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Controlled drug release properties of ionically cross-linked chitosan beads: the influence of anion structure

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Abstract

By adopting a novel chitosan cross-linked method, i.e. chitosan/gelatin droplet coagulated at low temperature and then cross-linked by anions (sulfate, citrate and tripolyphosphate (TPP)), the chitosan beads were prepared. Scanning electron microscopy (SEM) observation showed that sulfate/chitosan and citrate/chitosan beads usually had a spherical shape, smooth surface morphology and integral inside structure. Cross-sectional analysis indicated that the cross-linking process of sulfate and citrate to chitosan was much faster than that of TPP due to their smaller molecular size. But, once completely cross-linked, TPP/chitosan beads possessed much better mechanical strength and the force to break the beads was approximately ten times higher than that of sulfate/chitosan or citrate/chitosan beads. Release media pH and ionic strength seriously influenced the controlled drug release properties of the beads, which related to the strength of electrostatic interaction between anions and chitosan. Sulfate and citrate cross-linked chitosan beads swelled and even dissociated in simulated gastric fluid (SGF) and hence, model drug (riboflavin) released completely in 5 h; while in simulated intestinal fluid (SIF), beads remained in a shrinkage state and drug released slowly (release % usually $< 70\%$ in 24 h). However, swelling and drug release of TPP/chitosan bead was usually insensitive to media pH. Chitosan beads, cross-linked by a combination of TPP and citrate (or sulfate) together, not only had a good shape, but also improved pH-responsive drug release properties. Salt weakened the interaction of citrate, especially sulfate with chitosan and accelerated beads swelling and hence drug release rate, but it was insensitive to that of TPP/chitosan. These results indicate that ionically cross-linked chitosan beads may be useful in stomach specific drug delivery.

Keywords: Chitosan; Ionic cross-linking; Beads; Controlled drug release

1. Introduction

Chitosan, with excellent biodegradable and biocompatible characteristics, is a naturally occurring polysaccharide. Due to its unique polymeric cationic character and its gel and film forming properties, chitosan has been extensively examined in the pharmaceutical industry for its potential in the development of drug delivery systems (Chandy and Sharma, 1992, 1993; Yao et al., 1996; Patel and Amiji, 1996; Felt et al., 1998; Illum, 1998; Giunchedi et al., 1998; Gupta and Kumar, 2000).

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Recently, the use of complexation between oppositely charged macromolecules to prepare chitosan beads (or microspheres) as controlled drug release formulation, especially for peptide and protein drug delivery, has attracted much attention because this process is very simple and mild (Huguet et al., 1994; Polk et al., 1994; Liu et al., 1997; Dumitriu and Chornet, 1998). In addition, reversible physical cross-linking by electrostatic interaction instead of chemical cross-linking is applied to avoid possible toxicity of reagents and other undesirable effects.

Compared to polyanions, the preparation of cross-linking chitosan matrices using anions was found to be simpler and milder. For example, tripolyphosphate (TPP) cross-linked chitosan beads can be prepared simply by dropping chitosan droplets into TPP solution and this procedure was found to be useful in the pharmaceutical industry (Kawashima et al., 1985a,b; Bodmeier et al., 1989; Shirashi et al., 1993; Sezer and Akbuga, 1995; Aydin and Akbuga, 1996; Calvo et al., 1997; Aral and Akbuga, 1998). Unfortunately, up to now, only a few ionic cross-linked chitosan beads have been reported.

TPP/chitosan bead usually had poor mechanical strength, which limited its usage in drug delivery. In our previous studies, a novel approach was developed to improve the mechanical strength of TPP/chitosan beads by more than ten times (Shu and Zhu, 2000). Furthermore, other anions (sulfate and citrate etc.) were also found to interact with chitosan by electrostatic force (Shu and Zhu, 2001; Shu et al., 2001), therefore sulfate and citrate cross-linked chitosan films were prepared and their controlled drug release properties were investigated (Shu and Zhu, 2001; Shu et al., 2001).

In this paper, we aimed to prepare sulfate and citrate cross-linked chitosan beads and investigate the model drug controlled release performances (especially pH and ionic strength responsive properties) of sulfate, citrate and TPP cross-linked chitosan beads, which was discussed in view of the difference in molecular structure of the anions.

2. Materials and methods

2.1. Materials

Chitosan was obtained from Tianbao Chitosan Co. Ltd. (Ningbao, People's Republic of China) and refined twice by dissolving in dilute HAc solution and precipitating from dilute ammonia, the degree of deacetylation was 86%, Mv was 460,000. Gelatin (type B, ≈ 225 Bloom) was obtained from Sigma (St. Louis, MO). Riboflavin (Mw 376.37) was purchased from Aldrich (Milwaukee). Sodium sulfate, sodium citrate, sodium tripolyphosphate (TPP) and other reagents were all commercially available and used as received.

2.2. Preparation of chitosan beads

Sulfate, citrate and TPP cross-linked chitosan beads were prepared according to our recently reported method (Shu and Zhu, 2000) and described as follows.

Model drug (riboflavin) were dispersed in double-distilled water and added to an acetic acid (4% w/v) solution of chitosan containing gelatin at 37 °C under agitation. The component concentration in the solution (w/v): chitosan 4%, gelatin 4% and model drug 1%. Some 2 ml of the above mixture solution (37 °C) was dropped through a syringe needle (0.45 mm in diameter) into 250 ml cold sesame-seed oil (4 °C) to induce the coagulation of gelatin. After 30 min, the oil was discarded and 100 ml cold cross-linking solution was added under gentle agitation at 4 °C. The cross-linking solution contained sodium sulfate, sodium citrate or TPP with a concentration of 0.25–5.0% (w/v) (pH 7.0). After a certain time, the beads were separated and washed with cold double-distilled water (4 °C), then used in the following experiments or dried under vacuum at room temperature.

During the bead preparation process, the aqueous phase was collected and the drug content in aqueous phase was determined by UV-visible spectrophotometer at 444 nm. Usually $> 95\%$ model drug was loaded in the beads.

2.3. Bead mechanical strength determination

The mechanical strength of beads was evaluated using a similar method described by Daly and Knorr (1988). It was expressed as the value that the maximum force used when the bead was broken under uniaxial compression at a crosshead speed of 0.5 cm/min (Model TM, Instron Corp., Canton, MA).

2.4. Morphology observation

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

To investigate the cross-linking process, the wet beads in certain cross-linking time were collected during preparation, cut into two halves with blades and the uncross-linked part was washed out with 30 °C distilled water. Then, the cross-sectional morphologies in a wet condition were examined using an optical microscope (XJZ-6, Jiangnan Optical Instrument Plant, People's Republic of China).

2.5. Swelling ratio measurements

The swelling ratio in the release media during the drug release test was estimated by measuring the bead size using an optical microscope at three different positions and expressed as D_t/D_0, where D_t was the bead diameter at time t and D_0 was the initial bead diameter and the average values were taken for at least 20 beads.

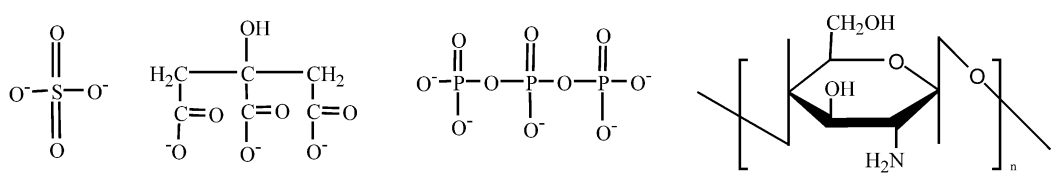
2.6. Model drug release studies

A certain amount of beads containing ≈ 10 mg model drugs were suspended in a glass bottle containing 100 ml release media and incubated on a shaking water-bath at 37 °C, 50 rpm. Release media: 0–3.6% (w/v) NaCl solution; simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) without enzyme (USP XXII). At appropriate intervals, 4 ml samples were withdrawn and riboflavin content in the medium determined by UV-visible spectrophotometer at 444 nm. An equal volume of the same dissolution medium was added back to maintain a constant volume.

3. Results and discussion

3.1. Morphology observation

Sulfate, citrate and TPP carry a maximum of two, three and five negative charges, respectively (Scheme 1). The electrostatic interaction between TPP and chitosan had been reported and exploited in the pharmaceutical industry to prepare TPP cross-linked chitosan beads, for long time (Kawashima et al., 1985a,b; Bodmeier et al., 1989; Shirashi et al., 1993; Sezer and Akbuga, 1995; Aydin and Akbuga, 1996; Calvo et al., 1997; Aral and Akbuga, 1998). Our recent results revealed that there was also electrostatic interaction of sulfate and citrate with chitosan (Shu and Zhu, 2001; Shu et al., 2001), therefore we managed to prepare sulfate and citrate cross-linked chitosan beads.



sulfate **citrate** **tripolyphosphate** **chitosan**

Scheme 1. The structure of sulfate, citrate, TPP and chitosan.

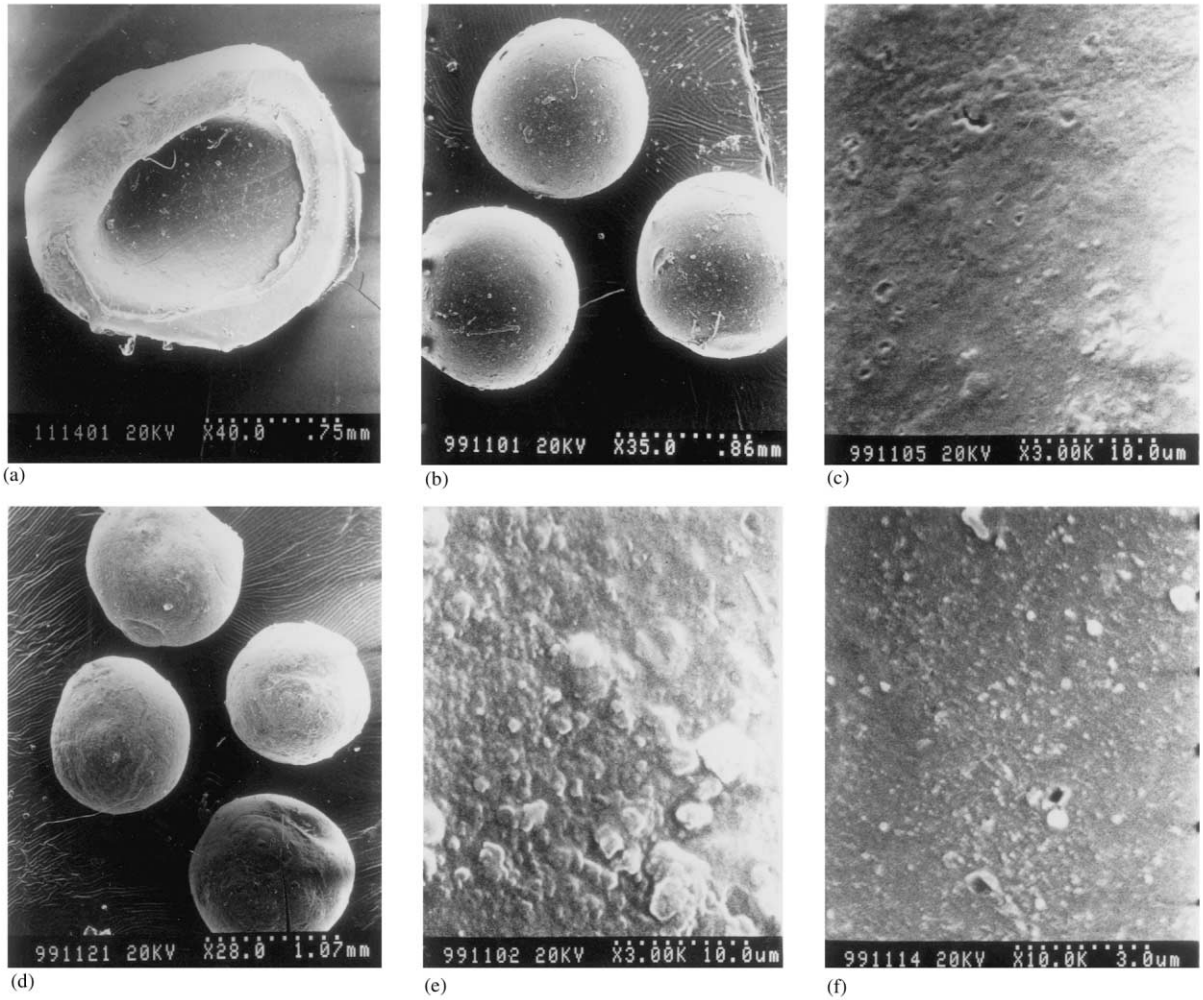


Fig. 1. SEM micrographs of TPP/chitosan beads (a); citrate/chitosan beads (b) and its surface morphology (c); sulfate/chitosan beads (d) and its surface morphology (e); the cross-sectional morphology of citrate/chitosan beads (f). The beads were prepared with anion concentration 1.0% (w/v) and cross-linking time of 20 min.

However, in the conventional method, i.e. dropping chitosan solution into TPP solution directly, only fragile beads were obtained; and as for the sulfate and citrate solution, only amorphous precipitations formed. By adopting our recently developed procedure, i.e. chitosan/gelatin droplet coagulated at low temperature and then cross-linked by anions, the cross-linked chitosan beads with relative good mechanical strength were prepared. The shape of these beads in wet state was very spherical (≈ 2.5 mm in diameter) and even after drying, the spherical shape could usually be retained, but was affected by anion concentration, cross-linking time and the nature of the anions. With anion concentration of 1.0% (w/v) and cross-linking time in 20 min, dried TPP/chitosan beads were similar to a disc with a collapsed center (Fig. 1a). The increase of TPP solution concentration and the prolonging of the cross-linking time were of benefit to keeping the

spherical bead shape. However, as for sulfate and citrate, under the same preparation conditions, the obtained beads were very spherical (Fig. 1b,d) ($\approx$ 1.3 mm in diameter) and the surface was very smooth (Fig. 1c,e).

Cross-sectional analysis revealed that the coagulated chitosan/gelatin droplet was cross-linked step by step with the diffusion of anions into droplets that influenced the cross-linking of beads seriously (Shu and Zhu, 2000). Due to its largest molecular size (Scheme 1), the diffusion of TPP into the droplet was much slower than that of sulfate or citrate, so the cross-linking speed was much slower, which resulted in the poor shape of TPP/chitosan beads with short cross-linking time (Fig. 1a).

In dried state, with anion concentration 1.0% (w/v) and cross-linking time 30 min, the inside structure of sulfate/chitosan and citrate/chitosan beads was very integral (Fig. 1f), while that of TPP/chitosan beads was a little porous (picture not shown).

3.2. Mechanical strength

According to cross-sectional analysis, with 30 min of cross-linking time for sulfate and citrate and 60 min for TPP (anion concentration 1.0% w/v), the cross-linking process of beads was usually completed. Further prolongation of cross-linking time just slightly improved bead mechanical strength. But the effect of anion nature on bead mechanical strength was significant (Fig. 2). Though the cross-linking process of TPP/chitosan beads needed longer time, they showed the most excellent mechanical properties in un-dried state when completely cross-linked (force to break was approximately ten times higher than that of sulfate/chitosan or citrate/chitosan beads). This result was probably related to the stronger interaction of TPP with chitosan due to its more charge numbers and the higher charge density (Scheme 1).

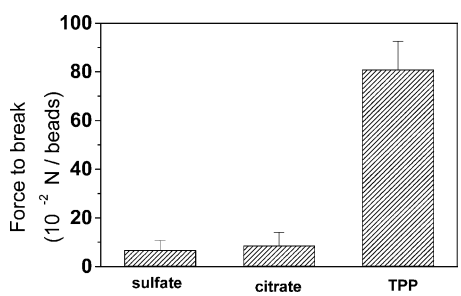


Fig. 2. The mechanical strength of sulfate, citrate and TPP cross-linked chitosan beads in an un-dried state, anion concentration 1.0% (w/v) and cross-linking time of 60 min.

3.3. Drug release studies

3.3.1. Release media pH effect

Charge density is an important factor in electrostatic interaction and mainly depends on solution pH. The charge numbers of sulfate, citrate, TPP and chitosan were all related to solution pH and the electrostatic cross-linking of anions to chitosan was also influenced by solution pH (Shu et al., 2001). Therefore, ionic cross-linked chitosan matrices should exhibit pH-responsive properties. For example, TPP/chitosan beads were usually exploited for stomach specific drug delivery.

Fig. 3 shows the pH-dependent release of riboflavin from chitosan beads in SGF and SIF. Sulfate/chitosan and citrate/chitosan beads exhibited a similar release profile for model drug. In SIF, beads were kept in a shrinkage state and drug released slowly (release % in 24 h usually $< 70\%$) due to the electrostatic attractive interaction between anions and chitosan; while in the acidic condition (SGF), part of the charge of sulfate and most of the charge of citrate were protoned and hence, electrostatic interaction weakened or disappeared, therefore beads swelled significantly and even dissociated rapidly, resulting in the quick model drug release (in < 5 h drug released completely) (Fig. 3a,b) (Shu and Zhu, 2001; Shu et al., 2001). As for TPP/chitosan beads, the pH-responsive drug release performance was poor and in 24 h riboflavin release percent in SGF and SIF was 59 and 49%, respectively (Fig. 3c).

Though to date some authors observed the pH-sensitivity of TPP/chitosan matrixes (Shirashi et al., 1993; Remuñãn-López and Bodmeier, 1997), our result indicated that this pH-sensitivity

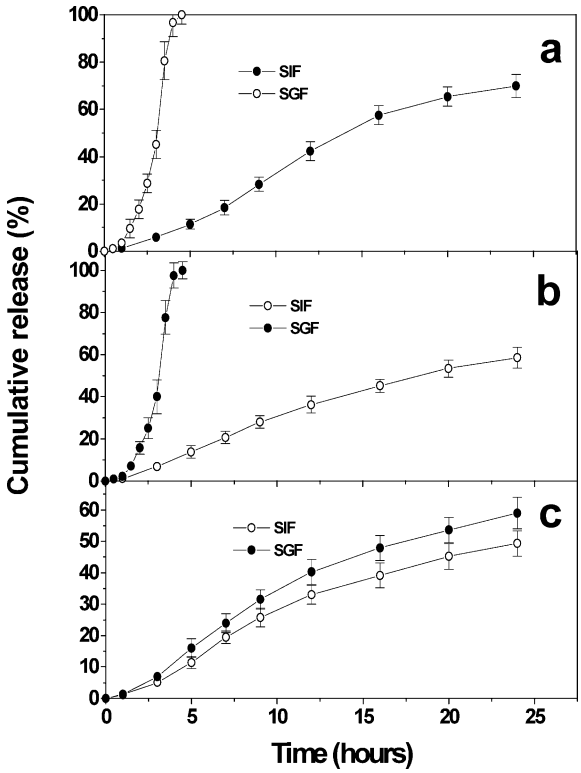


Fig. 3. The release curves of riboflavin in SIF and SGF from sodium sulfate (a), sodium citrate (b) and sodium tripolyphosphate (c) cross-linked chitosan beads. The concentrations of sodium sulfate, sodium citrate and sodium tripolyphosphate were all 5.0% (w/v) and cross-linking time of 1.0 h ($n = 3$).

was poor. Further investigation revealed that cross-linking time and TPP concentration during the preparation process played an important role on the pH-responsive properties of TPP/chitosan beads. Only those beads with both short cross-linking time (< 30 min) and low TPP concentration ($< 1.0\%$ w/v) possessed excellent pH-dependent swelling and drug release properties, which probably indicated that only the beads were not completely cross-linked, did pH-responsive behavior occur. Turbidimetric titration also indicated that due to the strongest interaction of TPP to chitosan, the formed TPP/chitosan complex could not be dissociated even in pH < 1.0, which possibly caused the poor pH-dependent drug release profile in Fig. 3(c).

However, with low TPP concentration and limited cross-linking time, the obtained TPP/chitosan beads were fragile and the shape was poor after dried (Fig. 1a). At the same time, from Fig. 3(a,b) it can also be seen that the drug release in SGF from sulfate/chitosan and citrate/chitosan beads was very quick. Therefore, we managed to prepare chitosan beads cross-linked by a combination of TPP and citrate (or sulfate). These beads had a spherical shape, no matter whether in wet or dried state (pictures not shown) and at the same time, the drug release in SGF was prolonged significantly.

Fig. 4(a,b) shows the pH-dependent swelling and riboflavin release, respectively, in SGF and SIF from chitosan beads prepared with 5.0% (w/v) citrate and 1.0 or 0.5% (w/v) TPP (w/v). The swelling ratio of these beads in SGF was much larger than that in SIF. In 3 h with 0.5% (w/v) TPP/5.0% (w/v) citrate and 1.0% (w/v) TPP/5.0% citrate (w/v), the swelling value was ≈ 1.65 both in SIF, while under the same conditions it was 2.9 and 2.5 in SGF, respectively (Fig. 4a). In accordance with this, in 12 h with 0.5% (w/v) TPP/5.0% (w/v) citrate and 1.0% (w/v) TPP/5.0% citrate

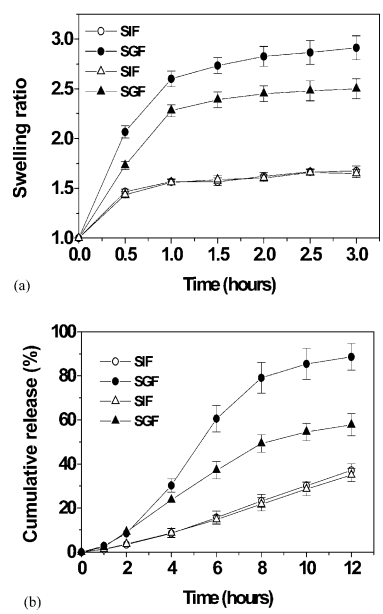


Fig. 4. The swelling curves (a) and riboflavin release curves (b) in SIF and SGF. Circle symbol curves: 0.5% sodium tripolyphosphate and 5.0% sodium citrate; up triplicate symbol curves: 1.0% sodium tripolyphosphate and 5.0% sodium citrate. Cross-linking time of 1.0 h ($n = 3$).

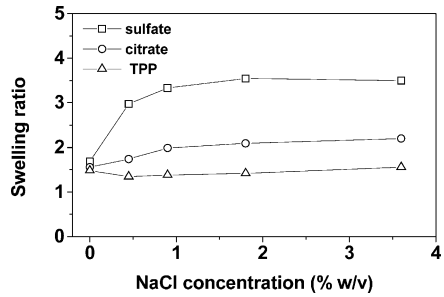


Fig. 5. The swelling ratio of ionic cross-linked chitosan beads after incubation in different NaCl solution for 5 h. The concentrations of sodium tripolyphosphate, sodium citrate and sodium sulfate were all 5.0% (w/v), cross-linked time of 1.0 h. (Error bars were less than the symbol) ($n = 3$).

(w/v) both $\approx 37\%$ drug released in SIF and under the same conditions, the release percent was 88 and 58% in SGF, respectively (Fig. 4b). These results mean that these chitosan beads may be useful in stomach specific controlled drug release.

3.3.2. Ionic strength effect

Ionic strength was another important factor influencing electrostatic interaction and salt usually had a shielding effect on ionic cross-linking. Fig. 5 shows the influence of NaCl concentration on the swelling ratio of ionic cross-linked chitosan beads. The swelling ratio usually increased with the increase of NaCl concentration, which was more significant for sulfate/chitosan beads. However, a further increase of NaCl concentration higher than 1.8% (w/v) caused the slight shrinkage of sulfate/chitosan beads. With NaCl concentration 0, 0.45, 0.9, 1.8 and 3.6%, the swelling ratio after incubation in release media for 5 h was 1.68, 2.98, 3.33, 3.55 and 3.50, respectively. The least acceleration and even retardation of NaCl to the swelling of TPP/chitosan beads was observed. With NaCl concentration 0, 0.45, 0.9, 1.8 and 3.6%, the swelling ratio in 5 h was 1.48, 1.35, 1.38, 1.42 and 1.56, respectively.

The different influence of salt to bead swelling may be related to ionic cross-linking capability of anions to chitosan. The weakest interaction of sulfate to chitosan resulted in the most significant shielding of salt, while the strongest interaction of TPP to chitosan caused the insensitivity of bead swelling to salt. The unusual shrinkage of beads in some cases may be related to the decrease of osmotic pressure with the increase of NaCl concentration, according to Donnan equilibrium (Moe et al., 1993). Similar results were also obtained in the case of sulfate, citrate and TPP cross-linked chitosan films (unpublished results).

In accordance with the results of swelling, riboflavin released from beads also in an ionic strength responsive way (Fig. 6). The acceleration of salt to drug release from sulfate/chitosan beads

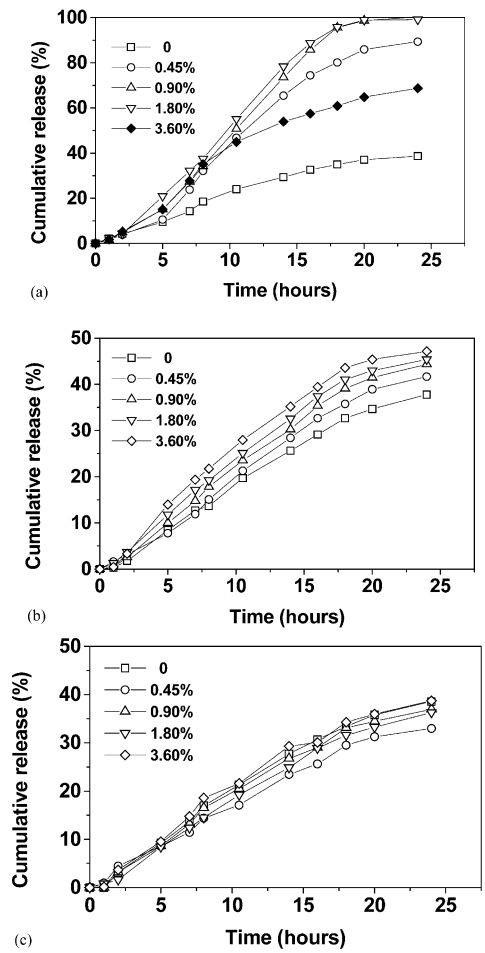


Fig. 6. The release curves of riboflavin from sodium sulfate (a), sodium citrate (b) and sodium tripolyphosphate (c) cross-linked chitosan beads in different concentration NaCl solution. The concentrations of sodium sulfate, sodium citrate and sodium tripolyphosphate were all 5.0% (w/v) and cross-linking time of 1.0 h. (Error bars for (a) $< 2\%$, for (b) and (c) less than symbol) ($n = 3$).

was the most significant and with NaCl concentration 0, 0.45, 0.9 and 1.8% (w/v) model drug release percent in 24 h was $\approx$39, 89, 100 and 100%, respectively, while a further increase of NaCl concentration to 3.6% (w/v) retarded drug release (release percent in 24 h was $\approx$69%) (Fig. 6a). In the case of citrate/chitosan beads, this acceleration of salt to drug release was more slight (Fig. 6b). While, as for TPP/chitosan beads, the model drug release was insensitive to NaCl concentration and even a retardation of salt to drug release was observed when NaCl concentration was low (Fig. 6c).

4. Conclusions

Sulfate, citrate and TPP cross-linked chitosan beads were prepared by our recently developed method. Due to their smaller molecular size, the cross-linking process of sulfate and citrate to chitosan was much faster than that of TPP; but once completely cross-linked, TPP/chitosan beads possessed much better mechanical strength than sulfate/chitosan and citrate/chitosan beads, due to its strongest interaction with chitosan. Release media pH and ionic strength seriously influenced the controlled drug release properties of beads, which related to the nature of anions. Sulfate and citrate cross-linked chitosan beads swelled and even dissociated in SGF and hence, model drug release completely within 5 h; while in SIF beads kept in shrinkage state and drug release slowly (release percent usually $<70\%$ in 24 h). On the other hand, swelling and drug release of TPP/chitosan beads to media pH was insensitive. Chitosan beads cross-linked by a combination of both TPP and citrate (or sulfate) not only had good shape, but also exhibited excellent pH-sensitivity. Salt weakened the interaction of citrate, especially sulfate with chitosan and accelerated beads swelling and hence, drug release rate, but it was insensitive to that of TPP/chitosan.

Acknowledgements

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