using a

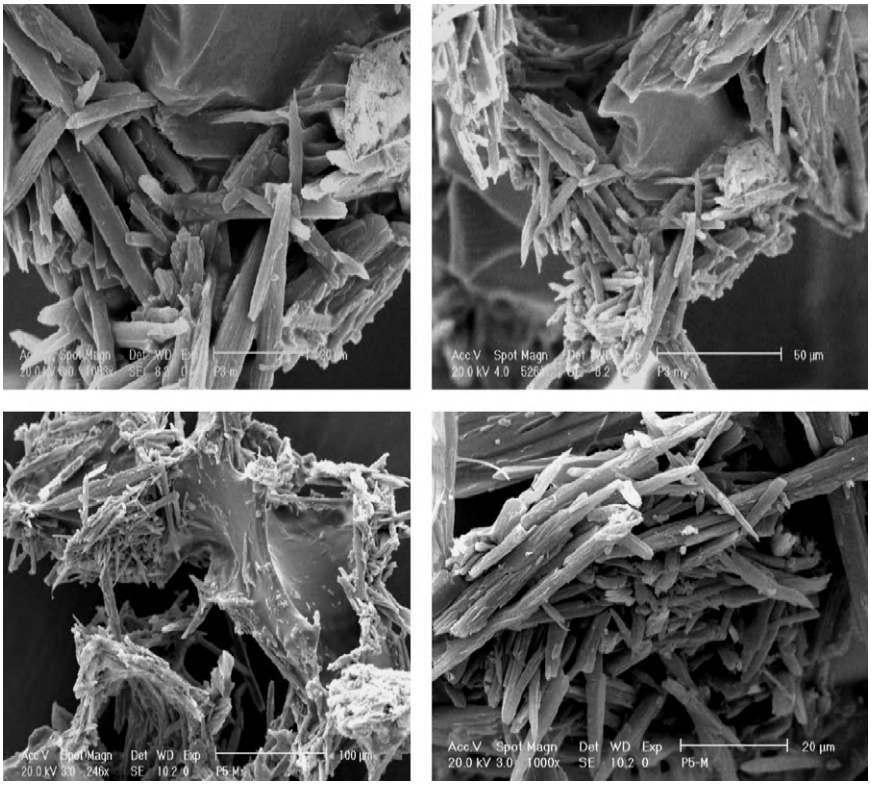

Fig. 4. SEM of chitosan nanoparticles with paclitaxel.

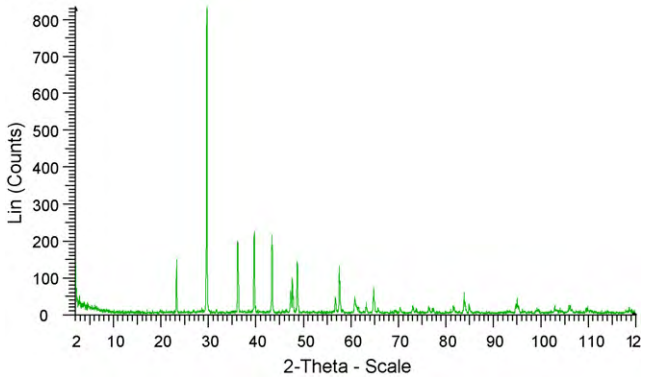

Fig. 5. XRD patterns of chitosan nanoparticles.

standard calibration curve obtained under the same conditions (Fig. 3).

3.5. Comparison of swelling ratio nano- and micro-carriers

It appears that the degree of swelling depends on their particle size. As shown in Fig. 2, a decrease in the molecular size of carriers increased the swelling rate.

3.6. Thermal analysis

The thermal behavior of a polymer is important in relation to its properties for controlling the release rate in order to have a suitable drug dosage form. The glass transition temperature (T_g) was determined from the DSC thermograms. The higher T_g values probably related to the introduction of cross-links, which would decrease the flexibility of the chains and the ability of the chains to undergo segmental motion, which would increase the T_g values. On the other hand, the introduction of a strongly polar carboxylic acid group can increase the T_g value because of the formation of internal hydrogen bonds between the polymer chains (Fig. 4).

3.7. X-ray diffraction studies

X-ray diffraction is also used to study the degree of crystallinity of pharmaceutical drugs and excipients. A lower 2θ value indicates larger d-spacings, while an increase in the number of high-angle reflections indicates higher molecular state order. In addition, broadness of reflections, high noise, and low peak intensities are characteristics of a poorly crystalline material. A broad hump in the diffraction pattern of chitosan hydogel extending over a large range of 2θ suggests that chitosan is present in amorphous state in the film. X-ray diffraction patterns of paclitaxel and chitosan-PMAA hydrogel film were obtained and compared, which revealed marked differences in the molecular state of paclitaxel (Fig. 5). X-ray diffractogram of paclitaxel and chitosan-PMAA hydrogel film shows several high-angle diffraction peaks were observed at the following 2θ values: 24.1°, 27.4°, 29.2°, 36.4°, 40.3°, and 44.6°. The 29.6° 2θ peak had the highest intensity as observed for hydrogel film.

4. Discussion

In order to develop a local delivery system for paclitaxel, a biodegradable N-chitosan-PMAA bead was attempted, which to our knowledge in the first effort of its kind. Potential formulation problems were anticipated since chitosan is only soluble in aqueous acidic solutions, whereas paclitaxel, being a hydrophobic drug, is insoluble under similar conditions. In early stages of formula optimization studies, it was observed that paclitaxel was incorporated into bead. The primary mechanisms for release of drugs from matrix systems in vitro are swelling, diffusion, and disintegration. In vitro degradation of chitosan-PMAA nanoparticles prepared by solution casing method occurred less rapidly as the degree 73% deacetylated showed slower biodegradation. Since the grade of chitosan used in the present study was of high molecular weight with a degree of deacetylation ≥85%, significant retardation of release of paclitaxel from nanoparticles is attributed to the polymer characteristics. In addition, diffusion of paclitaxel may have been hindered by increased tortuosity of polymer accompanied by a swelling mechanism.

Fig. 4 shows the SEM of paclitaxel and chitosan-PMAA hydrogel film that synthesized by chemical reaction. This hydrogel is very sensitive to the temperature that due to the interaction of electron and sample. Scanning electron micrography images were obtained from a diluted solution of the paclitaxel particle. The white spots are paclitaxel nanoparticles. The SEM image shows the presence of paclitaxel spherical particles in hydrogel matrix, which are homogenenously distributed throughout the hydrogel, which is also confirmed from ^{1}H NMR studies.

As observed from SEM photomicrographs, the crystals of paclitaxel have a different appearance than recrystallized paclitaxel. These nanoparticles do not have clearly defined crystal morphological features in the SEM photomicrographs. Hence, it appears that the irregularly shaped particle are surface deposited with poloxamer, which gives them an appearance resembling that of coated particles. X-ray diffraction is also used to study the degree of crystallinity of pharmaceutical drugs and excipients. A lower 2θ value indicates larger d-spacings, while an increase in the number of high-angle reflections indicates higher molecular state order. In addition, broadness of reflections, high noise, and low peak intensities are characteristic of a poorly crystalline material. A broad hump in the diffraction pattern of chitosan extending over a large range of 2θ suggests that chitosan is present in amorphous state in the nanoparticles.

5. Conclusions

The swelling and hydrolytic behavior of the hydrogel beads was dependent on the content of MAA groups and caused a decrease in gel swelling in SGF or an increase in gel swelling in SIF. Modified chitosan with different contents of MAA and CA by graft copolymerization reactions were carried out under microwave-radiation. The swelling of the hydrogels was dependent on the content of MAA groups and caused a decrease in gel swelling in SGF or an increase in gel swelling in SIF. Incorporation of MAA made the hydrogels pH-dependent and the transition between the swollen and the collapsed states occurred at high and low pH. The swelling ratios of the hydrogels beads increased at pH 7.4, but decreased at pH 1 with increasing incorporation of MAA.

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