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Formulation and characterization of pH sensitive drug carrier based on phosphorylated chitosan (PCS)

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Abstract

The new polyelectrolyte complex gel beads based on phosphorylated chitosan (PCS) were developed for controlled release of ibuprofen in oral administration. The PCS gel beads were readily prepared from soluble phosphorylated chitosan by using an ionotropic gelation with counter polyanion, tripolyphosphate (TPP), at pH 4.0. The beads were characterized by scanning electron microscopy (SEM) for morphological studies. The in vitro drug release behavior in various pH media was studied using ibuprofen as a model drug. Ibuprofen was highly loaded, around ~90%, in the PCS gel beads. The release percents of ibuprofen from PCS gel beads were found to be increased as the pH of dissolution medium increased. The release rate of ibuprofen at pH 7.4 was noticeably higher than the release rate at pH 1.4 due to the ionization of phosphate groups and high solubility of ibuprofen at pH 7.4. These factors suggest that the PCS gel beads may be useful for controlled drug delivery system through oral administration by avoiding the drug release in the highly acidic gastric fluid region of the stomach.

Keywords: Phosphorylated chitosan; Polyelectrolyte complex gel; Tripolyphosphate; Ibuprofen

1. Introduction

Recently, polyelectrolyte complex (PEC) gel matrices have been extensively investigated for design of drug delivery systems to release a pharmacologically active agent in a pre-determined, predictable and reproducible fashion. Successful oral drug delivery requires that the drug carrier is resistant both to the attack by enzymes and to the impact of pH gradients (pH changing from ~1–3 in the stomach to ~6–7 in the intestine) for the gastrointestinal (GI) transit time from mouth to caecum (3–16 h) depending on the state of stomach (Shalaby, Blevins, & Park, 1991).

Chitosan (CS) is a cationic biopolymer obtained industrially by hydrolyzing the acetamide groups of chitin, by alkaline treatment. Chitin is one of the most abundant natural polysaccharides and is the main component of shells of crustacean, e.g. crabs, shrimp, krill and the cuticle of insects. CS is a natural, non-toxic, biodegradable, biocompatible polysaccharide and has been used in biomedical areas in the form of sutures, wound healing materials and artificial skin, and for the sustained release of drugs (Chandy & Sharma, 1992; Gupta & Ravi Kumar, 2000; Sivakumar, Manjubala, & Panduranga, 2002) as well as in various industrial applications. CS is a very promising biomaterial for drug delivery; however, the use of CS polymer in oral administration is restricted by its fast dissolution in the stomach and limited capacity for controlled delivery system (Risbud, Hardikar, Bhat, & Bhonde, 2000). In order to overcome these disadvantages, many researchers have investigated the tripolyphosphate (TPP)/CS polyelectrolyte complex gel beads for sustained release performances (Ghanem & Skonberg, 2002; Mi, Shyu, Lee, & Wong, 1999; Mi, Sung, & Shyu, 2002; Shu & Zhu, 2000).

Phosphorylated chitosan (PCS) has attracted considerable interest because of their various advantages:

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anti-inflammatory property, formation of metal complexes and formation of anionic polyelectrolyte hydrogels (Lee & Shin, 1991; Nishi, Maekita, Nishimura, Hasegawa, & Tokura, 1987; Sakaguchi, Horikoshi, & Nakajima, 1981). PCS has both amino groups and phosphate groups in a molecule. While partially phosphorylated derivatives of chitosan were water-soluble, highly phosphorylated derivatives of chitosan were insoluble in water due to the formation of inter- or intra-molecular salt linkage between amino groups and phosphate groups.

In this study, partially phosphorylated chitosan was used for the preparation of PCS gel beads using TPP to improve the controlled release system in a GI fluid. This work focused on the characterization of the PCS gel beads by morphological observation and swelling study and the in vitro drug release profiles monitored in various pH media at 37 °C using ibuprofen as a model drug.

2. Experimental

2.1. Materials

Chitosan (weight-average molecular weight, 10^5; deacetylation degree, 92.4%) was a gift from Kyowa Tecnos Co. Ltd (Japan). Tripolyphosphate (TPP) sodium salt was purchased from Wako Pure Chemical Industries, Ltd (Japan). Ibuprofen was purchased from Sigma (USA). All other materials were of reagent grade.

2.2. Formulation of phosphorylated chitosan (PCS) gel beads

Phosphorylated chitosan with degree of substitution, 0.14 and molecular weight, 3.6×10^4 was synthesized in the phosphorus pentoxide-methane sulphonic acid system (Nishi et al., 1986). Phosphorylation degree was determined by measuring phosphorous concentration of PCS hydrolyzate using ICP spectrophotometer SPS 7800 (Seiko Instruments Inc., Japan). Molecular weight was determined by size exclusion chromatography with three columns of Tosoh TSKgel G3000PW$_{XL}$, TSKgel G2500PW$_{XL}$ (all 7.8 mm I.D.X 300 mm) and TSKguard column PWXL (6.0 mm I.D. X 40 mm) (Tosoh, Japan) in a series.

PCS gel beads were prepared by using ionotropic gelation process with counter polyanion TPP (Fig. 1). PCS (ca. 0.9 g) was dissolved in 10 mL of 2 wt% acetic acid and stirred for 1 d to obtain transparent, homogeneous solution. The PCS solution with or without drug was dropped through a syringe needle (0.5 mm in diameter) into the 10 wt% TPP aqueous phase. Then, solidified white beads were formed immediately and allowed to stand for 1 h in the solution with occasional agitation at room temperature. The pH of TPP aqueous phase was adjusted to pH 4.0 with 1 mol/L hydrochloric acid solution. The gel beads were filtered, washed with deionized water repeatedly and dried under vacuum at room temperature for 2 d.

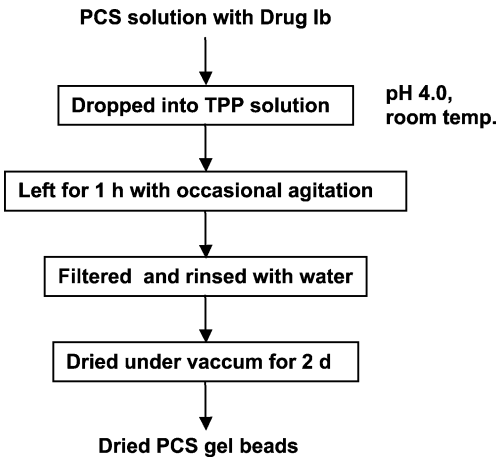

Fig. 1. Schematic diagram for preparation of Ib-loaded PCS gel beads.

For the preparations of drug-loaded PCS gel beads, two different amounts of ibuprofen (Ib): 10 and 20 mg (0.2 and 0.4 wt% with respect to the PCS solution) were dispersed in 10 g of PCS solution for 1 d, respectively. Ibuprofen (Ib) is a prominent non-steroidal anti-inflammatory drug used extensively in the treatment of various musculoskeletal disorders and painful conditions (Gallardo, Parejo, & Roman, 2001). CS gel beads with or without Ib were prepared from the same procedure.

2.3. Swelling tests

Dried PCS gel beads were carefully weighed and immersed in media (50 mL) with pH values from 1.2 to 7.4 at 37 °C. At pre-determined time intervals, swollen beads were taken out, and the excess water was blotted with filter paper from the surface, and then weighed on a sensitive balance (Shimadzu LIBROR AEU-210, Japan). The following equation was used to determine the swelling degree.

$$\text{Swelling degree}[\%] = \{(X_w - X_d)/X_d\} \times 100$$

Here, X_d and X_w represent the mass of dry and swollen beads, respectively.

2.4. Morphological study

The surface morphology of drug free PCS gel beads was examined using a scanning electron microscopy (JEOL JSM-5310 LV, Japan) at 15 kV under low vacuum, 30 Pa, without metal-coating.

2.5. Determination of drug loading efficiency

During the gelation process in TPP aqueous solution, Ib could be diffused out from PCS droplet into TPP solution. After gelation, the Ib concentration in the TPP solution was

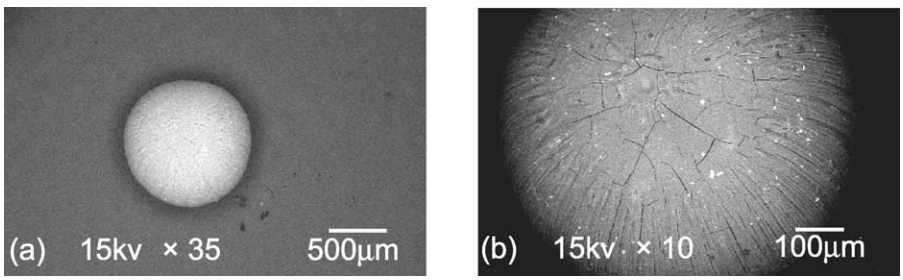

Fig. 2. SEM photographs of drug-free PCS gel beads at (a) × 35 and (b) × 100 magnification.

determined by UV spectrophotometer (Hitachi UV-3200, Japan) at 222 nm. Then, the loading efficiency during gelation process was calculated by the following formula:

$$\text{Loading efficiency}[\%] = \{(m - c \times v)/m\} \times 100$$

where m, c and v represent the initial Ib mass in PCS droplet, the Ib concentration in TPP solution and the volume of TPP solution, respectively.

2.6. In vitro drug release studies

The in vitro release tests were carried out on all formulations of drug-loaded CS beads and PCS beads. The known amount of drug-loaded gel beads were suspended in 100 mL of various pH media at 37 °C and placed in an incubated shaker at 120 rpm. At pre-determined time intervals, 1 mL aliquot of sample was withdrawn and after suitable dilution, the concentration of drug released was monitored by UV spectrophotometer at 222 nm. The dissolution medium was replaced with fresh buffer to maintain the total volume. The drug release percent can be determined as following equation:

$$\text{Drug release}[\%] = R_t/L \times 100$$

where L and R_t represent the initial amount of drug loaded and the cumulative amount of drug released at time t.

3. Results and discussion

3.1. Characteristics of PCS gel beads

Phosphorylation on chitin or chitosan can be carried out in the short period and under mild condition by using methane sulphonic acid-phosphorus pentoxiide system. Due to the strong acidic condition, the molecular weight of the polymer decreased from 10^5 to 3.6×10^4 during phosphorylation of CS polymer. PCSs with low DS are soluble in water while those with high DS are insoluble in water due to the formation of inter- or intra-molecular salt linkage between amino groups and phosphate groups. In this study, PCS with DS of 0.14 was used for the preparation of PCS gel beads by cross-linking with polyanion TPP at pH 4.0. The liquid–gel transition of PCS in TPP aqueous solution can be explained by the electrostatic interaction between positively charged amino groups of phosphorylated chitosan with negatively charged counteranion TPP (Mi et al., 1999).

Fig. 2 shows SEM images of drug-free PCS beads. The PCS gel beads have spherical shape, dense surface, homogeneous polymer matrix and the size varies from

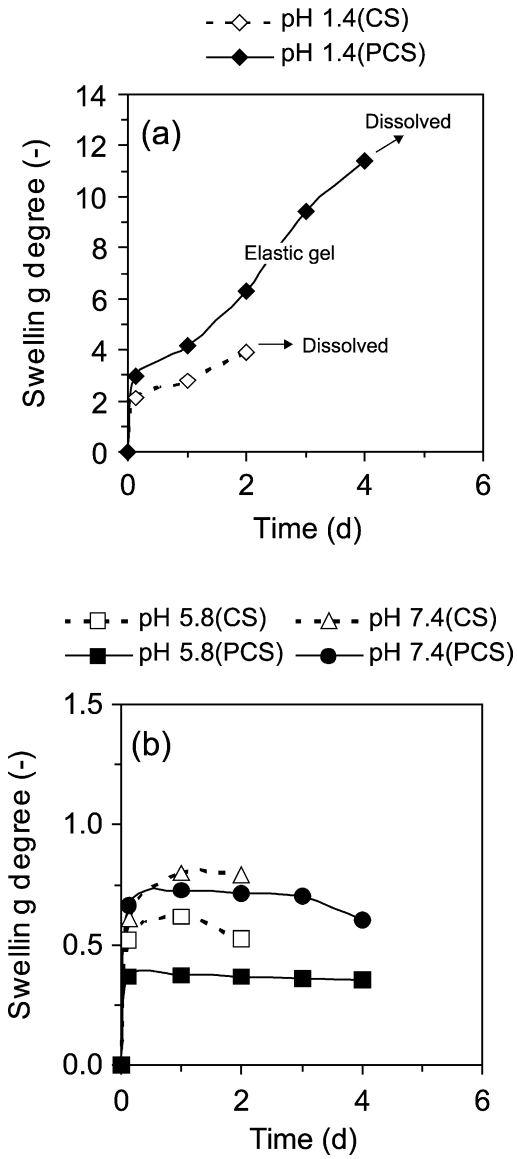

Fig. 3. Swelling behavior for CS and PCS gel beads in different pH media at 37 °C.

Table 1
Loading efficiencies for Ib-loaded CS and PCS gel beads ($n = 5$)

Formulation	Initial drug content (mg/g-beads)	Loading level (mg/g-beads)	Loading efficiency (%)
0.2% Ib-CS	28.6	27.24 ± 0.58	95.24 ± 2.04
0.4% Ib-CS	57.1	52.56 ± 2.71	92.05 ± 4.74
0.2% Ib-PCS	28.6	25.30 ± 0.22	88.45 ± 0.78
0.4% Ib-PCS	57.1	47.91 ± 6.70	83.90 ± 11.74

700–1000 μm. The PCS beads have a number of cracks on the surface.

In our investigation the swelling behavior of CS gel beads at pH 1.4 was found to disintegrate within 1 d and then dissolved after 2 d. On the other hand, PCS beads were found to maintain their spherical shape like elastic gel for 3 d and only after 4 d most of the beads were dissolved in the medium (Fig. 3). The successful oral drug carrier must be resistant to the impact of pH gradients due to pH changing from the strongly acidic medium in the stomach to the weakly alkaline medium in the intestine. From our investigation, it is obvious that PCS beads have higher stability than CS beads at pH 1.4 and may be suitable for oral drug delivery purposes. The high integrity of PCS gel beads at pH 1.4 could be explained by the inter- or intra-molecular cross-linkage between amino groups and phosphorous groups in PCS molecules.

3.2. Entrapment of drug in PCS gel beads

As seen in Table 1, a less water-soluble drug, Ib, was efficiently entrapped into PCS gel beads during ionotropic gelation process. The relatively high efficiency of loading could be explained by the less water-solubility of Ib as well as the ionic binding between negatively charged carboxyl groups in Ib and positively charged amino groups in PCS. It was reported that the carboxyl groups in Ib and the amino groups in PCS are ionized at pH 4 (Handgraft & Valenta, 2000; Sorlier, Denuziere, Viton, & Domard, 2001). In this work, the drug was not chemically attached to the polymer and there are only the electrostatic attractions as well as

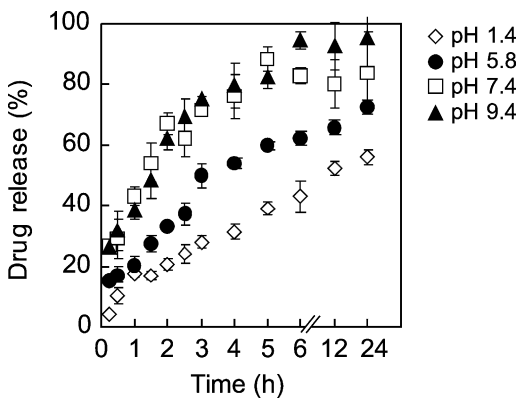

Fig. 4. Release profiles of Ib from 0.2% loaded-PCS gel beads in various pH media at 37 °C ($n = 3$).

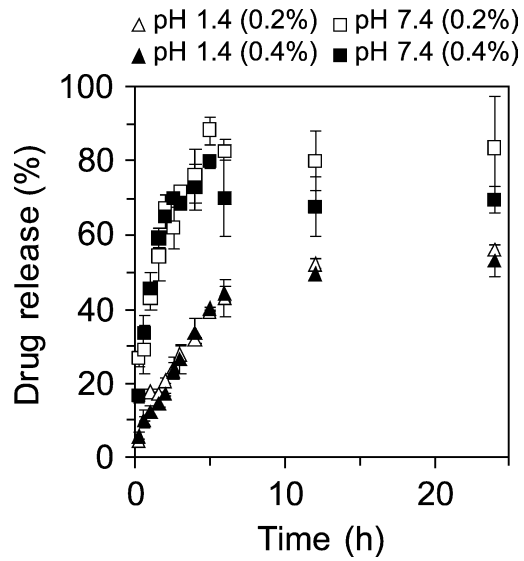

Fig. 5. Drug release from Ib-loaded PCS gel beads with different initial drug contents (0.2 and 0.4%) in simulated gastric fluid, pH 1.4, and simulated intestinal fluid, pH 7.4 ($n = 3$).

entrapment within the polymer matrix. Therefore, the drug remains in a biologically active form and can exert its effect upon the body as soon as it is released from the polymer matrix. Obviously, the major advantage of this formulation is that the drug in the polymer matrix is unaltered, after being released is the same as the native drug.

3.3. The in vitro release profiles of ibuprofen

Fig. 4 shows the release profiles of ibuprofen from 0.2% Ib-PCS beads at various pHs at 37 °C as a function of time. In general, the percentage of drug released increased with an increase in pH of dissolution medium. Within 3 h, 28% of Ib was released from PCS gel beads at pH 1.4 and 72% at pH 7.4. This behavior shows that drug release profiles of PCS gel beads are pH-sensitive. There are two factors to be considered in the noticeably higher release rate of ibuprofen

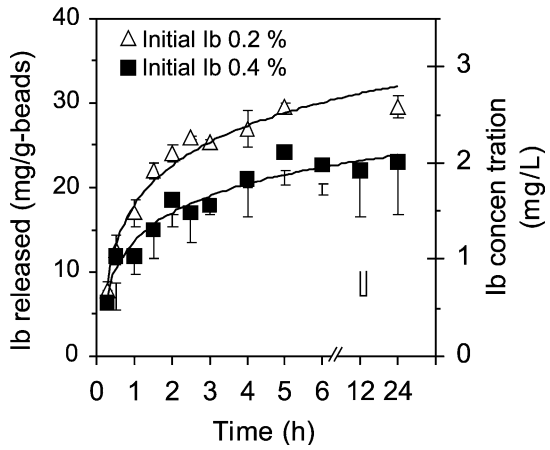

Fig. 6. Time course of Ib amount released from PCS gel beads and Ib concentration in dissolution buffer at different initial drug contents (0.2 and 0.4%) at pH 7.4 ($n = 3$).

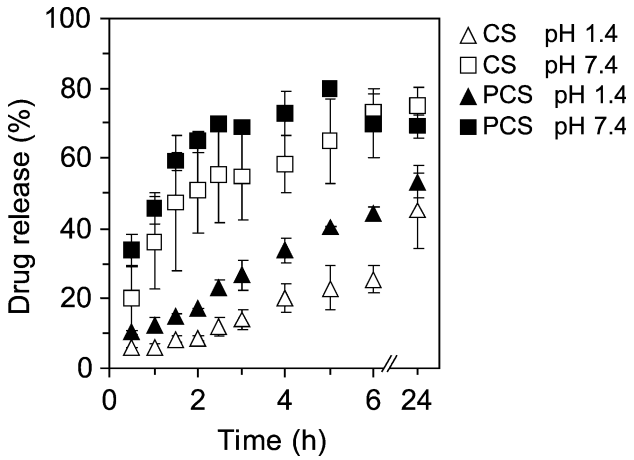

Fig. 7. Release profiles from 0.4% Ib-loaded CS and PCS gel beads in simulated gastric fluid, pH 1.4, and simulated intestinal fluid, pH 7.4 ($n = 3$).

at pH 7.4 than that at pH 1.4. The first factor is the high solubility of Ib in alkaline media (Table 1) (Handgraft & Valenta., 2000) and the later could be the electrostatic repulsion between negatively ionized carboxyl groups of Ib and phosphate groups in PCS in pH 7.4 media.

The influence of initial drug loading on the release percent of Ib from PCS gel beads at pH 1.4 and 7.4 is evaluated in Fig. 5. There is no substantially change between the release percents of Ib from 0.2 and 0.4% loaded PCS gel beads. For different initial drug loading, the amounts of Ib released per unit mass of PCS gel beads at pH 7.4 are shown in Fig. 6. The amount of drug released from high drug-loaded gel beads is higher than that from low drug-loaded beads. Increasing the initial drug content provides the larger equilibrium amount of drug released in each pH.

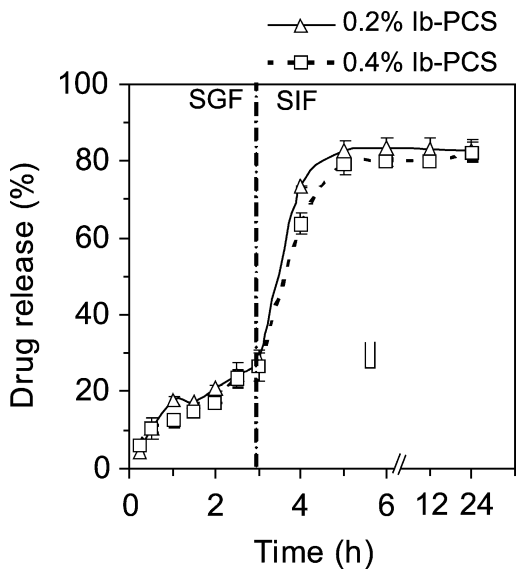

Fig. 8. Sequential release profiles from Ib-loaded PCS gel beads in simulated gastric fluid (pH 1.4) and simulated intestinal fluid (pH 7.4). The gel beads were suspended in pH 1.4 media for initial 3 h and then shifted into pH 7.4 media ($n = 3$).

The in vitro release profiles of Ib-loaded CS and PCS gel beads were investigated at pH 1.4 and 7.4 (Fig. 7). At pH 1.4, 14% of Ib was released from CS gel beads within 3 h while that from PCS gel beads is twice higher. Ib was released at 54% from CS gel beads at pH 7.4 within 3 h while 70% of Ib from PCS gel beads was released. The release percent of Ib from PCS gel beads was higher than that from CS gel beads. It is an advantage of PCS gel beads containing phosphate groups for successful oral drug delivery system.

In order to investigate the sequential release profile, Ib-loaded PCS gel beads were suspended in pH 1.4 buffer for 3 h and then shifted into pH 7.4 buffer. In Fig. 8 the release percents of Ib from both 0.2 and 0.4% Ib-loaded PCS beads were nearly same and were independent on the initial drug content as described above. The Ib released percent was 28% within 3 h in simulated gastric fluid (pH 1.4) and the equilibrium release percent in simulated intestinal fluid (pH 7.4) reached to nearly 80% of the initial drug contents in 2 h after changing media. Therefore, PCS gel beads can be successful to deliver the drug to the intestine without reducing the drug leakage in the stomach.

4. Conclusions

The PCS gel beads described in this paper were developed as a pH-sensitive and gastric fluid-resistant drug carrier. The gel beads were prepared under very mild condition at room temperature and pH 4.0 and the loading efficiency of a model drug, Ib, was over 80%. The water solubility of the drug, the swelling degree of beads and the ionization of phosphate groups were found to predominantly influence on release profiles of Ib at various pHs.

The release rate in simulated intestinal fluid (pH 1.4) is three times higher than that in simulated gastric fluid (pH 7.4), enabling the drug delivery or release to take place preferentially in the intestine with avoiding drug leakage in the stomach. All of these interesting features indicate that the PCS gel beads can be used as successful drug carrier for the controlled drug delivery in oral administration. We report on only in vitro release of Ib from PCS gel beads at simulated buffers. More detailed results on enzyme-mediated release profiles and in vivo experiments will be reported in forth coming papers.

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