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Biodegradation of polyelectrolyte complex films composed of chitosan and sodium cellulose sulfate as the controllable release carrier

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ABSTRACT

For the applications of polyelectrolyte complex (PEC) of chitosan and sodium cellulose sulfate (NaCS) as the controlled release drug carrier, the biodegradation characteristics of chitosan, NaCS and chitosan/NaCS PEC films were investigated with pepsin, trypsin, lipase, α-amylase, and cellulase. The results showed that pepsin, amylase, trypsin and lipase have the appreciable hydrolytic activity to chitosan, but cannot degrade NaCS. However, cellulase showed high cellulosic activity and low chitosanolytic activity. For the hydrolysis of chitosan/NaCS films, the degradation rates were greatly influenced by the molecular weights of chitosan and NaCS. In vitro tests showed that different formulations caused diverse disintegration time of the films through the gastrointestinal tract. The results indicated the PEC based on chitosan and NaCS showed good potential for the gastrointestinal delivery systems.

1. Introduction

The oral controlled release systems have become the focus of drug delivery in the recent years, due to the ability to target the gastrointestinal tract (Deshpande, Rhodes, Shah, & Malick, 1996; Langer & Peppas, 1983; Porter, 1989; Yang, Chu, & Fix, 2002). According to the target sites of drug delivery, the oral controlled release systems could be divided into the stomach-specific delivery (Deshpande et al., 1996), the intestinal delivery (George & Abraham, 2006), and the colon-specific delivery (Basit, 2005). A great number of polymers have been evaluated for the preparation of the oral controlled release, such as chitosan (Hejazi & Amiji, 2003), pectin (Liu, Fishman, & Hicks, 2007), alginate (Tonnesen & Karlsen, 2002), acrylic polymers (Deshpande, Shah, Rhodes, & Malick, 1997), cellulose derivatives (Porter, 1989) and so on.

Chitosan is a cationic polymer, chemically a poly-β-(1–4)-D-glucosamine, derived from natural chitin by alkaline deacetylation. Chitosan has been used for conventional and novel gastrointestinal drug delivery systems (Hejazi & Amiji, 2003; Tozaki et al., 1997) because of its nontoxic, biocompatible, mucoadhesive, and biodegradable properties (Onishi & Machida, 1999; Xia, Liu, & Liu, 2008). However, chitosan is easy to dissolve in the solution at low pH, resulting in the limitation of its applications. Hence, various synthetic polymers or polysaccharides have been evaluated for the modification of chitosan, such as alginate (George & Abraham, 2006), pectin (Bigucci et al., 2008), dextran sulfate (Serizawa, Yamaguchi, & Akashi, 2002), oxypullulan (Muzzarelli et al., 2002), and so on. Sodium cellulose sulfate (NaCS) is a biocompatible and hydrophilic cellulose derivative, which has been applied to prepare the bio-microcapsules for cell cultures (Chen, Yao, Guan, & Lin, 2005; Yao, Guan, & Lin, 2006; Zhang, Guan, Ji, & Yao, 2006; Zhang, Yao, & Guan, 2005a; Zhang, Yao, & Guan, 2005b; Zhao, Chen, & Yao, 2006) and increasingly used for the biomedical application (Dautzenberg et al., 1999; Fluri, Kemmer, Baba, & Fussenegger, 2008). Recently, NaCS has been utilized for the modification of chitosan to improve its properties. In aqueous solution, chitosan and NaCS can easily form a water-insoluble complex, since the former is polyanion and the later is polycation. Wang, Xie, Zheng, and Yao (2009) prepared a novel colon-specific drug delivery capsule based on chitosan and NaCS. By in vitro degradation tests, the chitosan–NaCS complex could be degraded by the colon microflora, and hydrolyzed in the simulated gastric fluid (SGF) and the simulated intestinal fluid (SIF). Xie, Wang, and Yao (2009) extended the chitosan–NaCS complex to form a microcapsules by means of layer-by-layer self-assembly with the shell thickness of about 6 nm. It could be deduced that the NaCS/chitosan complex would be a promising material for the oral controlled release system.

The degradation behavior of biomaterial is very important for the use of controlled release since it may affect not only the release process but also the host response. It has been

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known that chitosan could be degraded by many non-specific enzymes, including pepsin, lipase from the gastrointestine (Kumar & Tharanathan, 2004; Pantaleone, Yalpani, & Scollar, 1992). However, the degradation behaviors of chitosan/NaCS complex by different gastrointestinal enzymes were rarely investigated in the literature. Hence, it is necessary to investigate the degradation of chitosan/NaCS complex before its application for the oral controlled release systems.

In the present study, five enzymes existed in the gastrointestinal tract (GIT) (pepsin in the stomach; amylase, trypsin and lipase in the small intestine; cellulase from the colonic bacteria) were used to simulate the environment of GIT. Effects of chitosan and NaCS molecular weight (Mw) on the degradation of chitosan, NaCS and the chitosan/NaCS complex films by the enzymes mentioned above will be investigated. This work would not only expand the knowledge on the enzymatic degradation of insoluble carbohydrates, but also benefit the application of chitosan–NaCS complex to the controlled release systems.

2. Materials and methods

2.1. Materials

Chitosan with an 85% degree of deacetylation and Mw of 118.7 kDa, 135.3 kDa, 563.3 kDa and 723.2 kDa were supplied by Jinan Haidebei Co., Ltd. (China). NaCSs with degree of substitution (DS) among 0.3–0.6 and Mw ranged from 5.2 kDa to 710.8 kDa were prepared by the heterogeneous reaction as described previously (Yao, 2000). Enzymes from the gastrointestine (pepsin from porcine gastric mucous membrane; trypsin and lipase from porcine pancreas) were obtained from Sinopharm Chemical Reagent Co., Ltd. (China). α-Amylase from *Bacillus subtilis* was obtained from Shandong Longda Biological Engineering Co., Ltd. (China). Cellulase from *Trichoderma viride* was kindly gifted by Prof. Liming Xia (Institute of Biological Engineering, Zhejiang University). All other chemicals and reagents used were of analytical grade and were used without further purification.

2.2. Preparation of NaCS/chitosan complex films

NaCS (4%, w/v) and chitosan (1%, w/v) with different Mws were mixed by blending two polymer solutions at weight rate of 4:3, followed by stirring for 15 min at room temperature. In the reactor, NaCS and chitosan can form a polyelectrolyte complex. Then the mixtures were casted in the plastic petridishes and dried at 45 °C for 24 h. After cooling down, the NaCS/chitosan complex films were removed from the petridishes and stored in the desiccators at room temperature.

2.3. Biodegradation tests

The degradation of chitosan was conducted with 0.5% chitosan solution (w/v). The optimum pH was determined at the range of 1.5–7.4. The optimum protein concentration of enzymes was determined at the concentration from 40 to 100 mg/l. The degradation of NaCS was conducted with 0.5% NaCS solution and 44 mg/l cellulase. The chitosan/NaCS films with mean thickness in the range 80–120 μm was cut by 2 cm × 2 cm. The film samples were degraded in 20 ml solutions with appropriate enzymes.

In vitro tests were conducted with SGF, SIF and simulated colonic fluid (SCF). SGF was an aqueous solution of 12,000 U/l pepsin that was adjusted to pH 1.5 using 0.1 M HCl. SIF was prepared with the phosphate buffer (pH 7.4), 10,000 U/l α-amylase, 15,000 U/l trypsin, 8400 U/l lipase and 0.2% (w/v) $CaCl_2$. SCF was prepared with 1200 U/l cellulase and phosphate buffer (pH 6.4).

Table 1
The optimum pH, the chitosanolytic and the proteolytic activities of five enzymes tested.

Enzyme	Optimum pH	Chitosanolysis (U/mg)[a]	Proteolysis (U/mg)[b]
Pepsin	3.0	0.550	169
Amylase	5.0	0.060	175
Trypsin	6.0	0.340	221
Lipase	5.0	0.020	84
Cellulase	4.5	0.002	ND[c]

[a] Unit = μmoles of reducing equivalents released/min.
[b] Unit = μmoles of casein degraded/min.
[c] Not detected.

The activity of all the enzymes mentioned above was measured with the corresponding substrate.

2.4. Viscosimetry

The viscosity of chitosan solutions was measured with Ubbelohde viscometer. The decrease of viscosity with the enzyme treatment was determined as the function of reaction time. The data were normalized and plotted as the relative viscosity with respect to the initial value, taking an enzyme-free chitosan solution as the control.

2.5. Reducing end assay

The concentration of reducing ends following the degradation of chitosan, NaCS, and chitosan/NaCS films were measured according to the modified method of Schales (Roncal, Oviedo, de Armentia, Fernandez, & Villaran, 2007). The concentration of reducing ends was expressed as the concentration of reducing sugars equivalent to glucosamine from a calibration curve prepared with this monosaccharide as the standard.

3. Results and discussion

3.1. Degradation of chitosan

In order to evaluate the degradation of NaCS/chitosan films, the investigation of chitosanolysis is primary. Although there were some previous studies on the degradation of chitosan, the chitosanolytic activity and the kinetic parameters of enzymes from different sources (even the same origin) were not the same. E.g. Lipase from *Aspergillus oryzae* performed relatively low chitosanolytic activity, but lipase from *Aspergillus niger* was comparable to chitosanase (Pantaleone et al., 1992). Roncal et al. (2007) reported that the optimum pH of chitosanolysis by pepsin from porcine gastric mucosa was 4.5, while it was 5.0 according to Kumar and Tharanathan (2004). For five enzymes tested in the present work, Table 1 represents the optimum pH, enzyme concentration, the activity of enzymes to chitosan as well as the corresponding substrates. The pH was determined at the range from 1.5 to 7.4. Slight increase of chitosanolysis activity for pepsin was found by increasing pH from 1.5 to 3.0, but further increase would result in a great decrease of hydrolysis activity of pepsin. High activity of pepsin at low pH may be beneficial for chitosanolysis, since pepsin showed the highest chitosanolysis activity (0.55 U/mg) among five enzymes tested. When the pH was around/over 5.0, the chitosanolytic activities showed close relationship to the proteolytic activity. As shown in Table 1, trypsin showed the highest proteolytic activity (221 U/mg) and a relative high chitosanolysis activity (0.34 U/mg), followed by amylase and lipase. Similar results were also reported by Kumar and Tharanathan (2004). The reason might be that the proteolytic activity caused the depolymerization of chitosan. Based on the experimental data, it was

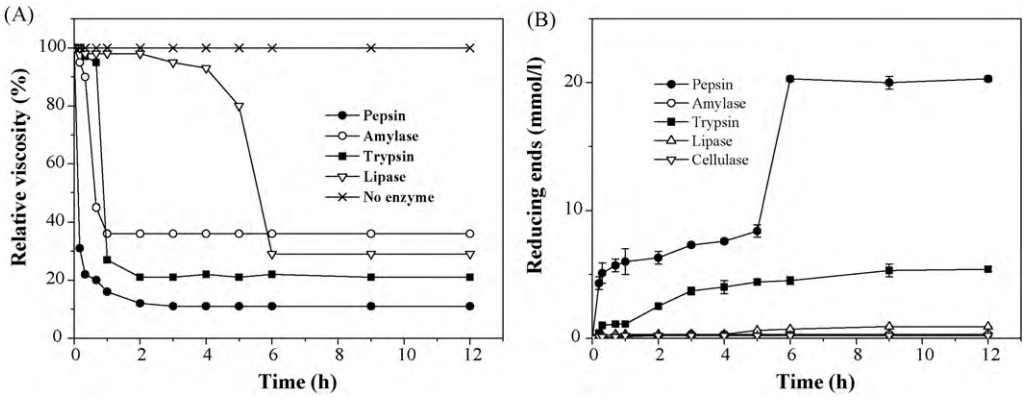

Fig. 1. Time course of the viscosity decrease of chitosan (A) and the reducing ends formation (B) with degradation by different enzymes.

evident that the activities of enzymes tested towards chitosan were much lower than that towards protein. Although several previous reports showed high chitosanolytic activities of cellulase (Ike et al., 2007; Liu & Xia, 2006; Xia et al., 2008), the cellulase tested in the present work has quite low hydrolysis activity towards chitosan (0.002 U/mg). This performance may be related to the composition of cellulase. The cellulases can be classified into three major categories (Beguin, 1990): (a) cellobiohydrolases (CBH), which attack cellulose molecules stepwise from the non-reducing end, liberating cellobiose subunits; (b) endoglucanases (EG), which cleave β-glucosidic bonds at random in the middle of cellulose molecules; (c) β-glucosidases, which hydrolyze cellobiose and low Mw cellodextrins into glucose. The low activity of β-glucosidase may account for low hydrolysis activity of cellulase to chitosan, since β-glucosidase was found to have high chitosanolytic activity (Zhang & Neau, 2002).

Fig. 1A showed the decrease of viscosity with the chitosanolysis by different non-specific enzymes tested. All enzymes tested, except for cellulase, showed appreciable chitosanolysis activity. Notable decrease of viscosity could be observed in 6 h. Pepsin showed the fastest initial viscosity decrease of chitosan solution at the beginning of hydrolysis, reaching a viscosity decrease greater than 80% after 1 h treatment. High chitosanolytic activity of pepsin was also reported by other researchers (Kumar & Tharanathan, 2004; Kumar, Varadaraj, & Tharanathan, 2007; Roncal et al., 2007). Amylase also caused fast viscosity decrease for initial 1 h hydrolysis, however, slight change was observed in the viscosity after 1 h, indicating the *endo*-action of the enzyme and the action of amylase on chitosan of specific Mw. Trypsin showed quite low chitosanolysis ability at beginning 40 min, but remarkable viscosity decrease was observed between 40 min and 1 h. It may be deduced that the initial action of trypsin on chitosan was *exo*-type. Lipase caused slow decrease of viscosity of chitosan solution for initial 4 h treatment, and gave a sharp decrease between 4 and 6 h, then no apparent change after 6 h. In addition, cellulase showed quite low chitosanolytic activity and gave a slight decrease of viscosity of chitosan solution.

Compared to the viscosity decreases during the chitosanolysis by the enzymes tested, the reducing ends formation was relative slower as shown in Fig. 1B. Pepsin catalysis produced the most reducing ends, and had different degradation rates around the time. For the first 20 min a relatively fast generation of reducing ends occurred, then slight increase until 5 h. Afterwards, the hydrolysis rates increased greatly again, and kept same after 6 h. It has been reported that pepsin is more prone to the action on –GlcN–GlcNAc– and –GlcNAc–GlcNAc-linkages, resulting in the products with GlcNAc for the non-reducing end type (Kumar & Tharanathan, 2004). Trypsin catalysis could also form some amount of reducing ends. In the first 20 min there was an increase of reducing ends, and a sharp increase could be found during 1–3 h. Amylase and lipase showed lower activity of reducing ends releasing, indicating that they hydrolyzed chitosan into low Mw fragments and released little reducing ends. Cellulase showed lowest activity of reducing ends releasing. It was worth noting that all enzymes tested showed two-step increase of reducing end for the degradation of chitosan. This behavior may be explained by four types of glycosidic linkages found in chitosan, GlcN–GlcN–, –GlcN–GlcNAc–, –GlcNAc–GlcN– and –GlcNAc–GlcNAc–. The occurrence probability of each type bonds depends on the deacetylation degree of the polymer.

Effects of chitosan Mw on the chitosanolysis by five enzymes tested were also determined (data not shown). The results indicated that the optimum Mw varied with different enzymes. For pepsin, lipase and cellulase, there were slight differences with the change of chitosan Mw. However, obvious variations in the chitosanolysis were observed for amylase and trypsin. The optimum chitosan Mw was 118.7 kDa for amylase while 723.2 kDa was not suitable. For trypsin, chitosan of 118.7 kDa showed the worst and 135.3 kDa was the most suitable one. This behavior also provided an evidence of the Mw specificity for trypsin and amylase.

The degradation behaviors of chitosan showed that main gastrointestinal enzymes, such as pepsin, amylase, trypsin, and lipase, have good chitosanolytic activity. Meanwhile, cellulase showed low hydrolytic activity to chitosan. It could be deduced that chitosan would be disintegrated by the gastrointestinal enzymes and could not keep intact through GIT. Okamoto et al. (2001) also reported that chitosan was unable to prevent destruction due to its high biodegradability in the GIT. Therefore, some modifications on chitosan are necessary to suppress its high degradability when it is used as drug carriers.

3.2. Degradation of NaCS

NaCS is a derivative of cellulose, and it would not be degraded by the enzymes in the upper GIT. However, NaCS could be degraded in the colon since there are enormous numbers of cellulolytic bacteria (Macy, Farrand, & Montgomery, 1982; Robert & Bernalier-Donadille, 2003). So the degradation of NaCS by cellulase should be investigated before its applications.

Fig. 2 showed the hydrolysis of NaCS by cellulase as the function of time. There was a sharp linear increase of reducing sugar for the first 5 min. After that, reducing sugar increased slowly until 40 min and the degradation rate was about 50%. This behavior may be explained by different types of enzymes contained in the cellulase. Cellulase is usually a mixture of cellobiohydrolases, endoglucanases, β-glucosidases or two of them. Endoglucanases and cellobiohydrolases hydrolyzed cellulose by the synergistic

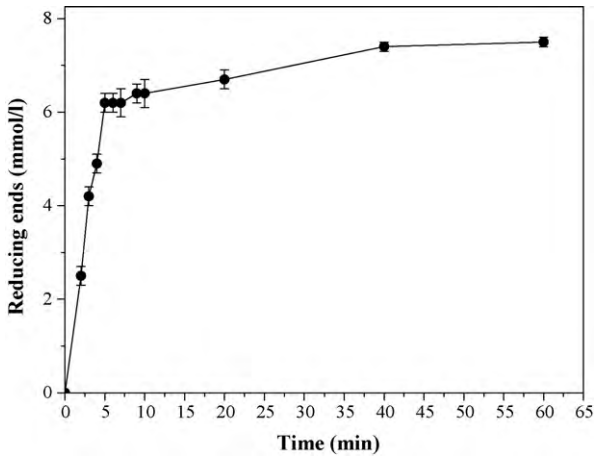

Fig. 2. Time course of NaCS hydrolysis by cellulase.

interaction to yield cellobiose, which is subsequently cleaved to glucose by β-glucosidase (Mansfield & Meder, 2003). For the first 5 min, NaCS was degraded into cellobiose quickly, due to the synergistic interaction of endoglucanases and cellobiohydrolases. After that, the cellobiose was cleaved to glucose slowly by glucosidases (Beguin, 1990). It may be deduced that the cellulase tested in the present work has low activity of glucosidases.

Fig. 3A shows the effects of Mw of NaCS on the degradation by cellulase. No obvious difference was observed by varying NaCS Mw from 5.2 kDa to 169.7 kDa. However, when increasing the Mw up to 632.6 kDa there was a sharp increase on the reducing ends releasing, and high hydrolysis rate of NaCS was maintained for higher Mw of 710.8 kDa. According to some previous studies (Greathouse, 1950; Reese, Siu, & Levinson, 1950), the degradation of cellulose

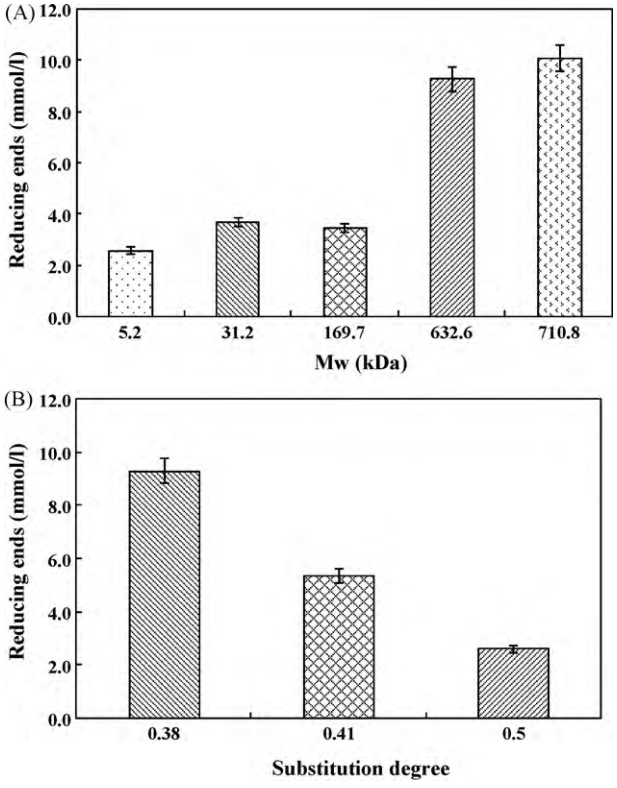

Fig. 3. Effects of molecular weight and the substitution degree on the degradation of NaCS by cellulase.

Table 2
Effects of chitosan molecular weight on the weight loss of chitosan/NaCS films after degradation[a].

	Mw of chitosan (kDa)			
	118.7	135.3	563.3	723.2
Pepsin (%)	5.8 ± 0.3	7.2 ± 0.5	6.7 ± 0.5	5.7 ± 0.1
Amylase (%)	5.0 ± 0.4	9.7 ± 1.3	3.2 ± 0.3	6.5 ± 0.2
Trypsin (%)	9.2 ± 0.4	13.1 ± 1.0	12.4 ± 2.5	10.3 ± 3.1
Lipase (%)	ND	ND	ND	ND
Cellulase (%)	5.0 ± 0.1	7.9 ± 0.5	7.5 ± 0.1	4.7 ± 0.5

[a] The degradation time was 3 h for pepsin and 6 h for other enzymes. And chitosan/NaCS films were prepared with 710.8 kDa NaCS and chitosan of different Mws.

derivatives is independent on the polymerization degree (DP) over a large range from 120 to 1675. The increase of degradation rate with increasing Mw may be due to the more cleavable linkages in NaCS with higher DP. The marked hydrolysis of NaCS with high Mw may be due to the excellent endoglucanase activity and relatively low β-glucosidase activity. The substitution degree (DS) of NaCS also had great influence on the degradation by cellulase. As shown in Fig. 3B, the degradation displays a sharp decrease by increasing the DS from 0.38 to 0.50. The reason may lie in the fact that the higher DS caused the stronger steric hindrance for the approach of cellulase to NaCS. Similar results were also observed on the hydrolysis of carboxymethyl cellulose (CMC) (Reese et al., 1950).

No degradation of NaCS by the enzymes in the upper GIT, such as pepsin and trypsin, was found, which would be helpful for preventing the chitosan/NaCS complex from the degradation in the upper GIT. Moreover, high susceptibility of NaCS to degradation by cellulase would accelerate the disintegration of the chitosan/NaCS complex in the colon.

3.3. Degradation of the chitosan/NaCS complex films

The evaluation of the films as a means to predict the properties of the chitosan/NaCS complex will be experimentally economical and statistically efficient (Ghaffari, Navaee, Oskoui, Bayati, & Rafiee-Tehrani, 2007). Variables such as the substrates and formulations are essential for film preparation. In addition, the films are easy to prepare by casting or spraying methods. So the degradation behaviors of chitosan/NaCS complex were investigated in the form of films.

The effects of chitosan Mw on the degradation of chitosan/NaCS films was presented in Table 2. Among five enzymes tested, the highest degradation rate was obtained by trypsin. With the chitosan Mw of 135.3 kDa trypsin showed the highest degradation rate (13.1%), and the lowest degradation rate of trypsin was obtained when the chitosan Mw was 118.7 kDa (9.2%). The results consisted with the chitosanolytic ability of trypsin. However, the degradation of chitosan/NaCS films differed from the chitosanolysis for amylase. Chitosan with the Mw of 135.3 kDa showed the highest degradation rate (11.2%), while that with 118.7 kDa got the lowest (4.9%) for amylase. Pepsin showed similar performance with amylase, although it gave no apparent difference in the degradation of chitosan. As shown in Table 2, lipase did not show the hydrolysis activity on the chitosan/NaCS films. As for cellulase, the highest and lowest degradation rates were obtained for chitosan with the Mw of 135.3 kDa and 723.2 kDa, respectively.

Compared with the degradation of chitosan, trypsin and pepsin showed a sharp decrease in the initial reducing ends release rate (70–95%). Moreover, the finial amount of reducing ends decreased by 20–50%, compared with the former. The results indicated that the presence of NaCS had great influence on the initial degradation rate of chitosan/NaCS films and inhibited the attack of the proteolytic enzymes to chitosan. It may be suggested that

Table 3
Effects of NaCS molecular weight on the weight loss of chitosan/NaCS films after degradation[a].

	Mw of NaCS (kDa)				
	5.2	31.2	169.7	632.6	710.8
Pepsin (%)	7.1 ± 0.4	12.2 ± 1.6	39.1 ± 5.1	4.4 ± 0.4	6.7 ± 0.5
Amylase (%)	2.0 ± 0.3	1.8 ± 0.1	1.6 ± 0.2	7.3 ± 0.4	3.2 ± 0.3
Trypsin (%)	48.0 ± 7.9	8.9 ± 0.1	25.2 ± 0.3	17.6 ± 2.3	12.4 ± 2.5
Lipase (%)	ND	ND	ND	ND	ND
Cellulase (%)	8.3 ± 0.5	3.3 ± 0.2	6.9 ± 0.5	5.7 ± 0.2	7.5 ± 0.1

[a] The degradation time was the same to that of Table 2. Chitosan/NaCS films were prepared with 563.3 kDa chitosan and NaCS of different Mws.

chitosan/NaCS PEC could be used for the gastrointestinal drug delivery, since the main enzymes in the GIT are proteolytic enzymes (Bernkop-Schnurch, 1998). For amylase, when the Mw of chitosan was higher than 118.7 kDa, it was surprising that the degradation of chitosan/NaCS films was easier than the degradation of chitosan, and the amounts of reducing ends were higher, too. The reason may be that the addition of chitosan to NaCS changes the conformation of some parts of NaCS from β- to α-type, thus promoting the degradation of chitosan/NaCS films by α-amylase. In contrast, the degradation rate of chitosan/NaCS films by cellulase was much lower than that of NaCS, indicating the high steric hindrance of chitosan for cellulase.

The effects of NaCS Mw on the degradation of chitosan/NaCS films were also investigated (Table 3). The degradation of chitosan/NaCS films changed with the variation of NaCS Mw. Similar to the results in Table 2, trypsin showed the relatively high hydrolytic activity to the films. And the highest degradation rate was achieved when the Mw of NaCS was 5.2 kDa (48%), followed by 169.7 kDa (25.2%) and 710.8 kDa (25.0%). The reason may be that NaCS with lower Mw would be easier for the attack of trypsin to chitosan. As for pepsin, the maximum degradation rates were obtained when the NaCS Mw was 169.7 kDa (39.1%), followed by 31.2 kDa. Amylase got the highest degradation rates at the NaCS Mw of 632.6 kDa, and the lowest one was 169.7 kDa. Cellulase has the highest degradation rate at the NaCS Mw of 5.2 kDa. Based on the data in Table 3, it could be concluded that the degradation rates of chitosan/NaCS films were significantly influenced by the Mw of NaCS. Therefore, the adjustment of NaCS Mw would control the degradation rate and change the targeted site for the chitosan/NaCS PEC as the carrier.

The effects of the DS of NaCS on the degradation of chitosan/NaCS films were also studied (Table 4). When the DS was 0.38, five enzymes tested showed the highest degradation rates, and the increase of DS from 0.38 to 0.41 resulted in the decrease of degradation rates. Increasing the DS from 0.41 to 0.50 led to an appreciable increase of degradation for all enzymes tested except pepsin. The results indicated that more compact PEC was formed when the DS was 0.41. This behavior was a bit different from that of Wang et al. (2009). Wang et al. reported that increasing DS would

Table 4
Effects of the substitution degree of NaCS molecular weight on the weight loss of chitosan/NaCS films after degradation[a].

	DS of NaCS		
	0.38	0.41	0.50
Pepsin (%)	6.7 ± 0.5	4.8 ± 0.3	3.2 ± 0.2
Amylase (%)	3.2 ± 0.3	2.0 ± 0.1	3.1 ± 0.2
Trypsin (%)	12.4 ± 2.5	9.5 ± 0.4	16.6 ± 0.4
Lipase (%)	ND	ND	ND
Cellulase (%)	7.5 ± 0.1	4.6 ± 0.1	7.0 ± 0.2

[a] The degradation time was the same to that of Table 2. Chitosan/NaCS films were prepared with 563.3 kDa chitosan and NaCS of different Mws.

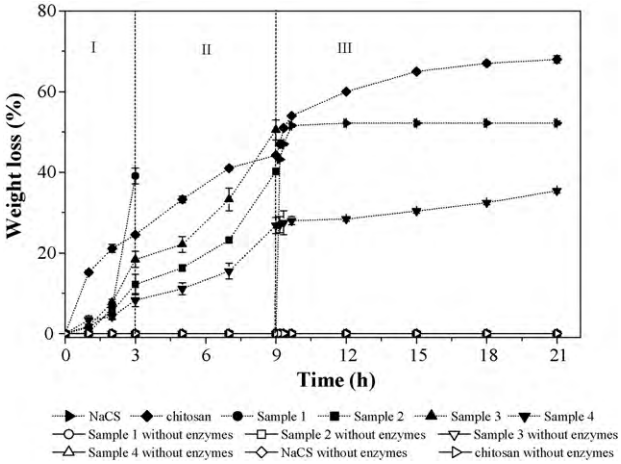

Fig. 4. Kinetic degradation of NaCS/chitosan films in vitro. I, SGF; II, SIF; III, SCF. Sample 1: 563.3 kDa chitosan and 169.7 kDa NaCS; sample 2: 563.3 kDa chitosan and 31.2 kDa NaCS; sample 3: 135.3 kDa chitosan and 710.8 kDa NaCS; sample 4: 563.3 kDa chitosan and 710.8 kDa NaCS.

cause the decrease of degradation of chitosan/NaCS complex. The reason may lie in the fact that the combination of chitosan and NaCS was related with not only the DS, but also the Mw of chitosan and NaCS.

The in vitro tests of the degradation of chitosan/NaCS films were carried out by immersing the film samples in the SGF, SIF, SCF, and the solutions of pH 1.5, pH 7.4 and pH 6.8 in sequence. Four samples (samples 1–4 with different compositions) were found to have four typical behaviors. The degradation process of the four samples is shown in Fig. 4. It was found that the weight loss of chitosan/NaCS films was much lower than that of chitosan in the first 2 h in SGF. However, the degradation of chitosan/NaCS films diversified in the following time. Sample 1 was disintegrated after 3 h treatment in the SGF, while samples 2–4 suffered a weight loss of 12.1%, 11.1% and 8.3%, respectively. During the treatment in SIF, the degradation rates of samples 2–4 increased gradually. Samples 2 and 3 showed great increases of the degradation rates as high as 40%, and were disintegrated after 6 h in SIF. However, sample 4 underwent the gradual degradation in SIF and showed no obvious change in the shape and surface. The following degradation in SCF caused further weight loss of sample 4 and small pores in the film could be found.

The results showed that the biodegradability of chitosan/NaCS films could be changed by varying the Mws of chitosan and NaCS. Meanwhile, it was found that chitosan/NaCS films were mucoadhesive and swellable upon the hydration. Therefore, chitosan/NaCS PEC may be potentially used for preparing the intestinal and colonic drug delivery systems due to its variable biodegradability, mucoadhesive and swelling properties. For example, the formulation of samples 2 and 3 may be suitable as the intestinal drug carrier since they could suffer through SGF with little weight loss. Moreover, chitosan could enhance the absorption of peptides and proteins across intestinal epithelia (Borchard et al., 1996). The formulation of sample 4 may be used for the colon-specific drug delivery systems because it could remain relatively intact after 3 h treatment in SGF and 6 h in SIF. The potential use of chitosan/NaCS complex for colon-specific drug delivery has been confirmed by Wang et al. (2009). Wang et al. (2009) investigated in vitro drug release of 5-aminosalicylic acid (5-ASA) with the chitosan/NaCS capsules. After 1 h treatment in SGF and 4 h in SIF, only 10% of 5-ASA was released. However, over 80% of drug was released from the capsules after 2 h in SCF. The results indicated the potential use of chitosan/NaCS complex for colon-specific drug delivery.

4. Conclusions

The biodegradation of chitosan, NaCS and chitosan/NaCS films were studied by the hydrolysis with five enzymes, including pepsin, amylase, trypsin, lipase and cellulase. It was found that the Mw of chitosan had great influence on the degradation of chitosan as well as the chitosan/NaCS films. The chitosan/NaCS films showed the highest susceptibility to the hydrolysis by pepsin, amylase and trypsin, when it was made with 135.3 kDa chitosan and 710.8 kDa NaCS. In addition, the degradation of NaCS by cellulase was affected by both the Mw and DS of NaCS. For the degradation of chitosan/NaCS films, pepsin showed the highest degradation rate on the film made of 169.7 kDa NaCS and 563.3 kDa chitosan, and trypsin showed the maximum activity towards the film made of 5.2 kDa NaCS and 563.3 kDa chitosan. In vitro degradation of chitosan/NaCS films in SGF, SIF and SCF indicated that the degradation rate and disintegrated sites of chitosan/NaCS films could be controlled by regulating the Mws of chitosan and NaCS, which demonstrated that the chitosan/NaCS PEC should be a possible candidate as drug carrier for oral controlled release.

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