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Controlled release of ciprofloxacin hydrochloride from chitosan/polyethylene glycol blend films

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Abstract

Films of chitosan and polyethylene glycol (PEG), with ciprofloxacin hydrochloride as model drug incorporated at different concentrations, have been obtained by a casting/solvent evaporation method. Interrelated chemical, morphological and mechanical characterizations included the component ratio of chitosan and PEG, the loaded amount of ciprofloxacin hydrochloride, the pH and ionic strength of the release solution, the thickness of the drug loaded films, the coating layer of sodium alginate and the cross-linking time with tripolyphosphate (TPP) and others. The results of controlled release tests showed that the amount of ciprofloxacin hydrochloride released increased with an increase in the proportion of PEG and decreased as the amount of drug loaded in the film increased; however, the cumulative release amount of the drug increased. The chitosan/PEG films were also sensitive to pH and ionic strength. In simulated intestinal fluid, the thickness of the film increased from 35 to 85 μm with a concomitant reduction of the ciprofloxacin hydrochloride concentration from 100% to 71%. Differing the concentration of sodium alginate coating solution reduced the release of ciprofloxacin hydrochloride by as much as 16% in simulated gastric fluid and 38% in simulated intestinal fluid. When the cross-linking time of these films in the TPP solution were 0, 5, 15 and 30 min, the drug release rate attained 100%, 100%, 70% and 42%, respectively, within 24 h.

Keywords: Chitosan; PEG; Ciprofloxacin hydrochloride; Blend films; Drug delivery systems

1. Introduction

Chitosan, the deacetylated derivative of chitin, is one of the most abundant naturally occurring polysaccharides. Recently, it has attracted much interest in the biomedical industry because of its excellent biodegradability, biocompatibility, antimicrobial activity and accelerated wound-healing properties (Hirano, Seino, & Akiyama, 1994; Malette, Euiglem, & Gaines, 1983; Qurashi, Blair, & Allea, 1992; Wel, Hudson, & Mayer, 1992). Chitosan has good gel and film forming properties. When it is dissolved in dilute acetic acid solutions, the amino groups become protonated and associated with acetate counter-ions, making the charged polymer soluble. Therefore, net negatively charged compounds such as DNA, glycosaminoglycans, and most proteins can be incorporated into chitosan without the use of harsh and denaturing organic solvents, such as methylene chloride, which are needed for film preparation of many biodegradable polymers. Therefore, chitosan has been investigated extensively in the pharmaceutical industry for its potential use in the development of controlled release implant systems (Aspden, Mason, Jones, Lowe, & Illum, 1997; Karlson, 1991; Mao, Troungle, & Janes, 2001; Oungbho & Muller, 1997; Wang, Du, & Fan, 2005).

Polyethylene glycol (PEG) is a biocompatible polymer with excellent biocompatibility and non-toxicity (Zhang, Gong, Zhao, & Zhang, 2002). It is often blended or compounded with other polymers to be used in the field of drug-controlled release (Chandy, Mooradian, & Ral, 1998; Won, Chu, & Lee, 1998; Won, Chu, & Lee, 1998).

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With regard to the excellent film forming properties of chitosan, many new and original films materials has been achieved (Tang, Du, Zheng, & Fan, 2001; Yu, Du, & Zheng, 1999). Drug-loaded film is one of the applications of those films in pharmaceutical technology. In addition, numerous controlled or sustained-delivery systems have been described in the literature, whereby the active ingredient has been dissolved or dispersed within these films (Carmen & Roland, 1997). For the development of film-based controlled release devices, tests carried out with them are very important and needed (Chad et al., 2001; Graeme, John, & John, 1999; Shu, Zhu, & Song, 2001). It was reported that when PEG was blended with chitosan to form films, it could promote the proliferation of cells. Most important, it could not reduce the activities of the protein in the cells (Zhang et al., 2002). In the present study, chitosan/PEG blend films were prepared and used in several controlled release applications to give a better overall understanding of their properties. Using ciprofloxacin hydrochloride as a model drug, some factors that may have influence the drug release from chitosan/PEG films as function of the ratio of chitosan and PEG used, the loaded amount of ciprofloxacin hydrochloride, the pH and ionic strength of the release solution, the thickness of the drug loaded films, the coating layer of sodium alginate and the cross-linking time with tripolyphosphate (TPP), have been studied. It is anticipated that the films reported herein may lead to a successful application for localized drug delivery in *in vivo* and *in vitro* environments.

2. Materials and methods

2.1. Materials

Chitosan from shrimp shell was purchased from Yuhuan Ocean Biochemical Co., Ltd (Zhejiang, China); the degree of deacetylation (*DD*) was 87%, and M_v was 8.0×10^5. The *DD* was measured by pH titration method (Lin, Jiang, & Zhang, 1992) and the M_v was measured viscosimetrically (Wang, Bo, & Qin, 1990). PEG6000 was purchased from Shanghai Chemical Reagent Co., Ltd (Shanghai, China). Ciprofloxacin hydrochloride was purchased from Jingxin Pharmacy Co., Ltd (Zhejiang, China) and used as model drug. Other reagents were all analytical grade.

2.2. Preparation of drug loaded films

Chitosan/PEG drug loaded films were produced by a casting/solvent evaporation technique. Solutions of chitosan and PEG, 2 wt%, were prepared with 2 wt% acetic acid solution and distilled water, respectively; and the PEG was dissolved in a higher temperature. These solutions were mixed in different proportions to obtain final PEG solution concentrations of 2.0, 3.5, 5.5 and 8.0 wt% of total. Ciprofloxacin hydrochloride (0.2 g) was dissolved, under stirring, in each one of these four resulting solutions (50 ml) to make them completely homogeneous. After that, they were sonicated in a sonication bath (FS-20, Jingrong Sonic Electronics Co. Ltd., Beijing, China), left to stand until trapped air bubbles were removed, and poured on a Teflon plate of $20 \times 15\,cm^2$. These films were dried in an oven (GDW-250, Saiou Test Machine Co. Ltd., Shanghai, China) at 37 °C for 48 h, and finally dried under vacuum at room temperature until constant weight. These dried films, with an average thickness of 55 μm determined by WHS-10A Portable Thickness Instrument (Tianfa Test Machine Co. Ltd., Jiangdou, China), were cut into $3 \times 3\,cm^2$ sections for tests. The several chitosan/PEG drug loaded films, prepared with ciprofloxacin hydrochloride, were designated as CP-1, CP-2, CP-3 and CP-4 (PEG contents were 2.0, 3.5, 5.5 and 8.0 wt%, respectively). The blank matrix film, without the drug, was marked with CP (PEG was 3.5 wt%).

Following the above method, different amount ciprofloxacin hydrochloride (0.1 and 0.3 g) was dissolved in solutions (PEG contents ratio was 3.5 wt%), producing drug loaded films designated as CPC-1 and CPC-2, respectively. By changing the volume of the forming solution of CP-2 poured onto the Teflon plate, drug loaded films were achieved with different thickness of 35 and 85 μm, marked as CPD-1 and CPD-2, respectively. Finally, CP-2 films dipped in sodium alginate coating solutions different concentrations (0.3 and 0.6 wt%) for 20 min were then dried. The thickness of the coating layer was determined to be 5 and 8 μm by aforementioned methods and they marked were as CPA-1 and CPA-2, respectively. Finally, a CP-2 film sample immersed in a 1 wt% TPP solution for different times to achieve different degrees of cross-linking. After being washed with distilled water, these films were dried using the aforementioned methods.

2.3. FT-IR analysis

The FT-IR spectra of pure chitosan, PEG, ciprofloxacin hydrochloride, CP and CP-4 films were recorded within KBr pellets on a Nicolet FTIR spectrometer, Model 170SX (USA).

2.4. X-ray diffraction studies

The X-ray diffraction patterns of pure chitosan, PEG, ciprofloxacin hydrochloride, CP and CP-4 films were determined on a Shimadzu Lab-XRD-6000X diffractometer (Japan), using Nickel-filtered CuKα radiation at 40 kV and 50 mA in the 2θ range of 5°–40°. From the results of CP and CP-4 films, the effects of ciprofloxacin hydrochloride on the degree of crystallization of the blend films were determined.

2.5. Morphology observations

The cross-sectional morphologies of the CP and CP-4 films were examined using scanning electron microscopy (SEM) Hitachi S-570 (Japan). Cross-sectional samples were prepared by fracturing films in liquid nitrogen. Prior to

observation, samples were arranged on metal grids, using double-sided adhesive tape, and coated with gold under vacuum before observation.

2.6. Mechanical properties

The tensile strength (σ_b) and the elongation at break (ε_b) for dried drug loaded films were determined on an electronic tester machine (CMT8502, Shenzhen SANS Test Machine Co. Ltd., China). The gauge length was 50 mm and crosshead speed was 50 mm/min. All samples were preconditioned at 20 °C and 65% relative humidity, for 24 h prior to mechanical testing.

2.7. Release studies

The drug loaded films were suspended in glass vessels containing 50 ml of medium, as specified below, and incubated on a shaking bed (HS-150, Saiou Test Machine Co., Ltd., Shanghai, China) at 37 °C, 130 rpm. At appropriate time intervals aliquots of the solutions were withdrawn and the amount of ciprofloxacin hydrochloride released from the drug loaded films were evaluated by UV spectrophotometry at 277 nm. Then an equal volume of the same dissolution medium was added back to maintain a constant volume. The medium for the controlled release studies were four typical solutions: pH 1.0 (0.1 M HCl solution, acts as simulated gastric fluid), pH 3.6 and pH 5.0 (10 mM solution acetate buffer), and pH 7.4 (10 mM NaH_2PO_4–Na_2HPO_4-buffered solution, acts as simulated intestinal fluid). The ionic strength of the above buffered solutions was carefully adjusted to a relatively level by adding an appropriate amount of NaCl. All the experiments were done in triplicate.

3. Results and discussion

3.1. Structure and morphology characterization

3.1.1. FT-IR analysis

Fig. 1 shows the FT-IR spectra of PEG, chitosan, and blank matrix film CP, whereas Fig. 2 shows the FT-IR spectra of blank matrix film CP, ciprofloxacin hydrochloride and drug loaded film CP-4. In pure chitosan, two characteristic absorption bands at 1637 cm^{-1} and 1564 cm^{-1} were detected and attributed to amide I(C═O) and amide II(N–H), respectively; 1383 cm^{-1} was attributed to the distorting vibration of C–CH_3 (Sannan, Kurita, & Ogura, 1978). The characteristic absorption band at 1100 cm^{-1} of PEG was attributed to the bending vibration of C–O, and two absorption bands at 1340 and 2890 cm^{-1} were attributed to the bending vibration and stretching vibration of C–N, respectively. Finally, the wide absorption band around 3421 cm^{-1} was due to the stretching vibration of O–H bonded to N–H.

From the FT-IR spectra of CP film, in Fig. 1, it can be seen that the characteristic absorption bands at 1564 and

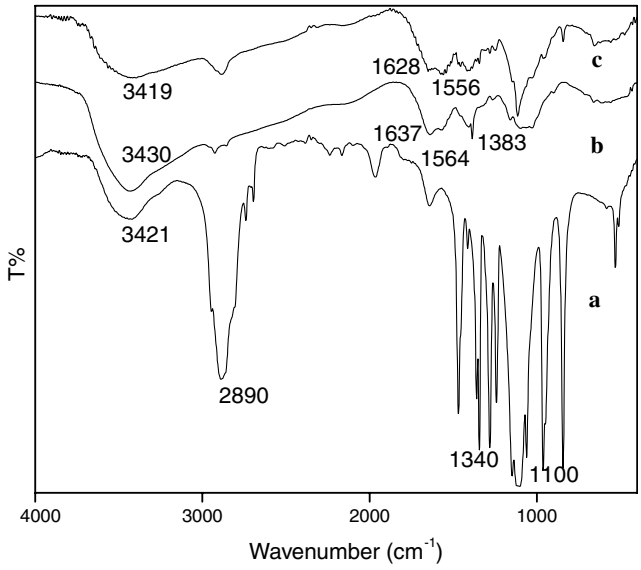

Fig. 1. IR spectra of PEG (a), chitosan (b) and blank matrix chitosan/PEG film CP (CP, chitosan/PEG is 96.5:3.5 wt%) (c).

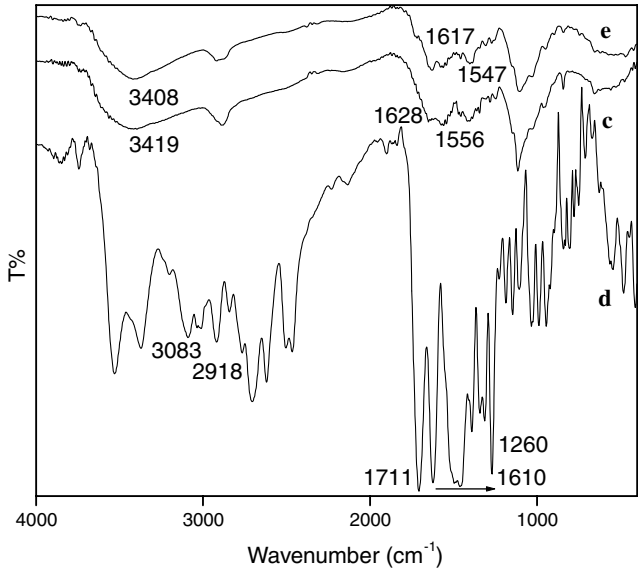

Fig. 2. IR spectra of blank matrix chitosan/PEG film CP (CP: chitosan/PEG is 96.5:3.5 wt%) (c), ciprofloxacin hydrochloride (d) and drug-loaded film CP-4 (CP-4, chitosan/PEG is 92:8 wt%) (e).

1637 cm^{-1} of chitosan shifted to lower wave number at 1556 and 1628 cm^{-1}. At the same time, the absorption band around 3430 cm^{-1} concerning the stretching vibration of N–H group bonded to O–H group shifted to a lower wave number at 3419 cm^{-1} and became wider, suggesting an increase in the hydrogen bonding (Yu et al., 1999). All those changes give a strong evidence for the intermolecular interactions and good molecular compatibility between chitosan and PEG. The characteristic absorption bands at 1260 and 1610 cm^{-1} of ciprofloxacin hydrochloride (Fig. 2) were due to the stretching vibration of C–F bond and the vibration of phenyl framework conjugated to –COOH, respectively; the stretching vibration at 1711 cm^{-1} was due to –COOH and, at 3083 and 2918 cm^{-1} were observed the stretching

vibrations of C–H from the phenyl framework. Through the FT-IR spectra of CP-4 film, Fig. 2, it can be seen that the characteristic absorption bands at 1628 and 1556 cm^{-1} of CP shifted to lower wave number at 1617 and 1547 cm^{-1}, respectively; and also that the characteristic absorption band at 3419 cm^{-1} had shifted to a lower wave number at 3408 cm^{-1}. All those results indicated that the model drug used in this work had strong hydrogen bonds and ionic bonds with the matrixes of the films. At the same time, there were no new characteristic absorption bands of drug-loaded films, permitting conclusion that there were no obvious chemical reaction between the drug and the matrix. As an important result, ciprofloxacin hydrochloride did not lose its activity in the drug-loaded films.

3.1.2. X-ray diffraction studies

It may be seen, in Fig. 3, the X-ray diffraction patterns of PEG, chitosan and blank matrix film CP, whereas Fig. 4. shows the X-ray diffraction patterns of blank matrix film CP, ciprofloxacin hydrochloride and drug loaded film CP-4.

The diffractogram of chitosan, Fig. 3, consisted of two typical crystalline peaks at $2\theta = 11.8°$ and 22.9°. PEG had typical crystalline peaks at $2\theta = 19.4°$, 23.6°, 26.6° and 36.3° because of its close molecular packing and regular crystallization. Ciprofloxacin hydrochloride, Fig. 4d, showed typical crystalline peaks at $2\theta = 8.9°$, 11.2°, 19.2°, 24.6°, 26.3° and 29.1° because of its close molecular packing and regular crystallization. From the X-ray diffraction patterns of CP film, Fig. 3, it may be seen that the diffraction peaks of chitosan at 11.8° and 22.9° rapidly weakened and new diffraction peaks appeared at 11.5°, 19.4° and 23.4° with increasing PEG content. This can be explained by the strong interaction between chitosan and PEG which has destroyed the close packing of the chitosan molecules for the formation of regular crystallites and has created new crystallites. In Fig. 4, it may be seen that ciprofloxacin hydrochloride also changed the diffraction patterns of the blank matrix film CP. Comparing the X-ray diffraction patterns of CP and CP-4, Fig. 4c and e, it is possible to verify that after the addition of the drug to the matrix film, the diffraction intensities of CP increased at 11.5° and decreased at 23.4°; also the diffraction of CP at 19.4° disappeared and a new diffraction was created at 25.7°. These results indicate that the addition of ciprofloxacin hydrochloride destroyed the ordered packing of the molecules of CP film to form the regular crystallites. In other words, the results of X-ray diffraction reinforce the existence of good compatibility between the matrix film and the drug used due to both kinds of strong interactions namely hydrogen bonds and ionic interactions.

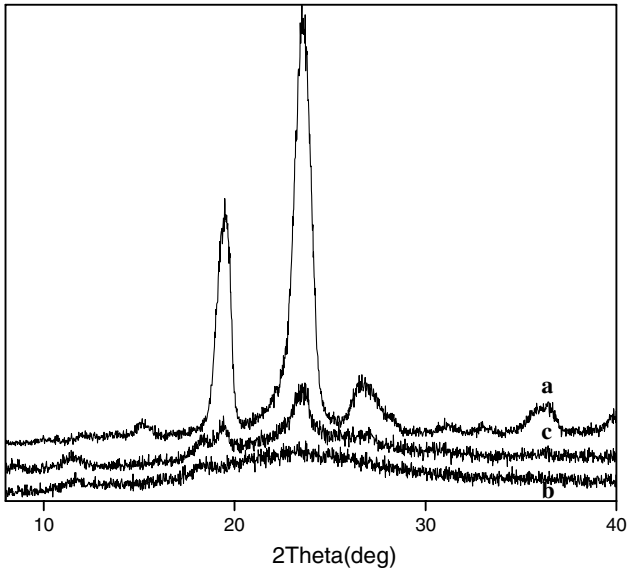

Fig. 3. XRD patterns of PEG (a), chitosan (b) and blank matrix chitosan/PEG film CP (CP, chitosan/PEG is 96.5:3.5 wt%) (c).

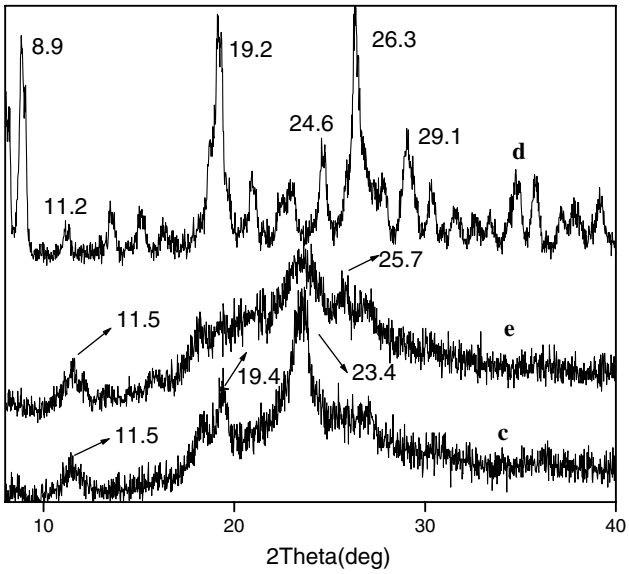

Fig. 4. XRD patterns of blank matrix chitosan/PEG film CP (CP, chitosan/PEG is 96.5:3.5 wt%) (c), ciprofloxacin hydrochloride (d) and drug-loaded film CP-4 (e) (CP-4, chitosan/PEG is 92:8 wt%).

3.1.3. Morphology observations

Analysis of the morphologies of CP and CP-4 films obtained by Scanning Electron Microscopy (SEM) (Fig. 5) shows that the cross-section of both is smooth and homogeneous, with absence of any micro phase separation. Again, the result obtained here indicates good compatibility between the matrix and the drug, ciprofloxacin hydrochloride.

3.2. Mechanical properties

From the mechanical properties of the drug loaded films (Fig. 6) it may be seen that the maximum value of tensile strength and elongation at breaking were both observed when the content of PEG was 5.5 wt%, indicating that blending is effective in improving the mechanical properties of the drug loaded films.

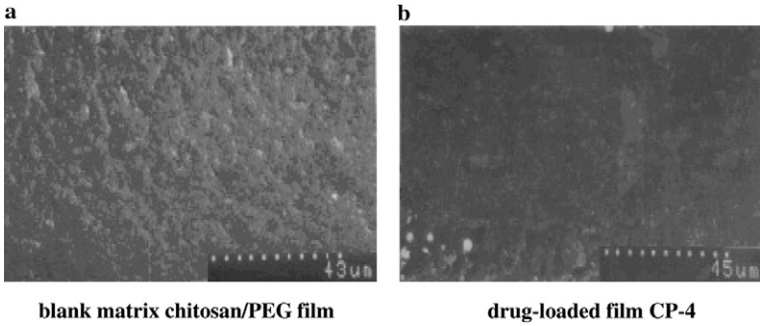

Fig. 5. SEM photographs of blank matrix chitosan/PEG film CP and drug loaded film CP-4. (a) Blank matrix chitosan/PEG film and (b) drug-loaded film CP-4.

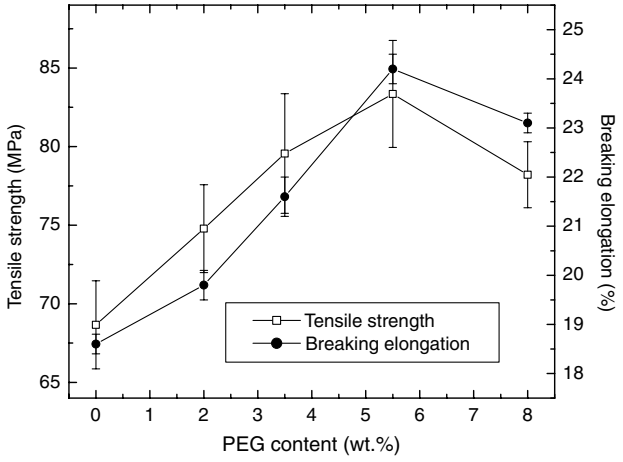

Fig. 6. Mechanical properties of drug-loaded chitosan/PEG films.

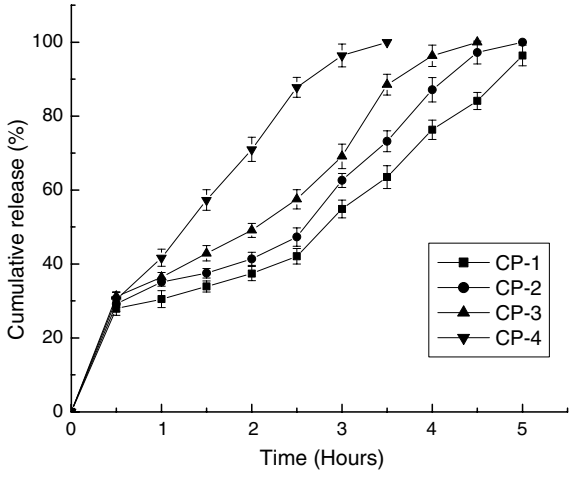

Fig. 7. Influence of the composition of drug-loaded chitosan/PEG films on the controlled drug release process. CP-1, chitosan/PEG is 98:2 wt%; CP-2, chitosan/PEG is 96.5:3.5 wt%; CP-3, chitosan/PEG is 94.5:5.5 wt%; CP-4, chitosan/PEG is 92:8 wt%;the drug loaded amount of ciprofloxacin hydrochloride in these three films is 0.2 g.

3.3. Release studies

3.3.1. Effect of the composition ratio of drug loaded film

The influence of the different composition ratios of chitosan and PEG in the drug loaded films CP-1, CP-2, CP-3 and CP-4 (2, 3.5, 5.5 and 8 wt% of PEG, respectively) on the release into 10 mM sodium phosphate buffer, pH 7.4 (ionic strength of 0.145 M) (Fig. 7), was such that the release rate of ciprofloxacin hydrochloride increased with increase content of PEG. Because PEG is somewhat soluble in these aqueous solutions, it dissolves and leaves pores that accelerate the release of the drug from the matrix film.

3.3.2. Effect of the drug loaded amount

Testing of films CPC-1, CP-2 and CPC-2 with different drug loaded amounts (0.1, 0.2 and 0.3 g, respectively) (Fig. 8) showed that the more drug was loaded, the lower the cumulative drug release rate was; but according to the fact that more drugs were loaded, the cumulative release amount is increasing. So more persistent release can be achieved by increasing the drug-loaded amount.

3.3.3. Effect of the thickness of drug loaded films

The drug release from films CPD-1, CP-2 and CPD-2 with different thicknesses (35, 55 and 85 μm, respectively), (Fig. 9) declined as the thickness of the films increased. This shows clearly that the thickness of the film changes the rate of the drug diffusion into the matrix film.

3.3.4. Effect of pH

The drug release from loaded film CP-2, in four different buffered solutions with pH values of 1.0, 3.6, 5.0 and 7.4, (ionic strength all adjusted to 0.145 M, by adding an appropriate amount of NaCl) (Fig. 10) was very sensitive to the pH of the medium. The release was accelerated with decrease of pH, because the electrostatic interaction between anions and chitosan was greatly influenced by solution pH (Shu et al., 2001). The decrease of pH weakened salt bonds and therefore, facilitated film swelling, thereby accelerating drug release. The pH also has a slight effect on the solubility of ciprofloxacin hydrochloride. A higher pH leads to a better solubility of ciprofloxacin hydrochloride, which results in higher drug release rate. But compared to the strong influence of pH on the film matrix, pH effects on ciprofloxacin hydrochloride could be neglected.

3.3.5. Effect of ionic strength

Adding an appropriate amount NaCl to the 10 mM NaH_2PO_4–Na_2HPO_4 buffer, pH 7.4, produced the four

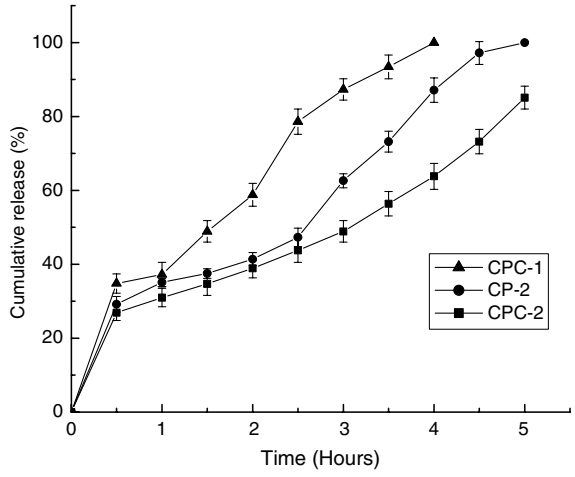

Fig. 8. Influence of the amount of drug-loaded in chitosan/PEG films on the controlled drug release process. Chitosan/PEG of these three films are all 96.5:3.5 wt%; the drug loaded amount of ciprofloxacin hydrochloride in CPC-1, CP-2 and CPC-2 is 0.1, 0.2 and 0.3 g, respectively.

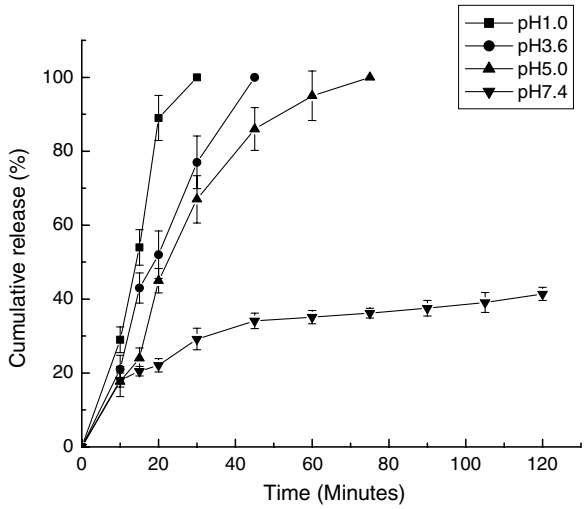

Fig. 10. pH influence of the release medium on the ciprofloxacin hydrochloride controlled release process. The drug loaded film CP-2 was used as release matrix.

different release media of different ionic strength. Fig. 11 showed that with the increase of ionic strength the drug release rate (based on drug loaded film CP-2) also increased. The result was possibly related to the decrease of osmotic pressure inside the film with the increase of the salt concentration and the weakened salt-bond between ciprofloxacin hydrochloride and film matrix by salt ion (Yin & Prudhomme, 1992).

3.3.6. Effect of sodium alginate coating of drug loaded film

The effects of sodium alginate coating layers of drug loaded films (Figs. 12 and 13) lead to the conclusion that the sodium alginate coating layer can prolong the drug's release efficiently both at high pH (pH 7.4) and at low pH (pH 1.0). The main reason was that there were strong electrostatic interactions between the molecules of chitosan and sodium alginate at high pH (Yao, Liu, Cheng, Lu, & Tu, 1996); furthermore, at low pH, the carboxyls of sodium alginate were protonated and rendering the alginate molecules insoluble, despite there already had no electrostatic interactions between the molecules of the matrix film (Alison, John, Martin, & Colin, 1995; Yoshihisa, Yoshioka, Segi, & Ikeda, 1991).

3.3.7. Effect of cross-linking time

Testing of drug loaded films (CP-2) cross-linked for different times (Fig. 14) showed that the longer the cross-linking process, the more slow was the drug released, due to a higher degree of cross-linking formed in the matrix, causing a delay in the diffusional release of drug.

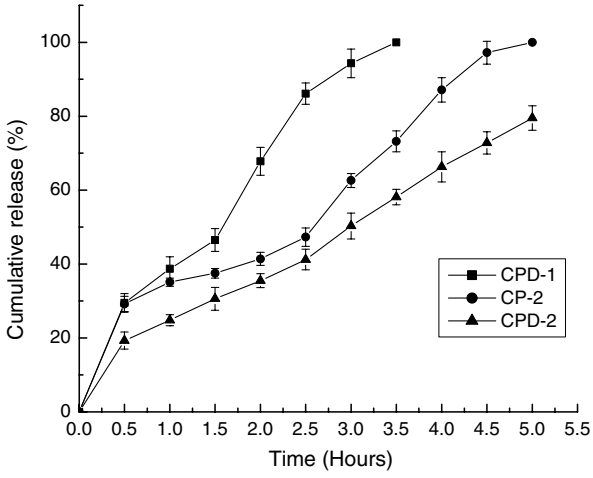

Fig. 9. Influence of film thickness of drug-loaded chitosan/PEG film on controlled drug release. Chitosan/PEG of these three films are all 96.5:3.5 wt%; the drug loaded amount of ciprofloxacin hydrochloride in these three films is 0.2 g; the thickness of CPD-1, CP-2 and CPD-2 is 35, 55 and 85 μm, respectively.

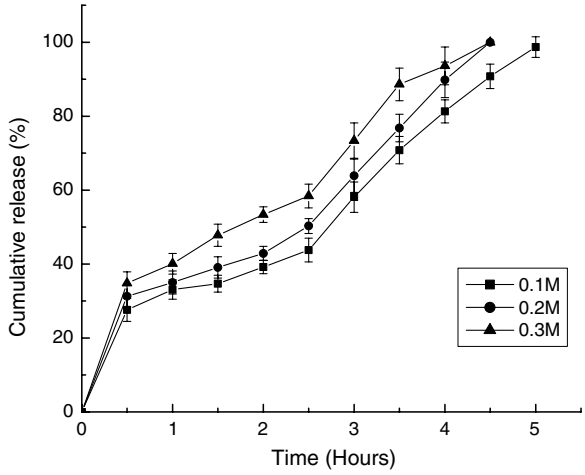

Fig. 11. Influence of ionic strength of the release medium on ciprofloxacin hydrochloride controlled release from chitosan/PEG films containing the drug. The drug loaded film CP-2 was used as release matrix.

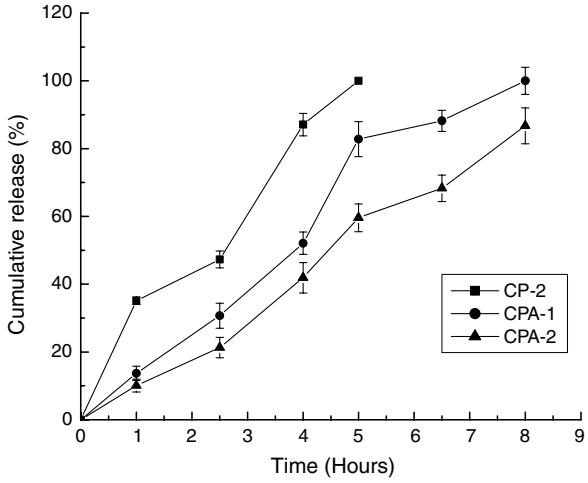

Fig. 12. Influence of sodium alginate coating layer on ciprofloxacin hydrochloride controlled release from chitosan/PEG films containing the drug into simulated intestinal fluid. The thickness of CP-2, CPA-1 and CPA-2 are 0, 5 and 8 μm, respectively.

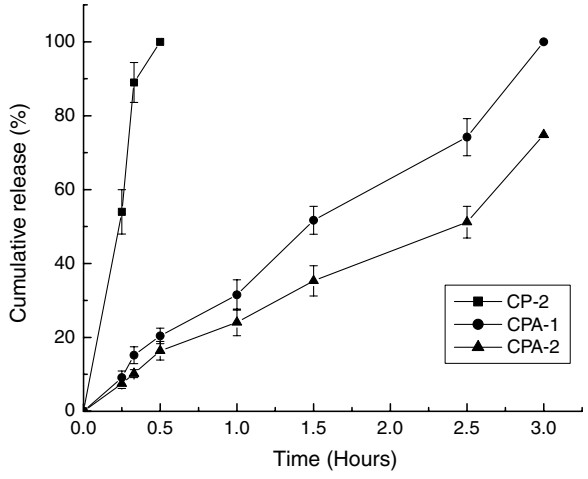

Fig. 13. Influence of sodium alginate coating layer on ciprofloxacin hydrochloride controlled release from chitosan/PEG films containing the drug in simulated gastric fluid. The thickness of CP-2, CPA-1 and CPA-2 are 0, 5 and 8 μm, respectively.

4. Conclusions

Drug loaded films based on chitosan and PEG, were produced by a casting/solvent evaporation method. With ciprofloxacin hydrochloride as a model drug, we studied the films' structures and characteristics, especially its potential capacity in drug delivery system. The chemical and morphological characterizations showed that there is a good compatibility between the matrix film and the drug used due to both kinds of strong interactions namely hydrogen bonds and ionic interactions. The films' mechanical property is also good. The results of controlled release tests showed that the amount of ciprofloxacin hydrochloride released increased with an increase in the proportion of PEG and decreased as the amount of drug loaded in the film increased; however, the cumulative release amount of

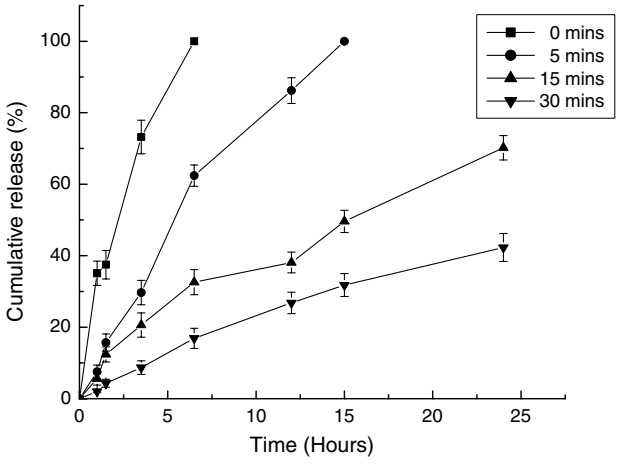

Fig. 14. Influence of cross-linking time in 1 wt% TPP solution on the ciprofloxacin hydrochloride controlled release from chitosan/PEG films. The drug loaded film CP-2 was used as release matrix.

the drug increased. The chitosan/PEG films were also sensitive to pH and ionic strength. The thickness of the film will slow the drug release process. The higher concentration of sodium alginate coating solution reduced the release of ciprofloxacin hydrochloride. Furthermore, the longer cross-linking time of these films in the TPP solution, the lower drug release rate attained within 24 h. Thus, we can control the drug release rate through changing some influential factors of the drug loaded film. The film can lead to a successful application for localized drug delivery *in vivo* or *in vitro* environment.

Acknowledgement

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