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Preparation and characterization of chitosan microparticles intended for controlled drug delivery

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Abstract

Chitosan microparticles were prepared with tripolyphosphate (TPP) by ionic crosslinking. The particle sizes of TPP-chitosan microparticles were in range from 500 to 710 μm and encapsulation efficiencies of drug were more than 90%. The morphologies of TPP-chitosan microparticles were examined with scanning electron microscopy. As pH of TPP solution decreased and molecular weight (MW) of chitosan increased, microparticles had more spherical shape and smooth surface. Release behaviors of felodipine as a model drug were affected by various preparation processes. Chitosan microparticles prepared with lower pH or higher concentration of TPP solution resulted in slower felodipine release from microparticles. With decreasing MW and concentration of chitosan solution, release behavior was increased. The release of drug from TPP-chitosan microparticles decreased when cross-linking time increased. These results indicate that TPP-chitosan microparticles may become a potential delivery system to control the release of drug.

Keywords: Chitosan; Microparticle; Tripolyphosphate; Drug delivery system

1. Introduction

Chitosan[poly(β-(1→4)-2-amino-2-deoxy-D-glucose)] is a natural cationic polysaccharide derived from chitin, which is copolymer, a glucosamine and an *N*-acetyl glucosamine units, combined together (Lee et al., 1997; Ravi Kumar, 2001). Chitosan has been extensively studied as carriers for drugs (Bayomi et al., 1998; Mi et al.,

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2001), protein carriers (Calvo et al., 1997) and gels for the entrapment of cells or antigens (Mi et al., 1999c) in the pharmaceutical industry. Moreover, chitosan has antacid and antiulcer characteristics, which prevents or weakens drug irritation in the stomach (Gupta and Ravi Kumar, 2000).

According to previous studies, drug release from chitosan microparticles could be controlled by crosslinking the matrix using chemical crosslinking agents such as glutaraldehyde (Jameela and Jayakrishnan, 1995; Genta et al., 1997, 1998; Blanco et al., 2000), NaOH (Chandy and Sharma, 1996; Lim et al., 1997; Vasudev et al., 1997) and ethylene glycol diglycidyl ether (Mi et al., 1999c). However, these chemical crosslinking agents have possibility of inducing undesirable effects. Chemically synthesized glutaraldehyde can cause irritation to mucosal membranes due to its toxicity (Lim et al., 1997; Mi et al., 2001; Shu et al., 2001).

To overcome this disadvantage of chemical cross-linking, ionic crosslinking interaction has been applied. For example, chitosan beads, micro or nanoparticles were produced by ionic crosslinking with tripolyphosphate (TPP) (Bodmeier et al., 1989; Shiraishi et al., 1993; Calvo et al., 1997). Shu and Zhu (2000, 2001) reported the chitosan bead, which was prepared with TPP, increased the drug loading efficiency as well as prolonging the drug release period, and they also showed that citrate cross-linked chitosan film possessed pH-sensitive swelling and drug controlled release properties. Mi et al. (2001) reported that the chitosan microspheres prepared with genipin, a naturally occurring crosslinking reagent, affected the release behavior of the drugs in microspheres.

TPP is nontoxic and multivalent anions. It can form gel by ionic interaction between positively charged amino groups of chitosan and negatively charged counterion of TPP (Aral and Akbuğa, 1998; Mi et al., 1999b; Shu and Zhu, 2000, 2001). This interaction could be controlled by the charge density of TPP and chitosan, which is dependent on the pH of solution. The chitosan matrix could be depended on molecular weight (MW) of chitosan. Puttipipatkhachorn et al. (2001) reported that the higher the MW and degree of deacetylation of chitosan, the lower the release rate of chitosan film.

In this study, the chitosan microparticle was prepared with TPP and chitosan of various MW. The objective of this study was to evaluate the effect of the preparation process on the release behavior of chitosan microparticles, which was prepared by various conditions such as (1) concentration and MW of wall material (chitosan), (2) pH and concentration of cross-linker (TPP) solution and (3) curing time. Felodipine ($C_{18}H_{19}C_{12}NO_4$, MW 384.3), the treatment of hypertension, was used as a model drug.

2. Materials and methods

2.1. Materials

Chitosan with different MW (2.5×10^6, 3.5×10^6, 6.5×10^6, the degree of deacetylation was 86.8, 86.4, 93.6%, respectively) were obtained from Biotech. Co. Ltd (Korea). Felodipine was obtained from Nivedita Chemical Pvt. Ltd (Mumbai, India). TPP and other reagents were all analytical reagents grade.

2.2. Preparation of TPP-chitosan microparticles

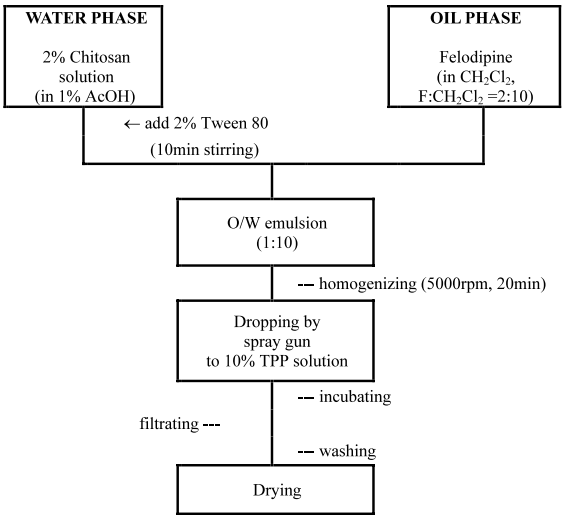
Various amounts of chitosan solutions were prepared by dissolving it in 1% acetic acid as shown in Table 1 and Tween 80 (2% v/v) was added into the solution as a surfactant. Core material, felodipine, was dissolved in CH_2Cl_2 (2:10) due to its water-insoluble behavior and then this oil phase was mixed with aqueous phase (chitosan solution) by homogenizer at 5000 rpm for 20 min. The ratio of oil and aqueous phase was 1:10. O/W emulsion was dropped into TPP solutions by spray gun. After the crosslinking time, microparticles were washed with distilled water repeatedly and then vacuum dried for 12 h. The preparation of TPP-chitosan microparticles are shown in Scheme 1.

2.3. Morphological characterization

The surface morphology of microparticles was observed by scanning electron microscopy (SEM). The microparticles were vacuum dried, coated

Table 1
Preparation of TPP-chitosan microparticles

TPP pH	TPP concentration (% w/v)	Chitosan MW	Chitosan concentration (% w/v)	Crosslinking time (min)
5	10	3.5×10^6	2	30
2	10	3.5×10^6	2	30
8.6	10	3.5×10^6	2	30
5	1	3.5×10^6	2	30
5	5	3.5×10^6	2	30
5	10	2.5×10^6	2	30
5	10	6.5×10^6	2	30
5	10	3.5×10^6	1	30
5	10	3.5×10^6	3	30
5	10	3.5×10^6	2	15
5	10	3.5×10^6	2	60



Scheme 1. Preparation procedures of TPP-chitosan microparticles.

with gold palladium and observed microscopically (JSM-5310Lv, JEOL, Japan). The particle size of the microparticles was measured by using standard sieves (Chung Gye Industrial MFG., Co., Korea) and SEM.

2.4. Drug content of microparticles

Encapsulation efficiency was studied by dissolving microparticles in 0.1N HCl for 24 h. Tween 80 (0.5%) was added into the solution to enhance the drug solubility. The amount of drug loaded was determined by spectrophotometer at 362 nm (Filipović-Grčić et al., 1996). All the experiments were carried out in triplicate.

$$\text{Loading efficiency}(\%) = [\text{Wa}/\text{Wt}] \times 100$$

where Wa is the actual felodipine content and Wt is theoretical felodipine content.

2.5. In vitro release studies

Microparticles (0.01 g) were suspended in 500 ml of phosphate-buffered saline (PBS) (pH 7.4) contained in a glass bottle, and maintained at 37 °C, 50 rpm. Tween 80 (0.5%) was added into the medium as an emulsifier. Samples (1 ml) were periodically removed and the volume of each sample was replaced by the same volume of fresh medium. The amount of released felodipine was analyzed with a spectrophotometer at 362 nm. The in vitro release studies were performed in triplicate for each of the samples.

3. Results and discussion

3.1. Properties of TPP-chitosan microparticles

TPP-chitosan microparticles were prepared by the ionic interaction between a positively charged amino group of chitosan and a negatively charged counterion of TPP. The ionization degree of TPP is dependent on the pH value of solution. In

original TPP solution (pH 8.6), TPP is dissociated into OH^- and TPP ions ($HP_3O_{10}^{4-}$ and $P_3O_{10}^{5-}$). However in low pH, only $P_3O_{10}^{5-}$ anions are. Moreover, chitosan is a weak polybase, and as pH of the solution decreased, the ionization of amine group of chitosan increased. Therefore, TPP-chitosan microparticles prepared in the original TPP solution are dominated by deprotonation and slightly ionic-crosslinking, but chitosan microparticles prepared in acidic TPP solution are completely ionic-crosslinking dominated (Mi et al., 1999a,b; Shu and Zhu 2000, 2001; Lee et al., 2001). The surface morphologies of chitosan microparticles are shown in Fig. 1. The chitosan microparticles were not completely spherical in shape, and had a rough surface. The microparticles prepared with TPP solution at low pH values (pH 2.5) had more spherical shape and smoother surface than those of microparticles prepared with pH 8.6 TPP solution due to high density of matrix. These results show that TPP-chitosan matrix formation is dependent on pH values of the TPP solution.

Fig. 2 shows the morphology of TPP-chitosan microparticles prepared with various MW of chitosan. The chitosan MW affects the morphology of microparticles surface. As the MW of chitosan increased, the viscosity of chitosan solution increased and it may result in formation of relatively strong walls of microparticles upon interaction with TPP. Therefore, the higher the MW of chitosan, the more spherical the shape of microparticles. The particle sizes of drug loaded chitosan microparticles were ranged mainly between 500 and 710 μm. (The data were not shown.)

Table 2 shows the loading efficiency in chitosan microparticles. Generally, the loading efficiencies were more than 90%. Since felodipine is nonsoluble in water, it was not dissolved in solution during crosslinking and hardening process. Therefore, the loss of felodipine from microparticles was minimal during the hardening and washing process.

3.2. Drug release behavior

Fig. 3 shows release behavior of felodipine from chitosan microparticles prepared with TPP solutions at various pH levels. As pH of TPP solution decreased, the release profile of felodipine from TPP-chitosan microparticles decreased. This result showed that the ionic reaction of chitosan-TPP complex is dependent on the pH of TPP solution. As previously described, the results demonstrated

Table 2
Encapsulation efficiency of TPP-chitosan microparticles

Variables		Actual drug content (%, w/w)	Efficiency (%)
TPP pH	2	1.87±0.2	93.7±11
	5	1.94±0.3	98.2±5
	8.6	1.98±0.1	98.7±5
TPP Concentration (%)	1	1.92±0.2	92.7±11
	5	1.82±0.4	93.7±8
	10	1.94±0.3	98.2±5
Chitosan MW	2.5×10^6	1.95±0.1	97.5±8
	3.5×10^6	1.94±0.4	98.2±5
	6.5×10^6	1.76±0.1	88.1±4
Chitosan Concentration (%)	1	1.96±0.1	97.8±7
	2	1.94±0.3	98.2±5
	3	1.48±0.1	72.9±4
Crosslinking time (min)	15	1.82±0.1	90.9±8
	30	1.94±0.3	98.2±5
	60	1.82±0.1	91.2±7

Data shown are the mean±standard deviation ($n = 3$).

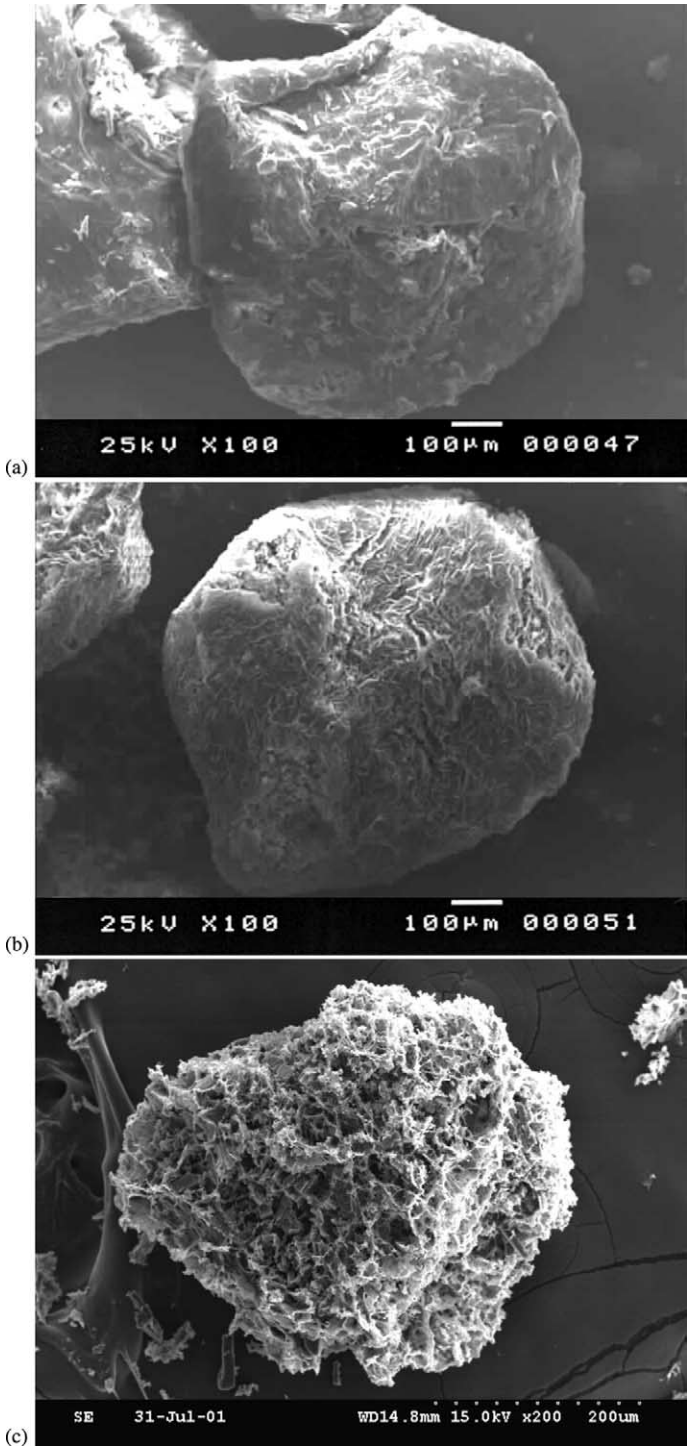


Fig. 1. SEM pictures of felodipine loaded chitosan microparticles (TPP conc., 10%; chitosan MW, 3.5×10^6; chitosan conc., 2%; crosslinking time, 30min); (a) morphology of the TPP-chitosan microparticles crosslinked in TPP solution of pH 2, (b) pH 5 and (c) pH 8.6.

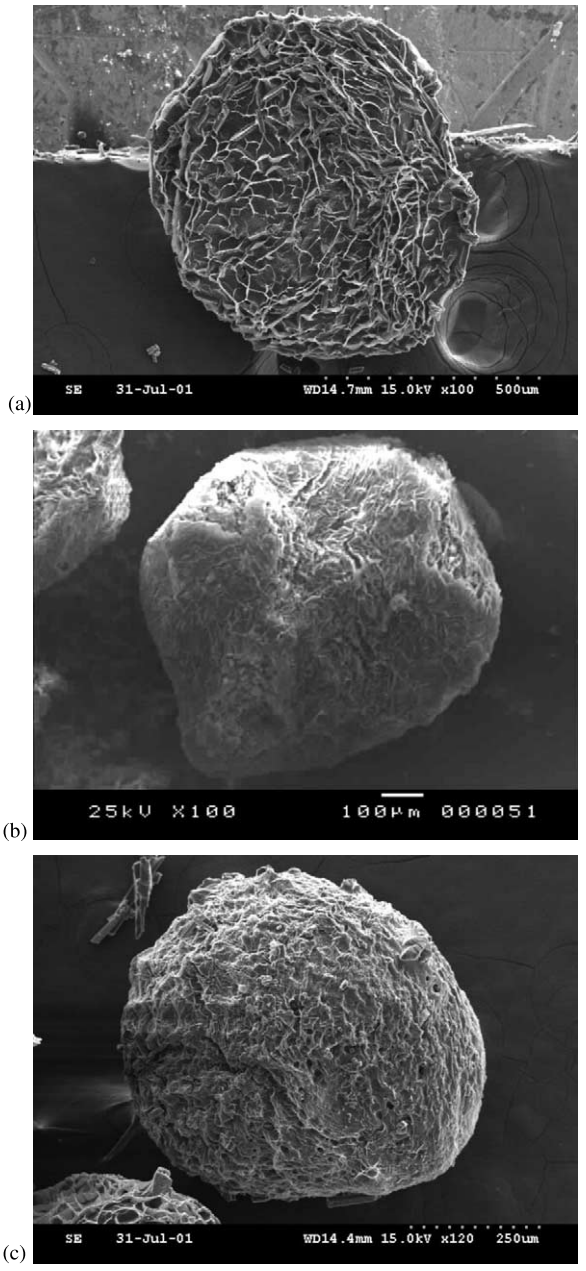


Fig. 2. SEM pictures of felodipine loaded chitosan microparticles (TPP pH 5; TPP conc., 10%; chitosan conc., 2%; crosslinking time, 30 min); (a) morphology of the TPP-chitosan microparticles prepared with chitosan MW 2.5×10^6, (b) chitosan MW 3.5×10^6 and (c) chitosan MW 6.5×10^6.

that the ionic-crosslinking density of TPP-chitosan microparticle was improved by the curing in acidic TPP solution. In the lower pH region, ionization degree of TPP and chitosan are high and chitosan forms gel with completely ionic-crosslinking without deprotonation. (Mi et al., 1999c; Shu and Zhu, 2000, 2001; Lee et al., 2001). Mi et al. (1999a,b) reported that the pore structure of chitosan microparticle was modified by the change of pH of TPP solution and open porous structure was observed when the chitosan microparticles were prepared in TPP solution of pH 8.6. This porous structure, i.e. low density structure, is more degradable than high density structure, therefore the release behavior of felodipine from microparticle prepared in TPP solution of pH 8.6 was much faster than those of microparticles prepared in pH 2 and 5.

Fig. 4 shows the effect of the crosslinking agent (TPP) amount on the felodipine release behavior. In general, the release profile of drug from TPP-chitosan microparticles decreased with the increased crosslinking agent concentration. It also depends on the density of TPP-chitosan matrix. Remuñán-López and Bodmeier (1997) reported that the diffusion of drug from chitosan films decreased as the concentration of the TPP solution increased. In addition, they showed that the swelling and permeability characteristics of chitosan films were dependent on pH and concentration of crosslinking agent.

The viscosity of chitosan solution is important for the formation of microparticles. As the MW of the chitosan solution increased, the release behavior of felodipine decreased significantly (Fig. 5). The release of drug from microparticles prepared with the 2 and 3% chitosan solution were lower than those produced with the 1% chitosan solution (Fig. 6). These results indicate that the release behavior of drug is relative to viscosity of chitosan solution. The increased viscosity of chitosan solution forms relatively strong walls of microparticles upon interaction with TPP. High crosslinking density of TPP-chitosan matrix resulted in less swelling ability, therefore the release of drug decreased. These results were similar to the study of Lim et al. (1997). They showed that the chitosan

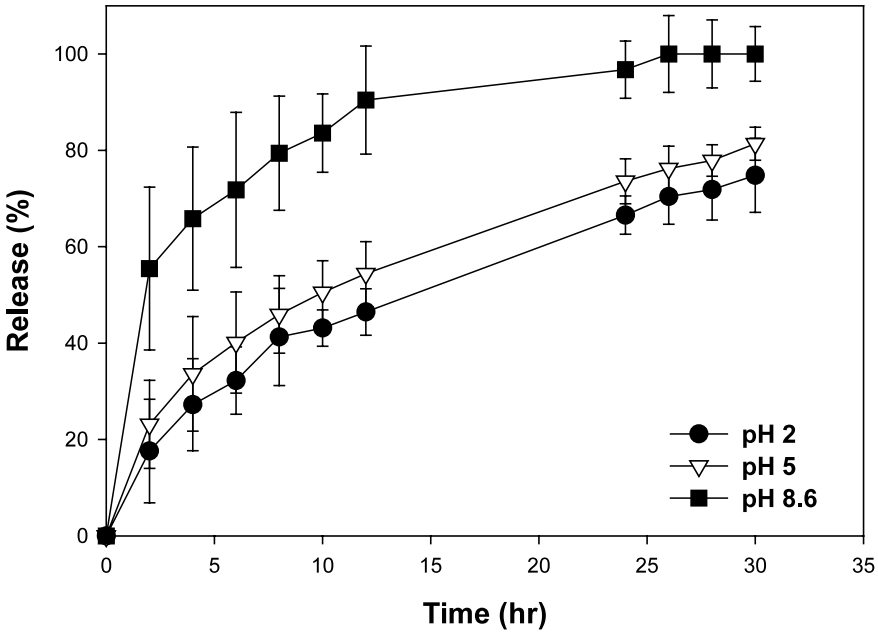


Fig. 3. The influence of TPP solution pH on the felodipine release behavior from TPP-chitosan microparticles (TPP conc., 10%; chitosan MW, 3.5×10^6; chitosan conc., 2%; crosslinking time, 30 min).

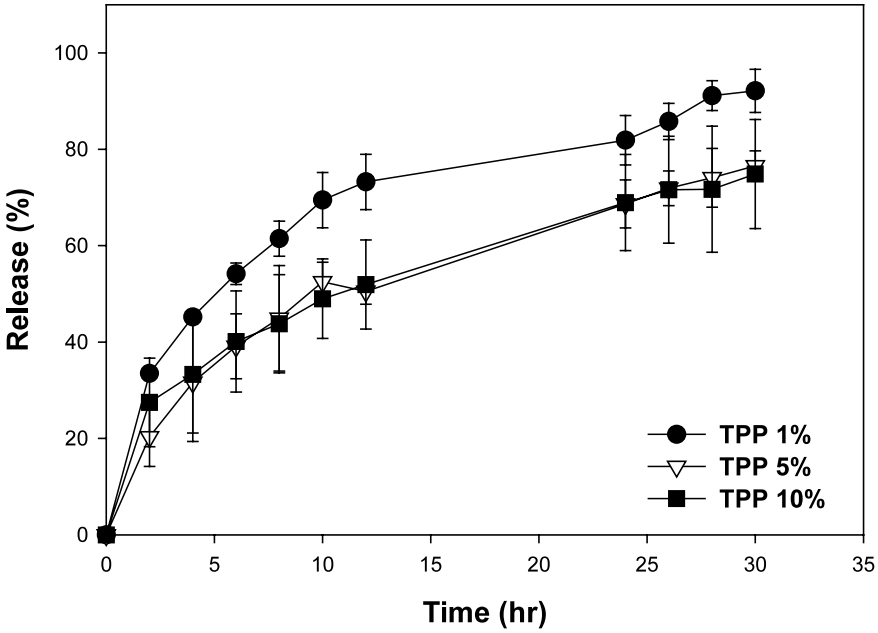


Fig. 4. The influence of the concentration of TPP on the felodipine release behavior from TPP-chitosan microparticles (TPP pH 5; chitosan MW, 3.5×10^6; chitosan conc., 2%; crosslinking time, 30 min).

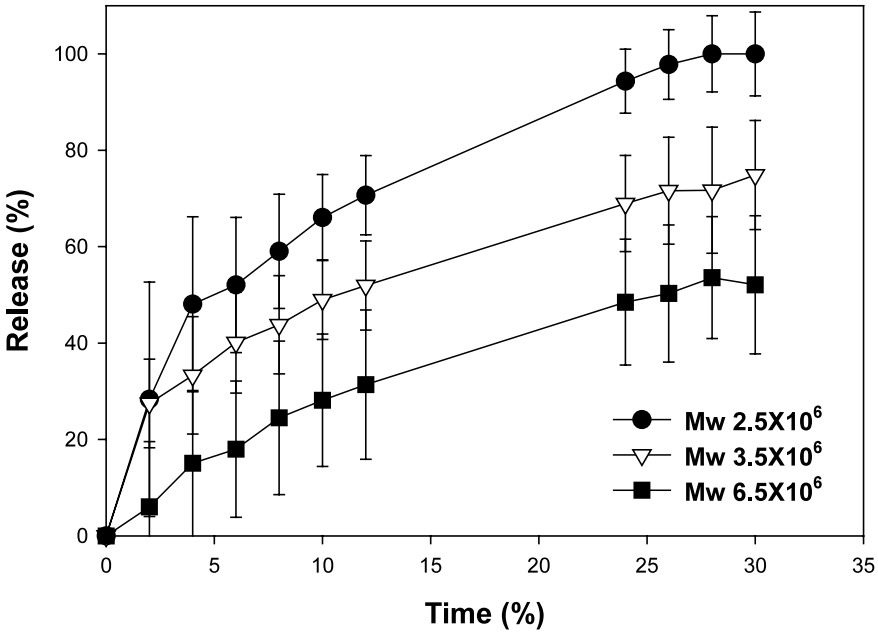


Fig. 5. The influence of the chitosan MW on the felodipine release behavior from TPP-chitosan microparticles (TPP pH 5; TPP conc., 10%; chitosan conc., 2%; crosslinking time, 30 min).

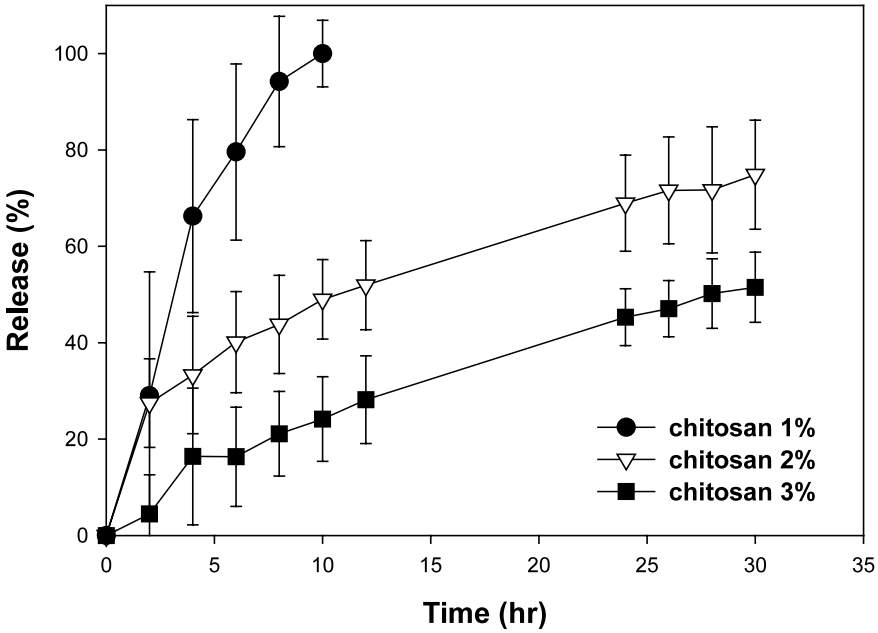


Fig. 6. The influence of the concentration of chitosan solution on the felodipine release behavior from TPP-chitosan microparticles (TPP pH 5; TPP conc., 10%; chitosan MW, 3.5×10^6; crosslinking time, 30 min).

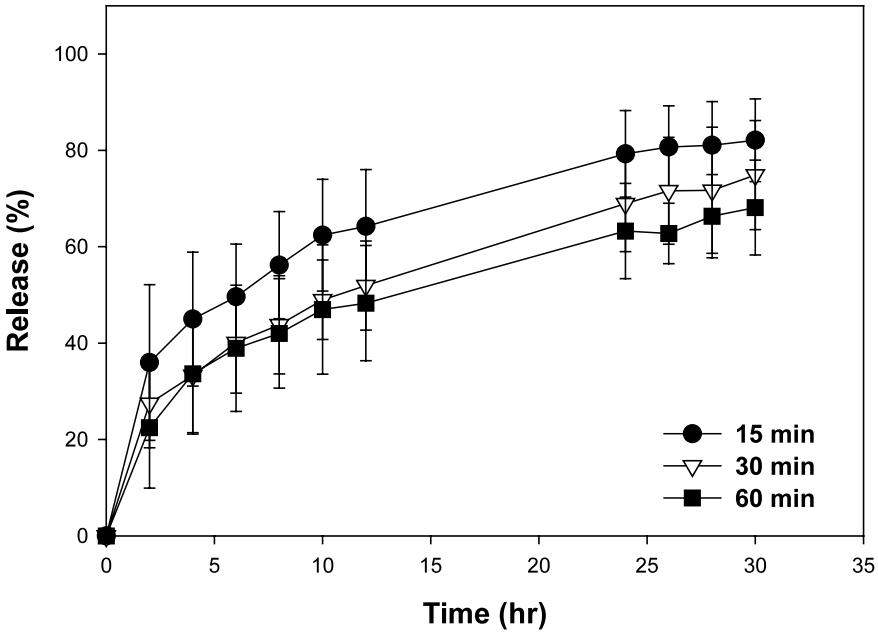


Fig. 7. The influence of crosslinking time on the felodipine release behavior from TPP-chitosan microparticles (TPP pH 5; TPP conc., 10%; chitosan MW, 3.5×10^6; chitosan conc., 2%).

solution at low concentration formed weak microspheres with NaOH.

As crosslinking time increased from 15 to 60 min, the release of drug also decreased (Fig. 7). When chitosan solution was dropped into the TPP solution, the OH^- ions competed with tripolyphosphoric ions to react with amino group of chitosan immediately by ionic interaction, and then $P_3O_{10}^{5-}$ ions diffused into chitosan drops to interact with amine groups of chitosan (Mi et al., 1999a,b). Therefore, short curing time might not give the sufficient interaction time for the TPP-chitosan matrix. As shown in Fig. 7, the microparticle prepared in TPP solution for 15 min showed faster release behavior than those of microparticles prepared by the long curing time (30 and 60 min).

4. Conclusion

TPP-chitosan microparticles were modified by various factors to control the release of felodipine. The results shows that the pH and concentration of the crosslinking agent solution, MW and concentration of wall material and curing time play major roles on the TPP-chitosan matrix density: as the MW and concentration of chitosan solution and curing time increased, the release behaviors of felodipine decreased significantly. Also, lower pH and higher concentration of TPP solution resulted in slower felodipine release from microparticles. Controlled preparation of TPP-chitosan microparticles is a prerequisite for the controlled release of drugs.

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