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Preparation and Evaluation of Chitosan/Carrageenan Beads for Controlled Release of Sodium Diclofenac

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ABSTRACT

The polyelectrolyte complex (PEC) hydrogel beads based on chitosan (CS) and carrageenan (CR) have been studied as a controlled release device to deliver sodium diclofenac (DFNa) in the simulated gastrointestinal condition. Various factors potentially influencing the drug release (ie, CS/CR proportion, DFNa content, types and amount of cross-linking agents) were also investigated. The optimal formulation was obtained with CS/CR proportion of 2/1 and 5% (wt/vol) DFNa. The controlled release of the drug from this formulation was superior to other formulations and was able to maintain the release for ~8 hours. Upon cross-linking with glutaric acid and glutaraldehyde, the resulting beads were found to be more efficient for prolonged drug release than their non-cross-linking counterparts. The bead cross-linked with glutaraldehyde was able to control the release of the drug over 24 hours. The difference in the drug release behavior can be attributed to the differences in ionic interaction between the oppositely charged ions and to the concentrations of the drug within the beads, which depends on the compositions of the formulation and the pH of the dissolution medium. The release of drug was controlled by the mechanism of the dissolution of DFNa in the dissolution medium and the diffusion of DFNa through the hydrogel beads.

KEYWORDS: Sodium diclofenac, chitosan/carrageenan, controlled delivery, hydrogel bead.

INTRODUCTION

The use of hydrogel systems for controlling the release of drugs has increasingly become important in the formulation of pharmaceuticals. It is well known that hydrogel can respond to surrounding conditions such as pH, ionic strength, temperature, and electric current. The pH-sensitivity of hydrogel is an important factor in designing polymers for controlled drug release in the gastrointestinal tract, which has a variation of pH from the stomach to the intestine. Hydrogels from natural polymers, especially polysaccharides have been widely used because of their advantageous properties such as nontoxicity, biocompatibility, and biodegradability.[1] Among these polymers, chitosan, the N-deacetylated product of the polysaccharide chitin, has gained considerable attention. In recent years, chitosan has been proposed as a useful excipient for obtaining sustained release of water-soluble drugs[2] and for enhancing the bioavailability of poorly water-soluble compounds.[3] Moreover, chitosan has also been presented as a useful polymer for colon-specific drug delivery because it is biodegradable by colonic bacteria.[4] These properties have been considered for using this polymer to control the release of drugs for oral administration.

According to previous studies, polyelectrolyte complex (PEC) in the form of beads and microspheres that are formed by cationic polymer(s) and anionic polymer(s) could enhance the controlled or prolonged release of a drug. Examples of PEC for controlling drug release include alginate/chitosan,[5] chitosan-cellulose multicore microparticles,[6] chitosan-coated pectin,[7] chitosan/poly(acrylic acid) complexes,[8] poly(vinyl alcohol)/sodium alginate blend beads,[9] poly(methacrylic acid-g-ethylene glycol) particles.[10]

Carrageenan is an anionic polymer extracted from marine red algae. Its structure is a linear heteropolysaccharide with ester sulfate groups. The main chain consists of alternative copolymer of 1,4-α and 1,3-β-D-galactopyranose and 3,6-anhydro-D-galactopyranose. Because of its gelling, viscosity enhancing, and proven safety properties,[11] carrageenan can be used as a sustained-release composition.

Chitosan/carrageenan PEC has been developed for controlled release of drugs. Tomida et al[12] suggested that κ-carrageenan/chitosan membrane spherical capsules could release theophylline as a model drug from the capsules. It is noted that this model drug demonstrates zero-order kinetics.

Tapia et al[13] evaluated the possibility of using mixtures and/or polyelectrolyte complexes of chitosan/carrageenan in a tablet form as a prolonged drug release system, using diltiazem hydrochloride as a model drug. The release of the drug was

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controlled by the capacity of carrageenan to promote the entry of water into the matrices.

Genta et al[14] described that glutaraldehyde as a cross-linking agent for chitosan can moderate the release of theophylline from chitosan microspheres. Bodnar et al[15] investigated novel biodegradable nanoparticles based on chitosan for biomedical application using natural dicarboxylic and tricarboxylic acid for intramolecular cross-linking of the chitosan linear chains.

Sodium diclofenac (DFNa, $C_{14}H_{10}Cl_2NO_2Na$) is a widely used nonsteroidal anti-inflammatory drug (NSAID) that exhibits antirheumatic, analgesic, osteoarthritis, and antipyretic activities. It has a short half-life in plasma (1-2 hours). The daily dose varies between 75 and 200 mg/person, given in 3 or 4 divided portions depending on the route of administration. The most common adverse effects of the drug are gastritis, peptic ulceration, and depression of renal functions.[13,16] Because of the short biological half-life and associated adverse effects, it is considered an ideal model drug for controlled drug delivery.

The purpose of this study was to prepare and evaluate the chitosan/carrageenan gel beads as a new controlled drug release system for diclofenac. Another purpose is to investigate the conditions in which the polymer bead is formed, and hence the dependence of the drug release on bead formation. This study reports the effects of matrixing agents on in vitro dissolution profile of the controlled-release bead of DFNa, in comparison with commercial capsule dosage forms.

Materials and Methods

Materials

The following materials were obtained from the indicated suppliers and used as received: sodium diclofenac (available from Center for Chitin-Chitosan Biomaterial, Bangkok, Thailand); chitosan (food grade, deacetylation 90% minimum, molecular weight (MW) 50 000 to 300 000, BFM, Bangkok, Thailand); carrageenan (food grade, KL-805 type, Union Chemical, Bangkok, Thailand); hydrochloric acid 35%; sodium chloride; sodium hydroxide; sodium hydrogen phosphate; potassium chloride; potassium dihydrogen phosphate; and potassium bromide (Merck & Co, Whitehouse Station, NJ), acetic acid glacial (Scharlau, Barcelona, Spain), glutaric acid (Aldrich, St Louis, MO) and glutaric dialdehyde solution in water 25% (Acros Organics, Geel, Belgium).

Hydrogel Beads Preparation

Coagulant Conditions

The coagulant conditions were determined to prepare the well-formed beads with high loading efficiency. A mixture of 1:1 (wt/vol) chitosan:carrageenan containing DFNa 1% (wt/vol) was dropped through an 18-gauge needle into coagulant solutions that vary in concentrations of NaOH (2.5%-7.5% wt/vol) and KCl (0-0.5 M). The formed gel beads were left in the solution with various immersion times (3 hours, 5 hours, and overnight) and temperatures of coagulant (10°C and room temperature). After gelation process, the beads were filtered, rinsed with water, and freeze-dried for 24 hours. The DFNa contents in the coagulant solutions and in the beads were determined by UV-Visible spectrophotometer at 276 nm. All experiments were performed in triplicate. The conditions of coagulant in this preliminary study were evaluated by drug loading efficiency (LE) according to the following equation:

$$LE(\%) = \frac{The\ Drug\ Given - The\ Drug\ Loss}{The\ Drug\ Given} \times 100\% \quad (1)$$

Once the optimum coagulant condition was obtained, this condition was used for the following preparation hydrogel beads.

Preparation of the Hydrogel Beads With Various Ratios of Chitosan:Carrageenan

To the solution of 2.5% wt/vol carrageenan dissolved in deionized water at 70°C± 5°C, a constant 1% (wt/vol) of DFNa was added. After the drug was thoroughly dissolved, the solution of 2.0% (wt/vol) chitosan in 2.0% (vol/vol) acetic acid was added to the mixture of carrageenan and DFNa solution at the specific chitosan:carrageenan ratio (wt/wt) of 1:0, 3:1, 2:1, 1:1, 1:2, 1:3, and 0:1. Then, the volume was adjusted to 40 mL for each formulation. The mixtures were further stirred until becoming homogeneous.

Forty milliliters of the mixture (with the exception of the formulation of chitosan solution [A] and carrageenan solution [G]) was extruded in the form of droplets, using an 18-gauge needle, into 100 mL of 0.3 M KCl/5.0% (wt/vol) NaOH as coagulant solution. The formulation of chitosan solution (A) and carrageenan solution (G) were extruded into 5% NaOH solution and 0.3 M KCl solution, respectively. The solutions were maintained at 10°C for 5 hours to let the beads hardened. Then, the beads were filtered and washed with cold deionized water to remove excess NaOH and potassium ion. Finally, the hydrogel beads were freeze-dried at −42°C for 24 hours.

Preparation of the DFNa-Loaded Beads With Various DFNa Content

As a result of the %LE obtained from the beads prepared in the prior study, the 2:1 chitosan:carrageenan yielded the best release behavior. Therefore, this ratio was selected for further study by varying the DFNa content of 1% to 5% wt/vol

Table 1. Drug Loading Efficiency of the Chitosan/Carrageenan Bead in Preliminary Study of Coagulant Solutions*

Formulation	NaOH Concentration (%wt/vol)	KCl Concentration (M)	Temperature (°C)	Time (hours)	%LE† ± SD
P1	2.5	—	10	5	88.4 ± 0.2
P2	5.0	—	10	5	94.9 ± 0.3
P3	7.5	—	10	5	96.5 ± 0.5
P4	5.0	—	RT	5	88.1 ± 0.3
P5	5.0	0.1	10	5	95.6 ± 0.1
P6	5.0	0.3	10	5	96.2 ± 0.9
P7	5.0	0.5	10	5	96.2 ± 0.3
P8	5.0	0.3	10	3	—‡
P9	5.0	0.3	10	overnight	91.7 ± 0.2

*LE indicates loading efficiency; SD, standard deviation; RT, room temperature.

†Determined using the indirect method.

‡Not determined because the beads were broken after being dried.

(referred to as Formulation C, I to L, given in Table 1). The hydrogel bead preparations were performed by using the same manner as described above.

Preparation of the Cross-Linking Hydrogel Beads

The effect of types and amounts of cross-linking agents (Formulations M to S) were also studied based on the best formulation obtained from the previous study. The detailed compositions of each formulation are given in Table 2. In this case, 2 methods for cross-linking the hydrogel beads were investigated: the first method was to cross-link the hydrogel before forming hydrogel beads in a coagulant solution; the second method was to mix a cross-linking agent with a coagulant solution before forming hydrogel beads in the solution.

Table 2. Properties of Various Chitosan/Carrageenan Beads*

Formulation	CS:CR Ratio	DFNa Content (%wt/vol)	Cross-Linking Agent (%wt/vol)		Bead Size ± SD (mm)	%EE ± SD
			GA	GD		
Non-Cross-Linked Beads						
A	1:0	1	—	—	2.1 ± 0.2	79.6 ± 1.6
B	3:1	1	—	—	2.4 ± 0.1	89.70 ± 0.7
C	2:1	1	—	—	2.4 ± 0.1	84.7 ± 2.0
D	1:1	1	—	—	2.5 ± 0.3	89.2 ± 0.3
E	1:2	1	—	—	ND	—†
F	1:3	1	—	—	ND	—†
G	0:1	1	—	—	ND	—†
H	2:1	—	—	—	2.3 ± 0.2	—‡
I	2:1	2	—	—	2.0 ± 0.3	96.9 ± 1.9
J	2:1	3	—	—	2.2 ± 0.2	76.4 ± 3.5
K	2:1	4	—	—	2.3 ± 0.1	89.3 ± 8.4
L	2:1	5	—	—	2.7 ± 0.2	77.7 ± 8.5
Cross-Linked Hydrogel Solution Before Dropping Beads (The first method)						
M	2:1	5	1.00	—	ND	—†
N	2:1	5	—	1.00	ND	—†
Cross-Linked Beads in Coagulant (The second method)						
O	2:1	5	0.25	—	2.6 ± 0.2	90.3 ± 0.2
P	2:1	5	0.50	—	2.6 ± 0.2	84.0 ± 1.4
Q	2:1	5	0.75	—	2.6 ± 0.4	92.0 ± 1.4
R	2:1	5	1.00	—	2.9 ± 0.2	93.6 ± 0.7
S	2:1	5	—	5.00	3.1 ± 0.1	65.4 ± 2.5

*CS indicates chitosan; CR, carrageenan; DFNa, sodium diclofenac; GA, glutaric acid; GD, glutaraldehyde; SD, standard deviation; %EE, percentage of encapsulation efficiency; —, components not included in formulations; ND, not determined.

†Not determined because the beads were not successfully prepared.

‡The bead without DFNa.

In the first method (Formulations M and N), 1.00% (wt/vol) of either glutaric acid or glutaraldehyde as a cross-linking agent was added to the mixture of 2:1:5 chitosan:carrageenan: drug hydrogel. The bead preparations were carried on in the same manner as the previous method.

In the second method (Formulations O to S), 1.00% (wt/vol) of either glutaric acid or glutaraldehyde as a cross-linking agent was added into a coagulant solution containing 0.3 M KCl/5.0% (wt/vol) NaOH. The beads were formed in the same manner as the previous method.

Observation of Scanning Electron Microscope

The surfaces and cross-section morphologies of the beads were observed using a microscope and a scanning electron microscope (SEM) (JSM-5800 LV, JEOL, Tokyo, Japan). In preparation of SEM examination, the samples were mounted on metal grids and coated by gold under vacuum before observation. The photomicrographs were taken at different magnifications.

Measurement of Fourier Transform Infrared Spectroscopy

The infrared spectra of all formulations were recorded with Fourier transform infrared spectroscopy (FTIR) (Impect 4.1, Nicolet ThermoFisher Scientific, Waltham, MA). FTIR spectra were taken in the wavelength region 4000 to 400 cm^{-1} at ambient temperature.

Measurement of Differential Scanning Calorimetry

The thermal behavior of the different bead components was characterized by differential scanning calorimetry (DSC) (DSC7, NETZSCH Inc, Exton, PA). Approximately 3 to 6 mg of the dried beads were weighed into an aluminum pan. The samples were heated from 25°C to 350°C at a heating rate of 10°C/min.

Measurement of Thermogravimetric Analysis

The compositions of the beads were determined by thermogravimetric analysis (TGA) (409 C/CD, NETZSCH). The weight of the dried beads for the TGA experiment was between 13 and 16 mg. The experiments were conducted using closed aluminum pans with a cover hole. The sample was examined under a nitrogen flow rate of 20 mL/min at a scan rate 10°C /min with the range from 25°C to 550°C.

Determination of Encapsulation Efficiency

The drug content in the DFNa-loaded hydrogel beads was quantitatively determined by immersing the dried beads (100 mg) in 250 mL of phosphate buffer saline pH 7.4 to dissolve the drug dispersed inside the beads.[16] After sonication, the solution was collected and the drug content entrapped inside the beads was determined by UV-Vis spectrophotometry at 276 nm. The encapsulation efficiency (EE) was calculated according to the following equation. All experiments were performed in triplicate.

$$EE(\%) = \frac{Actual\ Drug\ Content}{Theoretical\ Drug\ Content} \times 100\% \quad (2)$$

Swelling Study

The swelling behavior of the chitosan/carrageenan beads were studied in 3 dissolution systems as follows: 0.1N HCl (pH 1.2), phosphate buffer saline pH 7.4, and the pH-change system.[17,18]

Beads were immersed in either 0.1N HCl (pH 1.2) or phosphate buffer saline pH 7.4. The diameter of swollen beads was determined for 5 hours: every 10 minutes for the first 30 minutes, every 15 minutes for the next 1 hour, every 30 minutes until the third hour, and then every hour after that.

For the pH-change system, the beads were immersed in 0.1N HCl (pH 1.2) for 2 hours. Then, the dissolution medium was changed to phosphate buffer saline pH 7.4. In this solution, the diameters of the beads were observed for 5 hours.

The swelling behavior was determined by measuring the change of the diameter of the bead using a microscope with a micrometer. The swelling ratio for each sample determined at time t was calculated using the following equation.[19]

$$S_w = \frac{D_t}{D_0} \quad (3)$$

where D_t is the diameter of the beads at time (t), and D_o is the initial diameter of the dried beads.

The experiments were performed in triplicate and represented as a mean value.

In Vitro Release Study

The DFNa release study of the beads from each formulation was performed in the simulated gastrointestinal condition by the pH-change method at 37°C.[5,10] The media of pH 1.2 (0.1N HCl) was chosen to represent the gastric condition; pH 6.6 was a compromise condition between the pH of the gastric and the small intestine, and the condition in the small intestine was represented by pH 7.4. One hundred milligrams of beads were enclosed in a teabag and placed into a beaker that contained 250 mL of the dissolution medium. The beaker was placed on a horizontal shaking water bath at a speed of 50 rpm and incubated at 37°C±2°C. In the dissolution model

with pH-change, the pH of the dissolution medium was kept at 0.1N HCl (pH 1.2) for the first 2 hours (at 30-minute time intervals). Then, the dissolution medium was changed to phosphate buffer saline pH 6.6 for 1 hour (at 15-minute time intervals). At different time intervals, 5 mL of the dissolution medium was withdrawn. Finally, the release dissolution medium was changed to pH 7.4 and maintained up to 24 hours. In this solution, at various time intervals, 2 mL of the dissolution medium was withdrawn. Each sample solution was centrifuged and diluted to a suitable concentration if necessary. The release rate of DFNa was assayed by UV-Vis spectrophotometry at 276 nm. All experiments were performed in triplicate. The amount of DFNa released was calculated by interpolation from a calibration curve containing increasing concentrations of DFNa. A cumulative correction was made for the previously removed sample to determine the total amount of drug release.

Results and Discussion

Coagulant Conditions

The percentage of loading efficiency (%LE) in each chitosan/carrageenan bead formulation is given in Table 1. The results indicated that %LE of the beads increased along with increasing NaOH concentration (Table 1, Formulations P1 to P3). In spite of this, the concentration of NaOH solution should not exceed 7.5% (wt/vol) as it becomes difficult to remove excess NaOH and also difficult to neutralize it. Therefore, in this study, the optimum concentration of NaOH was 5.0% (wt/vol). The temperature of the coagulant solution maintained at 10°C, the %LE (94.9%) was better than the one maintained at room temperature (88.1%).

For the effects of KCl concentration in the coagulant solution, the concentrations of KCl were varied from 0 to 0.5M in 5% NaOH, at 10°C for 5 hours (Table 1, Formulations P2, P5, P6, and P7). The results showed that the highest %LE (96.2%) was obtained after 0.3M KCl was added (Table 1, Formulation P6), corresponding to the preparation of pure carrageenan beads reported by Cassidy et al[20] and López et al.[21]

Furthermore, the %LE also depends on the immersion time. The results showed that the beads immersed in the coagulant for 5 hours produced the most well-formed beads and also showed the least drug loss. Thus, the suitable coagulant condition used in the further experiment was 5% NaOH/0.3M KCl maintained at 10°C and the bead immersion time of 5 hours.

Characterization and Physical Properties

Morphology

The observation of size, shape, and surface topography of the dried beads was done by SEM. The size of the beads for each formulation ranged from 2 to 3 mm (Table 2), depending on the effect of the compositions of each formulation.

The SEM photomicrograph of the beads with chitosan:carrageenan ratio of 1:1 and 2:1 containing 1% (wt/vol) of DFNa are shown in Figure 1A, B, C, and D. The shapes of the beads were not completely spherical, and the surface was rough and folded; these findings are because the beads shrank during the freeze-drying process. Furthermore, the cross-section view showed that the cavities inside the beads increased when the ratio of carrageenan in the beads increased. Figure 1E and F show the SEM photomicrographs of beads with 2:1 chitosan:carrageenan containing 2% (wt/vol) of DFNa. When the DFNa content was varied, a higher content of DFNa led to an increase in the size of the beads, and their surface was covered with irregular crystals of DFNa.

The photomicrographs of the non-cross-linked bead, the bead cross-linked with 0.75% (wt/vol) glutaric acid, and 5.00% (wt/vol) glutaraldehyde are shown in Figure 2A to 2C. The beads cross-linked with a glutaric acid at different concentrations showed irregular shapes and rough moon-like surfaces. Meanwhile the bead cross-linked with 5.00% (wt/vol) glutaraldehyde exhibited spherical shape and larger size than

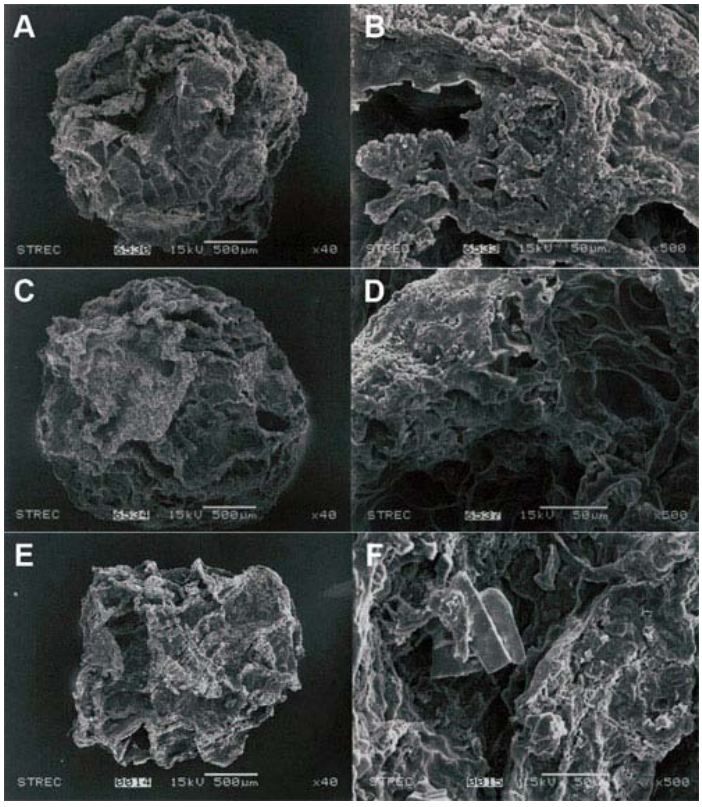

Figure 1. Scanning electron photomicrographs (×40) and the cross-section (×500) of the beads with CS:CR ratio of (A-B) 1:1, 1% wt/vol DFNa; (C-D) 2:1, 1% (wt/vol) DFNa; (E-F) 2:1, 2% wt/vol DFNa. CS indicates chitosan; CR, carrageenan; DFNa, sodium diclofenac.

Figure 2. Scanning electron photomicrographs (×40) of the 2:1 CS:CR with 5% wt/vol DFNa beads: (A) non-cross-linked; (B) cross-linked with 0.75% wt/vol glutaric acid; (C) cross-linked with 5.00% wt/vol glutaraldehyde. CS indicates chitosan; CR, carrageenan; DFNa, sodium diclofenac; GA, glutaric acid; GD, glutaraldehyde.

the others, presumably because the bead slightly shrunk during the freeze-drying process. The surface of the bead was smooth and had less porous than the others, resulting in the difficulty for dissolution medium to penetrate into the bead. Therefore, this may also reduce the efficiency of the drug release.

FTIR spectroscopy

The IR spectrums are shown in Figure 3. The IR spectrum of chitosan/carrageenan beads (Figure 3C) showed a new absorption band at 1447 cm^{-1} which is assigned to $–NH_3^+$ group. A decreased in intensity of $–SO_4^{2–}$ group absorption band at 1018 cm^{-1} is the evidence of the forming of strong polyelectrolyte complex (PEC).

DFNa (Figure 3D) showed that the principle IR peaks at 1280 and 1303 cm^{-1} resulted from C-N stretching and the peak at 1501 and 1571 cm^{-1} resulted from C=C stretching and C=O stretching of carboxylate group, respectively. The IR peaks of DFNa/chitosan/carrageenan beads (Figure 3E) displayed a combination of the unshifted principal peaks from both polymer matrix and the drug. It could be concluded that interactions between the polymer matrix and the drug were unlikely to occur.

The IR spectrum of the DFNa-loaded chitosan/carrageenan bead cross-linked with glutaric acid (Figure 3F) showed an increase in peak intensity of the C=O group at 1575 cm^{-1} and a decrease in peak intensity of $–NH_3^+$ group at 1451 cm^{-1}, which indicated the ionic cross-linking between $–NH_3^+$ groups of chitosan and $–COO^–$ groups of glutaric acid.

The IR spectrum of the DFNa-loaded chitosan/carrageenan bead cross-linked with glutaraldehyde (Figure 3G) showed a decrease in peak intensity of $–NH_3^+$ of chitosan at 1451 cm^{-1} and a new peak at 1692 cm^{-1} appeared. These results indicate the formation of imine linkage (C=N) between the amino groups of chitosan and the carbonyl groups of glutaraldehyde.

Differential Scanning Calorimetry

The DSC thermograms of the pure chitosan, the pure carrageenan, and the chitosan/carrageenan beads are shown in Figure 4A, B and C. The pure chitosan bead revealed an exothermic peak at 262°C. This peak represents the degradation

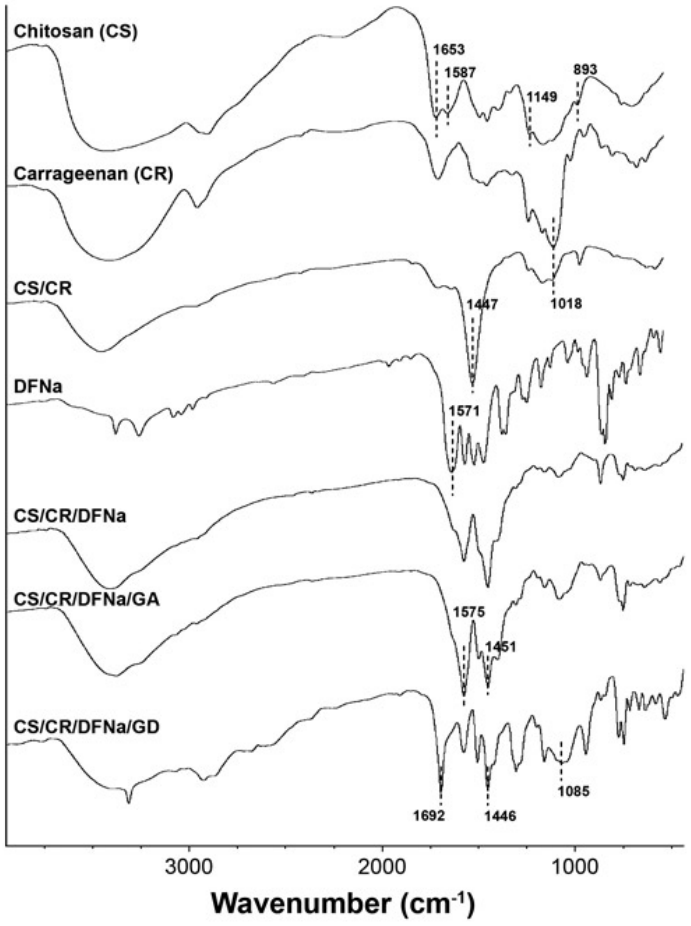

Figure 3. Fourier transform infrared spectroscopy of the beads: (A) chitosan; (B) carrageenan; (C) CS/CR; (D) DFNa; (E) CS/CR/DFNa; (F) CS/CR/DFNa/GA; and (G) CS/CR/DFNa/GD. CS indicates chitosan; CR, carrageenan; DFNa, sodium diclofenac; GA, glutaric acid; GD, glutaraldehyde.

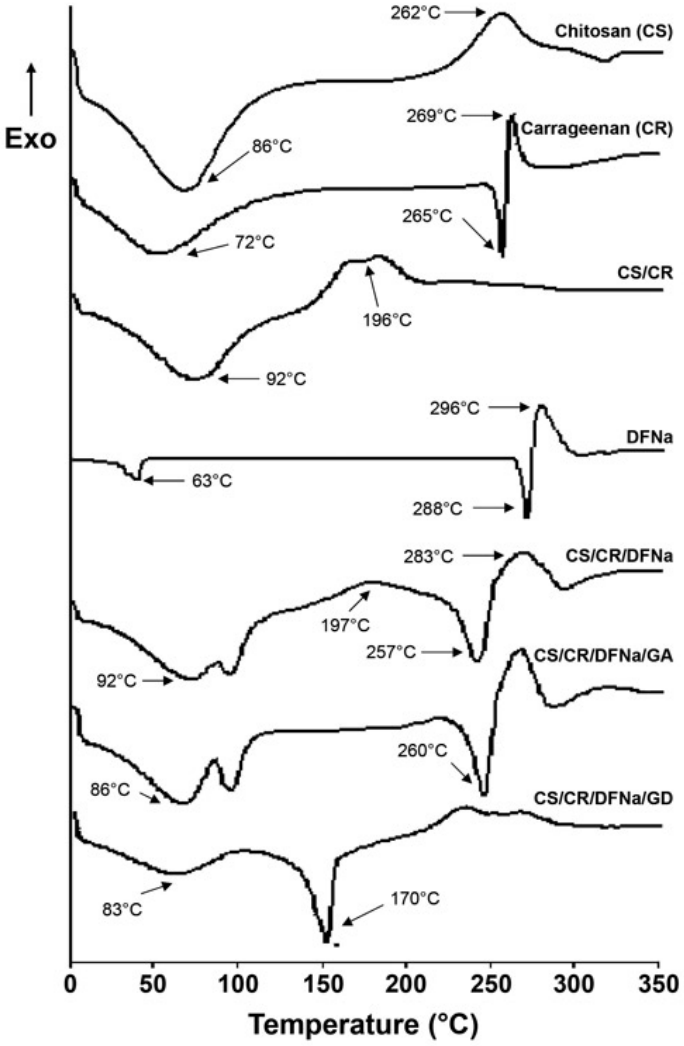

Figure 4. Differential scanning calorimetry thermograms of (A) chitosan; (B) carrageenan; (C) CS/CR; (D) DFNa; (E) CS/CR/DFNa; (F) CS/CR/DFNa/GA; and (G) CS/CR/DFNa/GD. CS indicates chitosan; CR, carrageenan; DFNa, sodium diclofenac; GA, glutaric acid; GD, glutaraldehyde.

of chitosan. The pure carrageenan bead showed a sharp endothermic peak at 265°C before fusion with degradation at 269°C. The chitosan/carrageenan bead showed a lower exothermic peak than the pure chitosan and pure carrageenan bead at 196°C. This phenomenon indicated that the PEC between chitosan and carrageenan formed. Similarly, the thermal stability behavior was observed by Maciel et al.[22]

The DSC thermogram of pure DFNa showed 2 endothermic peaks (Figure 4D). The first small endothermic peak at 63°C was due to water loss. The second sharp endothermic peak at 288°C and an exothermic peak at 296°C indicated the fusion of the solvated crystals and the oxidation reaction between DFNa and oxygen in air environment fusion, respectively.[23]

The DSC thermogram of the DFNa-loaded chitosan/carrageenan bead showed that the characteristic peak of the chitosan/carrageenan bead and DFNa were also still presented but slightly shifted from their original positions (Figure 4E). That finding indicated that the interaction between drug and polymer bead did not occur.

The characteristic peak of the non-cross-linked bead and the cross-linked bead with glutaric acid showed no difference in the DSC thermogram pattern, except that the exothermic peak of polymer bead at 197°C had disappeared (Figure 4F and G). This finding indicated that the anionic interaction between chitosan and glutaric acid had occurred. Meanwhile, the bead cross-linked with glutaraldehyde showed a different pattern of the DSC thermogram with a broad endothermic peak at 83°C and a new endothermic peak at 170°C. Moreover, the decomposition peak of both polymer and DFNa had disappeared. These results indicated that the chemical interaction between glutaraldehyde and the compositions inside the bead were rather strong.

The observation of FTIR spectroscopy conformed to the results of the DSC thermograms. The PEC occurred when chitosan combined with carrageenan, while there was no interaction between the drug and the PEC. Furthermore, the results showed that the beads cross-linked with glutaraldehyde by covalent cross-linking, and cross-linked with glutaric acid by ionic cross-linking.

Thermogravimetric Analysis

The TGA of substances and beads is shown in Table 3. The pure chitosan beads showed a weight loss of ~22% (40°C to 153°C). Afterwards, the degradation of the chitosan occurred in the temperature range from 157°C to 550°C with the additional derivative thermogravimetric (DTG) peak at 19°C. The degradation of the pure carrageenan beads was presented in the temperature range from 200°C to 550°C with the additional peak of the DTG at 258°C.

The DTG peak of chitosan/carrageenan beads showed decreasing temperature from 191°C to 177°C and from 278°C to 231°C. This finding might be due to the degradation chitosan and carrageenan undergo to become the polyelectrolyte complex, and because the thermal stability of PEC was less than pure chitosan and carrageenan.

The TG curve of the DFNa-loaded chitosan/carrageenan showed the combination of polymer and drug in 3 degradation stages. The first stage was due to the loss of water. The second stage was due to the degradation of the polymer. The third stage in the temperature range from 232°C to 310°C with a peak in the first derivative at 278°C was due to the degradation of DFNa together with the polymer bead. The different decreasing slope of TG curve indicated that there was no interaction between the polymer bead and the drug.

Table 3. Thermogravimetric Analysis of Substances and the Beads Obtained by Varying Compositions*

Composition	Temperature Range (°C)	Weight Loss (%)	DTG Peak (°C)
Pure CS bead	40-153	22	91
	157-550	19	191
Pure CR bead	35-220	9	74
	200-550	43	258
DFNa	35-97	2	58
	220-310	44	30
CS/CR (Formulation H)	40-201	22	113
	201-550	26	231
CS/CR/DFNa (Formulation L)	40-130	7	110
	130-232	3	194
	232-550	22	278
CS/CR/DFNa/GA (Formulation O)	40-134	4	106
	134-550	30	280
CS/CR/DFNa/GD (Formulation S)	40-113	2	75
	113-281	28	233
	281-550	34	375

*DTG indicates derivative thermogravimetric; CS, chitosan; CR, carrageenan; DFNa, sodium diclofenac; GA, glutaric acid; GD, glutaraldehyde.

The DTG peak of polymer at 194°C disappeared in the DFNa-loaded chitosan/carrageenan cross-linked with 0.25% glutaric acid. The results indicated that glutaric acid formed a strong interaction with chitosan, which caused an increase in temperature of the polymer and possibly combined with the degradation stage of DFNa at 280°C.

The degradation of the DFNa-loaded chitosan/carrageenan with 5.00% glutaraldehyde showed a weight loss at the high temperature of 375°C, which could suggest that glutaraldehyde formed a very strong interaction with chitosan.

Encapsulation Efficiency of Beads

The percentages of encapsulation efficiency (%EE) of the DFNa-loaded beads prepared from various compositions are shown in Table 2. The %EE was obtained in the range of 65% to 97%. Formulation I gave the highest %EE of 96.9% and Formulation S gave the lowest %EE of 65.4%. The chitosan/carrageenan beads showed the %EE to be around 84.7% to 89.7%, which was higher than the pure chitosan bead (Formulation A, 79.6%). The results might indicate that the $-SO_4^{2-}$ group of carrageenan exhibited electrostatic interactions with the $-NH_3^+$ group of chitosan in the PEC form, resulting in the drug entrapped in PEC forming better than the pure chitosan bead. For Formulations E to G, the %EE of the beads could not be determined because the obtained beads were fragile, and the shape was not well formed, hence they were broken into pieces after being dried.

The %EE was increased from 84.7% to 96.9% when the DFNa content was increased from 1% to 2% (wt/vol). It was because the drug was better entrapped in the viscous hydrogel. But when the drug content was increased from 3% to 5% (wt/vol), the %EE was decreased from 76.4% to 89.3%. This is probably because the excess drug could not be entrapped in the beads.

The effect of cross-linking agent concentration on the %EE was shown in Formulations M to S. The %EE of beads from Formulation M to N could not be determined because the beads could not be formed successfully owing to the extremely high viscosity of the hydrogel solution. The % EE of the cross-linked beads with glutaric acid at 0.25% to 1.00% (wt/vol) of 84.0% to 93.6%, respectively, was higher than that of the non-cross-linked beads (77.7%). The high %EE is likely the result of the formation of strong ionic cross-link between glutaric acid and chitosan.

The cross-linked bead with glutaraldehyde showed that the %EE of 65.4% was less than that of the non-cross-linked bead (77.7%). This result is because glutaraldehyde formed a covalent cross-link with chitosan, which consequently made the bead more rigid and decreased the free volume space within the bead, thus reducing the %EE.

Swelling Study

The swelling behavior study of the bead was performed in 3 dissolution systems: (1) 0.1N HCl (pH 1.2); (2) phosphate buffer saline pH 7.4; and (3) the pH-alternating system. The chitosan:carrageenan (CS:CR, 2:1) bead with 5% (wt/vol) DFNa content was selected for this preliminary test because it showed an optimum DFNa release profile.

The swelling ratio of the bead in 0.1N HCl (pH 1.2), phosphate buffer saline pH 7.4, and the pH-alternating system was not significantly changed. These results agree with Sakiyama et al who reported that the swelling of chitosan and carrageenan gel was not observed at pH below 9.[24]

The beads were not significantly swollen and eroded in the 3 dissolution systems. Thus, from these results, it could be assumed that the drug release was not under the control of the swelling behavior but rather was controlled by the dissolution of DFNa in the dissolution medium and diffusion of DFNa through polymer matrix.

Dissolution Study

Dissolution Profiles

The release profiles of DFNa from the commercial products and Formulations A to S in the pH change systems are shown in Figures 5, 6, and 7. The release profiles of DFNa exhibited a sigmoidal profile. From the figures, it is evident

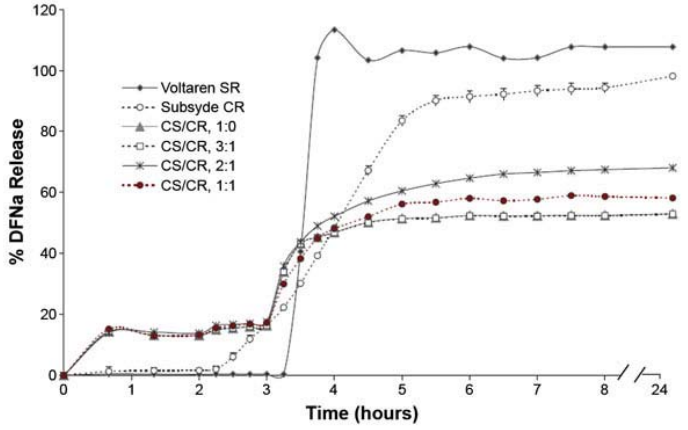

Figure 5. The dissolution profiles of sodium diclofenac (DFNa) from the beads with various chitosan:carrageenan (CS:CR) ratios in the pH-change system.

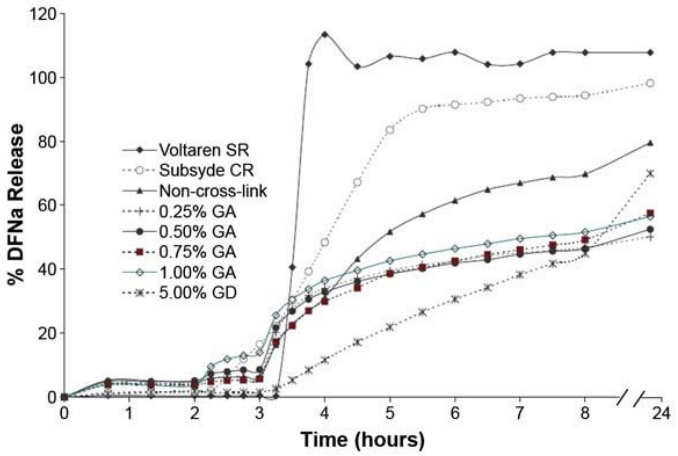

Figure 7. The dissolution profiles of sodium diclofenac (DFNa) from the chitosan:carrageenan (CS:CR, 2:1) beads with 5% (wt/vol) DFNa content prepared at different concentration of cross-linking agent in the pH-change system. CS indicates chitosan; CR, carrageenan; DFNa, sodium diclofenac; GA, glutaric acid; GD, glutaraldehyde.

that the release rate of DFNa is lower for all formulations than that of the commercial products. The DFNa release is low for the first 2 hours in acidic condition (HCl pH 1.2). At this pH, DFNa exists in its acidic form, which is well known to be practically insoluble in the stomach.[25,26] When the dissolution was changed to phosphate buffered saline pH 6.6 for 1 hour, the drug release slightly increased; this is possibly because the diclofenac acid was partially converted to diclofenac salt, which is a soluble form. Then, the beads were subjected to the phosphate buffer saline pH 7.4 and were continuously tested up to 24 hours. In pH 7.4, a rapid increase of the drug release from all formulations was clearly observed because an insoluble form of diclofenac was completely converted to a soluble form in this medium.

Moreover, the results obtained showed good consistency with the cross-linking results. When the beads were prepared without using a cross-linking agent, the dissolution medium could easily diffuse into the beads, and hence a higher amount of drug was released (around 15%) (Formulations A, B, C, and D). When using glutaric acid as a cross-linking agent (Formulations O, P, Q, and R), the cumulative DFNa release was reduced to less than 5%, and when the beads were cross-linked with glutaraldehyde (referred to as Formulation S), the released drug was less than 1.5%.

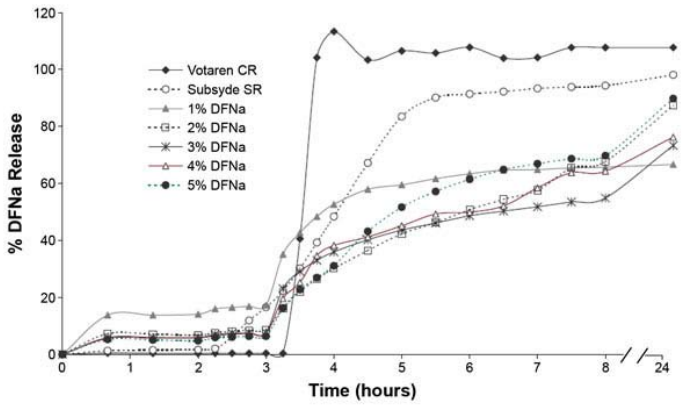

Figure 6. The dissolution profiles of sodium diclofenac (DFNa) from the chitosan/carrageenan (CS:CR, 2:1) beads with various drug content (%wt/vol) in the pH-change system.

Effect of Various Polymer Ratios on Dissolution Profiles

The dissolution profiles of DFNa from the beads with varying CS:CR ratios are shown in Figure 5. When the proportions of carrageenan in the formulations increased from the chitosan:carrageenan ratio of 3:1 to 2:1 and 1:1, the percentage of DFNa release within 24 hours increased from 52.9% to 68.0% and 58.2%, respectively. This finding indicated that the amounts of carrageenan had an effect on the drug release. Because carrageenan, a hydrophilic polymer, can promote the entry of the solution into the beads, it greatly improves the solubility of the drug and thus accelerates the dissolution. As for the formulation prepared using the CS:CR ratio of 1:1, the percentage of DFNa released within 24 hours was less than the CS:CR ratio of 2:1. The reason for this result was the difference in electrostatic interaction within the beads, which helped to control the drug release.

Hence, the CS:CR ratio of 2:1 was chosen for further bead preparation in the following studies as it showed the optimal release profile and the highest percentage of drug release within 24 hours.

Effect of Drug Content on Dissolution Profiles

Figure 6 shows the dissolution profiles of DFNa from the chitosan:carrageenan (ratio of 2:1) beads with varying DFNa

content (1%-5% (wt/vol)). In pH 1.2 and pH 6.6 medium, the percentage of drug released from Voltaren SR tablet (Novartis Canada, Dorval, Quebec, Canada) was negligible (less than 0.5%); whereas the CS:CR beads demonstrated a drug release ~5% to 7%. This difference might be attributed to the unencapsulated drug. In phosphate buffer saline pH 7.4, all formulations presented an initial burst effect due to the dissolution of drug from the surface. Afterwards, the drug was slowly released from the diffusion of drug through the hydrogel bead. After 24 hours of dissolution, the release of DFNa from all formulations was not complete. The reason might be because the polymer chains in the beads were entangled together with the strong ionic interactions. Thus, the penetration of dissolution medium into the hydrogel beads was difficult.

Effect of Cross-Linking Agent on Dissolution Profiles

The dissolution profiles of DFNa from the beads with varying concentrations of cross-linking agent are shown in Figure 7. The beads cross-linked with glutaric acid at different concentrations (0.25%-1.00% (wt/vol) showed no difference on drug release pattern. Due to the effect of the interactions between the $-NH_3^+$ groups of chitosan and the $-COO^-$ groups of glutaric acid, the amounts of drug release in the dissolution medium pH 7.4 from the cross-linked beads were lower than the non-cross-linked beads.

The beads cross-linked with 5.00% (wt/vol) glutaraldehyde showed the slowest drug release rate compared with other formulations. This finding might imply that the beads cross-linked with glutaraldehyde succeeded in the prolonging release behavior, which is very beneficial for maintaining the drug concentration in the therapeutic range.

CONCLUSION

This study showed that it is possible to control the release rate of diclofenac over a wide time scale by using the 2:1 chitosan: carrageenan with 5% (wt/vol) DFNa. The beads cross-linked with glutaric acid showed better results than the non-cross-linked beads, and the beads cross-linked with glutaraldehyde were the best with regards to the effectiveness for prolonged release of the drug over 24 hours. It was also observed that the release of diclofenac is slower in pH 1.2 and pH 6.8 and much higher in pH 7.4. This finding showed that the release system is effective as a controlled release system for colon-specific drug delivery.

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