Research Article

Effect of Acidic Medium on Swelling and Release Behaviors of Chitosan-Reinforced Calcium Pectinate Gel Beads

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Abstract

Chitosan-reinforced calcium pectinate (ChCP) gel beads were prepared by ionotropic gelation method. The swelling of ChCP gel beads and release behavior of indomethacin from the beads were investigated and compared with conventional calcium pectinate (CP) gel beads. The factors, such as molecular weight of chitosan, concentration of chitosan, and release medium, which can have a significant effect on the swelling and release behaviors from the beads, were discussed in this study. The mechanical test showed that the ChCP beads have slightly higher strength than that of CP beads. The swelling index of the ChCP beads in acidic medium was much lower than that in neutral medium. The release of indomethacin from ChCP beads under conditions mimicking intestinal transit were evaluated in pH 7.4 Tris buffer. The acid pretreatment caused a faster drug release from ChCP beads. The less swelling in acidic medium and faster drug release of acid-pretreated ChCP beads may be due to the dissolution of chitosan from the beads in acidic medium, as no fluorescence signal was seen at the shell of the beads. The results suggested that the acid, which essentially found in stomach, influenced the swelling and release behaviors of ChCP beads.

***Key Words*:** Chitosan; Pectin; Calcium pectinate; Beads; Drug release; Acid pretreatment

Introduction

An oral dosage form is the preferred route of administration of drugs because it provides easy, low cost administration. However, patient compliance becomes an important factor to consider in conjunction with oral administration of a drug. One method to maximize patient compliance is to reduce the number of dosage forms a patient must take to attain effective therapy by using sustained release formulations which have been numerously described in the many literatures (e.g., Colombo et al., 1996; Uhrich et al., 1999). Many of these formulations are comprised of a solid, polymeric matrix throughout which a drug has been dispersed. After the formulation is ingested, the active drug will slowly release from the polymer matrix, resulting in prolonged release of the active agent (Uhrich et al., 1999).

Natural polysaccharides such as pectin and chitosan have been widely investigated for applications in coating membranes, controlled release drug delivery, and biomaterials (Skaugrud, 1995; Sriamornsak, 2003). Pectin, an anionic polysaccharide, is predominantly a linear polymer of mainly α-(1-4)-linked D-galacturonic acid and its methyl esters (Figure 1). Pectin can form gels by cross-linking with calcium ions. Intermolecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the pectin molecules, called an 'egg-box' conformations with interstices in which the calcium ions may pack and be coordinated (Braccini and Pérez, 2001). Recently, calcium pectinate (CP) beads prepared by the ionotropic gelation method (Aydin and Akbuga, 1996; Sriamornsak and Nunthanid, 1998) have been investigated as a sustained release drug delivery system; however, the use of pectin beads has some drawbacks due to their rapid *in-vitro* release. The drug release from CP beads has been modified by changing the type of pectin or divalent cations as well as using hardening agent (El-Gibaly, 2002; Sriamornsak et al., 1998; Sriamornsak and Nunthanid, 1999).

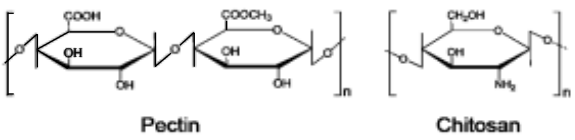

Figure 1 Chemical structure of pectin and chitosan.

Chitosan is a natural cationic polysaccharide made from alkaline N-deacetylation of chitin, consists of N-acetyl-glucosamine and glucosamine residues (Figure 1). It has biocompatible, biodegradable, nontoxic and mucoadhesive characteristics (Skaugrud, 1995). It can form interpolymeric or polyelectrolyte complexes (PEC) with anionic polymers, such as sodium alginate or pectin (e.g., Hiorth et al., 2005; Tao et al., 2006). The properties of composite particles composed of chitosan and pectin have been reported recently (Chang and Lin, 2000; Sriamornsak and Puttipipatkhachorn, 2004). In those cases, chitosan was used as an additive for the bulk modification of the particle structure, i.e., forming composite particles by complex formation. The addition of chitosan altered the drug release behavior of hydrogel beads and modified their swelling behavior of complex particles (Chang et al., 2000; Sriamornsak and Puttipipatkhachorn, 2004). Kim et al. (2003) used chitosan coated pectin beads as an oral delivery system for albumin, a model protein drug. They investigated the effect of some factors, e.g., concentration of calcium chloride, molecular weight of chitosan, pH of chitosan, on the release of albumin. However, the effect of some factors influencing the release behavior has not been examined in detail.

In the present study, the release behavior of chitosan-reinforced calcium pectinate (ChCP) gel beads was further studied and compared with the conventional CP beads, using indomethacin as a model for small molecular drug. Indomethacin has been the successful non-steroidal anti-inflammatory agent available for the treatment of rheumatoid arthritis, osteoarthritis, as well as other inflammatory diseases. As the pKa of indomethacin is about 4.5, the official monographs of indomethacin extended-release capsules in the United States Pharmacopeia (USP 30, 2007) recommended using pH 6.2 or 6.8 phosphate buffer as dissolution medium. However, by oral administration, the dosage forms would pass through stomach, which is in acidic environment, before reaching the small intestine. The effect of acid pretreatment on the release of indomethacin from ChCP gel beads was then investigated. The effects of chitosan molecular weight and chitosan concentration on drug release, morphology and mechanical properties of ChCP gel beads were also studied.

Experimental

Materials

Chitosans with high molecular weight (MW 419 kDa) and low molecular weight (MW 191 kDa) and degree of N-acetylation of 93% and 97%, respectively, were purchased from Aqua Premier (Thailand) and were referred as ChH and ChL, respectively. Pectin with degree of esterification of 28 % (GENU Pectin, type LM-104 AS-FS) was the generous gift of CP-Kelco (Denmark). Indomethacin and calcium chloride ($CaCl_2$) were of standard pharmaceutical grade and all chemical reagents used were of analytical grade and used as supplied without further purification.

Preparation of CP and ChCP gel beads

CP gel beads were prepared by ionotropic gelation method (Sriamornsak et al., 1998; Sriamornsak et al., 1999). Pectin was dispersed in water (5% w/w) with agitation and indomethacin was added to aqueous solution. The dispersions were dropped using a nozzle of 0.80-mm inner diameter into 5% (w/v) $CaCl_2$. The beads formed were allowed to stand in the solution for 1 hour, separated and washed with distilled water, then screen-filtered and dried at 37 ^{o}C for 12 hours. ChCP gel beads were prepared by the same method except the mixture of chitosan (0.1-2.0% w/w of ChH or ChL) and $CaCl_2$ (5% w/v) was used instead of $CaCl_2$ alone (Sriamornsak and Puttipipatkhachorn, 2004).

Determination of mechanical properties of gel beads

The mechanical properties of the CP and ChCP gel beads were determined using a Texture Analyzer (model TA.XT plus, Stable Micro Systems, UK) equipped with a stainless steel cylindrical probe (6-mm diameter). The bead sample, after gentle drying on filter paper, was placed centrally under cylindrical probe and the compression test was commenced. The maximum force required to break the bead structure was taken. All analyses were performed on 10 replicate samples under identical conditions.

Morphological examination of gel beads

Morphological examination of the surface and internal structure of the CP and ChCP beads was carried out using a scanning electron microscope (model Maxim 2000S, CamScan Analytical, UK) at the accelerating voltage of 12 kV. The internal structure of the beads was examined by cutting them in half with a steel blade.

Swelling studies

Both simulated gastric fluid USP, pH 1.2 (SGF) and simulated intestinal fluid (pH 7.4 Tris buffer) (SIF) were used as the test medium, in order to see how the pH of testing medium influence the swelling behavior of CP or ChCP beads. Thirty dried beads were placed in a beaker to which 200 mL of test medium (37°C) were added, and then stirred with a magnetic stirrer at a speed of 100 rpm. At predetermined time, the swollen beads were observed and measured under an optical microscope (model BH-2, Olympus, Japan). The magnitude of swelling (i.e., swelling index) was presented by the ratio of the mean volume of swollen beads to the mean volume of the dried beads before the test.

Fluorescence studies

Fluorescein thiocyanate (FITC)-labeled chitosan was synthesized according to the method by Qaqish and Amiji (1999). Briefly, one gram of chitosan was dissolved in 100 mL of 0.2M acetic acid. To the chitosan solution, 100 mL of dehydrated methanol was slowly added with continuous stirring. FITC, dissolved in methanol at 2 mg/mL concentration, was slowly added to the chitosan solution. The reaction between the isothiocyanate group of FITC and the primary amine group of the D-glucosamine residue was allowed to proceed for 3 hours in the dark at room temperature. FITC-labeled chitosan was precipitated in 0.5M sodium hydroxide solution. The precipitate was washed extensively with distilled water until there was complete absence of free FITC fluorescence signal in the washing medium.

The FITC-labeled chitosan was used to prepare ChCP beads, in the same manner as described above. The ChCP beads made of FITC-labeled chitosan were hydrated in SGF (pH 1.2) or SIF (pH 7.4 Tris buffer). After 1 hour, the swollen beads were washed with distilled water and then took off from the washing medium for observation under an inverted microscope (model Eclipse TE2000-U, Nikon Instruments, Japan) with fluorescence filter.

Determination of drug content and *In-vitro* release studies

Prior to the determination of indomethacin content, the beads must be dissolved by Tris buffer (pH 7.4) containing 5mM ethylenediamine tetraacetic acid. The content of indomethacin was later assayed by UV-spectrophotometer (Hitachi U-2000, Japan) in pH 7.4 Tris buffer at 318 nm. The determinations were made in triplicate.

The drug release behavior from the beads was evaluated using the rotating basket dissolution method (USP dissolution apparatus 1, Erweka, Germany). The baskets were rotated at 100 rpm at 37°C. The dissolution medium used was SIF (pH 7.4 Tris buffer). The beads were acid-pretreated by soaking in SGF for 1 hour before drug release test (to simulate the travel of dosage form through the stomach). In some cases, the release tests with no pretreatment were performed and compared with those pretreated in SGF. The analytical wavelength was 318 nm and Beer's law was obeyed over the range of 0–100 mg/L. The drug release was measured from accurately weighed amounts of the beads, equivalent to 75 mg of indomethacin, added to 750 mL of dissolution medium. All dissolution runs were performed in triplicate.

Statistical analysis

Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS version 10.0 for Windows (SPSS Inc., USA). *Post hoc* testing ($p<0.05$) of the multiple comparisons was performed by either the Scheffé or Games-Howell test depending on whether Levene's test was insignificant or significant, respectively.

Results and Discussion

Being an anionic polysaccharide, pectin can be cross-linked with cations such as calcium ions (Sriamornsak and Kennedy, 2006). In this study, an aqueous solution of pectin containing indomethacin was dropped into calcium chloride solutions and gelled spheres were formed instantaneously by ionotropic gelation in which intermolecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the pectin molecules, as previously described (Sriamornsak et al., 1998; Sriamornsak et al., 1999). To develop the ChCP beads, chitosan was added into calcium chloride solution. Pectin formed the gelled cores with calcium ions, whereas chitosan constituted the majority of the outer layer. Calcium ions and chitosan molecules had to diffuse into pectin droplets to form the gel beads. As a consequence, their concentrations decreased towards the center of the beads. Due to the higher diffusivity of the small molecule of calcium in solution, it is most likely that the concentration of chitosan in the inner layer was lower than that of calcium. The above factors led to a softer core with cavity inside and a more resilient surface layer for freshly formed gel beads. Upon air drying, the drug-loaded beads of all formulations become small and dense (2.3 – 2.6 mm) and, in which, drug particles are embedded (Sriamornsak and Thirawong, 2003).

Table 1 shows the indomethacin content in the CP and ChCP gel beads. As the chitosan concentration increased, the drug content decreased. The contents of indomethacin in ChCP beads using ChH and ChL were insignificantly different. The exception is for the high concentration of chitosan (i.e., 1 and 2%) in which the drug content in ChCP beads using ChH was significantly higher than those using ChL. The mechanical properties (i.e., breaking strength) of CP and ChCP beads are shown in Table 2. The ChCP beads have slightly higher breaking strength than the

Table 1 Drug content (mg/100mg of dry beads) in CP gel beads reinforced with different concentrations of low (ChL) and high (ChH) molecular weight chitosans (n=3).

	ChL	ChH
0% chitosan	21.43 ± 0.11	
0.1% chitosan	24.27 ± 1.82*	21.95 ± 1.08
0.5% chitosan	17.14 ± 1.29*	17.21 ± 0.32*
1% chitosan	15.78 ± 0.08*	17.66 ± 0.15*
2% chitosan	15.94 ± 1.31*	19.67 ± 0.35*

*, $p<0.05$, significantly difference from CP beads with 0% chitosan.

Table 2 Breaking strength (kPa) of some CP gel beads reinforced with low (ChL) and high (ChH) molecular weight chitosans (n=10).

	ChL	ChH
0% chitosan	124.73 ± 22.86	
0.1% chitosan	134.99 ± 22.53	133.28 ± 17.18
2% chitosan	145.46 ± 39.92	170.62 ± 29.36

CP beads, ranged from 133.28 to 170.62 kPa. It is obvious that the chitosan helped to strengthen the ChCP beads. The higher the concentration of chitosan, the higher the breaking strength of ChCP beads. Higher molecular weight chitosan seemed to improve the mechanical properties of ChCP beads, especially at 2% ChH.

The SEM images of CP and ChCP beads are shown in Figure 2. Upon air drying, the conventional CP gel beads became small and dense with rough surface. The back-scattered electron images of cross-sectional CP beads clearly showed a net-like structure of calcium pectinate and uniform distribution of drug particles (Sriamornsak and Thirawong, 2003). The CP and ChCP beads gave similar SEM images. The reinforced layer (or coating layer) of chitosan could not be distinguished by the SEM observation. It seemed that the ChCP beads using ChH (Figure 2c) were the densest, corresponded with the mechanical data.

When dried ChCP beads were placed into SGF, the beads swelled slightly, which is similar to the CP

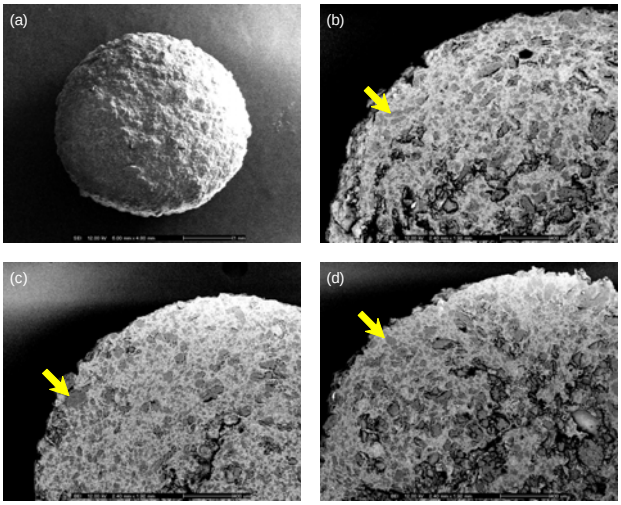

Figure 2 Scanning electron micrographs of (a-b) CP gel beads, and ChCP gel beads using (c) 2% high molecular weight chitosan, ChH and (d) 2% low molecular weight chitosan, ChL. The arrows indicate indomethacin particles.

beads (Figure 3). This suggested that, in acidic medium, chitosan in the outer layer dissolved out from the beads and swelling index was resulted from the cores made of calcium pectinate. It has been suggested that, in acidic medium, the calcium pectinate beads were converted to the insoluble pectinic acid beads which had a low swelling index (Sriamornsak and Kennedy, 2008). This transformation also influenced

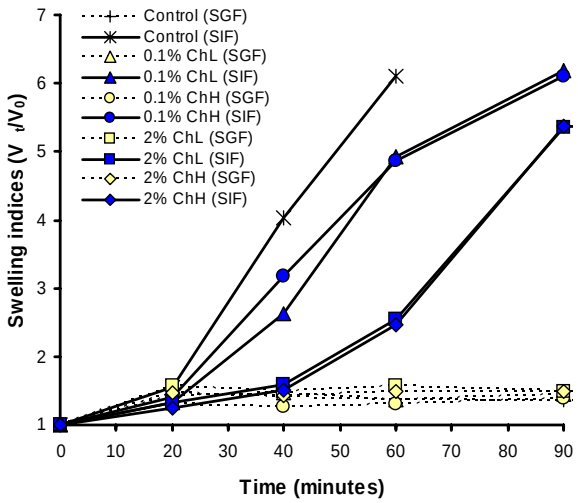

Figure 3 Swelling indices of CP and ChCP gel beads in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4).

the mechanical properties of the CP beads exposed to SGF (data not shown). In SIF (pH 7.4), however, the CP and ChCP beads swelled to a greater extent. The ChCP beads showed lower swelling indices, compared to the CP beads. This may be due to the less solubility of chitosan (in the outer layer) at higher pH restricted them from further expansion. The higher chitosan concentration gave the lower swelling index but the type of chitosan insignificantly influenced the swelling of ChCP beads at pH 7.4.

Fluorescence images of CP and ChCP beads after swelling for 1 hour in SGF and SIF are shown in Figure 4. The results agreed with the swelling data discussed above. The ChH and ChL were labeled with fluorescence marker by chemical reaction. After swelling test, it could be seen that only ChCP beads that tested in SIF (pH 7.4) showed the florescence signal at the shell of the beads under the microscope (Figures 4d and 4f). On the other hand, in SGF, no florescence signal was seen (similar to that of CP beads, Figure 4a), owing to the dissolution of the chitosan layer on the surface of ChCP beads, as discussed above.

The release behavior of indomethacin from ChCP gel beads was investigated *in-vitro*. The beads were soaked in SGF for 1 hour prior to test in SIF. The drug release, in SIF, from gel beads could be retarded by adding chitosan into gelation medium (Figure 5). This is similar to that of chitosan-reinforced calcium alginate gel beads reported by Murata and colleagues (Murata et al., 1993). The higher concentration and lower molecular weight of chitosan showed a slower drug release. It is probable that smaller molecules of chitosan (i.e., ChL) formed strong interaction between chitosan and pectin during beads formation and resulted in stronger outer layer of the ChCP beads, than those of larger molecules (i.e., ChH). Marudova et al. (2004) also suggested that chitosan oligomer can act as an effective cross-linker of pectin networks and the gel stiffness increased with increasing chitosan concentration. The smaller molecule of chitosan oligomer produced the stronger chitosan-pectin gels.

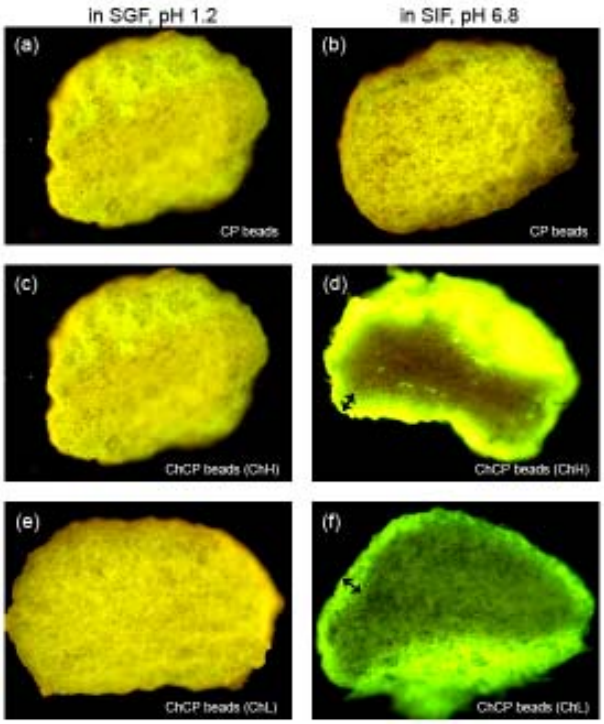

Figure 4 Fluorescence images of the cross-section of (a and b) CP gel beads, and ChCP gel beads using (c and d) 2% high molecular weight and (e and f) 2% low molecular weight chitosan, after swelling for 1 hour in simulated gastric fluid (SGF, pH 1.2) (left column) or simulated intestinal fluid (SIF, pH 7.4) (right column). The arrows indicate the layer of chitosan.

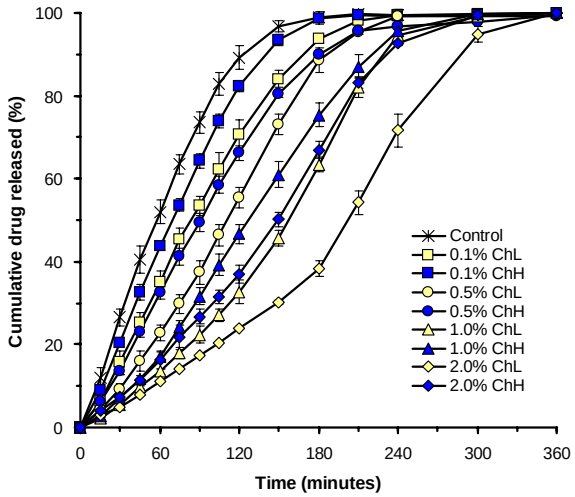

Figure 5 Effect of molecular weight and concentration of chitosan on drug release from CP and ChCP gel beads in simulated intestinal fluid (SIF, pH 7.4).

The comparison of the drug release profiles in SIF with and without acid pretreatment was shown in Figure 6. With acid pretreatment, as mentioned above, the drug release from CP beads was much faster than that tested in SIF without acid pretreatment. It is likely that the transformation of calcium pectinate to pectinic acid reduced the gel formation (as shown by lower swelling in acidic medium) and increased drug permeability. Sriamornsak and Kennedy (2008) reported the higher diffusion coefficient values of theophylline in calcium pectinate films equilibrated in acidic medium than those in neutral medium. The drug release from ChCP beads was much slower in SIF (without acid pretreatment), suggesting that the excess chitosan dissolved out in acidic medium prior to test in SIF, as confirmed by the fluorescence images of swollen ChCP beads (Figure 4). The results were also suggested that the ChH could form weaker interaction to pectin so that more excess chitosan dissolved from the beads.

The results demonstrated that the acid, which essentially found in stomach, influenced the swelling and release behaviors of ChCP beads although the dissolution test in acidic medium for indomethacin formulations is not required in the official monographs.

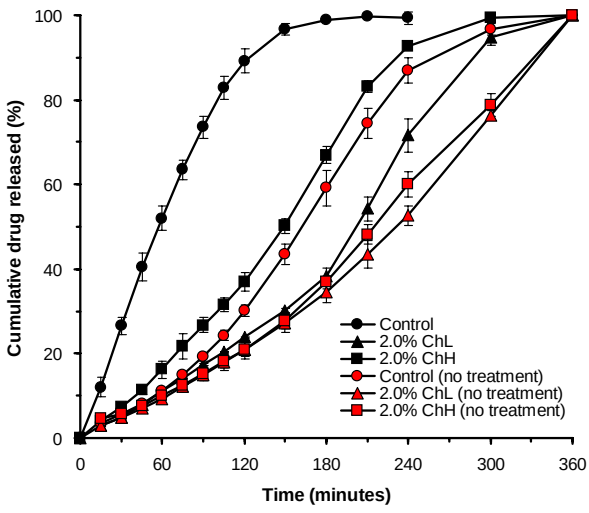

Figure 6 Effect of the acid pretreatment (prior to test) on the drug release from CP and ChCP gel beads in simulated intestinal fluid (SIF, pH 7.4).

Conclusions

The morphology, swelling and release behaviors of ChCP beads were investigated in this study. The swelling index of the ChCP beads in acidic medium was much lower than that in neutral medium. The effect of acid pretreatment on the drug release (in pH 7.4 Tris buffer) revealed that the drug release faster. The less swelling in acidic medium and faster drug release of acid-pretreated ChCP beads was likely due to the dissolution of chitosan from the beads in acidic medium, as confirmed by fluorescence images. The results indicated that the acid influenced the swelling and release behaviors of ChCP beads.

Acknowledgements

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