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Quaternized Chitosan (QCS) Nanoparticles as a Novel Delivery System for Ammonium Glycyrrhizinate

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The aim of this study was to generate a new type of nanoparticles made of quaternized chitosan (QCS) and poly(aspartic acid) via the ionotropic gelation technique and to evaluate their potential for the association and delivery of ammonium glycyrrhizinate (GLA). The effects of the pH value of nanoparticles, QCS molecular weight (Mw) and poly(aspartic acid) concentration on GLA encapsulation were studied. Suitably pH value of nanoparticles, moderate QCS MW, optimal concentration ratio of poly(aspartic acid) and QCS favored higher GLA encapsulation efficiency. The release of GLA from nanoparticles was pH-dependent. Fast release occurred in 0.1 M phosphate buffer solution (PBS, pH = 7.4), while the release was slow in 0.1 M HCl (pH = 1.2). The results showed that the new QCS/poly(aspartic acid) nanoparticles have a promising potential in GLA delivery system.

Keywords: Quaternized Chitosan, Poly(Aspartic Acid), Nanoparticles, Ionotropic Gelation, Ammonium Glycyrrhizinate, Delivery.

1. INTRODUCTION

Polymer nanoparticles consisting of biodegradable and biocompatible polymers are good candidates as drug carriers for deliver drugs, because they are expected to be adsorbed in an intact form in the gastrointestinal tract after oral administration.[1]

Chitosan (CS) has shown favorable biocompatibility, biodegradability characteristics and the ability to increase membrane permeability.[2–4] But chitosan is only soluble in acid medium. Quaternized chitosan, with improved solubility, enhanced positive charge intensity and the absorption across mucosal epithelia even in neutral environment.[5]

Poly(aspartic acid) and its derivatives possess extensive applications in the field of biomedicine, due to their excellent biocompatibility and biodegradability.[6]

Glycyrrhetic acid shows anti-inflammatory, antitumorigenic and anti-hepatotoxic activities,[7] but the oral absorption of glycyrrhetic acid (or its salt) was extremely ineffective.

The aim of this study was to combine the virtue of nanoparticles consisting of QCS and poly(aspartic acid) and to study the possibility to associate hydrophilic GLA.

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2. EXPERIMENTAL DETAILS

The quaternary chitosan salt (QCS) was prepared by a modified method proposed by Spinelli et al.[8] The mol ratio of glycidyl trimethyl ammonium to chitosan (deacetylation degree (DD) of 90%, Mw: 360 kDa, Jinqiao Biochemistry, Zhejiang, China) was 4. Mws of QCS were measured by the GPC method and the degree of substitution (DS) was determined by potentiometry. QCS with different Mw was obtained by H_2O_2 degradation.

QCS/poly(aspartic acid) nanoparticles were prepared via ionotropic gelation between QCS and poly(aspartic acid). Briefly, 3 mL of an aqueous solution of poly-L-aspartic acid sodium salt (0.5–2 mg/mL, Mw 5 kDa–15 kDa, Sigma Chemical (USA)) was added drop by drop to 6 mL of a QCS solution (1–8 mg/mL) under magnetic stirring at room temperature for 30 min to allow the complete stabilization of the system. The nanoparticles were isolated by ultracentrifugation at 35,000 rpm for 30 min at 16 °C.

The GLA was dissolved in heat distilled water. The GLA-loaded nanoparticles were obtained by adding GLA solution into QCS solution before the adding of poly(aspartic acid).

The yield of the nanoparticles was dry weight of nanoparticles precipitate.

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The encapsulation efficiencies (EE) of GLA were determined by ultracentrifuging the nanoparticles suspension at 35,000 rpm at 16 °C for 30 min. The precipitate separated from the supernatant was dried at 40 °C for 12 h and weighed. The amount of free GLA in the supernatant was measured by HPLC (Shimadzu LC-4A, Kyoto, Japan; 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 GLA encapsulation efficiency (EE) in the nanoparticles was calculated as follows:

$$EE = [(\text{total GLA} - \text{free GLA})/\text{total GLA}] \times 100$$

All measurements were performed in triplicate.

In vitro release studies were performed as follows: About 25 mg of the dried GLA-loaded nanoparticles were suspended in 5 mL of 0.1 mol/L HCl or 0.1 mol/L PBS of pH 7.4, and then incubated at 37 °C on the SHZ-88 Constant Temperature Water-bath Shaker (China) at the rate of 120 rpm. At predetermined intervals, samples were ultracentrifuged. 2 mL of the supernatant was taken out and the free GLA was determined by HPLC.

3. RESULTS AND DISCUSSION

To investigate the formation of QCS/poly(aspartic acid) nanoparticles, IR spectra (Perkin-Elmer, America) was investigated (data not shown). The new peak at 1483 cm^{-1} (methyl groups in the ammonium) in the spectra of QCS, which corresponded to an asymmetric angular bending of methyl groups of quaternary hydrogen. The 1599 cm^{-1} peak of $-NH_2$ bending vibration shifted to 1570 cm^{-1} and the absorption peak of 2876 cm^{-1} disappeared. The new peak 1430 cm^{-1} (salt of carboxyl) appeared in the spectra of QCS/poly(aspartic acid) nanoparticles. The results indicated that the presence of the electrostatic interactions between carboxyl groups of poly(aspartic acid) and $-N^+(CH_3)_3$ groups in QCS.

Figures 1(A, B) showed the morphological characteristics and size distributions of GLA-loaded nanoparticles. It could be seen that the nanoparticles were regular spherical in shape and had a narrow size distribution.

The pH value of the nanoparticles slowly decreased from 6.49 to 6.02 as the ratio of QCS to poly(aspartic acid) decreased from 8:1 to 1:1. The positive values of the zeta potential (Zetasizer 3000 HS, Malvern, United Kingdom) of nanoparticles decreased from 63.15 ± 2.89 to 49.18 ± 2.56 as the ratio of QCS to poly(aspartic acid) decreased from 8:1 to 1:1 (pure QCS (Mw = 65 kDa) was +65.9 mV, pH = 6.58). The result indicated that an electrostatic interaction between QCS and poly(aspartic acid) has taken place.

Figure 2(A) illustrated that the GLA encapsulation efficiency increased with the increased pH value of nanoparticles. The significantly increased nanoparticle zeta potential should be the main reason for the enhancement of GLA encapsulation efficiency along with the increased pH value of nanoparticles with different QCS/poly(aspartic acid) ratios. It was difficult to encapsulate GLA molecules completely within nanoparticles.[9] Hence, there must be a great part of GLA molecules absorbing around the surfaces of the nanoparticles, owing to the electrostatic interaction between the nanoparticles and GLA chains. In this way, for a certain weight of nanoparticles, high zeta potential of nanoparticles should absorb a larger amount of GLA than lower.

In Figure 2(B), GLA encapsulation efficiency increased first and then decreased along with the gradual increase of QCS Mw. Some researchers believed that higher Mw of CS led to higher encapsulation efficiency because longer chain of CS molecule could entrap more drugs.[8] The positive effect of high QCS Mw on GLA encapsulation was reasonable. On the other hand, QCS Mw also affected the size of the nanoparticles. The size of the nanoparticles might further effect their GLA encapsulation. In this way, the enlargement of nanoparticles might lower encapsulation efficiency due to the diminishment of total surface area.

Figure 2(C) showed that GLA encapsulation efficiency increased within the QCS concentration range of

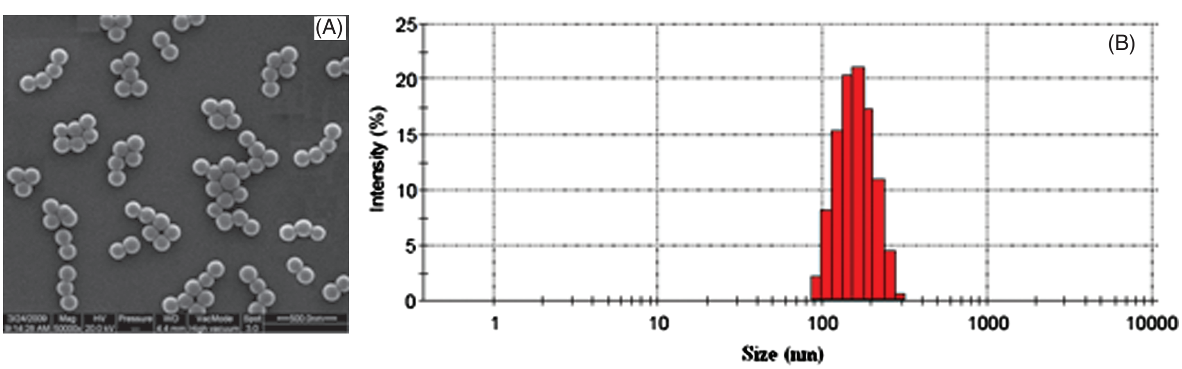

Fig. 1. ESEM image (A) and the particle size distributions, (B) of GLA-loaded QCS/poly(aspartic acid) nanoparticles. (QCS = 2 mg/mL, poly(aspartic acid) = 1 mg/mL, GLA = 1 mg/mL, QCS Mw = 65 kDa).

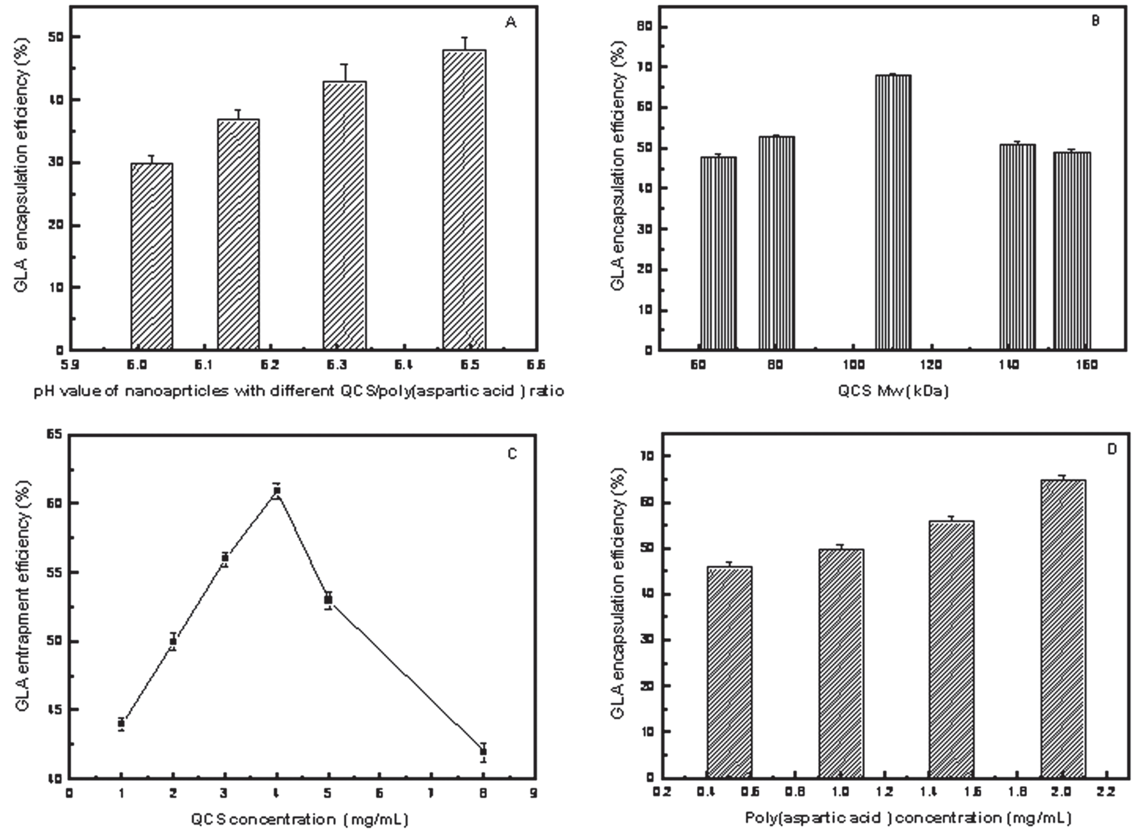

Fig. 2. Effects of pH value of nanoaprticles (QCS Mw = 65 kDa) (A), QCS Mw (B) (QCS = 2 mg/mL, poly(aspartic acid) = 1 mg/mL), QCS concentration (C) (poly(aspartic acid) = 1 mg/mL, QCS Mw = 65 kDa), and poly(aspartic acid) concentration (D) on GLA encapsulation efficiency.

1–4 mg/mL and then decreased after that point. The formation of QCS/poly(aspartic acid) nanoparticles depended on the electrostatic interaction between negatively charged poly(aspartic acid) and positively charged QCS. Poly (aspartic acid) acted as an ionic crosslinking reagent in the formation of nanoparticles. In the crosslinking, there was an optimal ratio between QCS and poly(aspartic acid) to form the QCS/poly(aspartic acid) nanoparticles. For a certain type of nanoparticles, their encapsulation ability should be closely related to their quantity. More nanoparticles yield could encapsulate more drugs. GLA encapsulation efficiency increased with the QCS concentration from 1 to 4 mg/mL (Fig. 2(C)). The property of GLA should be considered. GLA (acted actually as another ionic crosslinking reagent) with negatively charged and could electrostatically interact with positive QCS. Therefore, the initial increase of GLA encapsulation efficiency was reasonable within a certain range of QCS concentration, while the reduction of GLA encapsulation efficiency was inevitable when the nanoparticles yield continued to decrease (QCS concentration increased from 4 to 8 mg/mL, the nanoparticles yield decreased from 4.5 mg to 2.6 mg).

Figure 2(D) displayed the effects of poly(aspartic acid) concentration on GLA encapsulation efficiency. GLA encapsulation efficiency increased within the poly(aspartic acid) concentration range of 0.5–2 mg/mL and QCS concentration 2 mg/mL Above results illustrated that the increased of the nanoparticles yield (poly(aspartic acid) concentration range of 0.5–2 mg/mL and QCS concentration 2 mg/mL the nanoparticles yield increased from 2.8 mg to 11.5 mg) contributed to the enhancement of GLA encapsulation efficiency. Increasing both poly(aspartic acid) and QCS concentration at an optimal ratio was a good way to obtain a larger amount of the nanoparticles and higher GLA encapsulation efficiency.

In vitro release of GLA from the nanoparticles in both simulated gastric fluid (0.1 M HCl) and simulated intestinal fluid (0.1 M PBS, pH 7.4) at 37 °C was shown in Figure 3. The release profile was characterized by an initial burst effect followed by a continuous and slow release phase. The release of GLA from nanoparticles incubated in simulated intestinal fluid was much faster than that in simulated gastric fluid in the same period. Only 31% GLA was released in 0.1 M HCl, while more than 80% of GLA was eluted out in PBS from the nanoparticles. The accelerated release of GLA from nanoparticles incubated in the high pH media was more likely owed to the reduced electrostatic interactions between the polyion complexes and the nanoparticles at this pH. Two processes could explain the release of GLA from the nanoparticles:diffusion and erosion. Therefore, in 0.1 M HCl, the process of GLA

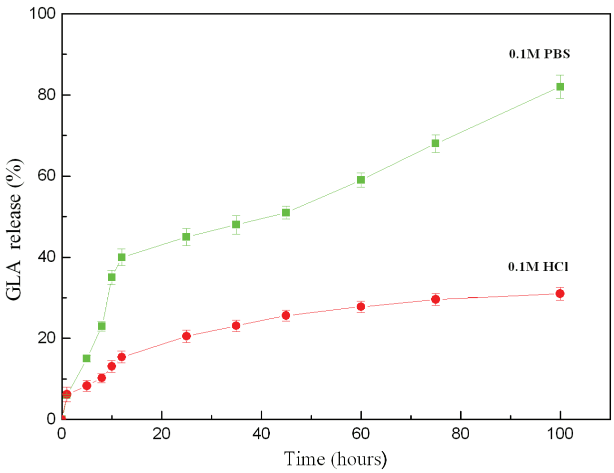

Fig. 3. *In vitro* release of GLA from QCS/poly(aspartic acid) nanoparticles with different media (a) in 0.1 M HCl and (b) in 0.1 M PBS (pH = 7.4) (QCS Mw = 65 kDa, $n = 3$).

release was mainly controlled by the diffusion process. In PBS, in addition to the diffusion process, the erosion of the nanoparticles caused greatly increased the GLA release rate.

4. CONCLUSION

A novel GLA-loaded QCS/poly(aspartic acid) nanoparticle system was successfully prepared via the ionotropic gelation method. Suitably pH value of nanoparticles, moderate QCS Mw, at an optimal ratio of poly(aspartic acid) and QCS concentration favored higher GLA encapsulation efficiency. The release of GLA from nanoparticles was pH-dependent. The results showed that the new QCS/poly(aspartic acid) nanoparticles have a promising potential in GLA delivery system.

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References and Notes

1. H. Zhang, O. Megan, A. Christine, and K. Eugenia, *Biomacromolecules* 5, 2461 (**2004**).
2. Y. Hu, X. Q. Jiang, Y. Ding, H. X. Ge, Y. Y. Yuan, and C. Z. Yang, *Biomaterials* 23, 3193 (**2002**).
3. J. S. Park and Y. W. Cho, *Macromolecular Research* 15, 513 (**2007**).
4. Y. Wu, W. L. Yang, C. C. Wang, J. H. Hu, and S. K. Fu, *Int. J. Pharm.* 295, 235 (**2005**).
5. S. M. Vander Merwe, J. C. Verhoef, J. H. Verheijden, A. F. Kotze, and H. E. Junginger, *Eur. J. Pharm. Biopharm.* 58, 22 (**2004**).
6. H. Arimura, Y. Ohya, and T. Ouchi, *Biomacromolecules* 6, 720 (**2005**).
7. S. Takeda, K. Ishihara, Y. Wakui, S. Amagaya, M. Maruno, T. Akao, and K. Kobashi, *J. Pharm. Pharmacol.* 48 , 902 (**1996**).
8. V. A. Spinelli, M. C. M. Laranjeira, and V. T. Fa/vere, *React. Funct. Polym.* 61, 347 (**2004**).
9. Y. M. Xu and Y. M. Du, *Int. J. Pharm.* 250, 215 (**2003**).

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