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Chitin-based fast responsive pH sensitive microspheres for controlled drug release

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ABSTRACT

Ionically crosslinked chitin microspheres were prepared by using electrostatic droplet method and the drug release from the microspheres was controlled by adjusting external pH. Specifically, chitin was dissolved in NaOH/urea solution at low temperature and functionalized with acrylamide moieties. Acrylamide-modified chitin microspheres with a diameter of 400–500 μm can be obtained at the voltage of 6 kV and the pump speed of 5 mL h^{-1}. The formed microspheres were ionically crosslinked with Fe^{3+} and then complexed with polycationic polysaccharide chitosan. The structure of the microspheres was characterized by FT IR, SEM, and EDS analyses. Besides, the drug release behavior of the microspheres exhibited a fast responsive and pH sensitive release of vancomycin. It took 30 h for complete release of vancomycin in pH 4.0 buffer and pH 1.2 HCl solution while a fast release (10 min) occurred in pH 7.4 buffer. The results showed that the microspheres could be used in drug delivery.

1. Introduction

Chitin is one of the most abundant natural polysaccharide after cellulose (Pillai, Paul & Sharma, 2009) and has appealing intrinsic properties, such as biocompatible, biodegradable and biofunctional (Oylum, Yilmaz, & Yilmaz, 2013). Chitin is considered to be of great futuristic potential with immense possibilities for structural modifications to impart desired properties and functions (Khor, 2002; Miyatake, Okamoto, Shigemasa, Tokura, & Minami, 2003; Prashanth & Tharanathan, 2007; Rinaudo, 2006; Sashiwa & Aiba, 2004). Chitin as insoluble material in most organic solvents has limited application, this can be attributed to the strong intra and intermolecular hydrogen bondings (Duan et al., 2013; Kurita, 2006; Rinaudo, 2006).

Recently, our lab has developed a new and environmental friendly solvent to dissolve chitin (Hu et al., 2007; Li et al., 2010). Chitin from various sources can be dissolved in 8% NaOH/4% urea (Li et al., 2010) and can be further functionalized homogenously. Chitin grafted with quaternized groups exhibits water solubility and excellent antibacterial property (Benhabiles, Salah, Lounici, Drouiche, Goosen, & Mameri, 2012; Ding, Shi, Li, Cai, Duan, & Du, 2012; Huang et al., 2013; Kumar, 2000). In another work, chitin was functionalized with acrylamide through Michael addition in NaOH/urea aqueous solution (Ding et al., 2013). The acrylamide-modified chitin showed double sensitive property (i.e. pH and cationic sensitive). Herein, we explored the drug release properties of acrylamide-modified chitin in the form of microspheres.

Due to the potential of chitin microsphere in drug delivery and biomedicine (Hata, Onishi, & Machida, 2000; Mi, Lin, Wu, Shyu, & Tsai, 2002; Yusof, Lim, & Khor, 2001), various methods have been developed for the preparation of microspheres, including emulsion (Hata, Onishi, & Machida, 2000), suspension (Ji, Wolfe, Rodriguez, & Bowlin, 2012), homogenization (Oylum, Yilmaz, & Yilmaz, 2013; Zhou, Zhang, Zhou, & Guo, 2004), freeze-drying (Ratanajiajaroen & Ohshima, 2012), hammer milling (Nakagawa et al., 2011), spray drying (Cervera et al., 2011) and electrostatic droplet method (Taniguchi, Torii, & Higuchi, 2002). Among these methods, electrostatic droplet method is an easy and continuous way. Well-shaped and high drug encapsulation microspheres from chitosan and alginate have been made by electrostatic droplet method (Ramakrishna, Fujihara, Teo, Yong, Ma, & Ramaseshan, 2006). At a critical voltage between a needle capillary outlet and a collector, the polymer droplets were formed by the electrostatic repulsion force of surface charges and sprayed into the collector. The prepared microsphere has a narrow distribution and can be mass-produced.

In this paper, acrylamide-modified chitin microspheres are prepared by electrostatic droplet method. The microspheres are ionically crosslinked with Fe^{3+} and then complex with polycationic polysaccharide chitosan. The water solubility of acrylamide-modified chitin provides a mild condition for drug encapsulation.

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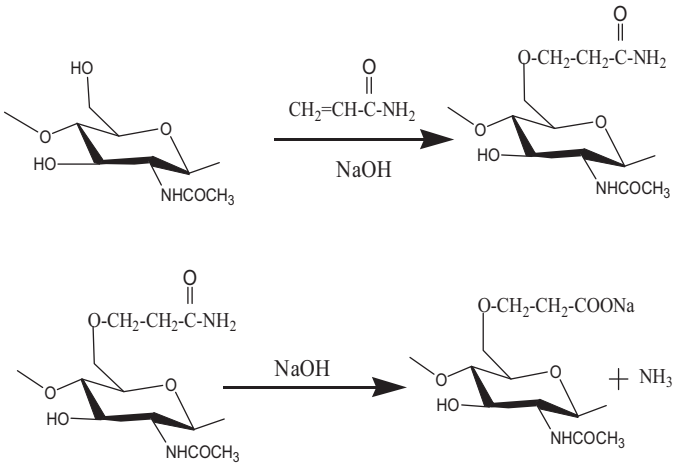


Scheme 1. The formation of acrylamide-modified chitin in NaOH/urea aqueous solution.

The pH dependent release behaviors of vancomycin from the microspheres are studied. In pH 4.0 buffer and pH 1.2 HCl solution, it takes 30 h for complete release of vancomycin, while it only takes 10 min in pH 7.4 buffer solution. The fast responsive and pH sensitive drug release behavior is particularly important for chitin based drug release system. We envision the acrylamide-modified chitin microsphere can be used as a potential candidate for intestinal drug delivery.

2. Experimental

2.1. Materials

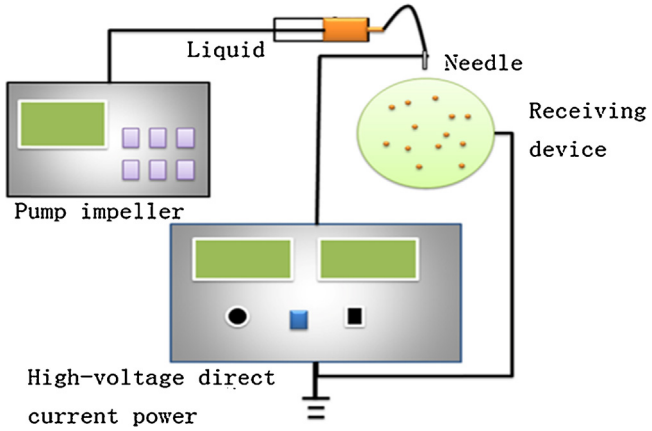
Chitin powder was provided by Jinke Chitin Co., Ltd (Zhe-jiang, China). The degree of acetylation (DA) was 0.98 and the molecular weight (Mw) was 5.0×10^5 Da (Chang, Chen, & Zhang, 2011). Vancomycin hydrochloride (molecular weight 1485.71) was purchased from Amresco. Acrylamide and $FeCl_3 \cdot 6H_2O$ was purchased from Shanghai Reagent Co. All other reagents were of analytical grade and were used without further purification.

2.2. Preparation of acrylamide-modified chitin

Acrylamide-modified chitin was prepared according to our previous work as shown in Scheme 1 (Ding et al., 2013). Chitin (4 g) was dispersed in 8 wt% NaOH/4 wt% urea solution (200 mL) and stored at −20 °C for 36 h. It was stirred twice manually during this period. After it thawed to room temperature, transparent chitin aqueous solution (2 wt%) was obtained. Acrylamide (12.605 g) was added into 200 g chitin solution and stirred at 15 °C for 24 h. Then the mixture was neutralized with HCl (7%) and dialyzed against water for 4–5 days. Finally, the acrylamide-modified chitin was obtained by freeze-drying process.

2.3. Preparation of acrylamide-modified chitin microspheres

Acrylamide-modified chitin microspheres were prepared by electrostatic droplet method as shown in Scheme 2. Acrylamide-modified chitin was dissolved in water to get a concentration of 1.5 g L^{-1}. The pump impeller drove the acrylamide-modified chitin solution through a needle with a diameter of 0.7 mm. A high voltage was imposed between the needle and the receiving solution ($FeCl_3$ dissolved in water). The high voltage electrostatic field shaped the acrylamide-modified chitin solution into small droplets and drove the droplets to the receiving solution. The size and shape



Scheme 2. Schematic diagram of experimental set-up.

of microspheres are affected by the experimental parameters. The optimal conditions for acrylamide-modified chitin microspheres preparation are as follows: the voltage was 6 kV; the concentration of $FeCl_3$ solution was 2 g L^{-1}; the distance between the needle and collecting solution was 3.5 cm and the driving speed was 5 mL h^{-1}. The prepared microspheres were further immersed in 0.05 wt% chitosan solution under stirring for 2 h to prepare chitosan coated acrylamide-modified chitin microspheres.

2.4. Characterization of microspheres

Fourier transform infrared spectroscopy (FT IR, Nicolet Corporation 5700) of chitin, acrylamide-modified chitin, ferric crosslinked acrylamide-modified chitin and chitosan coated acrylamide-modified chitin were recorded using KBr pellets method.

The photographs of the microspheres prepared at varying voltages were taken by a stereo microscope (Shunyu SZM45, Jiangsu, China). The morphologies of the surface and cross-section were observed by scanning electron microscopy (SEM, VEGA3 LMU, TESCAN).

The content of ferric ions in microspheres was obtained by phenanthroline method (Jin et al., 2012). The absorbance at 510 nm was measured by UV spectrophotometer (UV2000, Shanghai). The distribution of ferric ions in the microsphere was measured by Energy Dispersive Spectrometer (EDS).

2.5. Dissolution rate of microspheres

The dissolution rate of the microspheres was performed in 0.1 M HCl solution (pH 1.2) and buffer solutions (pH 4.0 and 7.4). At predetermined time, the microspheres were removed from the solution and weighed. Each sample was analyzed in triplicate. The dissolution rate (DR) of microspheres was calculated as follows:

$$\mathrm{DR}\,(\%) = \frac{W_0 - W_t}{W_0} \times 100$$

where W_0 is the initial weight of microspheres, W_t is the weight of microspheres at time t.

2.6. Drug release

Drug release study was carried out in 0.1 M HCl (pH 1.2) and buffer solutions (pH 4.0 and 7.4). Microspheres containing vancomycin (40 mg) were immersed in 50 mL release medium and rotated at 100 rpm at room temperature. The concentration of vancomycin in released buffer was measured spectrophotometrically at 280 nm. Each release study was performed in triplicate.

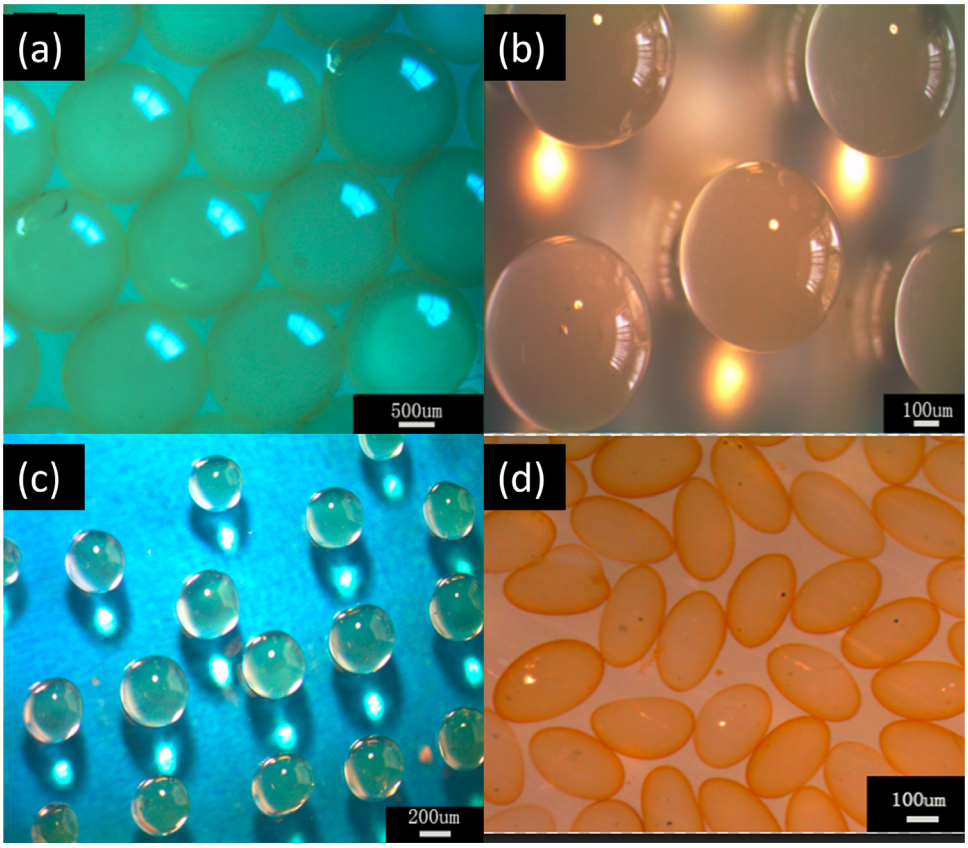


Fig. 1. Micrographs of microspheres. The voltage conditions: no voltage (a), 2 kV (b), 6 kV (c), 8 kV (d).

3. Results and discussion

3.1. Formation of acrylamide-modified chitin microspheres

Chitin was dissolved in NaOH and urea solution and functionalized with acrylamide groups. The acrylamide-modified chitin was obtained with the molar ratio of acrylamide to chitin monomer as 9:1. Since the reaction happened at low temperature, it had little effect on the degree of acetylation of chitin (Hu et al., 2007). The molecular weight of acrylamide-modified chitin was 1.35×10^5 analyzed by GPC (Ding et al., 2013). The DS of carboxyl groups (DS_{COO^-}) was 0.35 and the DS of acylamino groups (DS_{CONH_2}) was 0.32, as determined by titration methods described in our previous work (Ding et al., 2013). The substitution of COO^- and $CONH_2$ groups confered chitin with water solubility and strong chelation to metal ions. Iron is a metal that is important to human body and it has been commonly used as ionic crosslinker (Mohammadi, Cole, & Berkland, 2009). In addition, Fe^{3+} is a typical example of a "hard" metal cation and preferentially binds oxygen atoms in negatively charged ligands such as carboxylate groups (Jin et al., 2012). The acrylamide-modified chitin droplet generated by the high voltage electrostatic field formed spherical microsphere instantly in ferric chloride solution ($2\,g\,L^{-1}$). Acrylamide-modified chitin solution with high viscosity will lead to irregular microsphere. The appropriate concentration of acrylamide-modified chitin was 1.5 wt%.

The optical images of ferric-crosslinked microspheres prepared under different voltages were shown in Fig. 1. The size of the microspheres was uniform and decreased gradually with the increase of imposed voltage. However, the microsphere showed an ellipsoid shape when 8 kV voltage was applied. At the voltage of 6 kV, microspheres with a diameter of 300 μm could be obtained.

3.2. Structure of microspheres

The FT IR spectra of ferric crosslinked microsphere and chitosan coated microsphere were compared with chitin and acrylamide-modified chitin, as shown in Fig. 2. In the spectrum of chitin, the typical amide I band split into two peaks at 1660 cm^{-1} and 1629 cm^{-1}. This indicated that the chitin was α type. The band at

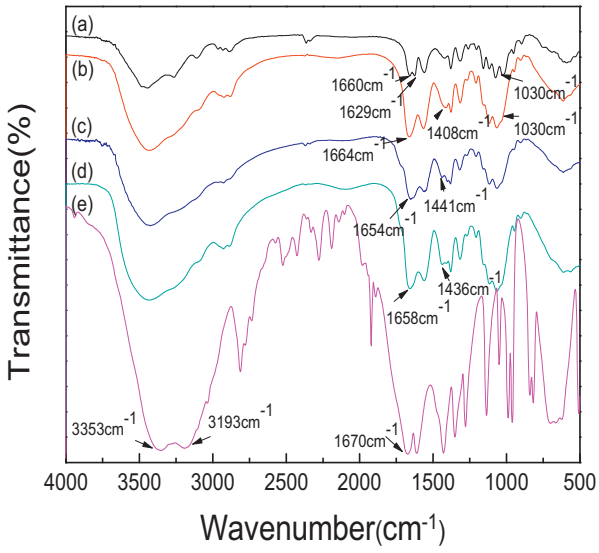


Fig. 2. FT IR spectra of chitin (a), acrylamide-modified chitin (b), ferric crosslinked acrylamide-modified chitin (c), chitosan coated acrylamide-modified chitin (d) and acrylamide (e).

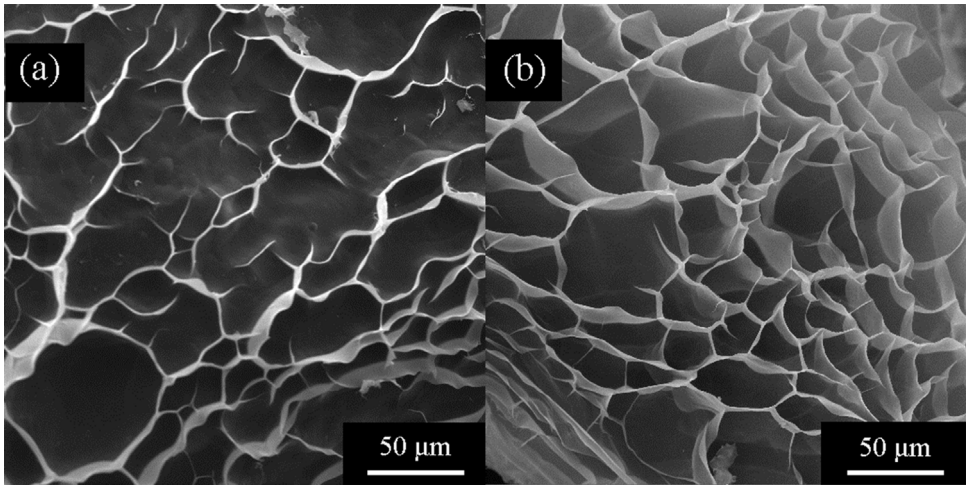


Fig. 3. The scanning electron microscopy (SEM) images of the ferric crosslinked acrylamide-modified chitin microsphere. Microphotographs of the surface (a) and cross-section (b).

1030 cm^{-1} in the spectrum of chitin which was corresponded to $-CH_2OH$ group, and it weakened in the spectrum of acrylamide-modified chitin. The change of the adsorption of $-CH_2OH$ group suggested that chitin had reacted with acrylamide. In the spectrum of acrylamide, it showed double peaks of primary amine at 3193 cm^{-1} and 3353 cm^{-1} and carbonyl peak at 1670 cm^{-1}. It should be noted that there was a new peak at 1408 cm^{-1} in the spectrum of acrylamide-modified chitin, which could be assigned to the stretching vibration of $-COO^-$ group, suggesting the hydrolysis of acylamino groups to carboxyl groups. This band moved to 1441 cm^{-1} after crosslinked with Fe^{3+}. Meanwhile, the band at 1664 cm^{-1} shifted to 1654 cm^{-1}. The changes of the adsorption of carboxyl groups suggested that acrylamide-modified chitin was crosslinked with Fe^{3+}, which led to the formation of microspheres. Compared the spectra of ferric crosslinked acrylamide-modified chitin and chitosan coated acrylamide-modified chitin, no notable peak difference could be discerned. It suggested that chitosan coated the microsphere by electrostatic interactions.

The morphologies of the surface and cross-section of the ferric crosslinked acrylamide-modified chitin microsphere were observed by SEM. The microphotograph in Fig. 3(a) showed that the surface was compact without visible pores. The wrinkle on the surface might be caused by the shrinkage of the microsphere during drying process. The morphology of the cross-section was shown in Fig. 3(b). The inner part of the microsphere had a honeycomb-like structure and some large pores over 100 μm could be seen.

The distribution of ferric ions across the microsphere was studied by EDS. Before the observation, microspheres were embedded in epoxy resin and ultrathin sliced. The distribution of irons inside the microsphere was scanned laterally as shown in Fig. 4(a).

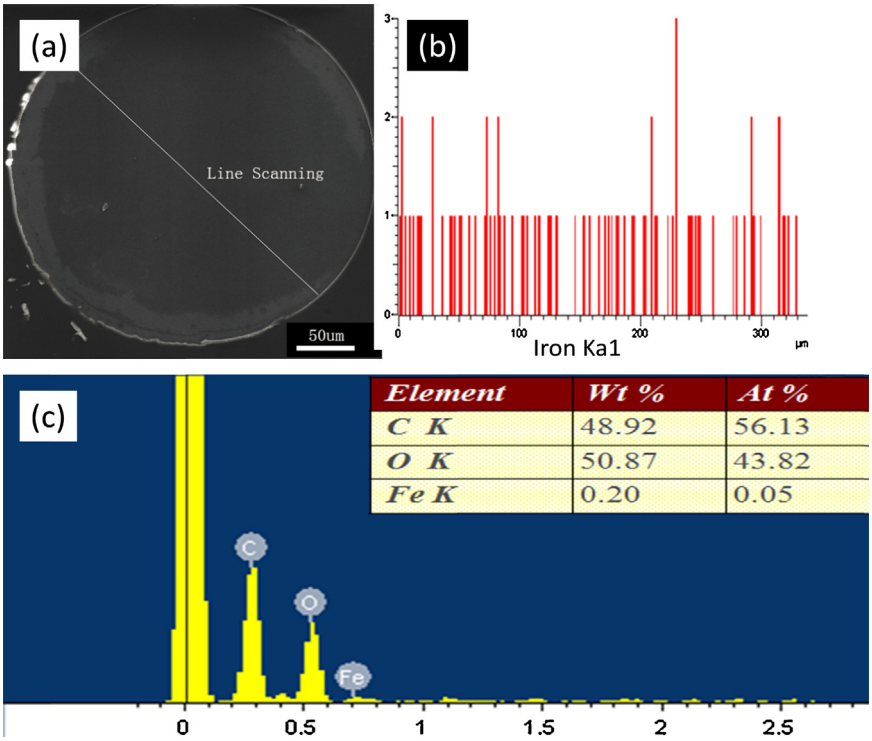


Fig. 4. The distributions of iron ions in the section of microspheres. Line scanning cross the microsphere embedded in epoxy resin (a), the iron signals in the diameter direction (b) and element analysis of the microsphere (c).

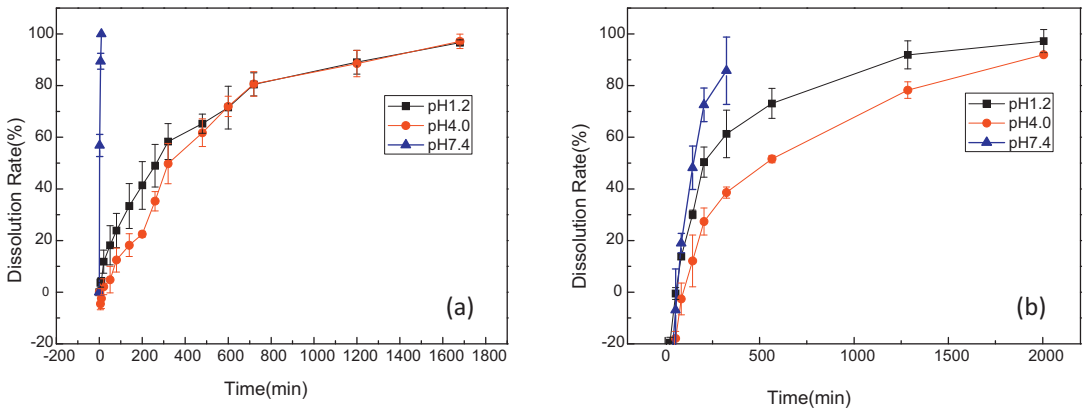


Fig. 5. Dissolution rate of acrylamide-modified chitin microspheres under different pHs (a) and dissolution rate of chitosan coated acrylamide-modified chitin microspheres under different pHs (b).

Fig. 4(b) showed the iron signals along the scanning line. It could be seen that there was no much difference of iron content in the diameter direction. Fig. 4(c) showed the elemental analysis of the microsphere. The C and O signals belonged to the acrylamide-modified chitin and Fe signal was caused by ferric crosslinking. It was found that the iron signal is weak, suggesting low iron content in the microsphere. The content of iron in the microspheres was further determined by phenanthroline method. The amount of iron ions was 0.138%. This low iron content excludes the concerns of toxicity caused by high doze taken.

3.3. Dissolution of microspheres

The dissolution rate of acrylamide-modified chitin microspheres under different pHs provided the information of the pH sensitivity of the microspheres. Fig. 5(a) showed the dissolution rate of ferric crosslinked microspheres in different pHs (pH 1.2 HCl, pH 4.0 and pH 7.4 buffers). The microsphere was relatively stable in pH 4.0 buffer. This pH is beneficial for the crosslink between Fe^{3+} and acrylamide-modified chitin. The dissolution was relatively faster in pH 1.2 HCl. Compared with the slow dissolution in pH

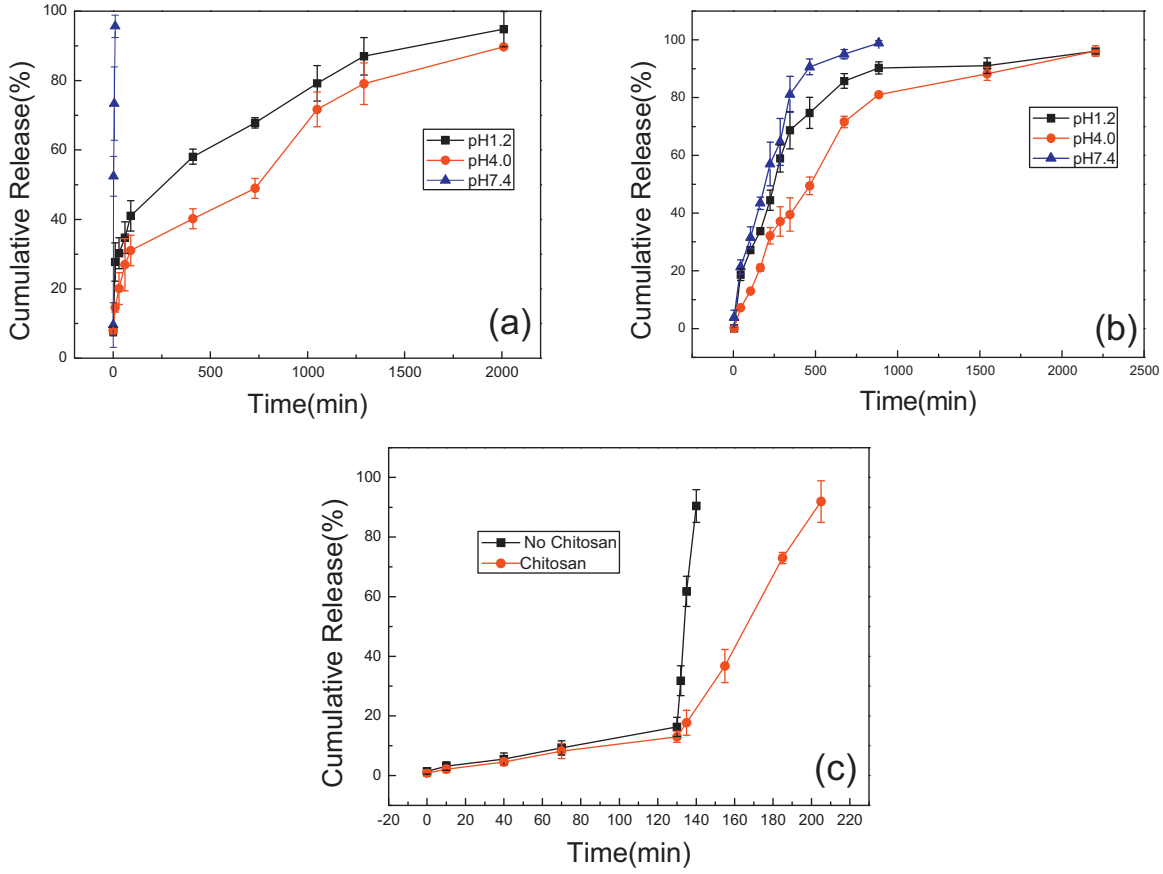


Fig. 6. Drug release of acrylamide-modified chitin microspheres under different pHs (a) and drug release of chitosan coated acrylamide-modified chitin microspheres under different pHs (b). Phased release curves of vancomycin of the ferric crosslinked microspheres and the chitosan coated microspheres in pH 1.2 HCl for 2 h and then transferred to pH 7.4 buffer (c).

1.2 and 4.0 buffer, the microsphere dissolved quickly in pH 7.4 buffer. It took 10 min for the microsphere to complete dissolution, which could be explained by the increase of electrostatic repulsions and reduction of hydrogen bonds in the microspheres, for carboxyl groups were mainly in the form of $-COO^-$ in pH 7.4 buffer solution. The formation of chitosan outlayer on the microsphere dramatically decreased the dissolution rate under all the investigated pHs (Fig. 5(b)), suggesting the complex with chitosan could change the drug release behavior of ferric crosslinked acrylamide-modified chitin microsphere.

3.4. Release and phased release of the drug

The release behaviors of vancomycin in acrylamide-modified chitin microspheres and chitosan coated microspheres at different pHs were shown in Fig. 6. The release curves in Fig. 6(a) indicated that complete vancomycin release needed 30 h in pH 1.2 HCl and pH 4.0 buffer solutions, while it only needed 10 min in pH 7.4 buffer solution. The release behavior is consistent with the dissolution rate of the microspheres, suggesting the microsphere dissolution plays a critical role in vancomycin release. Fig. 6(b) showed that chitosan coating sustained the vancomycin release, especially for the release in pH 7.4. The complete release of vancomycin in pH 7.4 prolonged from 10 min to longer than 10 h. The release profiles of the microspheres fit well with intestinal target delivery. To demonstrate this possibility, microspheres containing vancomycin were placed in pH 1.2 HCl solution for 2 h, and then transferred to pH 7.4 buffer solution. The drug release curves were shown in Fig. 6(c). For the ferric cosslinked and chitosan coated microspheres, less than 20% drug release was found within 2 h in pH 1.2 HCl. When the pH increased to 7.4, nearly linear release could be obtained. It took 10 min for the ferric crosslinked microspheres and 2 h for the chitosan coated microspheres to a release of 80%. The results demonstrate a typical phase release behavior of the microspheres and a fast pH response. In addition, the chitosan coating modifies the drug release behavior to meet the occasions when sustained drug release in intestine is required.

4. Conclusions

In this study, we showed that acrylamide-modified chitin microspheres could be prepared by electrostatic droplet method using ferric crosslinking and chitosan coating. The uniform microspheres with a diameter of 300 μm exhibited a fast pH responsive dissolution rate and drug release profiles. Using vancomycin hydrochloride as a model drug, we showed that a phased release could be obtained with slow release in low pH and fast release in pH 7.4. All these results suggested that the acrylamide-modified chitin microspheres studied in this work might have a certain potential application in intestinal drug delivery.

Acknowledgments

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