

Research Article

Chitosan-Carrageenan Polyelectrolyte Complex for the Delivery of Protein Drugs

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A chitosan-carrageenan polyelectrolyte complex (PEC) was prepared by salt induced impeding of polyplex formation method and was encapsulated with bovine serum albumin (BSA) to study the potential to be tailored to the pH responsive oral delivery of protein drugs. The FTIR spectra showed the successful formation of the PEC under the experimental condition. The release kinetics of BSA from the PEC was studied in the simulated gastrointestinal fluids with and without digestive enzymes. The prepared PEC showed the nature of pH-sensitivity. A typical controlled release of BSA from the PEC (180 μg of BSA from 3 mg of PEC) was obtained in the simulated intestinal fluid (SIF, pH 7.5), which was due to the significant swelling and disintegration of PEC, but little amount of BSA was released (11 μg of BSA from 3 mg of PEC) in the simulated gastric fluid (SGF, pH 1.2), confirming acidic stability of the prepared PEC. The presence of digestive enzymes was found not to affect the response of PEC to ambient pH value, but to speed up the release of BSA from carriers.

1. Introduction

With the rapid development in biotechnology and recombinant technology, more and more therapeutic peptides and proteins have been produced as drugs at commercial scale. Among various routes for administrating the drugs, oral route is preferred due to its high levels of patient acceptance and long term compliance [1]. Moreover, the dosage forms can be formulated with relative ease and are manufactured without sterile conditions [2]. However, this delivery route faces ineffectiveness for the peptide and protein drugs because the drugs can be easily hydrolyzed by the extreme acidity in the stomach and proteolytic enzymes in the gastrointestinal (GI) tract. The penetration barrier also prevents them from crossing the intestinal and then basal membranes for entry into the blood [3]. To improve the oral bioavailability from less than 1% to at least 30–50% for a polypeptide drug delivery through the GI tract has attracted intensive research [4].

Polyelectrolyte complexes (PEC) are formed by the ionic interactions as ionically cross-linked networks when two oppositely charged polyelectrolytes bind each other in an aqueous solution [5]. The net charge fixed on the complex, which is an important factor determining the swelling and the induced volume change of the PEC, is affected by pH value of ambient solution due to the variation in the degree of ionization of functional groups [6]. Thus, the nature of highly pH-sensitive swelling brings PEC to the application of oral drug delivery [7, 8] because the pH varies at each organs or the diseased part of human body. Due to the consideration of biocompatible and nontoxic substance as the components of polyelectrolyte complexes in such applications, several chitosan-based PEC have been studied for controlled release formulations in combination with either synthetic or natural polyanions, such as alginate [9], polyacrylic acid [10], carboxymethyl-cellulose [11], dextran sulfate [12], xanthan [13], and collagen [14]. One of the natural polyanions under investigation is κ -carrageenan, an acidic polysaccharide

with sulfate groups [15]. In an acidic solution, the negatively charged sulfate groups of κ -carrageenan bind to the positively charged amino groups of chitosan and form an acid-base type PEC. Upon increasing pH, the amino groups are deionized and the binding affinity between two polyelectrolyte molecules becomes weaker, which would lead to the swelling and disintegration of the PEC and finally the release of loaded drug [16, 17]. By adjusting factors that cause the swelling properties of PEC, it is possible for us to precisely modulate the drug release to the target site. Because of mucoadhesive properties of chitosan [18], chitosan-based PEC might give added advantage to enhance the intestinal absorption of drugs, to prevent the presystemic metabolism of peptides and to increase the residence time of the delivery system. Consequently, it will result in the increased oral bioavailability of peptide and protein drugs [19].

It is known in the prior art that pH-sensitive chitosan-carrageenan PEC and encapsulation of drug can be prepared by simple mixing of two aqueous solutions of polyelectrolytes together with drug solutions under optimal conditions. However, limited success has been reported on the stability of chitosan-carrageenan PEC in acidic gastric pH [9, 20]. It has been found that the solution properties of carrageenan differ upon degree of sulfation, the presence of salts, and its concentration [21]. Moreover, being a polyelectrolyte, rheology of chitosan solutions are affected by the presence of salt [22]. In this study, the chitosan-carrageenan PEC was prepared by mixing aqueous solutions of chitosan and κ -carrageenan in the presence of NaCl. It is expected that, due to the presence of electrolyte counterions, the interaction between two polyelectrolytes will be delayed and the characteristic of PEC formed will have different properties. This process is named as the "salt induced impeding of polyplex formation" method. Bovine serum albumin (BSA) was used as a model protein drug to investigate the release kinetic in simulated gastrointestinal tract environments.

2. Experiments

2.1. Materials. Chitosan powder with 90% deacetylation degree (MW~180,000) was obtained from Bio-line Co., Ltd., Thailand. κ -carrageenan, bovine serum albumin, pepsin, pancreatin, and Bradford reagent were purchased from Sigma-Aldrich. Other materials were of analytical grade from Merck. Simulated gastric fluid (SGF pH 1.2), which contains 7 mL of hydrochloric acid (37.4%), 2 g of sodium chloride in 1 L of deionized water, was prepared with and without 3.2 g of pepsin. Similarly, simulated intestinal fluid (SIF pH 7.5), which contains 6.8 g of potassium dihydrogen phosphate and 190 mL of 0.2 M sodium hydroxide in 1 L of deionized water, was prepared with and without 10 g of pancreatin [23].

2.2. Methods

2.2.1. Preparation of BSA Loaded Chitosan-Carrageenan Polyelectrolyte Complex. BSA was loaded into the chitosan-carrageenan polyelectrolyte complex matrix using incorporation method. 500 mL of 0.3% (w/v) chitosan solution was

prepared by dissolving 1.5 g of chitosan powder in 1% acetic acid. 400 mL of κ -carrageenan solution was prepared by dissolving 1.5 g of κ -carrageenan in deionized water with 25 g of sodium chloride at 90°C and cooled to room temperature. 100 mL of BSA solution was prepared by dissolving 0.5 g of BSA in deionized water. The κ -carrageenan solution and BSA solution were mixed gently to obtain 0.3% (w/v) κ -carrageenan solution with BSA of 1000 μ g/mL. Subsequently, chitosan solution was added dropwise to the κ -carrageenan solution at the rate of 3 mL/min under stirring. When the resultant particles settled, the supernatant was decanted and 0.01% acetic acid solution was added to the particles to wash away the unreacted free polymer and sodium chloride in the suspension. This step was repeated everyday for a week. After that, the suspension was freeze-dried to obtain the agglomerates. The freeze-dried PEC agglomerates were ground into particles, which were then subjected to the structural characterization and the release of BSA.

2.2.2. Characterization. The PEC particles were coated with platinum under vacuum and their scanning electron micrographs were obtained using a JEOL (JSM 5600) scanning electron microscope (SEM).

FTIR spectra were recorded using KBr pellet in a Perkin Elmer Spectrum One FTIR with ATR spectrophotometer. Chitosan, κ -carrageenan, and chitosan-carrageenan PEC were finely ground together with KBr, respectively. FTIR spectra were recorded in the range of 4000–500 cm^{-1} . The number of scans was set at 16 and the spectral resolution was 4 cm^{-1} .

2.2.3. Protein Release in SGF and SIF. Firstly, the release profiles of BSA from the encapsulated PEC particles in SGF and SIF were investigated, respectively. Each sample containing 3 mg of BSA encapsulated particles and 1 mL of simulated fluids was incubated in an orbital shaker incubator (TU-900) at 37°C with a stirring rate of 200 rpm. After the predetermined time intervals, the fluids was filtered with 0.45 μ m membrane and the BSA concentration in the filtrate was determined with Bradford reagent using a Nicolet Evolution 500 UV spectrophotometer.

Next, the release profiles of BSA from the encapsulated PEC particles were investigated under the mimic physiological gastrointestinal conditions (in SGF followed by in SIF). After the encapsulated particles were incubated in SGF at 37°C with a stirring rate of 200 rpm for 4 hours, the particles were centrifuged at 20,000 g for 10 min before transferred to SIF and incubated at the same conditions. The release medium in SIF was taken out for the analysis using the method described above.

Finally, the release profiles of BSA encapsulated particles in simulated GI tract with digestive enzymes were investigated. All the experiments were conducted in triplicates.

3. Results and Discussions

3.1. BSA Loaded PEC Particles. The presence of electrolyte counterions from NaCl (Na^+ and Cl^-) can effectively prevent

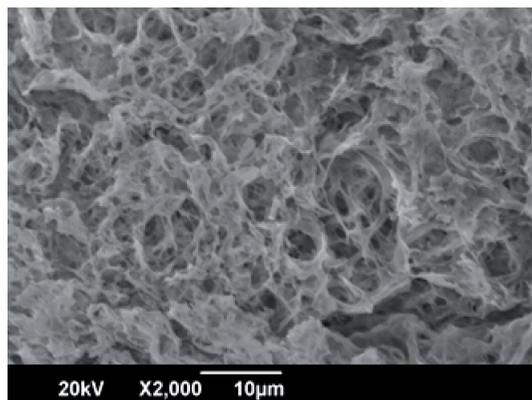


FIGURE 1: SEM image of the BSA loaded chitosan- κ -carrageenan agglomerate.

the immediate interaction of the oppositely charged chitosan and κ -carrageenan due to the weaker ionic attraction induced by the counterion atmosphere around the polyelectrolytes. With this principle, a homogeneous chitosan-carrageenan PEC hydrogel was prepared and found to be stable in acidic pH with substantial swelling [15]. In this study, the BSA loaded chitosan-carrageenan particles were formed in suspension in the presence of NaCl. The concentrations of chitosan, κ -carrageenan, the ratio between the two solutions, and the flow rate of chitosan solution were optimized (with a number of trials) to get homogeneous particle suspension under visual judgment. After washing away the unreacted components and freeze-drying concentrated particle suspension, dried particle agglomerates were obtained.

3.2. Characteristics of the PEC. The SEM image of the BSA loaded chitosan-carrageenan PEC particles was shown in Figure 1. It is seen that the particles presented interconnected pores of micrometer scale with some lumpy areas. The FTIR results indicated the successful formation of chitosan-carrageenan polyelectrolyte complex.

FTIR spectra of κ -carrageenan (a), chitosan (b), and polyelectrolyte complex of chitosan and κ -carrageenan (c) were shown in Figure 2 (wavelength 1800–600 cm^{-1}). The sulfate groups of κ -carrageenan attributed to the peaks at 1261, 924, and 851 cm^{-1} . The amide I peak of chitosan appeared at 1655 cm^{-1} . The broad bands around 1200–1000 cm^{-1} in both chitosan and carrageenan are from stretching of C–O–C. After the formation of chitosan-carrageenan polyelectrolyte complex, a new absorption band at 1525 cm^{-1} due to $-\text{NH}_3^+$ groups appeared. Moreover, the amide I peak from chitosan at 1655 cm^{-1} was shifted to 1641 cm^{-1} and the reduction of sulfate peak intensity at 1267 cm^{-1} was observed. It indicates the interaction between protonated amine groups of chitosan and sulfates groups of κ -carrageenan in the successful formation of chitosan-carrageenan polyelectrolyte complex.

3.3. Release of BSA in SGF and SIF as the Separated Medium. The release behaviors of BSA from the PEC particles in SGF

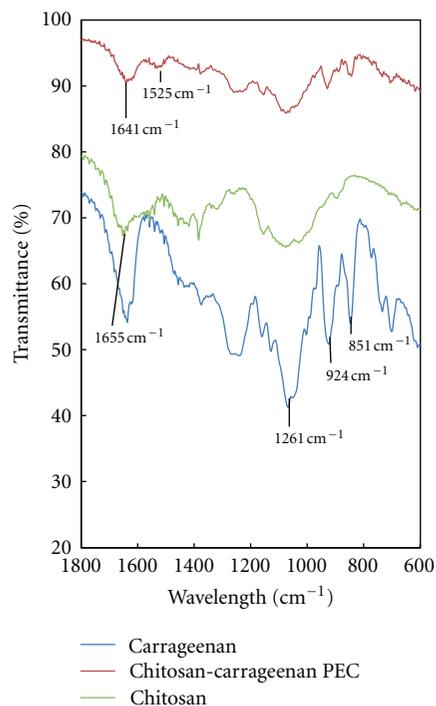


FIGURE 2: FTIR spectra of (a) κ -carrageenan, (b) chitosan, and (c) polyelectrolyte complex of chitosan and κ -carrageenan from 1800 to 600 cm^{-1} .

and SIF without digestive enzymes were measured and shown in Figure 3. The typical fast first-order followed by zero-order release kinetics was observed in both SGF and SIF media. However, the initial burst release observed in SIF was much faster than that in SGF. Within first 5 min, the release rate in SIF was 14 times faster than that in acidic SGF. Although no dramatic increase in BSA release was found in SGF after 5 min, BSA was further released in SIF for another one hour. Only 11 μg of BSA was released in SGF, while 180 μg of BSA was released in SIF.

Gan and Wang [24] had proposed the following protein release mechanisms: (a) desorption of protein molecules from the surface of particles, (b) diffusion through the swollen PEC, and (c) disintegration of the PEC. In acidic SGF medium (pH 1.2), chitosan and κ -carrageenan were oppositely charged and bound tightly. Therefore, further release was limited because of the absence of swelling or disintegration of PEC. The little amount of BSA released in SGF might be due to desorption of loosely bound BSA from the surface of particle. In SIF (pH 7.5), the ionic attraction between chitosan and κ -carrageenan became weaker because the pKa of chitosan is around 6.3 and little amino groups of chitosan got protonated. This will lead to significant swelling and disintegration of PEC, which allows large amount of encapsulated BSA to be released. As a result, the amount of BSA released in SIF was much higher than that in SGF. Those experimental results demonstrated that the chitosan-carrageenan polyelectrolyte complex can protect the loaded protein drug in acidic gastric condition and release it in higher pH of intestinal environment.

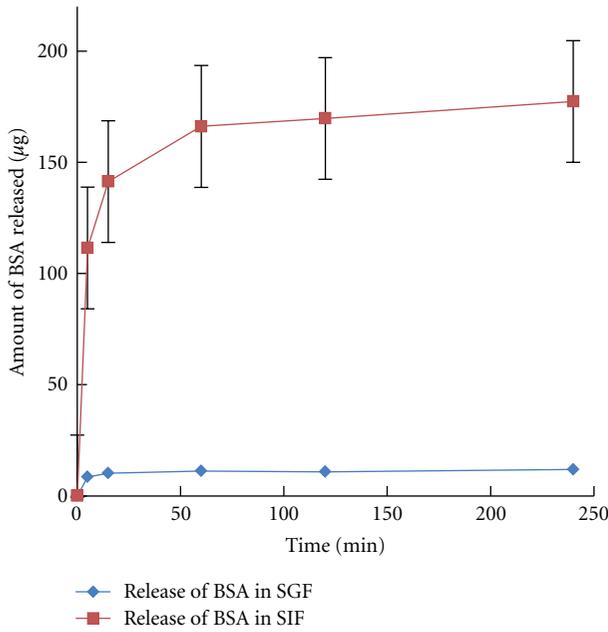


FIGURE 3: The release profile of BSA in SGF and SIF.

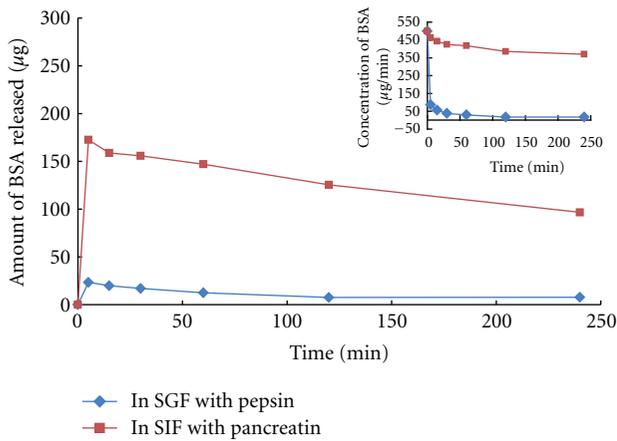


FIGURE 4: The release profile of BSA in SGF and SIF with digestive enzymes.

The release kinetics of BSA loaded PEC particles were also investigated in SGF and SIF in the presence of digestive enzymes. SGF was supplemented with pepsin and SIF with pancreatin. The results were shown in Figure 4. The trends of release kinetics were similar to those observed as in SGF and SIF without enzymes. In SGF with pepsin, maximum amount ($23 \mu\text{g}$) of BSA released was detected at 5 minutes, and then it decreased gradually to $8 \mu\text{g}$ at the end of 4 hours. In SIF with pancreatin, the maximum BSA release ($170 \mu\text{g}$) occurred at 5 minutes, and then it decreased to $95 \mu\text{g}$ after 4 hours. The release of BSA in SIF was again significantly higher than in SGF.

Compared with the BSA release profiles in SGF and SIF without digestive enzymes, more BSA was released in both SGF and SIF with enzymes in initial release period. It

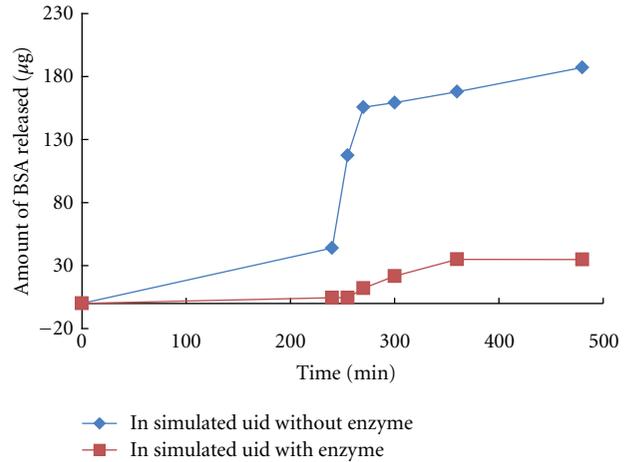


FIGURE 5: The cumulative release profile of BSA in SGF (4 hrs) and followed in SIF.

indicates that the digestive enzymes help to disintegrate the chitosan-carrageenan PEC and speed up the release from the carriers. The decreasing trends of protein concentration after the maximum release were attributed to the degradation of BSA by the digestive enzymes in the release media. Although no significant protein degradation was observed in plain SGF and SIF media, most of BSA was degraded in SGF with pepsin and up to 20% degradation of BSA was observed in SIF with pancreatin after 1 h (shown in Figure 4).

3.4. Release of BSA in SGF and Followed by in SIF as a Consecutive Release Medium. In order to mimic the physiological gastrointestinal conditions, the release study of the BSA loaded PEC was conducted in SGF for the first 4 hours and then the particles were transferred to SIF for another 4 hours. Similarly, the release was conducted in the media with and without digestive enzymes for comparison. The results were shown in Figure 5.

In the absence of enzymes, around $40 \mu\text{g}$ of BSA was released after 4 h of incubation in SGF. After transferring to SIF, about $65 \mu\text{g}$ of BSA was abruptly released in 5 minutes. It continued to increase with incubation time and reached $185 \mu\text{g}$ of cumulative release in a total of 8 hours incubation time. This amount was similar to the one released in 4 hours in SIF without pancreatin.

In the presence of digestive enzymes, only $5 \mu\text{g}$ of BSA was released after 4 hours of incubation in SGF with pepsin. After transferring to SIF with pancreatin, no increase of BSA concentration was detected for the next 15 minutes. Then, the amount of BSA released slowly increased to the maximum of about $35 \mu\text{g}$ in 2 hours and remained the same for the next 4 hours of incubation. It was believed that much more BSA was actually released in both SGF and SIF, but was digested by enzymes. When the particles were first incubated in SGF with pepsin, the enzymes could immobilize on the surface of the particles through electrostatic interaction. When the PEC was put in SIF with pancreatin, not only the loaded BSA protein but also the pepsin immobilized on the surface

of the PEC would release into the release medium of SIF. Apparently, the combined action of pepsin and pancreatin has synergistically degraded the released BSA protein. This would lead to the low amount of BSA detected in SIF although no significant amount of BSA was released in SGF in the presence of digestive enzymes.

Overall, only little amount of BSA was released from chitosan-carrageenan PEC in acidic SGF (with and without pepsin), but significant amount of BSA was released after the release medium was switched to SIF (with or without pancreatin). To our knowledge, this study is the first time to show that the chitosan-carrageenan PEC particles prepared in the presence of NaCl can be tailored to the pH responsive oral delivery of protein drugs.

The trends of the results are encouraging in response to pH value. However, the presence of digestive enzymes in the gastrointestinal tract is still detrimental to the activity of protein drugs because the released protein or peptide could be digested by the enzymes. The longer the residence time of drugs in the gastrointestinal tract, the higher the degree of the in situ degradation. Therefore, the released protein drug from the PEC should pass gastrointestinal mucosa as quickly as possible to reach the blood circulation to get the desired bioactivity. In the real life scenario, the presence of chitosan could potentially help the faster passage of the protein drug from the intestinal mucosa to the blood circulation because of the mucoadhesive properties of chitosan. Chitosan is known to interact with cystein-rich mucus to delay the mucus turnover rate and transiently open the tight junction of the intestinal wall. This process would help the released protein drug to transport across the intestinal barrier to reach higher bioavailability. In vivo studies are needed in the future to prove this speculation.

4. Conclusions

The pH responsive chitosan-carrageenan polyelectrolyte complex was successfully prepared by salt induced impeding of polyplex formation method. In the simulated gastric fluid (pH 1.2), only 11 μg of BSA was released from 3 mg of PEC, while in the simulated intestinal fluid (pH 7.5), 180 μg of BSA was released from 3 mg of PEC in four hours. In simulated gastrointestinal media (SGF followed by SIF), 185 μg of BSA was released from 3 mg of PEC, which is similar to the one released in SIF. However, in the presence of digestive enzyme the amount of released BSA increased in the first period and significantly decreased with the time going on. These results showed that the PEC matrix is suitable for oral delivery of protein and peptide drugs.

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