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Chitosan nanoparticles as a novel delivery system for ammonium glycyrrhizinate

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Abstract

The ammonium glycyrrhizinate-loaded chitosan nanoparticles were prepared by ionic gelation of chitosan with tripolyphosphate anions (TPP). The particle size and zeta potential of nanoparticles were determined, respectively, by dynamic light scattering (DLS) and a zeta potential analyzer. The effects, including chitosan molecular weight, chitosan concentration, ammonium glycyrrhizinate concentration and polyethylene glycol (PEG) on the physicochemical properties of the nanoparticles were studied. These nanoparticles have ammonium glycyrrhizinate loading efficiency. The encapsulation efficiency decreased with the increase of ammonium glycyrrhizinate concentration and chitosan concentration. The introduction of PEG can decrease significantly the positive charge of particle surface. These studies showed that chitosan can complex TPP to form stable cationic nanoparticles for subsequent ammonium glycyrrhizinate loading.

Keywords: Chitosan; Ammonium glycyrrhizinate; Nanoparticles

1. Introduction

Recently, polymer nanoparticles have been widely investigated as a carrier for drug delivery (Gref et al., 1994). Nanoparticles have a special role in targeted drug delivery in the sense that they have all the advantages of liposomes including the size property. But unlike liposomes, nanoparticles have a long shelf life and can entrap more drugs. Some investigators have also observed that the number of nanoparticles which cross the intestinal epithelium is greater than that of the microspheres (>1 μm) (Desai et al., 1996). Polymer nanoparticles from biodegradable and biocompatible polymers are good candidates for drug carrier to deliver drugs, because they are expected to be adsorbed in an intact form in the gastrointestinal tract after oral administration (Florence et al., 1995).

Chitosan (CS) is the second abundant polysaccharide and a cationic polyelectrolyte present in nature. CS has shown favorable biocompatibility characteris-

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tics (Knapczyk et al., 1989; Hirano et al., 1989, 1990) as well as the ability to increase membrane permeability, both in vitro (Aspden et al., 1997; Lehr et al., 1992; Dumitriu and Chornet, 1998) and in vivo (Takeuchi et al., 1996), and be degraded by lysozyme in serum. From a biopharmaceutical point of view, CS has the potential of serving as an absorption enhancer across intestinal epithelial for its mucoadhesive and permeability enhancing property (Janes et al., 2001). It has been proved that CS could enhance insulin absorption across human intestinal epithelial (Caco-2) cells without injuring them (Artursson et al., 1994; Schipper et al., 1996, 1997, 1999; Thanou et al., 2001). CS has been used in preparing films, beads, intragastric floating tablets, microspheres, and nanoparticles in the pharmaceutical filed (Berthold et al., 1996; Felt et al., 1998; Giunchedi et al., 1998; Calvo et al., 1997a; Illum, 1998; Wu et al., 2003). The research emphasized the importance of size and revealed the advantages of nanoparticles over the microspheres (>1 μm) (Meclean et al., 1998). With their easy accessibility in the body, nanoparticles can be transported via the circulation to different body sites. The hydrophilic nanoparticles generally have longer circulation in blood (Allemann et al., 1993). Such systems could not only control the rate of drug administration that prolongs the duration of the therapeutic effect but also deliver the drug to specific sites.

Glycyrrhetic acid (GLA) is an aglycone and an active metabolite of glycyrrhizin (GLZ). It shows various therapeutic effects such as anti-inflammatory, antitumorigenic and anti-hepatotoxic activities (Ichikawa et al., 1986; Ishida et al., 1989; Higuchi et al., 1992; Takeda et al., 1996). GLA is effective against chronic hepatitis but is also related to the side-effect aldosteronism. Since only GLA appears in the blood circulation after oral administration of glycyrrhizin (GLZ), GLA is considered to play an important role in the biological action of oral administration of GLZ (Oketani et al., 1985; Ishida et al., 1989). Many pharmacokinetic studies on GLA and GLZ have analysed their behaviour in the body, but the oral absorption of GLA (or its salt) was extremely ineffective. So far very few studies have reported on the entrapment of GLA into nanoparticulate carriers to improve the oral absorption.

Therefore, the major goal of the present work is to create a kind of new biodegradable nanoparticles for the incorporation of ammonium glycyrrhizinate, and to evaluate their potential as delivery systems. The present study confirms that chitosan can encapsulate appreciable quantities of ammonium glycyrrhizinate into stable nanoparticles. The factors that influence the preparation of nanoparticles were analyzed and the release property during incubation in phosphate buffer saline (PBS) was examined.

2. Materials and methods

2.1. Materials

Chitosan with the deacetylation degree (DD) of 95% and the molecular weight (Mw) of 360 kDa was purchased from Kabo Biochemical Co. (Shanghai, China). Chitosans of DD 95% with different Mws (7, 18, 24, 200 kDa) were prepared according to the reference (Qin et al., 2002). The Mws were measured through the viscometric method while the DDs were determined by elemental analysis. Ammonium glycyrrhizinate was bought from Tianshan Pharmaceutical Limited Company (China). PEG with Mw 2000 Da was obtained from Tiantai Chemical Company (Tianjin, China). All other chemicals were of reagent grade.

2.2. Preparation of chitosan nanoparticles and ammonium glycyrrhizinate-loaded nanoparticles

Chitosan nanoparticles were prepared according to the procedure first reported by Calvo et al. (1997b) based on the ionic gelation of CS with TPP anions. Chitosan was dissolved in acetic aqueous solution at various concentrations (1.0, 1.2, 1.44, 1.6, 2.0, 2.5, 3.0 mg/mL). The concentration of acetic acid in aqueous solution was, in all case, 1.5 times that of chitosan. Under magnetic stirring at room temperature, 4 mL sodium tripolyphosphate TPP aqueous solution with various concentrations (0.2, 0.4, 0.6, 0.8, 1.0 mg/mL) was added into 10 mL chitosan solution, respectively. Three kinds of phenomena were observed: solution, aggregates and opalescent suspersion. The zone of opalescent suspersion was further examined as nanoparticles. PEG-modified nanoparticles were formed spontaneously upon incorporation of 4 mL TPP solution (0.6 mg/mL) into 10 mL chitosan solution containing various concentrations of PEG (10.0, 20.0, 30.0, 40.0, 50.0 mg/mL).

For the association of the glycyrrhetic acid with chitosan nanoparticles, we selected ammonium glycyrrhizinate as a model glycyrrhetic acid. The ammonium glycyrrhizinate was dissolved in heat distilled water. Ammonium glycyrrhizinate-loaded nanoparticles were formed upon incorporation of 4 mL TPP solution (0.6, 1.0 mg/mL) into 10 mL chitosan solutions (1.44 mg/mL) containing ammonium glycyrrhizinate (0.1, 0.2, 0.3, 0.4, 0.5 mg/mL). Ammonium glycyrrhizinate concentration was 0.4 mg/mL for the preparation of PEG-modified nanoparticles loading ammonium glycyrrhizinate.

2.3. Physicochemical characterization of nanoparticles

Dynamic light scattering (DLS) (Malvern, Autoszer 4700) was used to measure the hydrodynamic diameter and size distribution (polydispersity index, PDI = $\langle\mu_2\rangle/\Gamma^2$) (Chu et al., 1991). All DLS measurements were done with a wavelength of 532 nm at 25 °C with an angle detection of 90°. The zeta potential of nanoparticles was measured on a zeta potential analyzer (Brookhaven, USA). For zeta potential measurements, samples were diluted with 0.1 mM KCl and measured in the automatic mode. All measurements were performed in triplicate.

Particle morphology was examined by transmission electron microscopy (TEM) (Hitachi, H-600). Samples were immobilized on copper grids. They were dried at room temperature, and then were examined using a TEM without being stained.

Chitosan nanoparticles separated from suspension were dried by a freeze dryer, and their FTIR were taken with KBr pellets on Nicolet, Magna-550 spectrum on FTIR.

2.4. Ammonium glycyrrhizinate encapsulation efficiency in nanoparticles

The encapsulation efficiency and loading capacity of nanoparticles were determined by the separation of nanoparticles from the aqueous medium containing non-associated ammonium glycyrrhizinate by ultracentrifugation at 35,000 rpm, 16 °C for 30 min. The amount of free ammonium glycyrrhizinate in the supernatant was measured by HPLC. Twenty microliters supernatant was injected into a chromatograph (Shimadzu LC-4A, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-10A) and reversed phase column (Inertsil ODS-3, 4.6 mm × 250 mm GL.Sciences). The mobile phase was a mixture of methanol:H_2O = 4:1 (adjusted to pH 3.5 by adding 3.6% acetic acid). The flow rate was 1.0 mL/min at 25 °C, and the wavelength was set at 254 nm. The ammonium glycyrrhizinate encapsulation efficiency (AE) and the ammonium glycyrrhizinate loading capacity (LC) of the nanoparticles were calculated as follows:

$$\mathrm{AE} = \frac{\text{total ammonium glycyrrhizinate} - \text{free ammonium glycyrrhizinate}}{\text{total ammonium glycyrrhizinate}} \times 100$$

$$\mathrm{LC} = \frac{(\text{total ammoniumcglycyrrhizinate} - \text{free ammoniumcglycyrrhizinate})}{\text{nanoparticles weight}} \times 100$$

All measurements were performed in triplicate.

2.5. Ammonium glycyrrhizinate releasing from the nanoparticles in vitro

In vitro Ammonium glycyrrhizinate release profiles of chitosan nanoparticles were determined as follows. The ammonium glycyrrhizinate-loaded chitosan nanoparticles were separated from the aqueous suspension medium through ultra-centrifugation. Ammonium glycyrrhizinate-loaded chitosan nanoparticles were re-dispersed in 5 mL 0.2 mol/L PBS solution (pH7.4) and placed in a dialysis membrane bag with a molecular cut-off of 5 kDa, tied and placed into 150 mL of PBS solution. The entire system was kept at 37 °C with continuous magnetic stirring. At appropriate time intervals, 3 mL of the release medium was removed and 3 mL fresh medium PBS solution was added into the system. The amount of ammonium glycyrrhizinate in the release medium was evaluated by HPLC. All measurements were performed in triplicate.

2.6. Stability of the ammonium glycyrrhizinate-loaded nanoparticles to salt

In brief, 0.1 mL of various nanoparticle suspensions was diluted with 0.9 mL 0.15 mol/L NaCl. Two different molecular weight chitosans (24, 200 kDa)

were selected for this series of experiments. Particle size was measured after being incubated at 37 °C for 40 min.

3. Results and discussion

3.1. Physicochemical characterization of chitosan nanoparticles

The molecular structures of glycyrrhetic acid, sodium tripolyphosphate and chitosan, are shown in Fig. 1.

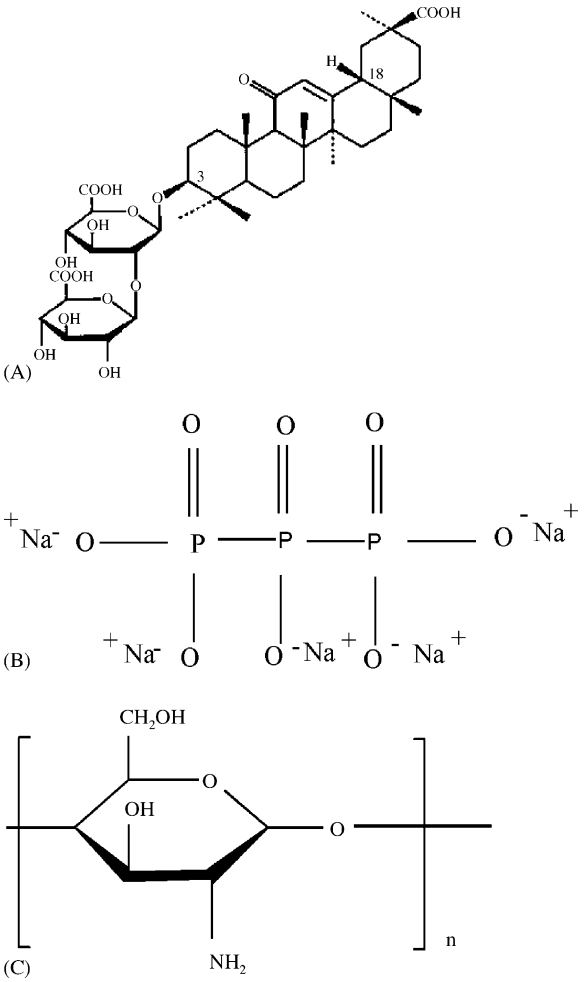


Fig. 1. Chemical structure of glycyrrhetic acid (A), sodium tripolyphosphate (B) and chitosan (C).

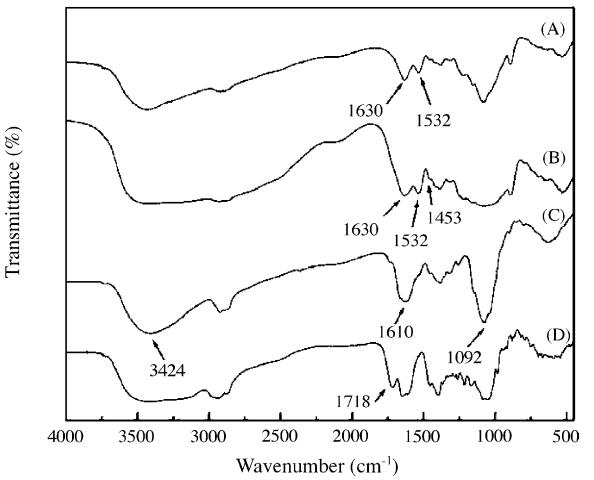
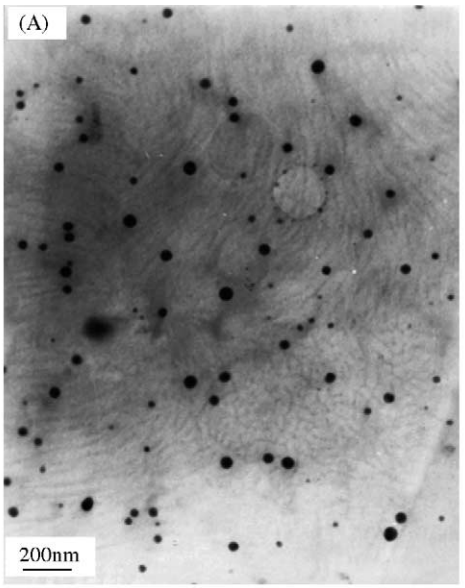


Fig. 2. FTIR of chitosan-TPP nanoparticles (A), ammonium glycyrrhizinate-loaded nanoparticles (B), chitosan (C) and ammonium glycyrrhizinate (D).

Fig. 2 shows FTIR spectra of chitosan, chitosan-TPP nanoparticles, ammonium glycyrrhizinate-loaded nanoparticles and ammonium glycyrrhizinate. There are three characterization peaks of chitosan (Fig. 2C) at 3424 cm^{-1} of ν(OH), 1092 cm^{-1} of ν(C–O–C) and 1610 cm^{-1} of $\nu(NH_2)$. The spectrum of chitosan-TPP nanoparticles (Fig. 2A) is different from that of chitosan matrix (Fig. 2C). In chitosan-TPP nanoparticles the peak of 3424 cm^{-1} becomes wider, indicating that hydrogen bonding is enhanced. In chitosan-TPP nanoparticles, the 1610 cm^{-1} peak of $–NH_2$ bending vibration shifts to 1532 cm^{-1} and a new sharp peak 1630 cm^{-1} appears. The FTIR spectrum is consistent with the result of chitosan film modified by phosphate, and it could be attributed to the linkage between phosphoric and ammonium ion (Knaul et al., 1999). So we suppose that the tripolyphosphoric groups of TPP were linked with ammonium groups of chitosan in nanoparticles. Compared with the spectrum of ammonium glycyrrhizinate (Fig. 2D), in the spectrum of ammonium glycyrrhizinate-loaded nanoparticles (Fig. 2B), the absorption peak of 1718 cm^{-1} (carboxyl group absorption peak) disappears and a new shoulder peak 1453 cm^{-1} (salt of carboxyl) appears. The results indicate that the presence of the electrostatic interactions between carboxyl groups of ammonium glycyrrhizinate and amino groups of chitosan.

Fig. 3 shows the morphological characteristic of nanoparticles. Chitosan-TPP nanoparticles (Fig. 3A)



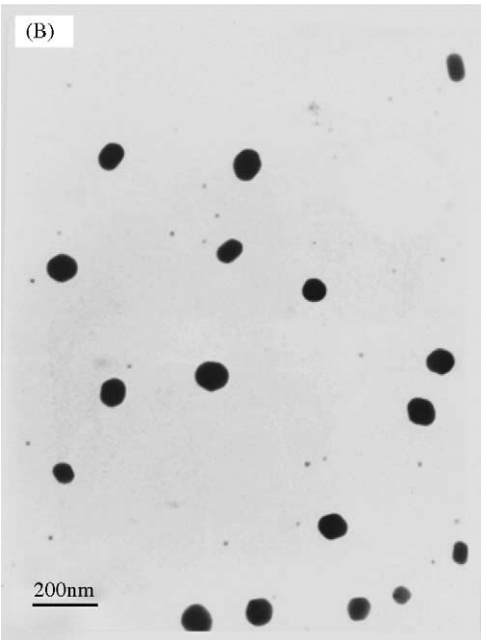


Fig. 3. TEM of chitosan-TPP nanoparticles (A) and the ammonium glycyrrhizinate loaded chitosan-TPP nanoparticles (B) (chitosan Mw = 200 kDa, 1.44 mg/mL, TPP 0.6 mg/mL, ammonium glycyrrhizinate 0.4 mg/mL).

and ammonium glycyrrhizinate-loaded chitosan-TPP nanoparticles (Fig. 3B) also take spherical shape. A similar morphology was also observed for low Mw chitosan system (data not shown). The size of these

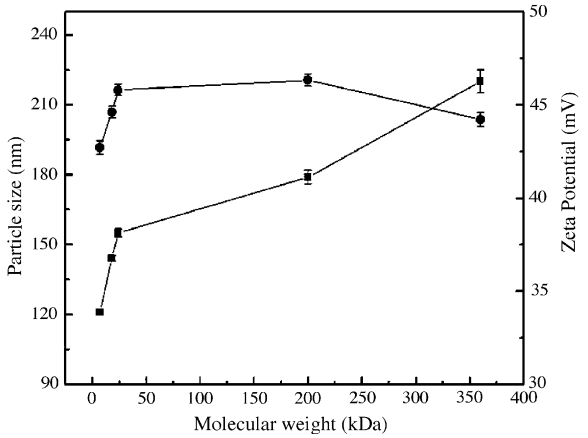


Fig. 4. The particle size (■) and zeta potential (●) measurements of chitosan-TPP nanoparticles (CS 1.44 mg/mL, TPP 0.6 mg/mL). All data are the mean ± standard deviation ($n = 3$).

nanoparticles (20–80 nm) is smaller than that determined by DLS (>120 nm) in water, presumably arising from the dry state of the TEM measurement.

3.2. Factors influencing the preparation of chitosan nanoparticles and encapsulation of ammonium glycyrrhizinate

3.2.1. Effect of chitosan molecular weight on the colloidal properties of chitosan nanoparticles

Fig. 4 shows the influence of chitosan molecular weight on the size and zeta potential values of nanoparticles. A gradual increase in the particle size with the increase in molecular weight was noted, but no significant change was observed in the zeta potential.

Although this trend may be explained by the fact that a higher molecular weight chitosan can interact with, and thus associate ammonium glycyrrhizinate more efficiently than a lower molecular weight chitosan. The factor is out-weighted by the fact that higher molecular weight chitosan is less soluble, and as a result, an increase in particle diameter or even aggregation may be obtained.

3.2.2. Effect of ammonium glycyrrhizinate concentration on the physicochemical properties of chitosan/TPP nanoparticles

Fig. 5A demonstrated that the particle sizes of ammonium glycyrrhizinate-loaded nanoparticles significantly increased as the concentration of ammonium

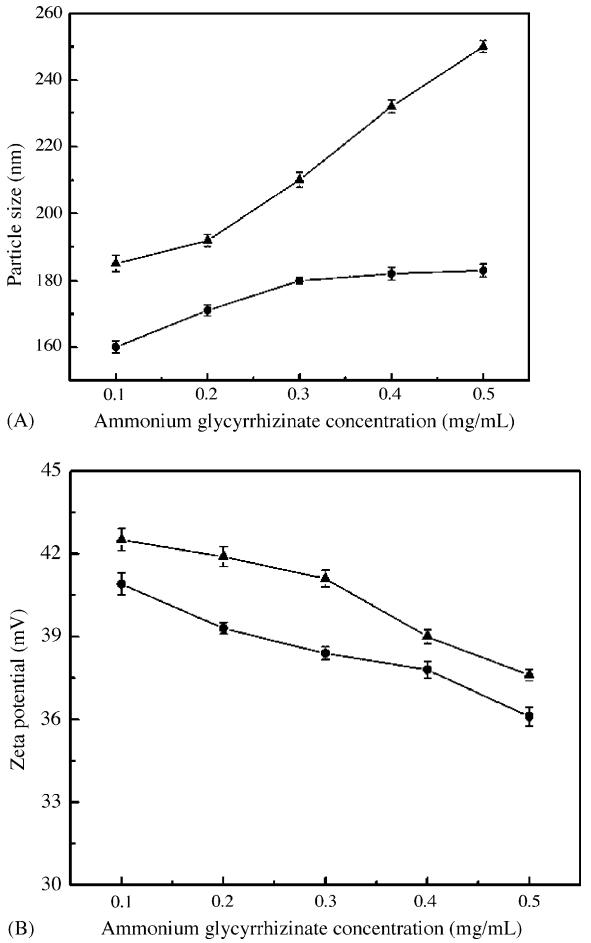


Fig. 5. The particle size (A) and zeta potential (B) of ammonium glycyrrhizinate-loaded nanoparticles as a function of the final ammonium glycyrrhizinate concentration added to chitosan nanoparticles. High Mw chitosan (Mw = 200 kDa) (▲) and low Mw chitosan (Mw = 24 kDa) (●) nanoparticles (CS 1.44 mg/mL, TPP 0.6 mg/mL). All data are the mean ± standard for $n = 3$ replicates.

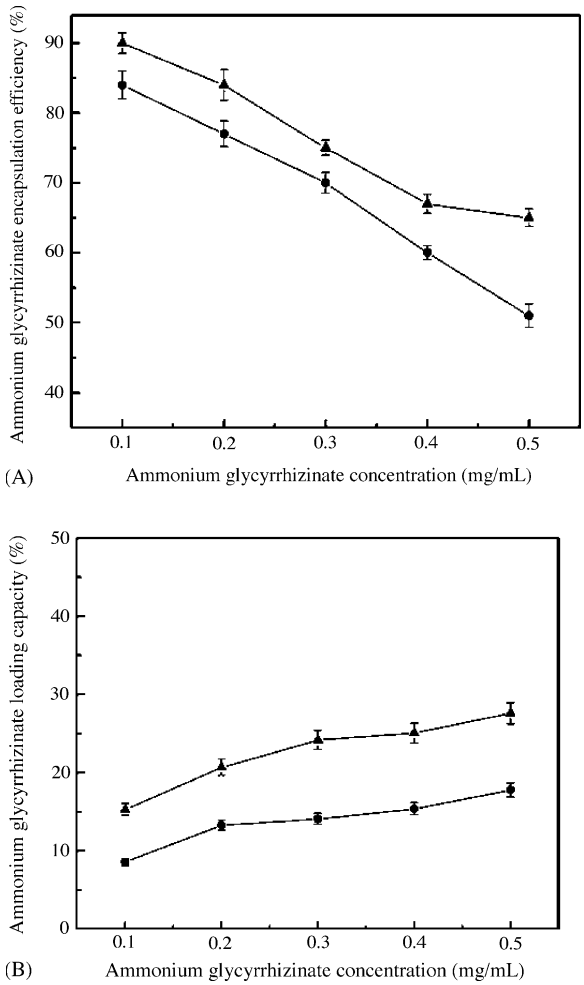


Fig. 6. Ammonium glycyrrhizinate encapsulation efficiency (A) and ammonium glycyrrhizinate loading capacity (B) of high Mw chitosan (Mw = 200 kDa) (▲) and low Mw chitosan (Mw=24 kDa) (●) nanoparticles (chitosan 1.44 mg/mL, TPP 0.6 mg/mL). Data shown are the mean ± standard deviation ($n = 3$).

glycyrrhizinate increased from 0.1 to 0.5 mg/mL. As expected, the zeta potentials decreased slightly when ammonium glycyrrhizinate concentration increased (Fig. 5B).

In general, the size of the nanoparticles increased when ammonium glycyrrhizinate was loaded on the surface. However, unlike the nanoparticles made with higher molecular weight chitosan (200 kDa), the particle size did not significantly increase as ammonium glycyrrhizinate of greater concentration was added to low Mw chitosan solution (Mw = 24 kDa). Although the reason for this is not clear, it is considered that the increased solubility of the low Mw chitosan may aid in the colloidal stability of nanoparticles in solution (Fernandez-Urrusuno et al., 1999).

As shown in Fig. 6A and B, encapsulation efficiency and loading capacity of the nanoparticles were affected by the initial ammonium glycyrrhizinate concentration in the CS solution and the amount of ammonium glycyrrhizinate incorporated. The increase of ammonium glycyrrhizinate concentration led to a decrease of encapsulation efficiency and an enhancement of loading

capacity. The mechanism of ammonium glycyrrhizinate association to chitosan nanoparticles was mediated by an ionic interaction between both chitosan and ammonium glycyrrhizinate. The electrostatic interactions between the carboxyl groups of ammonium glycyrrhizinate and the amino groups of chitosan played a role in association of ammonium glycyrrhizinate to the chitosan nanoparticles.

Fig. 6A and B also shows that the encapsulation and loading capacity of high Mws chitosan (200 kDa) is greater than that of low Mws chitosan (24 kDa). This is possibly attributed to their longer chains of high Mws chitosan, which can entrap greater amount of ammonium glycyrrhizinate when gelated with TPP.

3.2.3. *Effect of chitosan concentration on encapsulation efficiency*

When TPP concentration was 1 mg/mL, too high chitosan concentration (4 mg/mL) made encapsulation extremely difficult, and too low chitosan concentration (0.5 mg/mL) made some aggregates with large diameter form. The formation of nanoparticles is only possible within some moderate concentrations of chitosan and TPP. As for gelation between TPP solution of 1 mg/mL and chitosan solution of 1–3 mg/mL, we usually observed that some opalescent suspension was formed, which was further examined as nanoparticles. Fig. 7 shows that increase in chitosan concentration led to decrease of encapsulation efficiency of ammonium glycyrrhizinate. It has been previously reported that the highly viscous nature of the gelation medium hinders the encapsulation of drug in the study of chitosan microspheres (Vandenberg et al., 2001). So it was supposed that relatively lower viscosity of chitosan with lower concentration (such as 1–3 mg/mL) promotes the encapsulation of ammonium glycyrrhizinate and gelation between chitosan and TPP.

3.2.4. *Effect of PEG modification*

Polyethylene glycol (PEG) coated nanoparticles have been found to be potential in the therapeutic application for controlled release of drugs and drug delivery to specific sites (Quellec et al., 1998). Few studies have attempted to investigate chitosan nanoparticles coated with PEG. The PEG coated nanoparticles was conceived with the intention of making these nanoparticles more stable in physiological fluids.

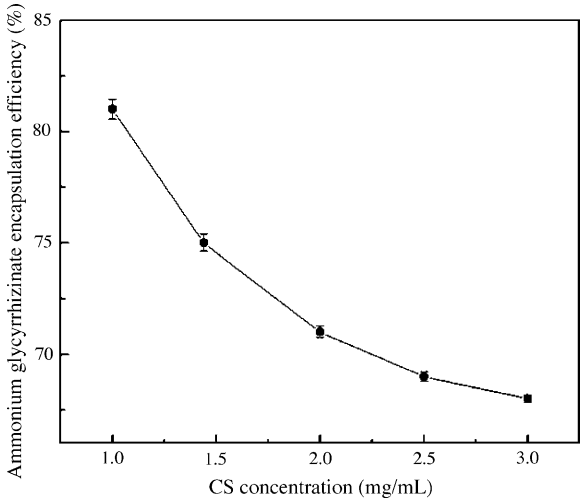


Fig. 7. The influence of chitosan concentration on ammonium glycyrrhizinate encapsulation efficiency (TPP 1 mg/mL, ammonium glycyrrhizinate 0.4 mg/mL). Data are the mean ± standard for $n = 3$ replicates.

Fig. 8 shows the morphological characteristic of PEG-modified nanoparticles. Compared with pure chitosan nanoparticles, the nanoparticle modified by PEG is of irregular shape.

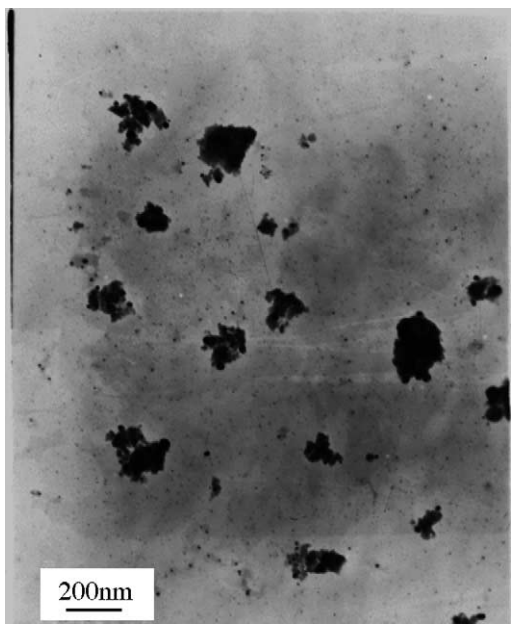


Fig. 8. TEM of chitosan/PEG nanoparticles (chitosan Mw=200 kDa, 1.44 mg/mL, TPP 0.6 mg/mL, ammonium glycyrrhizinate 0.4 g/mL, PEG30 mg/mL).

Table 1
Physicochemical properties of PEG modified chitosan nanoparticles loaded with ammonium glycyrrhizinate[a]

PEG (mg/mL)	Particle size (nm)	Polydispersity index ($\langle\mu_2\rangle/\Gamma^2$)	Zeta potential (mV)
0	182	0.17	37.8 ± 1.4
10.0	192	0.20	30.2 ± 1.1
20.0	204	0.23	23.8 ± 1.3
30.0	221	0.21	19.2 ± 1.2
40.0	252	0.24	17.4 ± 1.1
50.0	266	0.22	13.9 ± 0.8

[a] Low Mw chitosan (Mw = 24 kDa) 1.44 mg/mL, TPP 0.6 mg/mL, initial ammonium glycyrrhizinate 0.4 mg/mL.

Table 1 shows the size and zeta potential values of the PEG-modified nanoparticles. The increased size and reduced zeta potential of these nanoparticles is a good indication of the incorporation of PEG in the nanoparticle structure. It has been previously reported that the incorporation of PEG in the gel system is through intermolecular hydrogen bonding between the electro-positive amino hydrogen of CS and electro-negative oxygen atom of PEG, thus forming a CS/PEG semi-interpenetrating network (Kim and Lee, 1995). The interaction between the oxygen atom of PEG and amino groups of chitosan is weak, and it still has effect on the nanoparticles formation. The nanoparticle structure modified by PEG is looser, thus the size is larger than that of pure chitosan nanoparticles.

Consequently, it is not surprising that the increase in the concentration of PEG leads to an increase of the size and a decrease of the positive charge of the nanoparticles. Quellec also reported that the introduction of PEG can decrease significantly the positive surface charge of the particles, and noticeably improve their biocompatibility (Quellec et al., 1998).

Fig. 9 shows that when PEG concentration increased from 10 to 50 mg/mL, encapsulation efficiency of ammonium glycyrrhizinate decreased from 63 to 35%. PEG was added to chitosan solution prior to gelation. Without TPP incorporation, PEG cannot gelate with chitosan, but the amine groups of chitosan can be occupied by the oxygen atom of PEG, which may compete in their interaction with the amine groups of chitosan. Thus, the possibilities of ion interaction between the ammonium glycyrrhizinate and chitosan are reduced. The entanglement of PEG chain with chitosan molecule hinders ammonium glycyrrhizinate from encapsulating into the nanoparticles.

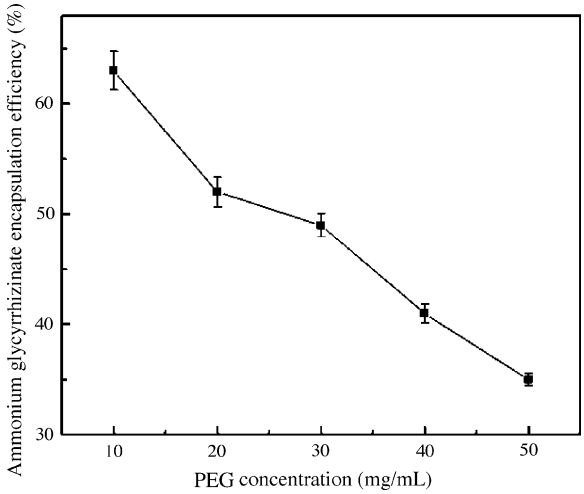


Fig. 9. The influence of PEG modification on ammonium glycyrrhizinate encapsulation efficiency (low Mw chitosan (Mw = 24 kDa) 1.44 mg/mL, TPP 0.6 mg/mL, initial ammonium glycyrrhizinate 0.4 mg/mL). Data shown are the mean ± standard deviation (n = 3).

3.2.5. *Effect of ionic strength on the stability of chitosan nanoparticles*

Since chitosan is a cationic polyelectrolyte, the effect of ionic strength of the medium on nanoparticles is important. As shown in Table 2, when nanoparticles are formed in distilled water, the mean diameter is 182 nm. The mean diameter increases with the increase of sodium chloride concentration. When the ionic strength was higher than 1 mol/L NaCl, some aggregations would form and the particle size distribution becomes wide.

As shown in Fig. 10, nanoparticles made from high Mw chitosan (200 kDa) were stable to 0.15 mol/L NaCl (physiological saline). However, nanoparticles made from low Mw chitosan (24 kDa) were not stable to

Table 2
Effect of ionic strength on the stability of chitosan nanoparticles[a]

NaCl (mmol/L)	Paticle size (nm)	Polydispersity index ($\langle\mu_2\rangle/\Gamma^2$)
0	182	0.17
1	184	0.18
10	228	0.19
50	389	0.27
150	506	0.45
1000	810	1.0

[a] Low Mw chitosan (Mw = 24 kDa) 1.44 mg/mL, TPP 0.6 mg/mL, initial ammonium glycyrrhizinate 0.4 mg/mL.

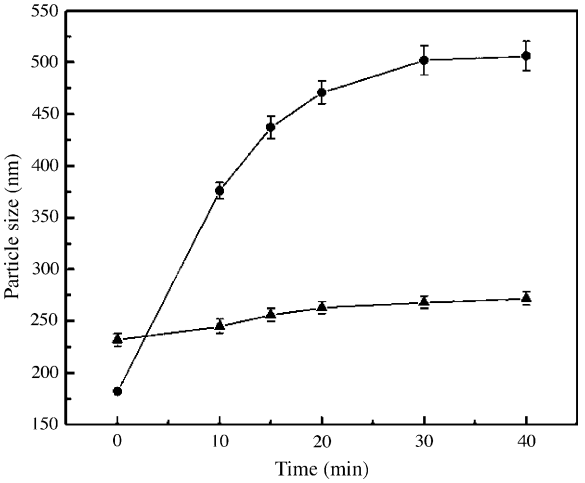


Fig. 10. The stability of the ammonium glycyrrhizinate-loaded nanoparticles to 0.15 mol/L NaCl. Ammonium glycyrrhizinate (0.4 mg/mL) loaded on low Mw chitosan (Mw = 24 kDa) (●) and high Mw chitosan (Mw = 200 kDa) (▲) nanoparticles (CS 1.44 mg/mL, TPP 0.6 mg/mL). All data are the mean ± standard for $n = 3$ replicates.

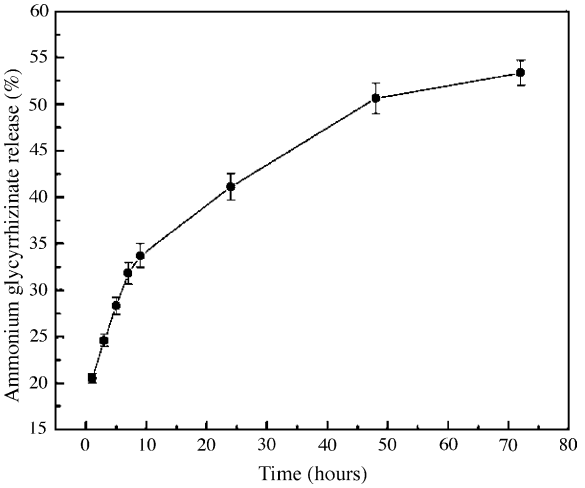


Fig. 11. Ammonium glycyrrhizinate release profile from ammonium glycyrrhizinate-loaded chitosan nanoparticles (low Mw chitosan (Mw = 24 kDa) 1.44 mg/mL, TPP 0.6 mg/mL, initial ammonium glycyrrhizinate 0.4 mg/mL).

0.15 mol/L NaCl. The facts may suggest that the low molecular weight chitosan (Mw = 24 kDa), which is relative weakly associated with the ammonium glycyrrhizinate, competes with salt ions for its small size. As the molecular weight of chitosan increases, it will associate with ammonium glycyrrhizinate more strongly, which is consistent with the salt effect. Thus, if an excess of positive charge is present in the nanoparticle, it is more difficult to replace the chitosan by the salt ions at a low concentration for its large size.

3.3. In vitro release of ammonium glycyrrhizinate from the nanoparticles

Fig. 11 displayed the release profile of ammonium glycyrrhizinate from chitosan nanoparticles. It was apparent that ammonium glycyrrhizinate release in vitro showed a very rapid initial burst, and then followed by a very slow drug release. Zhou et al. (2001) reported about microspheres and revealed that the release involves two different mechanisms of drug molecules diffusion and polymer matrix degradation. The burst release of drug is associated with those drug molecules dispersing close to the microsphere surface, which easily diffuse in the initial incubation time. The hypothesis is also suitable for ammonium glycyrrhizinate release from nanoparticles. Since the size of ammonium glycyrrhizinate molecule is much smaller than that of nanoparticles, ammonium glycyrrhizinate molecules diffuse easily through the surface or the pore of nanoparticles in a short time. Therefore, the rapid dissolution process suggests that the release medium penetrates into the particles due to the hydrophilic nature of chitosan, and dissolves the entrapped ammonium glycyrrhizinate. In addition, the nanoparticles with huge specific surface area can adsorb ammonium glycyrrhizinate, so the first burst release is also possibly due to the part of ammonium glycyrrhizinate desorbed from nanoparticle surface.

4. Conclusion

Chitosan nanoparticles had shown an excellent capacity for the association of ammonium glycyrrhizinate. Ammonium glycyrrhizinate can be loaded on these pre-formed nanoparticles with a final ammonium glycyrrhizinate concentration of up to 0.5 mg/mL. Adding PEG decreases the encapsulation and reduces the positive charge. The release profile of ammonium glycyrrhizinate from nanopaticles has an obvious burst effect and a slowly continuous release phase followed.

The nanoparticles may improve the oral absorption of ammonium glycyrrhizinate.

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