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Research Article

Chitosan/Polyethylene Glycol Beads Crosslinked with Tripolyphosphate and Glutaraldehyde for Gastrointestinal Drug Delivery

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Abstract. This study reports on the preparation of chitosan (CS)/polyethylene glycol (PEG) hydrogel beads using sodium diclofenac (DFNa) as a model drug. Following the optimization of the polymer to drug ratio, the chitosan beads were modified by ionic crosslinking with sodium tripolyphosphate (TPP). The CS/PEG/DFNa beads obtained from a (*w*/*w*/*w*) ratio of 1/0.5/0.5 with crosslinking in 10% (*w*/*v*) TPP at pH 6.0 for 30 min yielded excellent DFNa encapsulation levels with over 90% loading efficiency. The dissolution profile of DFNa from CS/PEG/DFNa beads demonstrated that this formulation was able to maintain a prolonged drug release for approximately 8 h. Among the formulations tested, the CS/PEG/DFNa (1/0.5/1 (*w*/*w*/*w*)) beads crosslinked with a combination of TPP (10% (*w*/*v*) for 30 min) and glutaraldehyde (GD) (5% (*w*/*v*)) were able to provide minimal DFNa release in the gastric and duodenal simulated fluids (pH 1.2 and 6.8, respectively) allowing for a principally gradual drug release over 24 h in the intestinal (jejunum and ileum) simulated fluid (pH 7.4). Thus, overall the CS/PEG beads crosslinked with TPP and GD look to be a promising and novel alternative gastrointestinal drug release system.

KEY WORDS: controlled release; chitosan/PEG beads; diclofenac sodium.

INTRODUCTION

The use of hydrogel systems for the controlled release of drugs has proven to be advantageous for many pharmaceutical formulations (1). The pH sensitivity of the hydrogel is one important factor in designing polymers for drug-controlled release in orally administered delivery vehicles, that is, within the gastrointestinal tract, which has a variation of pH from the strongly acidic stomach to the weakly alkaline intestine, as well as variation in catabolic enzymes, salts, and the level of secreted and ingested surfactants.

Hydrogels derived from natural polymers, especially polysaccharides, have been widely used due to their advantageous properties, such as their non-toxicity, biocompatibility, and biodegradability (1). Among these polymers, considerable interest in chitosan (CS), a copolymer of β-[1-4]-linked 2-acetamido-2-deoxy-D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, has occurred in recent years since this hydrophilic polymer has a good biocompatibility, biodegradability, and low toxicity, making it a potentially valuable device in the medical and pharmaceutical fields (2), while it is readily available, cheap, and sustainably produced.

Due to its cationic character, CS can readily form a polyelectrolyte complex (PEC) with anionic polymer(s) that can enhance the controlled or sustained release of a drug. Examples of PECs for controlling drug release include alginate/CS (3,4), CS/carrageenan (5), CS–cellulose multicore microparticles (6), CS-coated pectin (7), CS/poly(acrylic acid) complexes (8), poly(vinyl alcohol)/sodium alginate blend beads (9), and poly(methacrylic acid-*g*-ethylene glycol) particles (10).

In addition, the release rate of CS can be controlled by further crosslinking the matrix with certain chemical crosslinking agents, such as glutaraldehyde (GD) (11) and sodium hydroxide (12), or by using ionic crosslinking interactions with tripolyphosphate (TPP) (13). The CS beads prepared with TPP were shown to increase the drug loading efficiency and prolong the drug release period. For example, Ko *et al*. (14) demonstrated that CS/TPP microparticles could potentially be an interesting device as a drug delivery system. Moreover, CS hydrogel microspheres crosslinked with TPP and dextran sulfate were shown to successfully deliver a hydrophobic drug to the intestine without any significant level of prior loss in the stomach (15). Additionally, CS/TPP beads have been reinforced by other polymers to improve their properties as controlled release devices as well. Examples of such systems include chitosan/poly(ethylene oxide)-poly(propylene oxide)/TPP (CS/PEO-PPO/TPP) (16) and CS/alginate/TPP (17).

Polyethylene glycol (PEG) has been approved by the FDA for a wide range of biomedical materials. As a biocompatible, non-toxic, protein-resistant, non-immunogenic material with good water solubility, it has frequently been used in tissue engineering, organ protection, and pharma-

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ceutical applications (18). In particular, however, it is often blended or compounded with other polymers and utilized in the field of drug-controlled release (19).

CS/PEG has been developed for the controlled release of drugs in the form of films (18) and microspheres (20). Wang *et al.* (21) showed that the amount of ciprofloxacin hydrochloride released from CS/PEG blended films increased proportionally with the PEG content. Furthermore, a CS/PEG membrane was employed as an oral drug delivery system that leads to successful applications in the localized drug delivery to within the intestine (22). At least some CS/PEG bead formulations have been found to be suitable for the controlled release of the drug isoniazid in an oral sustained delivery system (23), while CS/PEG nanocapsules have been developed as oral peptide carriers (24), where they can enhance and prolong the intestinal absorption of salmon calcitonin. Based on these previous studies, CS/PEG hydrogels undoubtedly can lead to advances in oral drug delivery systems for the *in vitro* environment.

Sodium diclofenac (DFNa, $C_{14}H_{10}Cl_2NO_2Na$) is a widely used non-steroidal anti-inflammatory drug for the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis (25). It has a short mean elimination half-life of 1.2–1.8 h (26), requiring multiple daily administration at a dose between 75 and 200 mg/a day, given in three or four divided portions depending on the route of administration. The most common side effects of DFNa are gastric ulcers, gastrointestinal bleeding, blood dyscrasias, anaphylaxis, and depression of renal function (27,28). In addition, because of its short biological half-life and associated adverse effects, DFNa is considered as an ideal model drug for developing more controlled drug delivery systems. In this scenario, the ideal system will protect the drug with minimal release in the acidic gastric environment but with complete off loading as a sustained slow release once in the intestine.

Thus, the purpose of this study was to prepare and evaluate CS/PEG hydrogel beads crosslinked with tripolyphosphate as a gastrointestinal drug-controlled release system using DFNa as the model drug. This paper reports the factors that seemingly influence the drug release from the CS/PEG beads crosslinked with TPP, such as, the ratio of CS and PEG used, the amount of DFNa loaded into the particles, and the concentration and duration of TPP, as well as additional GD crosslinking. Moreover, the particle morphology, swelling, and *in vitro* DFNa release profiles were monitored in various pH media at 37°C and compared with a commercial drug delivery system.

MATERIALS AND METHODS

Materials

The following materials were obtained from the indicated suppliers and used as received: DFNa (available from the Center for Chitin-Chitosan Biomaterial, Thailand), CS (food grade, deacetylation 90% min., M.W. 50,000–300,000, BFM, Bangkok, Thailand), PEG (food grade, M.W. 6,000, Union Chemical, Bangkok, Thailand), TPP (food grade, Union Chemical, Bangkok, Thailand), hydrochloric acid 35%, sodium chloride, sodium hydroxide, sodium hydrogen phosphate, potassium chloride, potassium dihydrogen phosphate and potassium bromide (Merck, Germany), glacial acetic acid (Scharlau, Spain), and glutaric dialdehyde solution (GD) in water 25% (*v*/*v*; Acros Organics, USA). Diclofenac commercial tablets were obtained from Community Pharmacy Public Co., Ltd. (Thailand).

Hydrogel Beads Preparation

Coagulant Conditions

Various compositions of CS beads were prepared and named as described in Table I. Briefly, a CS/DFNa mixture was dropped through an 18-G needle into a coagulant solution of varying concentrations of TPP (1.0–10.0% (*w*/*v*)). The gel

Table I. The Composition, Size, and %EE of the Various CS/TPP Beads

	Ratio of compositions							Swelling ratio at 24 h	
Formulation	CS	PEG	DFNa	Conc. TPP (% *w*/*v*)	Time (min)	Bead size (mm)	% EE	pH 1.2	pH 7.4
A0	1	0	0	–	20	2.2±0.1	ND	–**	1.08±0.04
A1	1	0	1	–	20	2.4±0.2[a]	55.8±0.5	–**	1.01±0.04[b]
B	1	0	0	1	20	2.4±0.1[a]	ND	–**	0.94±0.02[b]
D	1	0	0.5	1	20	2.4±0.2[a]	92.0±1.9[d]	2.21±0.30	1.06±0.03[a]
F	1	0	1	1	20	2.3±0.1[a]	87.4±4.3[d]	2.72±0.27[f]	1.05±0.04[a]
H	1	0	2	1	20	2.4±0.1[a]	58.2±3.6[c]	1.72±0.07[f]	1.03±0.03[a]
J	1	0	0.5	5	20	2.4±0.2[a]	95.0±2.7[d]	2.00±0.14[e]	0.94±0.03[a]
K	1	0	0.5	10	20	2.2±0.1[a]	90.4±4.5[d]	1.92±0.12[e]	0.79±0.02[a]
N	1	0	0.5	10	30	2.2±0.2[a]	93.3±0.6[d]	2.30±0.24[e]	0.88±0.03[a]
O	1	0	0.5	10	60	2.3±0.1[a]	89.0±2.1[d]	2.29±0.16[e]	0.96±0.04[a]
P*	1	0	0.5	10	30	1.9±0.1[b]	93.4±0.8[d]	1.74±0.07[f]	1.01±0.03[a]

Data are shown as mean±1SD and are derived from three independent repeats
*Beads formed using a 22 G needle
**Data not available because the beads eroded after 24 hours
[a] Not significant (*P vs.* A0; ANOVA followed by LSD test)
[b] Significant (*P vs.* A0; ANOVA followed by LSD test)
[c] Not significant (*P vs.* A1; ANOVA followed by LSD test)
[d] Significant (*P vs.* A1; ANOVA followed by LSD test)
[e] Not significant (*P vs.* D; ANOVA followed by LSD test)
[f] Significant (*P vs.* D; ANOVA followed by LSD test)

beads were left in the solution for certain immersion times (20, 30, and 60 min) (12). After gelation, the beads were then washed, filtered, and freeze dried for 24 h. The DFNa contents in the beads were determined by UV–Vis spectrophotometer at 276 nm. After the optimal condition for preparation of the beads was obtained, from the above tested parameters and ranges, the size of the resultant composite beads was further reduced by using a smaller sized syringe needle, i.e., 22 G.

Preparation of Hydrogel Beads with Various Ratios of CS/PEG

The hydrogel beads were prepared with the outlined compositions for CS/TPP beads in Table I (A0 to P) and CS/PEG/TPP with or without DG crosslinking in Table II. To the PEG solution dissolved in deionized water at room temperature, the desired amount of DFNa was added, thoroughly dissolved, and then the solution of 1.5% (*w*/*v*) CS in 1.0% (*v*/*v*) acetic acid was added to the PEG and DFNa mixture to a final CS/PEG ratio (*w*/*w*) of 1/0, 1/0.25, 1/0.5, 1/1, or 1/2. The DFNa ratio in each formulation was varied as described in Table II. The mixtures were further stirred until homogeneous.

Twenty milliliters of the mixture was extruded in the form of droplets, using a 22-G needle, into 50 ml of 10.0% (*w*/*v*) TPP pH 6.0 coagulant solution. The solutions were maintained at room temperature for the indicated time (Tables I and II) to allow the beads to crosslink completely. After that, the beads were filtered and washed with deionized water. Finally, the hydrogel beads were freeze dried at −42°C for 24 h.

Preparation of the DFNa-Loaded Beads with Various DFNa Content

Based on the drug entrapment efficiency of the beads prepared in the above section, a 2:1 (*w*/*w*) CS/PEG ratio was selected for further study with a varying DFNa ratio (referred to as formulations PEG5 to PEG7, given in Table II), prepared in the same manner as described above.

Preparation of the Crosslinking Hydrogel Beads

The effect of GD (0–7.5% (*v*/*v*)) as a crosslinking agent was studied based on the optimum formulation obtained from the above study (PEG5–PEG7), with the detailed compositions of each of the three GD formulations (GD2.5 to GD7.5) being given in Table II (GD2.5 to GD7.5). In this case, the hydrogel beads were crosslinked before and during formation in the solution by first mixing the GD into the TPP coagulant solution and then proceeding as described above.

Characterization

Scanning Electron Microscopy

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

Fourier Transform Infrared Spectroscopy

The infrared spectra of all formulations were recorded with a Fourier transform infrared spectroscopy (FT-IR; Impect 4.1, Nicolet). The dried sample was ground and mixed with potassium bromide in an agate mortar and pestle. The mixture was then transferred to a hydraulic pressing machine and pressed into a thin disk. The KBr disk was then measured within the wavelength range of 4,000–400 cm^{-1}.

Determination of Encapsulation Efficiency

The drug content in the DFNa-loaded CS-based composite hydrogel beads was quantitatively determined by immersing the dried beads (100 mg) in 250 ml of phosphate buffer saline pH 7.4 followed by sonication to completely dissolve

Table II. The Composition, Size, %EE and Swelling Ratio of the Various CS/PEG/TPP Beads with or without Additional GD Crosslinking

Formulation	Ratio of compositions			Conc. TPP (% *w*/*v*)	Time (min)	Bead size (mm)	%EE	Swelling ratio at 24 h	
	CS	PEG	DFNa					pH 1.2	pH 7.4
Q	2	0	1	10	30	1.9±0.1	93.3±0.8	1.74±0.07	1.01±0.03
PEG0	1	1	0	10	30	2.0±0.1[a]	ND	NA	NA
PEG1	1	0.25	1	10	30	2.0±0.2[a]	98.8±2.0[a]	NA	NA
PEG2	1	0.5	1	10	30	1.9±0.1[a]	92.1±3.2[a]	NA	0.98±0.02[a]
PEG3	1	1	1	10	30	2.0±0.1[a]	90.6±2.0[a]	NA	0.96±0.02[a]
PEG4	1	2	1	10	30	2.1±0.1[a]	98.9±2.5[a]	NA	0.97±0.03[a]
PEG5	1	0.5	0.25	10	30	2.0±0.1[a]	95.7±0.4[a]	NA	NA
PEG6	1	0.5	0.5	10	30	2.0±0.1[a]	92.1±3.2[a]	NA	0.97±0.03[a]
PEG7	1	0.5	1.5	10	30	2.1±0.1[a]	95.0±2.7[a]	NA	1.01±0.02[a]
GD2.5	1	0.5	0.5	10	30	2.0±0.1[a]	92.8±1.6[a]	1.13±0.03[b]	1.15±0.03[b]
GD5.0	1	0.5	0.5	10	30	2.0±0.1[a]	87.0±0.8[b]	1.14±0.03[b]	1.17±0.02[b]
GD7.5	1	0.5	0.5	10	30	2.0±0.1[a]	84.0±0.9[b]	1.16±0.01[b]	1.13±0.02[b]

Data are shown as mean±1SD and are derived from three independent repeats
nd Not determined
na Data not available because the beads eroded after 24 hours
[a] Not significant (*P vs.* Q; ANOVA followed by LSD test)
[b] Significant (*P vs.* Q; ANOVA followed by LSD test)

the drug dispersed inside the beads (29). The solution was collected, and the drug content was determined by UV–Vis spectrophotometry at 276 nm. The % encapsulation efficiency (%EE) was calculated according to the following equation:

$$\%EE = \frac{\text{Drug content}}{\text{Theoretical drug content}} \times 100$$

Swelling Study

The swelling behavior of the CS/PEG beads was studied in two dissolution systems; 0.1 M HCl (pH 1.2) and phosphate buffer saline, pH 7.4. The beads were immersed in the respective solution, and the diameters of the swollen beads were measured at specific time intervals for up to 24 h using a compound microscope equipped with an ocular micrometer. The swelling behavior was determined from the changes in the diameters of the beads, and the swelling ratio for each sample, determined at time t, was calculated using the following equation (30):

$$S_w = \frac{D_0}{D_t}$$

where D_t is the diameter of the beads at time (t) and D_0 is the initial diameter of the dried beads.

In Vitro Release Study

The release of DFNa from the composite beads of each formulation was assayed under a simulated gastrointestinal condition by an alternating pH scheme (4,31). A dissolution media of pH 1.2 (0.1 M HCl) was chosen to represent the gastric condition, while pH 6.8 was used to simulate the duodenal condition, and pH 7.4 for the jejunum and ileum. One hundred milligrams of the beads were enclosed in a teabag and placed into a beaker containing 250 ml of the dissolution medium. The beaker was then placed on a horizontal shaking water bath (50 rpm) and incubated at 37 ± 2°C. For the first 2 h, the beads were kept in 0.1 M HCl (pH 1.2) dissolution medium, and then this was changed to phosphate buffer saline, pH 6.8, for 1 h. Finally, the release dissolution medium was changed to pH 7.4, and the beads were maintained in this media for 24 h. From each of these solutions, at various time intervals, 2 ml of the medium were withdrawn, clarified of residual particles by centrifugation, and the supernatant harvested and diluted to a suitable concentration, if necessary. The release rate of DFNa was assayed by UV–Vis spectrophotometry at 276 nm. The amount of DFNa released was calculated by interpolation from a calibration curve containing DFNa standards. A cumulative correction was made for each of the previously removed samples, to determine the total amount of drug release. The release experiments were done in triplicate.

Statistical Analysis

Statistical analysis for determination of differences in the measured properties between groups was accomplished using one-way analysis of variance (ANOVA) followed by the least square difference test and determination of confidence intervals, which was performed using SPSS version 11.5 (SPSS Inc., Chicago, Illinois, USA). All data are presented as the mean value ± 1SD. Differences were considered to be statistically significant when the p values were less than 0.05.

RESULTS AND DISCUSSION

Characterization and Physical Properties

Morphology

The size, shape, and surface topography of the dried beads, as visualized by SEM, for each formulation obtained with the 18-G needle, revealed a relatively large diameter ranging from 2.04 to 2.57 mm, but with the means lying within the range of 2.2–2.4 mm (A0 to O, Table I), depending to some extent on the composition of each formulation. When the droplet diameter size was reduced by using a 22-G needle, the diameter of the resultant beads was reduced to 1.84–2.19 mm, with a mean range of 1.9–2.1 mm, across the different formulations (Table II), although this size change was not statistically significant (ANOVA, P=0.4).

Representative SEM micrographs of beads derived from a CS/PEG/DFNa ratio of 1/0.5/1 and 1/1/1 reveal that the shape of the beads was almost spherical and that the surface was rough, while cross-sectional views showed that there were cavities inside the beads (Fig. 1). Comparison of these micrographs to those obtained from beads with a CS/PEG/DFNa ratio of 1/0.5/1.5 (PEG7; Fig. 1) suggests that as the loading amount of DFNa was increased, more DFNa powder presented at the surface of the beads. It appears that in this formulation, the capacity of the polymer to encapsulate the loaded drug was exceeded.

The cross-section photomicrographs of the beads crosslinked with 0%, 2.5%, 5.0%, and 7.5% (v/v) of GD are shown in Fig. 2, where although the beads crosslinked with different concentrations of GD showed the same broadly spherical shape, the network inside the beads were formed in different structures. Beads crosslinked with 5.0% and 7.5% (v/v) GD exhibited a dense and layer-like network, presumably because the beads were completely crosslinked with GD, and this dense network of polymers may prolong the DFNa release from the beads.

Fourier Transform Infrared Spectroscopy

The IR spectra of formulated beads are shown in Fig. 3. The IR spectrum of CS displayed the characteristic absorption bands at 1,592 and 1,652 cm^{-1} which result from N–H bending and the C=O bond of the primary amide group of chitin (32), respectively.

The IR spectrum of DFNa reveals distinct bands at 1,293 and 1,302 cm^{-1} due to C–N stretching and a peak at 1,575 cm^{-1} from C=C stretching combined with C=O stretching of the carboxylate group. However, since DFNa can be converted into DFH under acidic conditions, such as that of the chitosan solution, then the IR spectrum of diclofenac acid (DFH) was also required to be investigated. The IR spectrum of DFH shows the characteristic stronger carboxylic acid C=O group peak at 1,690 cm^{-1}, and C=C

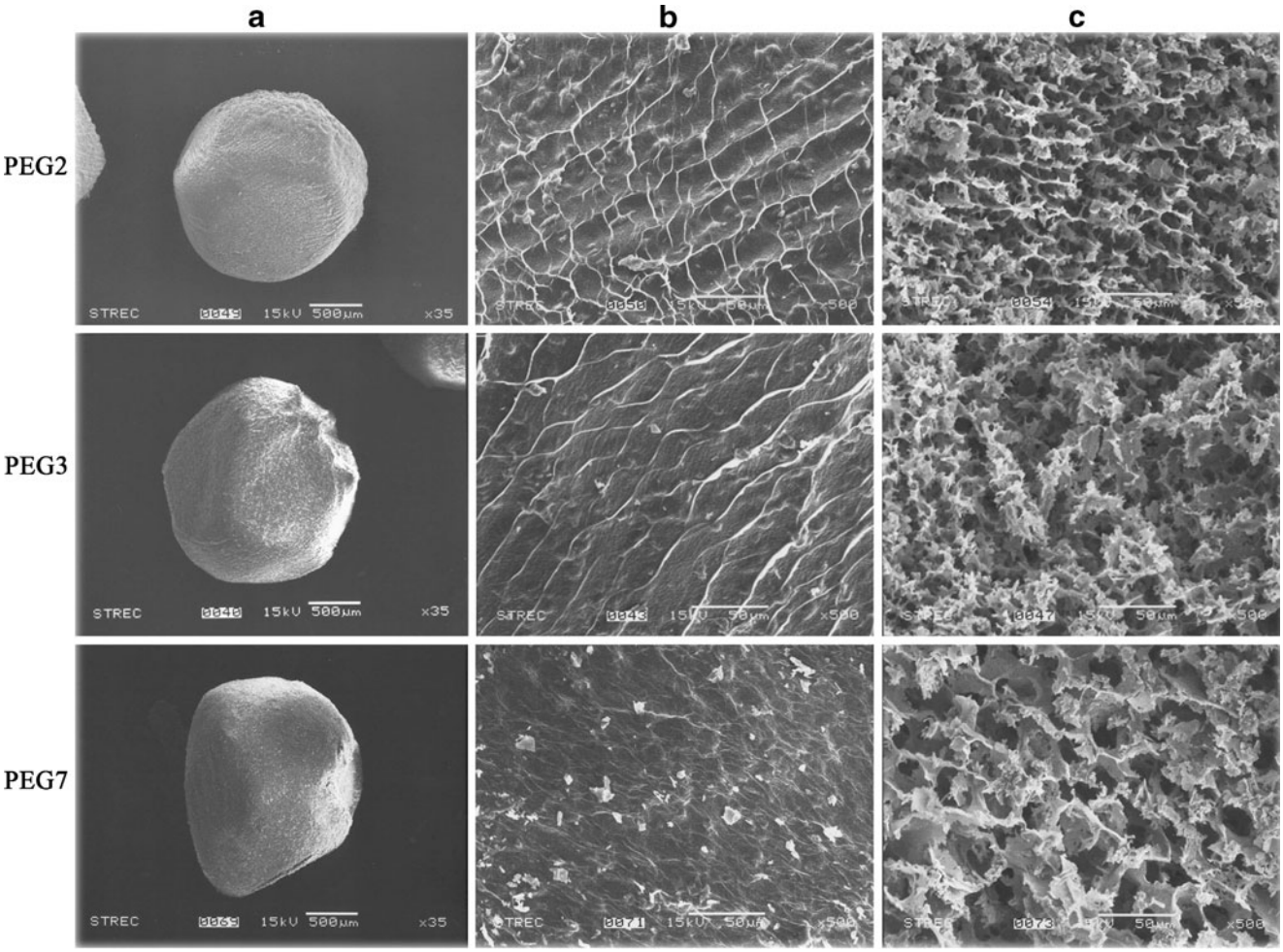

Fig. 1. Representative SEM micrographs of the CS/PEG beads showing **a** a typical whole bead (×35), **b** the surface (×500), and **c** the network inside the bead (×500). Beads were prepared with the same DFNa content and with the composition as listed in Table II

stretching was also present at 1,575 cm^{-1} The peak for the free –OH group was found at 3,313 cm^{-1}.

The IR spectrum of PEG revealed absorption bands at 1,102, 2,878, and a wide band at 3,421 cm^{-1} which are attributed to the bending vibration of C–O, the stretching vibration of C–H, and the stretching vibration of O–H bonded to N–H, respectively (21).

The representative FT-IR spectrum of DFNa-loaded CS/PEG crosslinked with TPP (PEG2, 1/0.5/1 (*w*/*w*/*w*) ratio of CS/PEG/DFNa) were consistent with the spectra of CS, PEG, and DFNa. The spectrum showed that the C=O bond of the primary amide group of chitosan was shifted from its original band at 1,652 to 1636 cm^{-1}, suggesting that the ionic interactions between CS and TPP occurred. There is a small peak of the carboxylic acid C=O group at 1,690 cm^{-1}, which implies that a small amount of DFNa was converted to DFH. Likewise, the representative FT-IR spectrum of DFNa-loaded CS/PEG beads crosslinked with glutaraldehyde that is GD5.0 beads (CS/PEG/DFNa/GD) was consistent with the main components of the beads. The C=O bond of the primary amide group of chitosan was slightly shifted from its original band at 1,652 to 1641 cm^{-1} due to the fact that chitosan was crosslinked with GD. Only a low amount of DFNa was converted to DFH, as can be seen from the very small broad peak of the carboxylic acid C=O group at 1,685 cm^{-1}.

Encapsulation Efficiency

Coagulant Conditions

The %EE for each CS-composite bead formulation with DFNa is given in Table I. The results indicated that the %EE of the beads prepared with 1%, 5%, and 10% (*w*/*v*) of TPP (formulations D, J, and K, respectively) were not significantly different (ANOVA, $p=0.5$). Therefore, the results from the swelling ratio (pH 1.2 and 7.4; Table I) were considered along with the %EE and bead size, and the optimum concentration of TPP coagulant was 10% (*w*/*v*), formulation K.

As summarized in Table I, the effect of varying the TPP crosslinking times was evaluated at 20, 30, and 60 min (formulations K, N, and O, respectively). Crosslinking for 20 and 30 min both resulted in an excellent drug EE at 90.4% and 93.3%, respectively, indicating that the networks inside the beads were largely to completely formed within 20–30 min of crosslinking time. On the other hand, excessive crosslinking (60 min) resulted in lower entrapment efficiency at 89.0%. PEG can dissolve in water, and consequently, the beads can swell, resulting in the drug leaking from the beads. Moreover, the sustained release pattern of the DFNa from CS–TPP composite beads reiterated that the optimum TPP crosslinking time was approximately 30 min (Fig. 4).

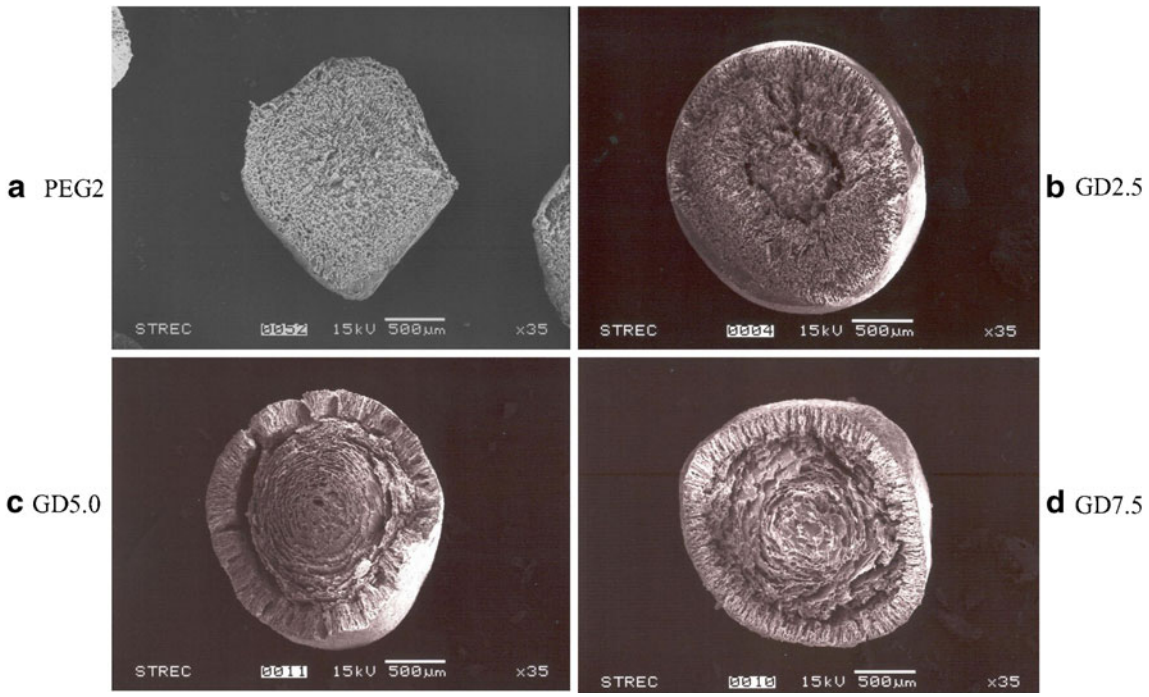

Fig. 2. Representative SEM micrographs showing the cross-sectional view of the CS/PEG beads crosslinked with varying levels of GD (composition as in Table II) at the same DFNa content

In general, the smaller size of the beads is of a greater advantage because smaller sized beads should be more convenient for application in pharmaceutical capsules. The smaller bore size syringe needle (22-gauge needle) was chosen for all subsequent preparations of the CS-composite beads, and thus, CS/TPP beads were hereafter prepared under the optimized conditions of crosslinked in 10% (*w/v*) TPP, pH 6.0, for 30 min, and formed through a 22-gauge needle.

Preparation of the Hydrogel Beads with Various Ratios of CS/PEG

PEG is another polymer commonly used in modifying nanoparticles for drug delivery and release and was evaluated in the present study for its ability to improve the DFNa release profile from CS/TPP-crosslinked beads. CS/PEG composite beads (PEG1 to PEG4) did not show a significantly different DFNa EE when compared to that of the CS/TPP beads (Table II, ANOVA, $p=0.09$). Therefore, the optimal CS/PEG ratio was determined based on the results of the % drug release, shown in Figs. 5 and 6, instead.

Swelling Study

Swelling Analysis

The swelling ratios of CS beads with or without PEG enhancement are summarized in Table II. The incorporation of PEG into the CS beads resulted in a higher degree of observed swelling in the simulated gastric fluid (SGF; data not shown). In addition, erosion of CS/PEG beads was observed after 6-h immersion in the SGF, which is probably due to the fact that the PEG can dissolve in an acidic medium, resulting in the enhanced swelling of CS/PEG beads.

Differing proportions of CS/PEG showed a significant difference in the level of observed swelling (Table II; ANOVA, $p<0.05$). The beads from the formulations PEG0 to PEG4 were eroded within 24 h in the SGF. This is because in the acidic condition (pH 1.2), the amino groups in chitosan can be protonated leading to electrostatic repulsion between the polymer chains and so allowing more water into the expanded hydrogel network and promoting the erosion of CS/PEG beads. The swelling behavior of beads from formulations PEG0 to PEG4 in the stimulated intestinal fluid (SIF) system (phosphate buffer saline pH 7.4) revealed that beads with DFNa and containing PEG at more than 50% (based on the total amount of CS and PEG) were not eroded before 24 h. At pH 7.4, most of the amino groups of chitosan are deprotonated and hence form the hydrogen bonds in the CS/PEG network. With a relatively small amount of PEG incorporation into the CS/PEG beads (lower than 50% of PEG), the PEG network will disrupt the hydrogen bonds among the amine groups of the chitosan, and this dissolution phenomena of PEG will dominate the erosion of the CS/PEG beads. When the amount of PEG is higher than 50%, based on the total amount of CS and PEG, the hydroxyl groups of PEG contributed to the formation of hydrogen bonds with the PEG networks and the amine groups of CS networks. Therefore, the beads were not eroded, but caused the swelling of the CS/PEG network. These observations outlined the pH-sensitive swelling behaviors of CS/PEG beads.

In Vitro Release Study

Effect of the Polymer Ratio (CS/PEG) on the Release Profiles

The dissolution profiles of various formulations of CS/PEG beads at various pH at 37°C as a function of time are

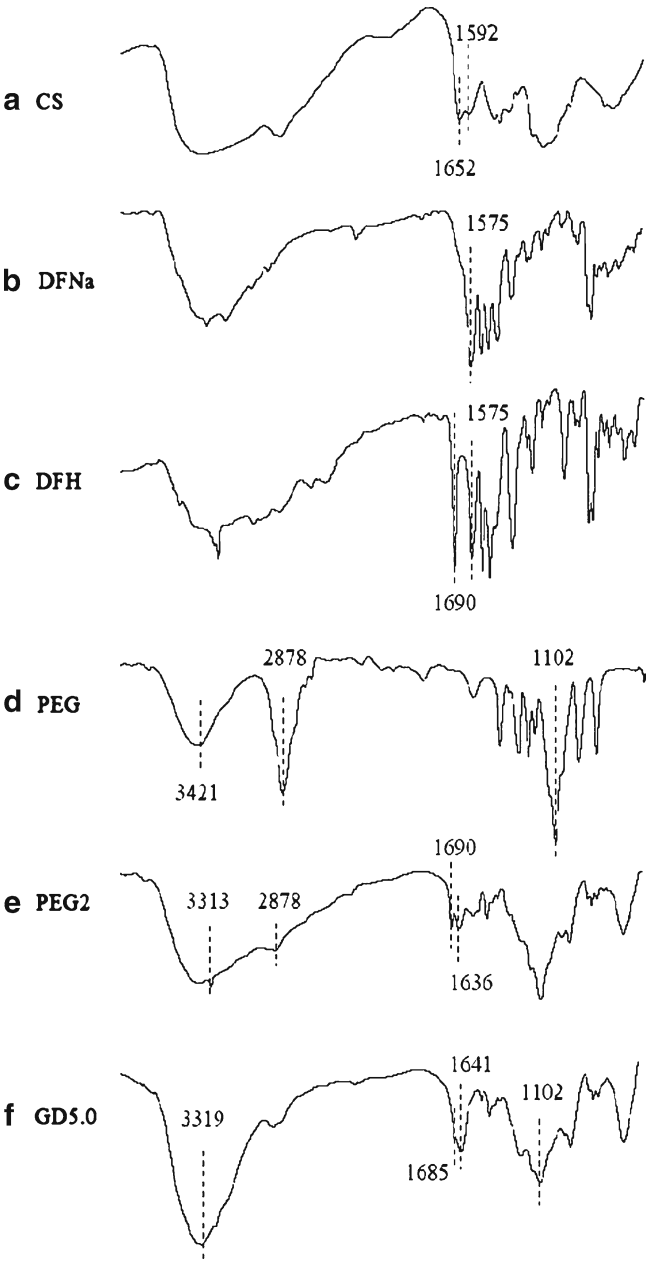

Fig. 3. FT-IR spectrum of CS beads; **a**–**d** the pure indicated compounds; **e** PEG2 (see Table II) and **f** GD5.0 (see Table II) beads

illustrated in Fig. 5. It can be seen that the percentage released increased with the increasing of pH of the medium. In order to simulate the sequential release profile, the beads were immersed in buffer at pH 1.2 for 2 h and then moved to buffer at pH 6.8 for 1 h. Release of diclofenac from the beads was lower than 5% at pH 1.2 and 6.8 and more higher than 75% at pH 7.4. The results indicated that the drug release profile from the beads is pH-dependent as described above. Even the chitosan can highly swell in the acidic condition because the amino groups of the chitosan are protonated at this pH and so cause electrostatic repulsion between chains and, as a consequence of the chains moving further apart, allow water molecules to penetrate more thoroughly in the chitosan network. However, the solubility of diclofenac sodium in acidic conditions is very low, leading to low amount

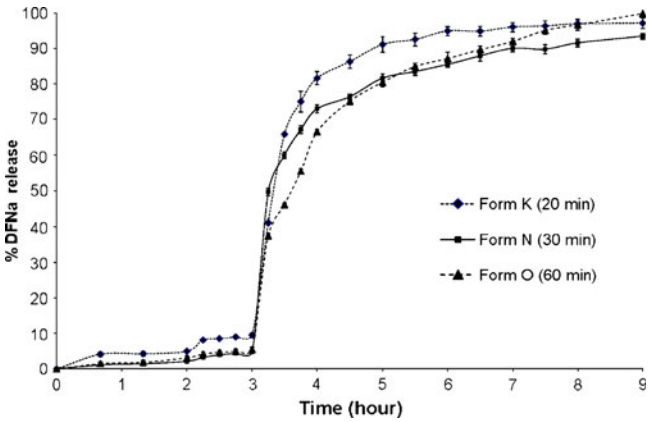

Fig. 4. The dissolution profiles of DFNa in the indicated pH-alternating scheme from CS–TPP beads without PEG formed with crosslinking times of 20 (*K*), 30 (*N*), and 60 (*O*) min. Data are shown as the mean±1SD and are derived from three independent repeats

of diclofenac sodium which was released from the bead (33). In the simulated gastric fluid (pH 1.2), the release of DFNa from the CS beads without PEG was similar to that from the CS/PEG beads. Within the range tested of pH 7.4, the release rate of DFNa from CS/PEG beads in simulated intestinal fluid (pH 7.4) reached more than 90% of the initial drug content 6 h after changing media. This suggested that the drug delivery preferentially takes place in the intestine with reduced drug leakage in the stomach.

The incorporation of PEG in the CS beads helped sustain release of the DFNa in the intestine, and this was somewhat dose-dependent with a slower release rate as the PEG content increased. The exception here is that for a CS/PEG ratio of 1:0.25, it clearly has a more marked reduction in the DFNa release rate than that seen for 1:0.5 and 1:1 (*w*/*w*) ratio CS/PEG preparations. It has been reported previously that the time required for 50% drug release in a 1:1 (*w*/*w*) CS/PEG formulation was over 4 h, which is an extended release time compared to other formulations, whereas this formulation was able to release over 80% of the drug within the

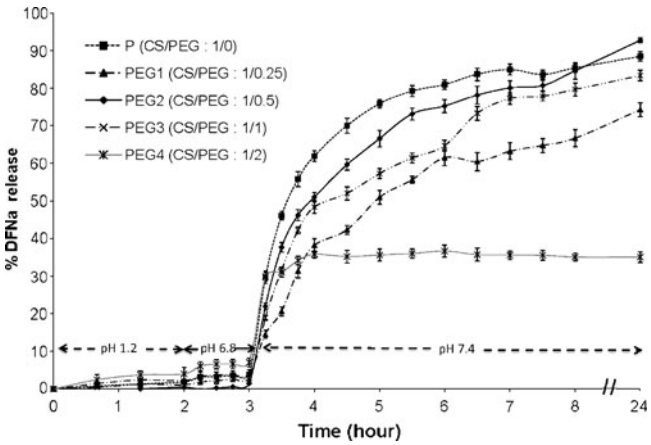

Fig. 5. The dissolution profiles of DFNa in the pH-alternating scheme from CS–PEG beads formed with varying CS/PEG ratios. Formulations are as per Table I and II. Data are shown as the mean± 1SD and are derived from three independent repeats

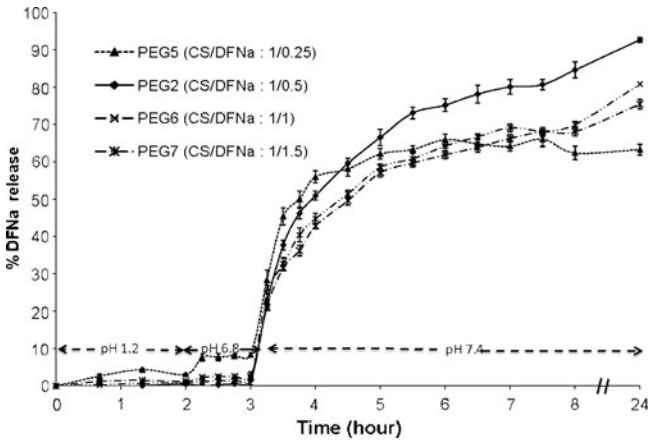

Fig. 6. The dissolution profiles of DFNa in the pH-alternating scheme from CS/PEG beads formed with different polymer to DS ratios. Formulations are as per Table II. Data are shown as the mean± 1SD and are derived from three independent repeats

extended period of 24 h. However, when the CS/PEG (*w*/*w*) ratio was higher still at 1:2 (PEG4), the initial release rate soon faded and only ~35% of the total DFNa was released, which is a low drug release level. This may be due to the fact that the beads contained a high content of water soluble PEG, resulting in fast swelling of the beads in SGF condition (1.5 times compared to the CS beads over the first 2 h and completely eroded after 6 h). Hence, the beads failed to firmly entrap the DFNa, leading to DFN diffusion out from the bead and subsequent conversion to diclofenac acid (acid–base reaction) and precipitation as diclofenac acid. When the beads were transferred to the SIF condition, only about 30% of the total amount of loaded DFNa remained in the beads and hence slowly released out from the beads. These results are also consistent with the observed swelling properties where the swelling ratio of the 1:2 CS/PEG beads in the first 2 h was 1.5, which is 20% higher than that of the of the CS beads (1.25). Formulation PEG2 (1:0.5 CS/PEG) can sustain the release of DFNa within 24 h with a high EE (92.1%) and can release virtually all of the encapsulated drug (92.7%).

Effect of the Polymer to Drug Ratio on the Release Profiles

With the "optimum" ratio of CS/PEG obtained, the next parameter to be considered was the amount of DFNa loaded. Here, CS/PEG polymer beads were made with a fixed CS/PEG ratio of 1:0.5 (*w*/*w*), and the ratio between the CS/PEG polymer and DFNa was varied between 1:0.25 and 1:1.5 (formulations PEG5, PEG2, PEG6, and PEG7; Table II).

The initial burst effect of all four formulations, and especially PEG2, PEG6, and PEG7, was reduced compared to that seen with PEG5. PEG2 gave the highest attained drug release level of 92.7% within 24 h, while PEG6 and PEG7 showed only 70.0% and 68.1% of the drug release, respectively (Fig. 6). As the amount of DFNa increases, the relative proportion of the DFNa that could be released is reduced (Fig. 6). PEG2 was optimal in that it could sustain the release of DFNa within 24 h with a high EE (92.1%) and high total release level (92.7%).

Effect of the Crosslinking Agent on the Release Profiles

The effect of the covalent crosslinking agent on the dissolution profiles was investigated. GD is commonly used as a covalent crosslinking agent for enhancing the dissolution property of CS beads and was selected as a model crosslinking agent. The dissolution profiles of CS/PEG crosslinked with 2.5%, 5.0%, and 7.5% (*w*/*w*) GD, respectively, under the pH-alternating scheme (pH 1.2 to 6.8 to 7.4), are presented in Fig. 7. In the first 2 h, when immersed in the SGF at pH 1.2, all three tested formulations of the CS/PEG/GD beads, plus the PEG2 sample as a no GD crosslinking reference sample, were stable yielding a similar and low drug (DFNa) release of 5.6 (2.5% GD), 6.6 (5.0% GD), and 4.7 (7.5% GD; Fig. 7). After that, the dissolution medium was changed to the simulated duodenal fluid (pH 6.8) for 1 h, and again all four samples revealed similar but low release rates of DFNa. Finally, the dissolution medium was changed to the SIF (pH 7.4). The release of DFNa increased in the first hour in SIF (Fig. 7) and continuously released more DFNa up to nearly 100% within 24 h in SIF in all four tested samples. However, the release rate of PEG2 was faster than that of the CS/PEG beads crosslinked with GD. After 3 h in the SIF (Fig. 7), the dissolution percentage of GD2.5, GD5.0, and GD 7.5 were 76.8, 69.6, and 69.5%, respectively, compared to ~77% for the reference PEG2 sample with no GD crosslinking. The release pattern of GD5.0 and GD7.5 was broadly similar, with GD5.0 and GD7.5 releasing most (99.6% and 97.1%, respectively) of the entrapped DFNa over 24 h. This result suggests that the presence of GD at over 5% in the reaction mixture did not increase the level of crosslinked amino groups of chitosan any further. In contrast, comparing the DFNa release rates between GD5.0 and GD7.5 with that from PEG2 (TPP alone), it was obvious that the release rate of DFNa was similar to that of by GD crosslinking. The reason of the CS/PEG beads crosslinked with TPP that could prolong release of DFNa in the similar way as the beads crosslinked with GD is that the amino groups of chitosan are protonated resulting in the electrostatic repulsions and solvation of ionic groups thus contributing to the swelling in

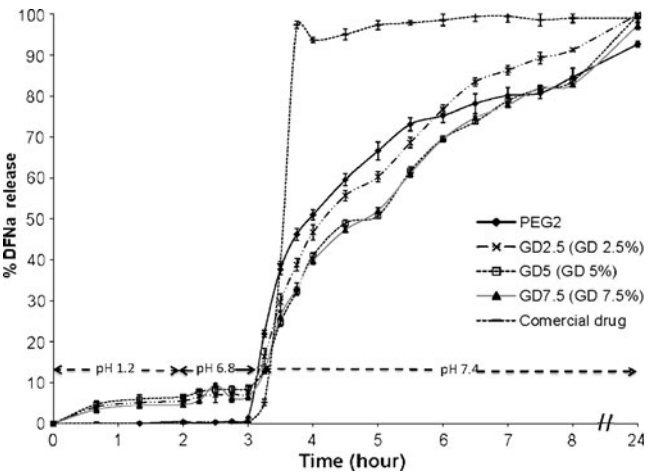

Fig. 7. The dissolution profiles of DFNa in the pH-alternating scheme from CS/PEG/TPP/GD beads with various concentrations of GD crosslinking and compared to a commercial drug. Data are shown as the mean±1SD and are derived from three independent repeats

an acidic environment. The release of DFNa from CS/TPP/DFNa is very low in pH 1.2 and 6.8, which can be negligible. Because, at pH 1.2, the amino group of chitosan was protonated to be $-NH_3^+$ group, resulting in the electronic repulsions and solvation of ionic groups thus contributing to the swelling. At a pH above 7, the concentration of $P_3O_{10}^{5-}$ and $HP_3O_{10}^{4-}$ is high; hence, the ions of macromolecular DFNa and $P_3O_{10}^{5-}$ should bind strongly to the $-NH_3^+$ group of chitosan, thereby forming a denser crosslinked bead structure that hinders the medium (OH^-) penetration into the chitosan sphere. Therefore, the DFNa could not be released from the beads in the lower pH (34). Whereas, the release rate of DFNa from the CS/GD/DFNa beads was higher than that of CS/TPP/DFNa in pH 1.2 and 6.8 conditions. Because the amino groups of chitosan can be protonated to be $-NH_3^+$ group, resulting in repulsion, hence there is more swell in the acidic condition. In addition, the drug release pattern of this formulation was far superior to that of the commercial drug under these *in vitro* trial conditions, which showed almost 100% DFNa release within just 1 h of exposure to the SIF.

CONCLUSION

Various proportions of CS, PEG, TPP, and DFNa contents in the CS-composite beads affected the rate and degree of release of DFNa (as the model drug), and this is presumably due to the differences in ionic interactions and drug concentrations within the beads. The optimum formulation was found to consist of CS/PEG/DFNa at a (*w*/*w*/*w*) ratio of 1/0.5/0.5, prepared by ionic coagulant and crosslinking with 10% (*w*/*v*) TPP at pH 6.0 for 30 min. In addition, the beads crosslinked with GD demonstrated a more sustained release profile than their non-GD crosslinked counterpart. The CS/PEG beads crosslinked with 5.0% (*w*/*v*) GD provided the best controlled release of the drug over 24 h.

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