Pluronic F127/chitosan blend microspheres for mucoadhesive drug delivery

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Abstract. Pluronic F127/chitosan blend microspheres were prepared via emulsification and cross-linking process using glutaraldehyde as a cross-linker. Compared with chitosan microspheres fabricated under the same experimental conditions, blend microspheres exhibited better physical stability and higher swelling capacity. Puerarin, a traditional Chinese medicine, was incorporated into microparticlesas the model drug. The in vitro release of puerarin from blend microspheres was reduced because of the improved compatibility of the drug with the matrices. According to the results from in vitro adhesion experiments, mucoadhesive behavior of blend microspheres on a mucosa-like surface was similar to that of chitosan microspheres, despite their good ability of anti-protein absorption in solution.

1. Introduction

In recent years, microspheres based on chitosan have been extensively investigated as carriers formucosaldrug delivery because of the unique physicochemical properties and the safety of chitosan [1]. Aiming to improve and optimize the characteristics of chitosan microspheres, several researches have been carried out on microspheres based on chitosan derivatives [2, 3]. In our previously published studies, PEGylated chitosan derivatives were synthesized and used to preparemicrospheres for the transmucosal delivery of puerarin [4]. The PEGylation of chitosan conferred valuable benefits to the propertiesof the microspheres even at a low degree of PEG substitution. Compared with the development of novel chitosan derivatives, polymer blending is a much more convenient approach for the desirable new properties of chitosan. Blends of chitosan with numerous natural or synthetic polymers, such as silk fibroin [5], polyurethane [6], polyethylene glycol [7] and polyvinylpyrrolidone [8], have been fabricated in the form of microspheres in order to enhance the physicochemical, mechanical, and functional properties for biomedical applications.

Yoo and colleagues used Pluronic F127 (F127), a hydrophilic ABA-type triblock copolymer consisting of polyoxyethylene units (A) and polyoxypropyleneunits (B), to prepared F127/chitosan blend microspheres via an ionic gelation process with tripolyphosphate for vaccine delivery [9]. Compared with chitosan microspheres, the blend microspheres had improved stability and immune activity, and also exhibited potential protection against infection. It has been reported that chitosan-based materials prepared using tripolyphosphate as the cross-linker have poor mechanical strength [10]. F127/chitosan blend microspheres fabricated by the emulsion-crosslinking method employing glutaraldehyde as a cross-linker were studied [11]. The published work investigated the influence of formulations on the incorporation and release behavior of 5-fluorouracil in the blend microspheres;

however, investigations focused on the mucoadhesiveness of F127/chitosan blend microspheres have yet to be reported.

Since the mucoadhesive behavior of the microspheres formulations is extremely important for a potential mucosal delivery system, the interactions between the F127/chitosan blend microspheres and mucin in vitro was investigated in this study. Puerarin, an effective traditional Chinese medicine treatment of cerebrovascular and cardiovascular diseases, was chosen as the model drug. Researches on swelling, dissolution, drug loading and release behavior of the blend microspheres were also carried out.

2. Materials and methods

2.1. Materials

Pluronic F127(F127) was purchased from Aldrich. Chitosan (200-400 mPa·s, >90%deacetylation) was obtained from Aladdin Reagent Co.. Puerarin (>98%) was provided by Nanjing Zelang Medical Technology Co.. Glutaraldehyde (50% in H_2O) were obtained from Sinopharm Chemical Reagent Co.. Mucin (from porcine stomach) was purchased from Amresco. All other reagents used were of analytical grade and used as received.

2.2. Preparation and characterization of microspheres

Microspheres were produced by emulsification and cross-linking process. Firstly, F127 and 50 mg of chitosan were dissolved in 3% (v/v) acetic acid solution to obtain a miscible polymer solution (aqueous phase). This solution (3.3 mL) was added slowly into liquid paraffin (100 mL, oil phase) containing 2% (v/v) span-80 under constant stirring at 1000 rpm for 10 min using a homogenizer (RW20 Digital Mechanical Overhead Stirrer, IKA, Germany). At the end of sufficient emulsification, the W/O emulsion was solidified by slowly dropping glutaraldehyde in 1,4-dioxane solution (5%, v/v, 1.25 mL) at 450 rpm for 1.5 h. The hardened microspheres were collected by filtration, separately washed with petroleum ether and isopropyl alcohol, and then dried in a vacuum oven overnight. Chitosan microparticles were prepared as a comparison.

The surface morphology of microspheres was examined under a scanning electron microscope (SEM, S-3000N, Hitachi, Japan). Microspheres were placed on a copper stub, coated with gold and observed.

The particle size analysis was performed on samples of the microspheres redispersed in distilled water (~1.0%, wt%) using the WQL particle size analyzer (LKY-2, SPSIC, China).

2.3. Swelling and dissolution experiments

The swelling properties of the microspheres were evaluated measuring the equilibrium water uptake of samples incubated in deionized water at 37 °C. At predetermined times (6 h, 12 h and 24 h), samples were taken out, and the excess surface adhered water was removed carefully. The swollen microspheres were weighed (W_s), and then oven dried at 60 °C until there was no change in the weight of the dried samples. The weight of dried microspheres was recorded (W_d). Each experiment was repeated six times andthe average valuewas used to calculate the swelling capacity (%) by the following equation.

$$\text{Swelling capacity (\%)} = \frac{W_s - W_d}{W_d} \times 100\% \quad (1)$$

The percentage dissolution of microparticles was calculated from the following equation. Each dissolution value was averaged from the six measurements.

$$\text{Dissolution (\%)} = \frac{W_0 - W_d}{W_0} \times 100\% \quad (2)$$

Where W_0 is the initial weight of microspheres and W_d is the remaining weight of dried samples.

2.4. Evaluation of puerarin-loading capacity of microspheres

Puerarin was dissolved directly into the polymer solution to form a drug-containingaqueous phase. Drug-loaded microspheres were prepared via the same emulsification and cross-linking process mentioned above. Ten milligrams of puerarin-loaded microspheres was dissolved in hydrochloric acid solution before the quantitative determination of the drug-loading capacity of samples using Agilent 1200 series HPLC (Chemstation System, DAD detector, Eclipse XDB-C18 column (4.6 mm × 150 mm, 5 μm)) as reported[12]. All measurements were performed in triplicate and averaged.

Drug-loading capacity (LC) was calculated as the following equation.

$$\text{LC}\ (\%) = \frac{\text{incorporated puerarin weight}}{\text{microspheres weight}} \times 100\% \quad (3)$$

2.5. X-ray diffraction analysis

X-ray diffraction patterns of pure puerarin, puerarin-loaded chitosan microspheres and puerarin-loaded F127/chitosan blend microspheres were obtained using an X-ray diffractometer (SHIMADZU XRD-7000, Japan) with Cu-Kα radiation source operating at 40 kV and 30 mA. Diffractograms were performed from the initial angle $2\theta = 10°$ to the final angle $2\theta = 80°$ with a step width of 0.02°.

2.6. In vitro release studies

Release studies of puerarinfrom microspheres were performed under sink conditions. Puerarin-loaded samples (10 mg) were suspended in 5 mL of pure water at 37 °C with horizontal shaking. At predetermined time points, samples were centrifuged at 4000 rpm for 4 min. Then, 1 mL of supernatant was withdrawn and immediately replaced with equal volumes of fresh water. The collected solutions were subjected to further HPLC analysis using the same conditions reported above. All experiments were carried out in triplicate.

2.7. Adsorption of mucin on microspheres

Mucin stock solutions with a concentration of 1 mg/mL in pure water were prepared. Ten milligrams of microspheres were dispersed in the above mucin aqueous solution (6 mL), and shaken at 37 °C for 2 h. Then, the dispersion was centrifuged at 4000 rpm for 2 min, and the supernatant was removed for the measurement of the free mucin content. The protein estimation was done by Periodic acid/Schiff colorimetric method[13]. All measurements were performed in triplicate and averaged. The adsorption of mucin on microspheres was assessed as follows.

$$\text{Adsorption ratio}\ (\%) = \frac{\text{amount of total mucin-amount of free mucin}}{\text{amount of total mucin}} \times 100\% \quad (4)$$

2.8. In vitro mucoadhesion studies

Mucoadhesion testing was conducted usingmucin saturated filter paper as the in vitro experimental model according to the previously described method[14]. Firstly, the filter paper (2.5 cm × 2.5 cm) was wetted completely with mucin solution (2% w/v in pure water) and fixed inside a tube under controlled conditions of humidity (75.8 ± 1.6%) and temperature (28 ± 1°C). Puerarin-loaded microspheres (10 mg) were spread out onto themucin saturated filter paper, and then a stream of air (6.2 ± 1 m/s) was blown over the filter paper with samples face downward for 15 s. Microparticles sticking to the surface were recovered by washing the filter paper fully with 3% (v/v) hydrochloric acid solution. The amount of drug in the remaining samples was quantified via HPLC as mentioned above. All measurements were performed in triplicate and averaged. The in vitro mucoadhesion behavior of the microspheres was expressed as the following equation.

$$\text{Mucoadhesivity}\ (\%) = \frac{\text{the amount of puerarin in the remaining microspheres}}{\text{the total amount of puerarin}} \quad (5)$$

3. Results

3.1. Morphology observation

Mixture of F127 and chitosan (1/5, 2/5 and 3/5, w/w) in acetic acid solution was used as the water phase for the preparation of blend microspheres (CS-10F, CS-20F and CS-30F). Blank chitosan microspheres (CS) were also prepared as a comparison. Both blend microspheres and CS had a spherical shape and a smooth surface (figure 1). Decreased degree of particle aggregation was observed in the SEM images of blend microspheres. The mean diameters of the CS, CS-10F, CS-20F and CS-30F were 3.42±0.21，3.50±0.27，3.69±0.18 and 3.20±0.10 μm, respectively.

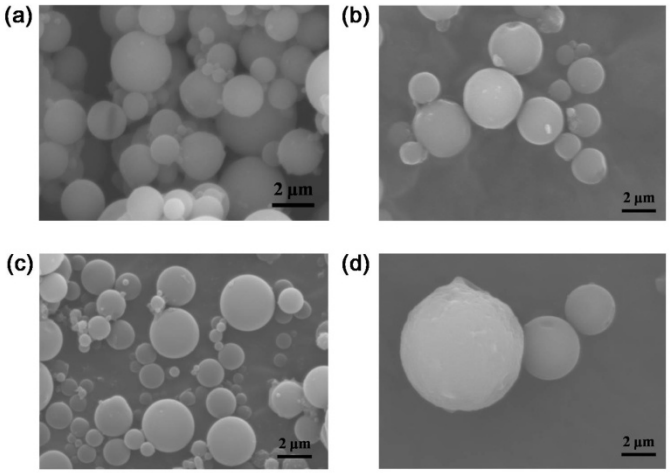

Figure 1. SEM images of (a) CS and (b) CS-10F, (c) CS-20F and (d) CS-30F.

3.2. Swelling behavior and dissolution assessment

All of the investigated samples exhibited considerable swelling properties, and a significant amount of water was absorbed within the first 6 h (figure 2). After immersion for 6 h, the swelling capacities of the CS, CS-10F, CS-20F and CS-30F were 172.22 ± 12.23, 245.45 ± 14.10, 249.65 ± 18.88 and 347.9 ± 18.32 %, respectively. After 24 h, the water uptake of microspheres approached saturation.

The dissolution values of the CS in water (37 °C) for 24 h was 11.7 ± 6.7 %. The dissolutions of composite microspheres CS-10F, CS-20F and CS-30F were 21.6 ± 3.6, 20.0 ± 2.8 and 22.2 ± 3.1 %, respectively.

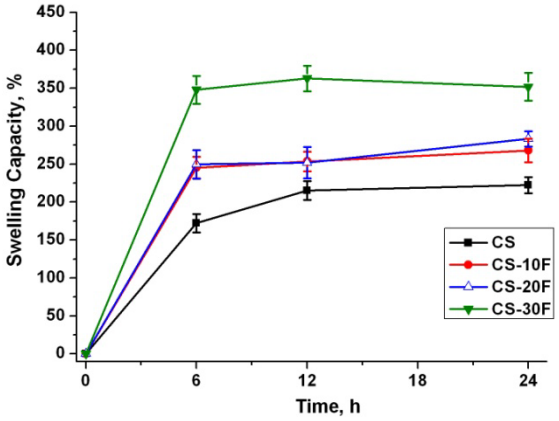

Figure 2. Swelling capacities of CS, CS-10F, CS-20F and CS-30F with respect to time.

3.3. Drug-loading capacity determination

The LC of the blank microspheres CS was 3.48±0.08%, which was 2.26±0.03%, 0.28±0.03% and 0.26 ±0.01% for the blend microspheres CS-10F, CS-20F and CS-30F.

3.4. X-ray diffraction analysis

X-ray diffraction patterns for puerarin alone, puerarin-loaded CS and puerarin-loaded CS-10F were shown in figure 3. The spectrum obtained from puerarin alone gave quite sharp and distinct diffraction peaks with a low and flat background as expected from a crystalline sample. The microspheres containing puerarin exhibited notable broadening and overlap of the diffraction peaks, and the sharp peaks corresponding to the drug in the crystalline state weakened and even disappeared. The peak intensity of CS-10F in the range of 12° - 30° was obviously weaker in comparison to that of CS.

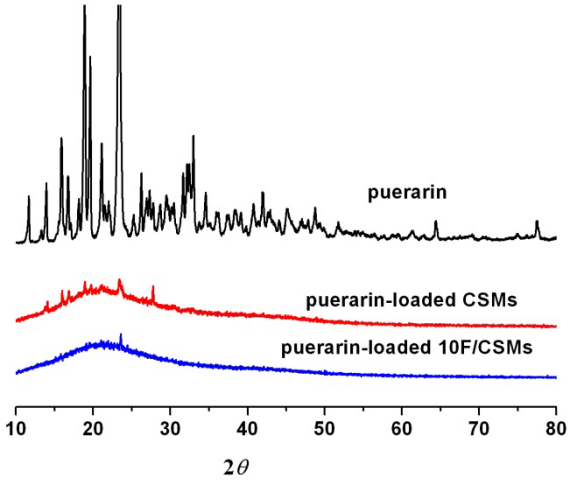

Figure 3. X-ray diffraction patterns of puerarin and puerarin-loaded microspheres.

3.5. In vitro release

In vitro release ratio studies from CS indicated severe initial burst releases, 75% within the first 15 min, and 81% at 2 h. The release rates of puerarin from CS-10F were obviously delayed, 53% at 15 min; the initial release was later followed by a more controlled release in the selected experimental time window, releasing accumulative 70% of drug from the microspheres (figure 4).

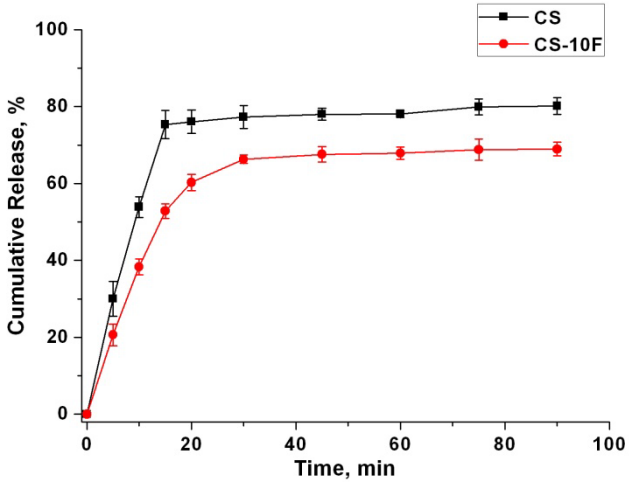

Figure 4. Release profiles of puerarin from CS and CS-10F.

3.6. In vitro mucoadhesivebehavior assessment

The amount of mucin adsorbed onto the surface of microspheres was determined from the change in the free concentration of mucin in the reaction solution according to equation (4). The average adsorption ratio of mucin on CS and CS-10F was 67.52±0.49% and 40.71±0.63%, respectively.

The amount of puerarin-loaded microspheres adhering to the mucin saturated surface after the application of a stream of air was further determined according to equation (5) via HPLC measurement of the concentration of the drug in the particles adhered on the surface. The average adhesion ratio of the CS and CS-10F on the mucosa-like surface was 73.97±1.09% and 72.10±1.25%, respectively.

4. Discussion

F127/chitosan blend microspheres were prepared by the emulsification and cross-linking method. The preparation parameters containing the stirring rate, cross-linking degree and the chitosan solution concentration, which generally affected the physicochemical and morphological properties of chitosan microspheres, remained constant based on our previous work. The influence of F127 content in polymer blends on microsphere morphology, drug loading and release profile characteristics, and mucoadhessiveness was investigated.

The presence of hydrophilic PEO chains of F127 hindered the self-aggregation of chitosan microspheres as expected, but increasing the concentration of F127 in the polymer blends solution did not affect the particle size. The particle size of microspheres was mainly determined by the droplets formed during emulsification which were later solidified in the presence of glutaraldehyde. It could be noted that the viscosity of polymer solution increased with increasing the amount of polymer blends, and highly viscous polymer solution was apt to form big droplets during emulsification [7]. Rokhade et al. reported that formulations containing higher amounts of F127 prepared under constant stirring at 400 rpm speed had bigger particle size [11]. In this work, the emulsion was violently stirred at a high speed of 1000 rpm to produce numerous stable and small droplets (about 20 times smaller than previously reported); thus the increase of the viscosity of polymer blends solution had no obviously effect on the particle size.

Results from swelling experiments demonstrated that blend microspheres had higher swelling rates than the blank CS, which were quite different from the reported ones [11]. On the basis of previously published researches on composite particles [15], it could be speculated that the formation of relatively loose structure of blend microspheres due to the entanglement of soft hydrophilic PEO chains of F127 with chitosan molecules resulted in an increase in the swelling capacity of microspheres. Herein, the maximum equilibrium water uptake value was obtained in the case of CS-30F.

F127 has been widely used as a pharmaceutical excipient for its surfactant and stabilizing properties. Because of the amphiphilic nature, F127 could self-assemble at high concentration, and exhibit its capability to increase the solubility of drugs. Puerarin-loaded microspheres formulated with a drug/chitosan mass ratio of 3/5 were preparedaccording to our previous research [4]. Blending of F127 with chitosan led to a significant decrease in the LC compared with the blank CS, especially in the case of CS-20F and CS-30F. The leak of puerarin from droplets during emulsification process attributing to the surfactant property of F127 perhaps was the main reason for the low LC of blend microspheres. CS-10F with acceptable drug loading was chosen tofurther investigate the properties of blend microspheres.

Drug release from the microspheres generally follows several types of mechanisms, including release from the surface of microspheres, diffusion through the swollen microspheres, and release due to the dissolution of microspheres. Hydration and dissolution behavior of the microspheres played an important role in their drug release profiles [7]. As mentioned previously, the presence of F127 promoted the water uptake of the microspheres. Chemical cross-linking with glutaraldehyde provided higher stability for chitosan microspheres, meanwhile, F127 diffusion from the polymer matrix occurred after immersion of blend microspheres in water at 37 °C. Both formulation CS and CS-10F showed an observed initial burst release which would be helpful in achieving the therapeutic plasma

concentration of the drug in a short time. Nevertheless, a significant slowdown of the drug release rate was observed in case of CS-10F. The results from X-ray analysis revealed a molecular rearrangement of puerarin in the polymer matrix and an amorphous state. Polymer blends increased the degree of miscibility between the drug and the polymer matrix. It could be hypothesized that a certain amount of puerarin was entrapped within the hydrophobic nanodomainsin the blend microspheres due to the self-assembly of F127 at high concentration. This was well in agreement with the results from the reported researches [11].

Because of the strong electrostatic interaction, mucin could be adsorbed onto the surface of the chitosan microspheres [16]. Extensive researches have shown that PEG-based polymers exhibit excellent protein resistant properties because of the PEG chain mobility and steric stabilization force [17]. In this study, the interaction between blend microspheres and mucin in the aqueous solution was decreased due to the presence of PEG chains on the surface of microspheres, which resulted in a dramatic decrease in the adsorption ratio of mucin on CS-10F as compared with CS. The mucoadhesive properties of chitosan microspheres were thought to be the results of the combined efforts of electrostatic interaction between chitosan and mucin, and increased viscosity of mucus via a dehydration process [18, 19].There was no obvious difference in the mucoadhesive properties of formulations CS and CS-10F, although the electrostatic interaction between CS-10F with mucin was significantly weaker. Combined with the results from the experiments of water uptake, it could be concluded that the improved dehydration of mucus played a considerable important role in the adherence of CS-10F onto the mucin saturated surface.

5. Conclusions

In this study, Pluronic F127/chitosan were prepared via an emulsification and cross-linking process. Blend microspheres exhibited more physical stability and drug-polymer compatibility than chitosan microspheres. Moreover, the excellent mucoadhesive properties of microspheres remained as F127/chitosan polymer blends instead of blank chitosan was employed in formulations. Therefore, blending of F127 with chitosan permit animproved sustained drug release from CS and these blend microspheres seem to be an acceptable mucosal drug delivery system.

Acknowledgements

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