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Research paper

The influence of multivalent phosphate structure on the properties of ionically cross-linked chitosan films for controlled drug release

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Abstract

The aim of this paper was to investigate the electrostatic interactions between multivalent phosphates (phosphate (Phos), pyrophosphate (Pyro) and tripolyphosphate (TPP)) and chitosan, as well as the influence of electrostatic interactions on the properties of chitosan films ionically cross-linked by the above mentioned phosphates. The charge number of Phos was too low to interact with chitosan, while Pyro and TPP with more negative charges showed a significant ability to ionically cross-link chitosan. Solution pH played an important role on the charge numbers carried by Pyro, TPP and chitosan, especially for Pyro/chitosan. For instance, at pH less than 2.0 the interaction between Pyro and chitosan disappeared, while for TPP/chitosan even in solutions at pH less than 0.5 it still existed. Media pH and ionic strength also had a significant influence on the properties of cross-linked chitosan film with multivalent phosphates. Usually these films swelled and drug was released quickly in acidic conditions (such as in simulated gastric fluid) while under neutral conditions (such as in simulated intestinal fluid) they remained in a shrinkage state and drug was released slowly. Compared to TPP/chitosan films, Pyro/chitosan films exhibited much better pH-sensitive swelling and controlled release properties due to their relatively weak electrostatic interaction. The same reasoning was used to explain the significant acceleration of Pyro/chitosan film swelling and model drug release observed on adding sodium chloride. These films may be promising for site-specific drug delivery in the stomach.

Keywords: Chitosan film; Phosphate; Pyrophosphate; Tripolyphosphate; Drug controlled release

1. Introduction

Chitosan with excellent biodegradable and biocompatible characteristics is a naturally occurring polysaccharide. Due to its unique polymeric cationic character, gel and film forming properties, chitosan has been extensively examined in the pharmaceutical industry for its potential use in the development of drug delivery systems [1–3].

Up to now, drug delivery formulations based on chitosan (such as films, beads, microspheres, etc.) were usually prepared by chemical cross-linking with glutaraldehyde, etc. [2,4,5]. These formulations were exploited for oral sustained drug delivery in the stomach due to their excellent pH-sensitivity, as well as for intragastric-floating that was used to prolong the retention of the formulations in the stomach [6–9]. To improve the pH-sensitive performance, blended chitosan films were prepared. For example, polyethylene oxide/chitosan film was reported to have excellent pH-sensitivity [9–11].

However, the chemical cross-linking agents possibly induce toxicity and other undesirable effects. To overcome this disadvantage, reversible physical cross-linking by electrostatic interactions was recently applied in the formulation preparation [2,12]. Polyanions were used as components to prepare chitosan films. For example, Yao et al. [13] reported the preparation of pectin/chitosan films by dissolving this polyelectrolyte complex in formic acid. Chu et al. [14] prepared xanthan/chitosan complex films in the presence of concentrated sodium chloride (ca. 0.5 M) and followed by treatment at high temperature.

Compared to polyanions, the use of low molecular weight anions to cross-link chitosan was found to be much simpler and milder. For instance, polyphosphate only binds on the surface of chitosan droplets [15], while tripolyphosphate (TPP) can diffuse into chitosan droplets or films freely to form ionically cross-linked chitosan beads or films [15–22]. Furthermore, strengthened TPP/chitosan beads and microspheres were also prepared by developed procedures in our laboratory using simple and mild conditions [23,24]. However, up to now very few types of anions were used

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to prepare chitosan formulations. Besides TPP and sulfate [24–26], only citrate/chitosan films have been made utilizing the electrostatic interactions between citrate and chitosan as reported by our laboratory recently [27]. These films exhibited excellent pH-dependent controlled drug release and are promising for stomach-specific drug delivery.

The properties of ionically cross-linked chitosan films were seriously influenced by the electrostatic interactions between anions and chitosan. Though TPP/chitosan matrices have been widely used in the pharmaceutical industry, the influence of the TPP/chitosan interaction on the formulation properties has still not been clarified. And as a result, some different phenomena have been reported [15–17].

In this paper, we aimed to prepare cross-linked chitosan films using other multivalent phosphates, i.e. phosphate (Phos), pyrophosphate (Pyro) and TPP. The electrostatic interactions of those phosphates with chitosan were investigated in view of the difference in their molecular structures, and the influence of electrostatic interactions on the controlled drug release properties of these films was also examined.

2. Materials and methods

2.1. Materials

Chitosan was obtained from Tianbao Chitosan Co. Ltd. (Ningbao, China), and refined twice according to the literature [27]. The degree of deacetylation was 86% (evaluated method referring to Ref. [28]); Mv was 460,000 (obtained from viscosity measurements and the Mark–Houwink relationship [29]). Riboflavin (Mw 376.37) was purchased from Aldrich (Milwaukee, USA). Coomassie brilliant blue R250 (BB, Mw 825) was purchased from Fluka A.G. (Switzerland) and used after sieving (less than 50 μm). Sodium tripolyphosphate, sodium pyrophosphate and sodium phosphate (analytical grade) and other reagents were all commercially available and used as received.

2.2. Turbidimetric titration

The interactions of Phos, Pyro and TPP with chitosan were investigated by turbidimetric titration according to the reported method [30,31]. Solutions of 1.0 g/l Phos (Pyro or TPP) and 0.2 g/l chitosan were prepared at pH 1.5 (except for TPP/chitosan at pH 0.5). Titrant (0.01–0.2 M NaOH) was delivered by a microburette into the solution with gentle stirring at 20 ± 0.2 °C, and pH was monitored by a digital pH meter with a precision of ±0.01. When the pH of mixed solutions reached ca. 8.5, titrant (0.01–0.2 M HCl) was added with a microburette to titrate back. Changes in turbidity were monitored at 420 nm with a UV-Vis spectrophotometer and reported as $100 - \%T$ which is linearly proportional to the true turbidity for $T > 0.9$. The time interval between turbidity measurements was ca. 2 min.

2.3. Potentiometric titration

Potentiometric titration was performed according to the method reported by Ikeda et al. [32] to evaluate the pH-dependent ionization degree of chitosan. A 0.1% (w/v) chitosan solution was neutralized by adding 0.1 M NaOH at 20 ± 0.2 °C with a microburette in a nitrogen atmosphere, and the solution pH was monitored by a digital pH meter with a precision of ±0.01.

The pH-dependent charge numbers of TPP, Pyro and Phos were calculated according to the reported pK_a as follows: TPP: $pK_1 = 1$, $pK_2 = 2$, $pK_3 = 2.79$, $pK_4 = 6.47$ and $pK_5 = 9.24$; Pyro: $pK_1 = 1.52$, $pK_2 = 2.36$, $pK_3 = 6.60$ and $pK_4 = 9.25$; Phos: $pK_1 = 2.12$, $pK_2 = 7.20$ and $pK_3 = 12.36$ [33].

2.4. Preparation of cross-linked chitosan films

Chitosan films were produced by a casting/solvent evaporation technique. Chitosan solutions (4.0%, w/v) containing model drug (BB or riboflavin 1.0% w/v) were prepared by dissolving chitosan and model drug (or dispersing) in 4.0% (w/v) acetic acid. Then 40 ml of the above solutions were sonicated, left to stand until the disappearance of trapped air bubbles, and poured on a glass plate (casting area: 10×10 cm^2). The films were dried for 48 h in an oven at 37 °C, then further dried under vacuum at room temperature to constant weight. The dried films were cut into 2×2 cm^2 test sections, and the thickness of drug loaded films was determined to be ca. 100 μm.

Cross-linked chitosan films were prepared by soaking the chitosan films (ca. 50 mg) in aqueous solution of TPP (or Pyro, or Phos) (100 ml) at 4 °C. The cross-linking conditions were: 1.0–10.0% (w/v) TPP (or Pyro or Phos); solution pH: 5.0–7.0; cross-linking time: 5 min to 4 h. The cross-linked chitosan films formed were then washed with distilled water, put on a glass plate and oven-dried at 37 °C for 48 h, and then dried under vacuum at room temperature to constant weight.

The model drug loss during the cross-linking process was determined by measuring the UV-Vis absorption, 590 nm for BB, 444 nm for riboflavin. Usually more than 95% of the model drug remained in the films.

2.5. Morphology observation

The surface and cross-sectional morphologies of cross-linked chitosan films were examined using scanning electron microscopy (SEM, S-590, HITACHI). Cross-sectional samples were prepared by fracturing films in liquid nitrogen. Prior to observation, samples were mounted on metal grids, using double-sided adhesive tape, and coated by gold under vacuum before observation.

2.6. Swelling ratio measurements

Cross-linked chitosan films (ca. 50 mg) were suspended

in glass bottles containing 250 ml different media, and incubated on a shaking water-bath at 37 °C, 50 rev./min. At the appropriate time point, the films were taken out, the excess water was carefully removed with filter paper from the film surface, and the films were then weighed immediately. The swelling ratio was calculated as W_t/W_0, where W_t was the film weight at time t and W_0 was the film initial weight. The media for swelling studies were described as follows. The buffered solutions were HCl (pH 1.0, 1.5 and 2.0), 10 mM acetic acid-sodium acetate (pH 3.5, 4.5 and 5.5), and 10 mM Tris buffered solution (pH 6.5, 7.4, 8.5 and 9.5), and the ionic strength of the above buffered solutions was carefully adjusted to 0.145 M by adding an appropriate amount sodium chloride. The sodium chloride solutions with different concentrations (0, 0.9, 1.8, 3.6 and 5.0% w/v), and enzyme free simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) (USP XXII) were also used for the test.

2.7. Drug release studies

The model drug release from chitosan films was performed under the same conditions described in swelling studies. At the appropriate time point, 4 ml solutions were withdrawn and the drug content was determined by measuring the UV-Vis absorption at 444 nm for riboflavin and 590 nm for BB. An equal volume of the same dissolution medium was added to maintain a constant volume. In some cases, chitosan leaching from the films was monitored by the phenolic-sulfuric acid color reaction [34,35].

3. Results and discussion

3.1. The interaction between anions and chitosan

The electrostatic interactions between anions and chitosan had an important influence on the properties of ionically cross-linked chitosan. Though TPP/chitosan matrices have been used in the pharmaceutical industry for many years, to our knowledge little information concerning the interaction between TPP and chitosan has been reported. Therefore, the electrostatic interactions of TPP, Pyro and Phos with chitosan were investigated.

Sufficient charge numbers (or density) are necessary for anions to cross-link chitosan by electrostatic force. TPP, Pyro and Phos are multivalent anions with a similar structure and they carry a maximum of five, four and three negative charges, respectively. On the other hand, chitosan used in our experiment is a weak polybase with a maximum of thousands of positive charges (Fig. 1). However, the charge number of the anions and chitosan are all mainly controlled by solution pH (Fig. 2). With the decrease of solution pH from neutral to acidic condition, the charge numbers of TPP, Pyro and Phos all decreased accordingly. At the same solution pH, the charge numbers of anions were ranked in the order TPP, Pyro and Phos. For example, at pH 4.0 the charge numbers of TPP, Pyro and Phos were ca. 2.8, 2.2 and 0.99,

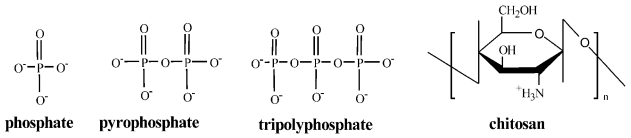

Fig. 1. The structure of Phos, Pyro, TPP and chitosan.

respectively. For chitosan, the increase of solution pH (especially near the pK_a of chitosan 6.3 [36]) resulted in the decrease of the ionization degree of amine groups greatly, and at pH higher than 7.5 less than 10% of the amine groups were ionized (Fig. 2).

The difference in the molecular structure of TPP, Pyro and Phos affected their interactions with chitosan significantly. The turbidimetric titration of Phos/chitosan solution revealed that obvious turbidity occurred only in neutral and basic conditions (pH >7.0), which was caused by the poor solubility of chitosan at this pH region. It indicated that the charge number of Phos was too low to cross-link chitosan through salt bonds.

On the other hand, obvious turbidity changes of TPP/chitosan and Pyro/chitosan solutions were observed during turbidimetric titration (Fig. 3), which was in accordance with the pH-dependent charge number of TPP (or Pyro) and chitosan (Fig. 2). In the low pH range (< ca. 1.2 for TPP/chitosan and < ca. 3.2 for Pyro/chitosan), the solutions were optically clear because of the low charge number of TPP and Pyro. However, with pH increased to a certain value (pH_ϕ where phase separation had taken place, ca. 1.4 for TPP/chitosan solution and ca. 4.0 for Pyro/chitosan solution), turbidity increased greatly and solutions began to separate into two phases. It could be attributed to the significant charges of TPP, Pyros and chitosan in these pH regions, and electrostatic interactions were strong enough to cause the precipitation of TPP/chitosan and Pyro/chitosan complexes. Further increase of solution pH over ca. 6.3 led to the greatly decreased charge density of chitosan, and

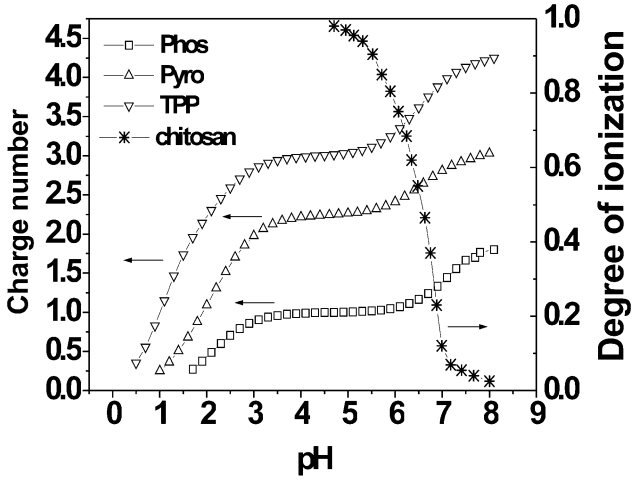

Fig. 2. The pH-dependent charge number of Phos, Pyro and TPP, and the degree of ionization of chitosan.

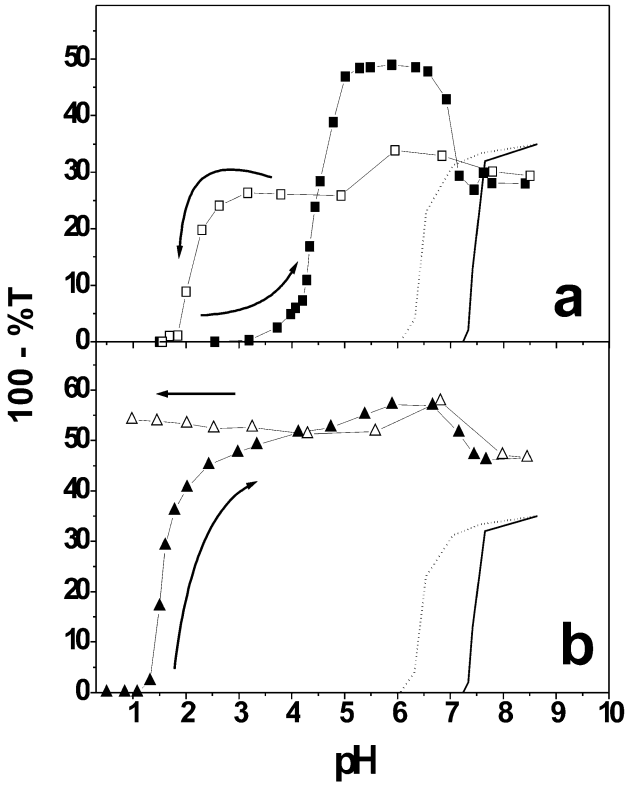

Fig. 3. The turbidity titration curves of Pyro/chitosan (square symbol curves) (a) and TPP/chitosan (triplicate symbol curves) (b) solutions at 420 nm (1.0 g/l Pyro or TPP, 0.2 g/l chitosan). The solutions were first titrated from low pH to high pH (solid symbol curves), and then titrated back from high pH to low pH (open symbol curves). The line curves are the turbidity titration curves of 0.2 g/l chitosan alone: solid line: from low pH to high pH; block line: from high pH to low pH.

hence a decrease in turbidity of both TPP/chitosan and Pyro/chitosan solutions. However, the poor solubility of chitosan in neutral and basic conditions interrupted the investigation of electrostatic interaction, and there was still significant turbidity even at solution pH higher than 8.0 where the ionization degree of chitosan was extremely low (less than 0.05) (Fig. 2) and the electrostatic interactions between anion and chitosan possibly absent.

However, the turbidity changes during turbidimetric processes exhibited some irreversible characteristics, i.e. once the complex precipitation occurred they could not be dissociated at the pH_ϕ, and significant turbidity was still observed at the pH region below pH_ϕ. The critical solution pH where complex precipitation dissociated was defined as pH_ϕ' (ca. 2.0 for Pyro/chitosan and less than 0.5 for TPP/chitosan) (Fig. 3). Under the same conditions, a similar result was also obtained in the case of citrate/chitosan solution, but it was not as serious as Pyro/chitosan and TPP/chitosan solution. The pH gap between $pH_\phi$and pH_ϕ' for citrate/chitosan solution was only ca. 0.8 while it was ca. 2.0 for Pyro/chitosan and TPP/chitosan complexes could not even dissociate in our experiments. We thought the high charge density of Pyro and TPP was responsible for their larger pH gaps between complexes formed and dissociated [37].

Besides solution pH, ionic strength also played an important role in electrostatic interactions. Fig. 4 shows the results of turbidimetric titration with the co-existence of different NaCl concentrations. Salt weakened the electrostatic interaction between Pyro and chitosan greatly. With the NaCl concentration increasing from 0 to 0.15 M, pH_ϕ also increased from ca. 4.0 to 5.2, and with 0.30 M NaCl the turbidity change of Pyro/chitosan solution was similar to that caused by the precipitation of chitosan alone and pH_ϕ moved to ca. 7.0 (Fig. 4a). As for TPP/chitosan solution, the shielding effect of salt on electrostatic cross-linking was much less. With NaCl concentrations of 0, 0.15, 0.30, and 0.60 M, the pH_ϕ was ca. 1.2, 3.1, 3.9, and 4.4, and even with NaCl concentrations as high as 1.0 M, electrostatic interaction still could not be shielded (pH_ϕ ca. 5.0) (Fig. 4b).

3.2. Morphology of anion cross-linked chitosan films

This was similar to citrate cross-linked chitosan films, the bottom surface of Pyro/chitosan and TPP/chitosan films was very smooth while the upper surface was relatively rough, which was in accordance with the morphology of chitosan films before cross-linking [27]. Pyro and TPP concentration,

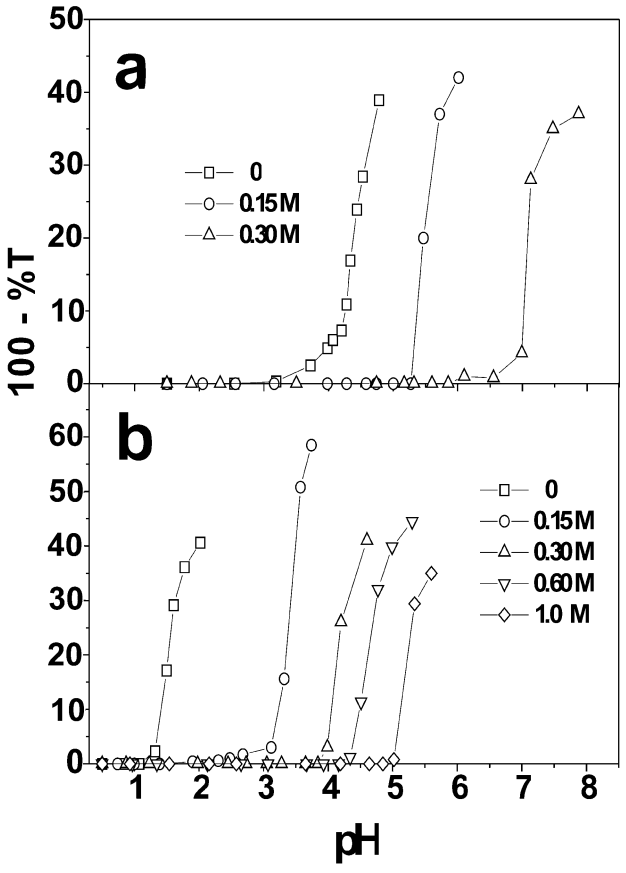

Fig. 4. The influence of NaCl concentration on the turbidity titration of Pyro/chitosan (a) and TPP/chitosan (b) solutions at 420 nm (1.0 g/l Pyro or TPP, 0.2 g/l chitosan).

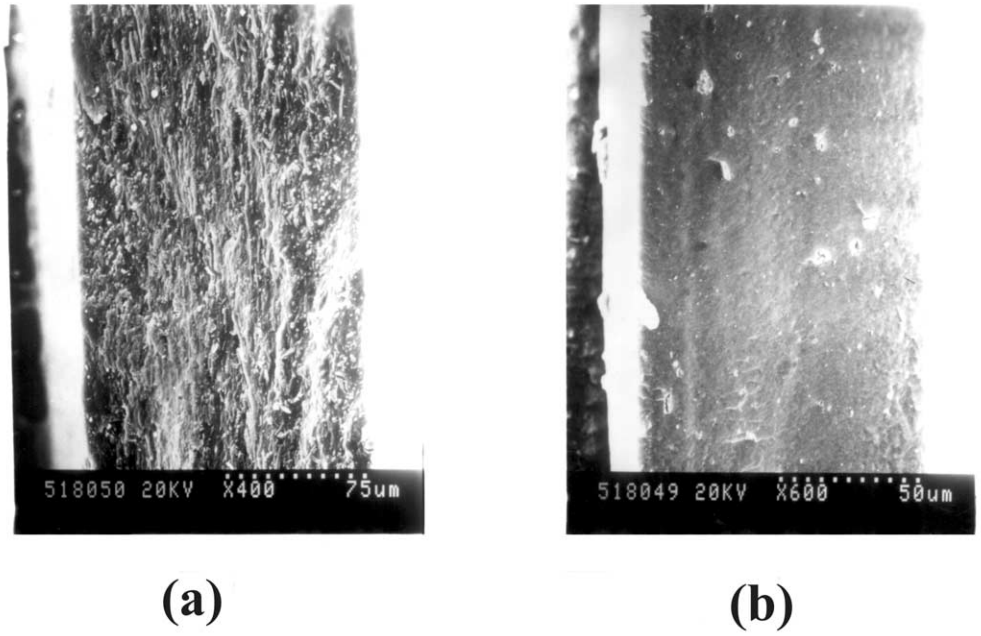

Fig. 5. The cross-section morphology of chitosan films: (a) loaded with riboflavin (ca. 25% w/w); (b) blank.

pH and cross-linking time had little effect on the surface morphology of films. The cross-section of the Pyro/chitosan and TPP/chitosan films was integral and dense (data not shown).

However, the incorporation of model drugs in chitosan films resulted in a significant change of the surface and cross-section morphologies. For example, large pores were observed on both the bottom and upper surfaces of riboflavin loaded chitosan films, and the cross-section was very loose while that of the blank chitosan films was quite dense (Fig. 5a,b). This porous structure of drug loaded chitosan film certainly facilitated the diffusion of Pyro and TPP into the inside of films and seriously affected film properties.

3.3. Swelling and drug controlled release properties

3.3.1. pH-sensitivity

From Figs. 2 and 3, it could be seen that the degree of ionization of Pyro, TPP and chitosan was mainly controlled by solution pH, and the electrostatic interaction between anions and chitosan was greatly influenced by solution pH; hence Pyro/chitosan and TPP/chitosan films should exhibit pH-dependent swelling, which is shown in Fig. 6. In the case of Pyro/chitosan film, at pH 5.5 and 6.5, the swelling ratio was very low (1.8–2.2) due to the significant electrostatic attraction between Pyro and chitosan. The decrease of pH weakened salt bonds and therefore facilitated film swelling (swelling ratio 2.9 at pH 4.5 and 4.0 at pH 3.5). Moreover, when pH was less than 2.0, Pyro/chitosan film swelled more significantly and usually dissociated within 24 h because very weak ionic cross-linking was observed in this pH region as revealed by turbidimetric titration (Fig. 3). On the other hand, the increase of pH over 6.5 also should weaken salt bonds, and result in a larger swelling ratio. However, at pH 7.4, 8.5 and 9.5 the films were kept in a shrunken state (swelling ratio ca. 1.8–1.9). It was reported that the swelling of polyelectrolyte complex films (such as pectin/chitosan film) under weakly basic conditions (pH 8–10) was very significant [13], mainly

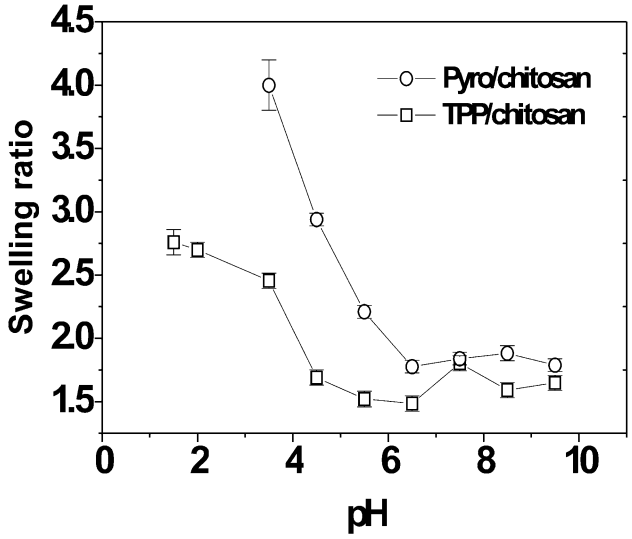

Fig. 6. The equilibrium swelling ratio of riboflavin (ca. 25% w/w) loaded Pyro/chitosan film and TPP/chitosan film in buffered solution with the same ionic strength (0.145 M) at 37 °C ($n = 3$). The films were prepared with 5.0% (w/v) sodium pyrophosphate or tripolyphosphate (pH 5.0) and a cross-linking time of 1.0 h. pH 1.0, 1.5 and 2.0 (HCl); pH 3.5, 4.5 and 5.5 (10 mM acetic acid-sodium acetate buffer); pH 6.5, 7.4, 8.5 and 9.5 (10 mM Tris-buffered solution).

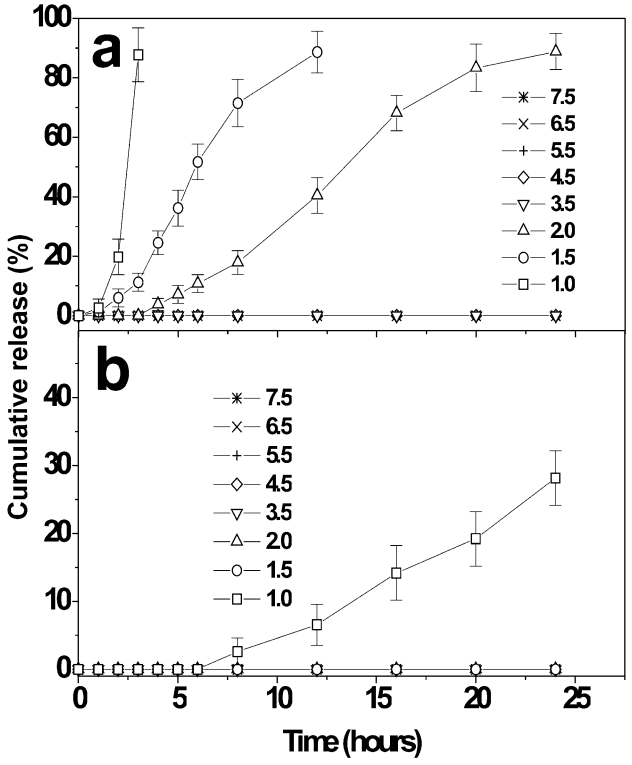

Fig. 7. The release of BB from Pyro/chitosan film (a) and TPP/chitosan film (b) in buffered solution with the same ionic strength (0.145 M) at 37 °C ($n = 3$). The films were prepared with 1.0% (w/v) sodium pyrophosphate or sodium tripolyphosphate (pH 7.0) and a cross-linking time of 1.0 h. pH 1.0, 1.5 and 2.0 (HCl); pH 3.5, 4.5 and 5.5 (10 mM acetic acid-sodium acetate buffer); pH 6.5, 7.4, 8.5 and 9.5 (10 mM Tris-buffered solution).

resulting from the dissociation of ionic cross-linking and the repulsion between negative charged carboxylic groups. But in our experiments, the dissociated Pyro at pH 8.5 or 9.5 may diffuse out from the films freely and no repulsive interaction between negatively charged groups existed inside the films, as well as other factors such as the hydrogen-bonding between amine groups of chitosan, attributed to the shrinkage of films in this pH region. Similar results have also been observed in the case of citrate cross-linked chitosan films [27].

TPP/chitosan films exhibited similar pH-sensitive swelling, but the swelling ratio was much smaller than that of Pyro/chitosan films and the stability in acidic condition was also much better because of the greater charge number of TPP and hence the increased ability to interact with chitosan (Fig. 6). Even when incubated in media with pH as low as 1.5, TPP/chitosan film still retained its integrity for more than 48 h (swelling ratio ca. 2.8), but at pH 1.0 the equilibrium swelling ratio of TPP/chitosan film could not be determined due to the serious and rapid degradation of chitosan [38].

Cross-linking time and anion concentration had an effect on the film swelling, and generally the prolongation of cross-linking time and the increase of anion concentration resulted in the decrease of the film swelling ratio. Chitosan film morphology also played an important role on the film swelling. Under the same conditions the swelling ratio of drug loaded films was less than that of blank film because the former had a porous structure that facilitated the diffusion of anions into the inside of film to form more cross-linking sites.

Fig. 7a,b shows the release of BB with poor water solubility from Pyro and TPP cross-linked chitosan films in buffered solution with different pH at the same ionic strength (0.145 M), respectively. In the case of Pyro/chitosan film, at pH 1.0 and 1.5, the films dissociated quickly (BB release percent higher than 80% in 12 h), while at pH 2.0, the release of BB was less than 50% in 12 h due to the relatively weak electrostatic attractive force between Pyro and chitosan (Fig. 3), and at pH 3.5, 4.5, 5.5, 6.5 and 7.5 little BB was released (Fig. 7a). As for TPP/chitosan film, only at pH 1.0 did significant release of BB occur and the release percentage in 24 h was less than 40% (Fig. 7b).

The leaching of chitosan from anion cross-linked chitosan films also exhibited pH-sensitivity (data not shown), which was in accordance with the pH-dependent BB release (Fig. 7a,b). These results were in accordance with the characteristics of electrostatic interaction between anions and chitosan (Fig. 3), i.e. little chitosan leached due to the electrostatic attractive force between anion and chitosan.

Fig. 8 shows the release of riboflavin from chitosan films in SIF and SGF. The model drug release from Pyro/chitosan exhibited excellent pH-sensitivity, and the drug release in SGF was much faster than in SIF (in SGF riboflavin released completely within 5 h while the release percent in SIF in 24 h was only ca. 23%). However, the pH-sensitive release of riboflavin from TPP/chitosan film was poor, and the release percentage in 24 h in SGF and SIF was ca. 40 and 20%,

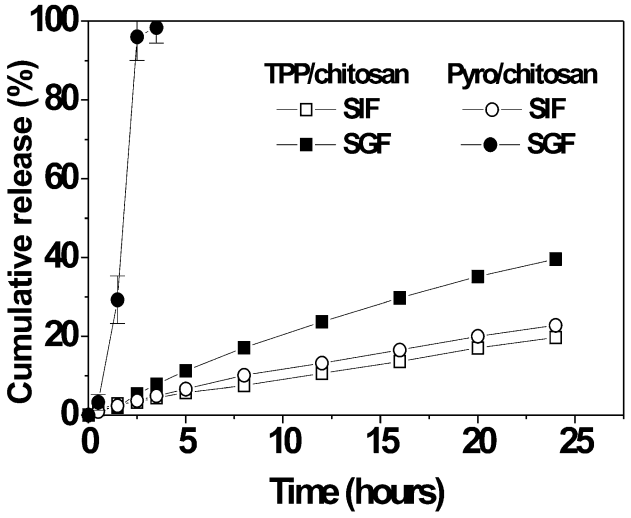

Fig. 8. The release curves of riboflavin in SGF and SIF from Pyro or TPP cross-linked chitosan films at 37 °C ($n = 3$). The films were prepared with 5.0% (w/v) sodium pyrophosphate or sodium tripolyphosphate (pH 5.0) and a cross-linking time of 1.0 h.

respectively. This result was in accordance with our previous discussion (Fig. 3).

However, some authors have reported that TPP/chitosan matrices possessed much better pH-sensitivity than that observed in our experiments [16]. We thought this disagreement might be caused by the incomplete cross-linking of the films. Turbidimetric titration had revealed that in SGF the ionic cross-linking between TPP and chitosan could not dissociate (Fig. 3) and therefore completely cross-linked TPP/chitosan film should have excellent stability in SGF. The result in Fig. 9 was in accordance with the above discussion. The prolongation of the cross-linking time greatly decreased the riboflavin release in SGF. With cross-linking times of 5, 10, 15, 30 and 60 min, the riboflavin release percentage in 24 h in SGF was 97, 86, 69, 51 and 40%, while in SIF under the same conditions the release percentage was always ca. 20–25%. Furthermore, with the same cross-linking time, the increase of TPP concentration from 1.0 to 10.0 (w/v) improved the stability of TPP/chitosan film in SGF because of the more rapid diffusion of TPP into the film. These results indicated that with more cross-linked sites formed (i.e. prolongation of cross-linking time or the increase of TPP concentration) the pH-sensitivity of TPP/chitosan weakened.

3.3.2. Ionic strength effect

In turbidimetric titration, a shielding effect of salt on the electrostatic force had been observed (Fig. 4). Fig. 10a,b shows the influence of the sodium chloride concentration on the swelling of Pyro/chitosan and TPP/chitosan films, respectively. In accordance with the results of the turbidimetric titration (Fig. 4), more significant swelling occurred in higher concentrations of sodium chloride solution, especially in the case of Pyro/chitosan film. However, the increase of the NaCl concentration over 1.8% (w/v) caused

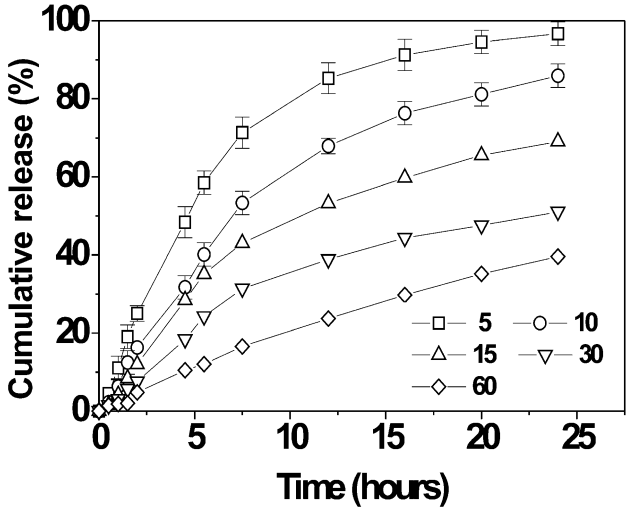

Fig. 9. The influence of cross-linking time on the riboflavin release from TPP cross-linked chitosan films in SGF at 37 °C ($n = 3$). The films were prepared with 5.0% (w/v) sodium tripolyphosphate, pH 5.0.

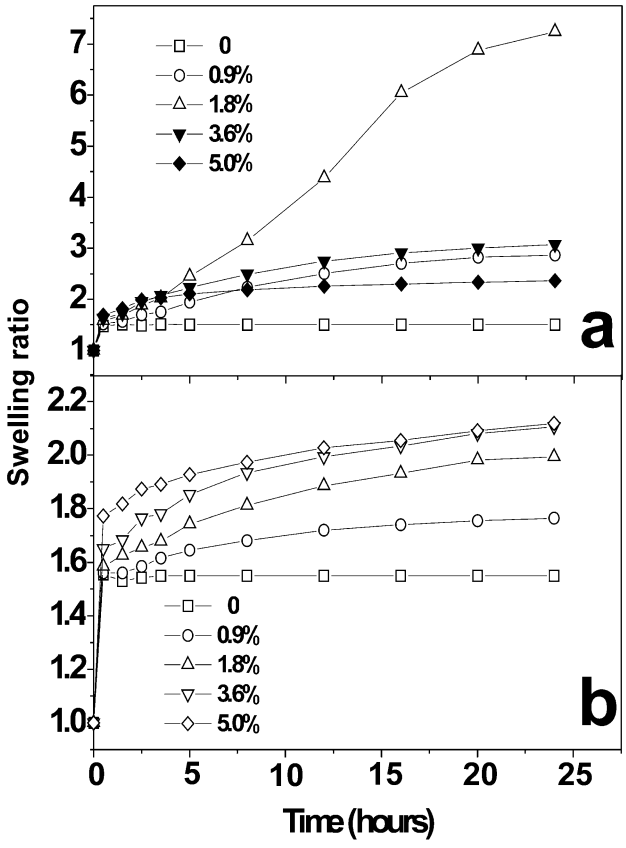

Fig. 10. The swelling curves of Pyro (a) and TPP (b) cross-linked chitosan films in different concentration NaCl solutions at 37 °C ($n = 4$). The films were prepared with 5.0% (w/v) sodium pyrophosphate or sodium tripolyphosphate (pH 5.0) and a cross-linking time of 1.0 h.

the decrease of the swelling ratio of Pyro/chitosan film, and in 24 h with sodium chloride concentrations of 0, 0.9, 1.8, 3.6 and 5.0% (w/v) the swelling ratio was 1.5, 2.9, 7.2, 3.1 and 2.4, respectively (Fig. 10a). This retardation by the salt of the film swelling was possibly related to the decrease of osmotic pressure inside the film with the increase of the salt concentration [39]. A similar phenomenon was observed in the case of sulfate and citrate cross-linked chitosan film (unpublished data). However, in the case of TPP/chitosan film this did not occur, which may be related to the stronger electrostatic interaction of TPP for chitosan, and the shielding effect and the osmotic effect of the salt on film swelling were compensated mutually. In agreement with that, the acceleration by salt of the swelling ratio of TPP/chitosan film was very slight, and in 24 h with sodium chloride concentrations of 0, 0.9, 1.8, 3.6 and 5.0% (w/v) the swelling ratio was 1.5, 1.8, 2.0, 2.1 and 2.1, respectively (Fig. 10b).

The model drug release from Pyro/chitosan and TPP/chitosan film also exhibited ionic strength-response similar to film swelling behavior (Fig. 11a,b). In 24 h with sodium chloride concentrations of 0, 0.9, 1.8, 3.6 and 5.0% (w/v) the riboflavin release percent from Pyro/chitosan film was 14.7, 29.2, 41.7, 28.3 and 25.5%, respectively (Fig. 11a),

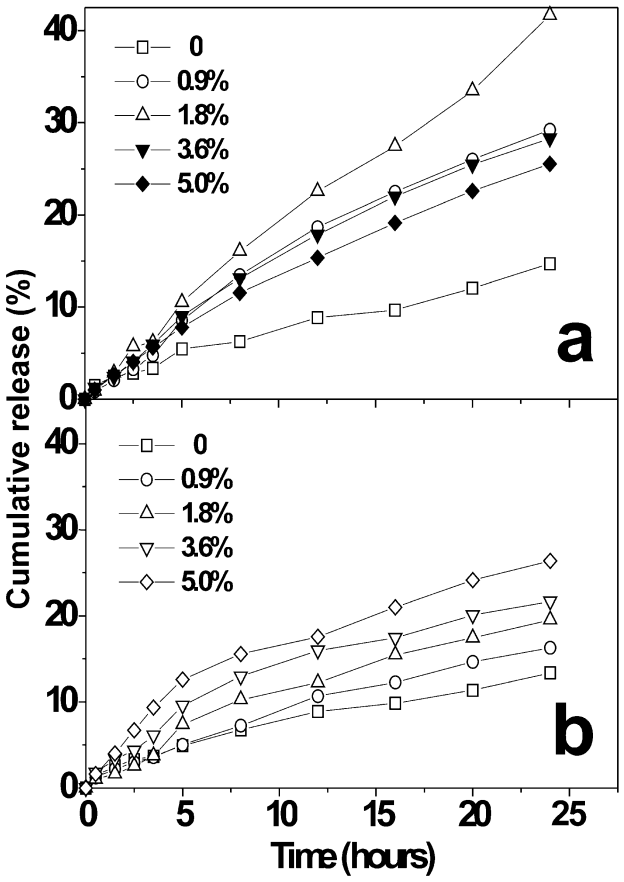

Fig. 11. The release curves of riboflavin from Pyro (a) and TPP (b) cross-linked chitosan films in different concentration NaCl solutions at 37 °C ($n = 4$). The films were prepared with 5.0% (w/v) sodium pyrophosphate or sodium tripolyphosphate (pH 5.0) and a cross-linking time of 1.0 h.

while in the case of TPP/chitosan film under the same conditions, the value was 13.4, 16.3, 19.5, 21.7 and 26.4% (Fig. 11b).

4. Conclusions

The molecular structure of multivalent phosphate strongly influenced the interactions with chitosan by salt bonds. The greater number of negative charges of TPP and Pyro resulted in their greater ability to ionically cross-link chitosan, while a weakly electrostatic attractive interaction between Phos and chitosan was observed. Solution pH played an important role in the charge numbers carried by TPP, Pyro and chitosan, and hence affected their electrostatic interactions, especially in the case of Pyro/chitosan. TPP and Pyro cross-linked chitosan films were prepared by dipping chitosan films into TPP or Pyro solution. In agreement with the electrostatic interaction between anions and chitosan, solution pH and ionic strength significantly influence the film properties. These films swelled and drug was released quickly in acidic conditions (such as in SGF) while under neutral conditions (such as in SIF) they remained in a shrunken state and drug was released slowly. Compared to TPP/chitosan, Pyro/chitosan films exhibited much better pH-sensitive swelling and drug controlled release properties due to their relatively weak interactions. The same explanation also accounted for the more significant acceleration effect of sodium chloride on Pyro/chitosan film swelling and drug release. These films are promising for use in site-specific drug delivery in the stomach.

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