Chitosan–Polyelectrolyte Complexation for the Preparation of Gel Beads and Controlled Release of Anticancer Drug. II. Effect of pH-Dependent Ionic Crosslinking or Interpolymer Complex Using Tripolyphosphate or Polyphosphate as Reagent

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ABSTRACT: Chitosan gel beads were prepared using an in-liquid curing method by ionotropic crosslinking or interpolymer linkage with tripolyphosphate (TPP) or polyphosphate (PP). The ionic interaction of chitosan with TPP or PP is pH-dependent due to the transition of "ladder-loop" complex structures. Chitosan gel beads cured in a pH value lower than 6 of a TPP solution was a controlled homogeneous ionic-crosslinking reaction, whereas chitosan gel beads cured in a lower pH PP solution was a nonhomogeneous interpolymer complex reaction due to the mass-transfer resistance for the diffusion of macromolecular PP. According to the results of FTIR and EDS studies, it was suggested that significantly increasing the ionic-crosslinking density or interpolymer linkage of a chitosan–TPP or chitosan–PP complex could be achieved by transferring the pH value of curing agent, TPP or PP, from basic to acidic. The swelling behavior of various chitosan beads in acid medium appeared to depend on the ionic-crosslinking density or interpolymer linkage of the chitosan–TPP or chitosan–PP complex, which were deeply affected by the in-liquid curing mechanism of the chitosan gel beads. By the transition of the in-liquid curing mechanism, the swelling degree of chitosan–TPP or chitosan–PP beads was depressed and the disintegration of chitosan–TPP or chitosan–PP beads did not occur in strong acid. The drug-release patterns of the modified chitosan gel beads in simulated intestinal and gastric juices were sustained for 20 h. These results indicate that the sustained release of anticancer drugs could be achieved due to the variation of the reaction mechanism of a chitosan–polyelectrolyte pH-dependent ionic interaction. J Appl Polym Sci 74: 1093–1107, 1999

Key Words: chitosan; polyelectrolyte complexation; controlled release; in-liquid curing

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INTRODUCTION

Chitosan is a useful polysaccharide derived from the marine bipolymer—chitin. Porous chitosan beads were applicated to the metal ion uptake, such as copper, cadmium, chromium, and uranium.[1–5] In recent years, chitosan was already reported to be suitable for biomedical implants, degradable sutures, and wound dressing,[6–8] especially for the slow-release delivery of drugs. Chitin and chitosan have been used for the encapsulation of drugs or biological agents, such as cells, albumin, and insulin.[9–14] In previous studies, chitosan or chitin having controlled-release characteristics was also prepared in our laboratory to be used as a vehicle for the sustained-release of drugs such as anticancer drugs, theophylline, or antibiotic agents.[15–17]

To improve the material properties used for controlled drug-delivery, chitosan gel beads should be chemically crosslinked using glutaraldehyde (GA) or ethylene glycol diglycidyl ether (EGDE).[18–24] However, neither GA nor EGDE was an ideal crosslinking agent due to their physiological toxicity. Recently, polyelectrolyte complexes (PECs) have been proposed for the design of drug-delivery systems. Chitosan could form gels with gentle and nontoxic multivalent counterions, tripolyphosphate (TPP), by ionic interaction.[25–28] However, the results of these investigations all displayed that chitosan–TPP gel without being crosslinked with GA was not suitable to be used as a sustained-release drug-delivery system in simulated gastric and intestinal juices, except for the releasing of an acidic or water-insoluble drug, such as indomethacin or sulfazadizine, which is severely dissolved in a lower pH dissolution medium.[29–30]

In this study, small molecular TPP or macromolecular polyphosphate (PP) was used to react with chitosan for the preparation of PECs. The

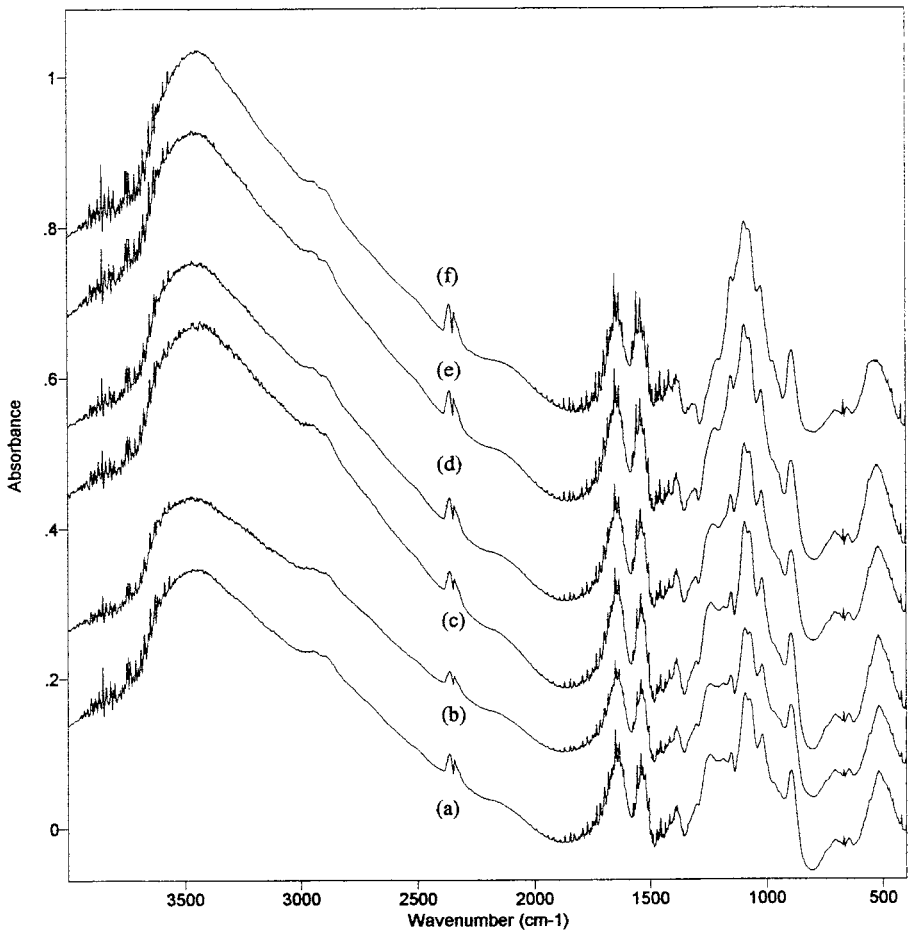


Figure 1 IR spectra of chitosan–TPP complex cured in pH (a) 1.2, (b) 2.0, (c) 3.0, (d) 4.0, (e) 6.0, or (f) 8.6 TPP solutions.

drug-release characteristics of the chitosan–TPP or chitosan–PP complexes could be improved by variation of the reaction kinetics of the PECs. FTIR and EDS analyses were used to confirm the mechanism transition in different pH values of a TPP or PP solution for the improvement of the ionic-crosslinking or interpolymer-complex characteristics of chitosan–TPP or chitosan–PP beads. The swelling properties of the chitosan–TPP or chitosan–PP beads were analyzed to examine their ionic-crosslinking or interpolymer-complex characteristics. This study also presents the results of the application of chitosan–TPP or chitosan–PP beads for swelling-controlled or diffusional-controlled release of 6-mercaptopurine (6-MP) in a simulated gastric (pH 1.2) or intestinal fluids (pH 6.8) solution. The effects of ionic crosslinking or an interpolymer complex of gel beads on the kinetics of drug release were also investigated.

EXPERIMENTAL

Materials

Chitosan, 6-MP, and sodium TPP were all purchased from Sigma (St. Louis, MO). The weight-average molecular weight and deacetylation degree of chitosan are 70,000 and 87%, respectively. All other materials were of reagent-grade purity.

Preparation of Chitosan–TPP or Chitosan–PP Gel Beads by pH-Dependent Ionic Crosslinking or Interpolymer Complex

Chitosan (15 g) was dissolved in 500 mL of dilute acetic acid (1.0% v/v) to prepare the chitosan solution. 6-MP (0.2 g) was dispersed in 10 mL of the chitosan solution. These suspensions were dropped into a gently agitated TPP or PP solution

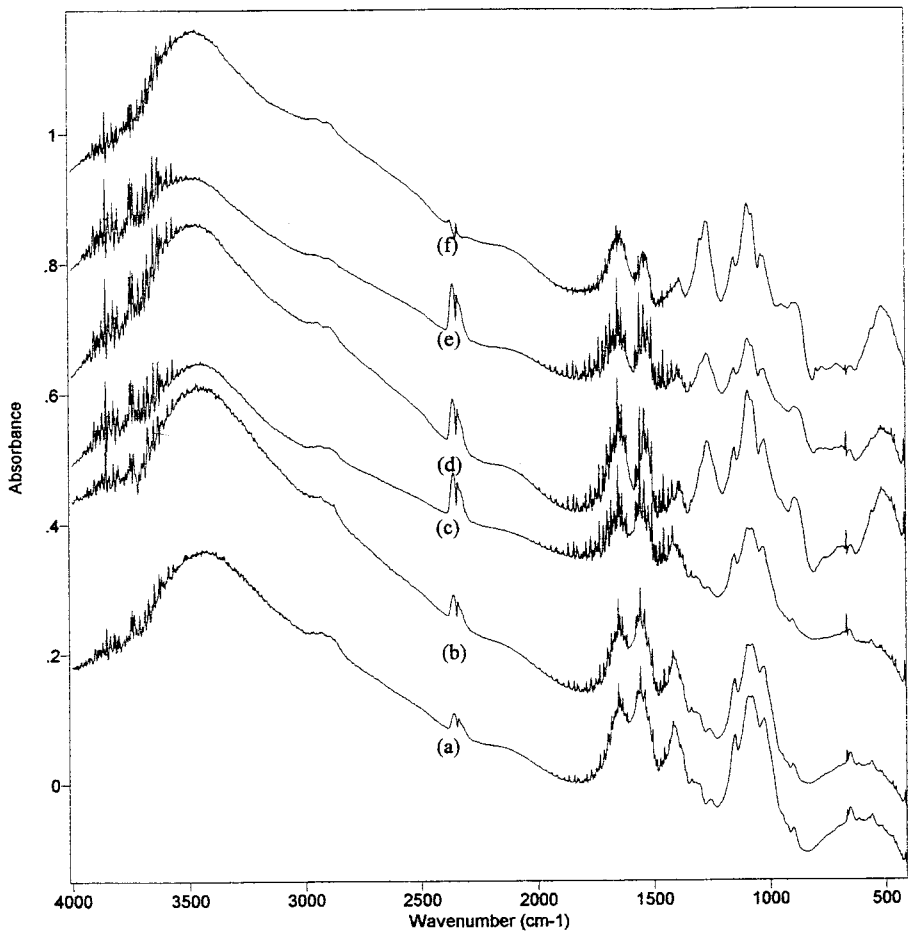


Figure 2 IR spectra of chitosan–PP complex cured in pH (a) 14.0, (b) 12.0, (c) 10.0, (d) 8.0, (e) 6.0, and (f) 4.0 TPP solutions.

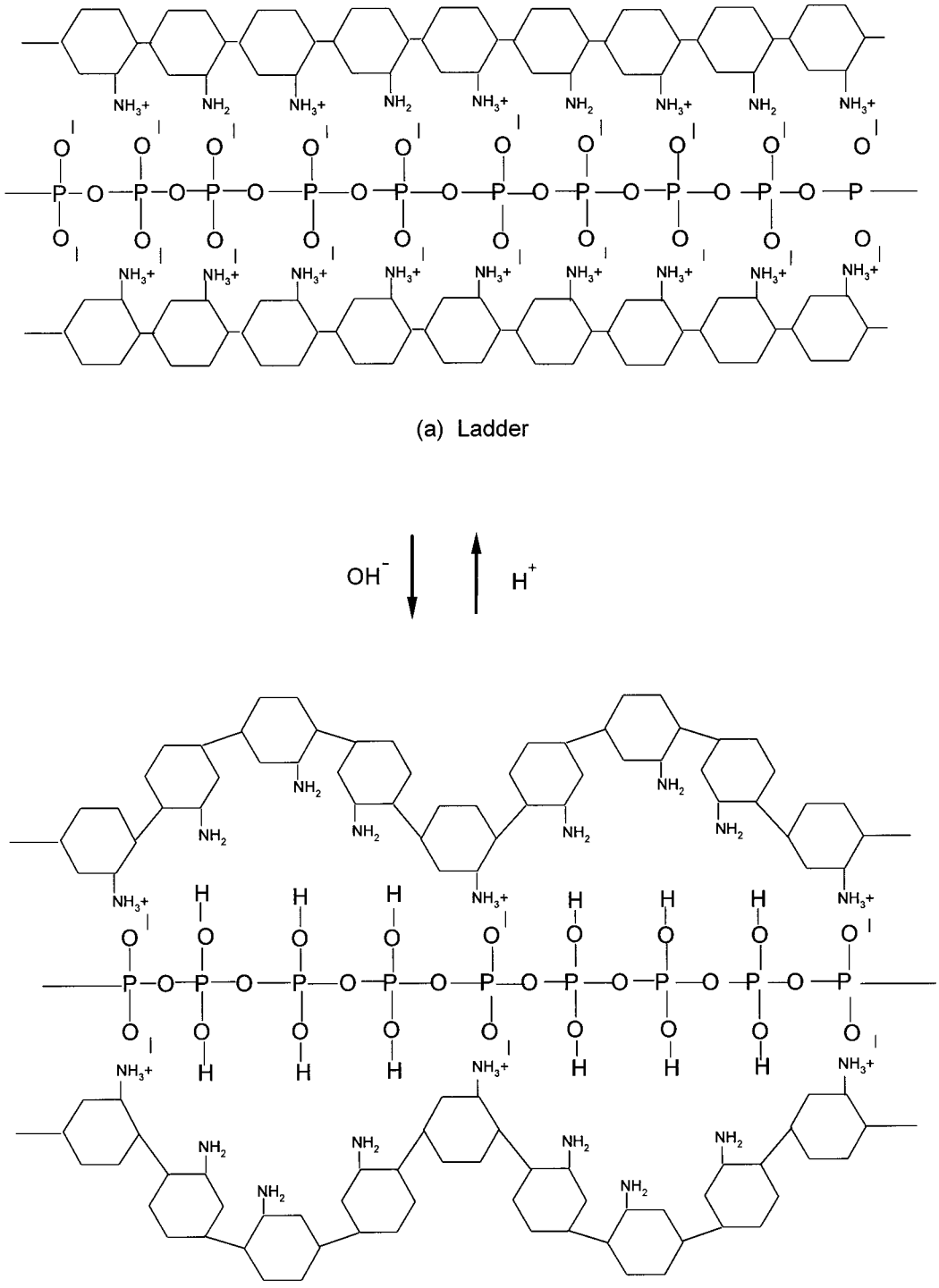


Figure 3 Ladder-loop transition of chitosan–PEC structures: (a) chitosan–PP complex; (b) chitosan–TPP complex.

(10% w/v) and they stood in the solution for 2–30 min. The pH value of the TPP solutions was adjusted from the original (pH 8.6) to pH 1.0, and the pH of the PP solutions was adjusted from the original (pH 4.0) to pH 14.0. The solidified beads were filtered and washed with 100 mL of deionized water repeatedly, then dried in a vacuum oven at 40°C for 24 h. The final products were stored in a dessicator for future drug-release analysis.

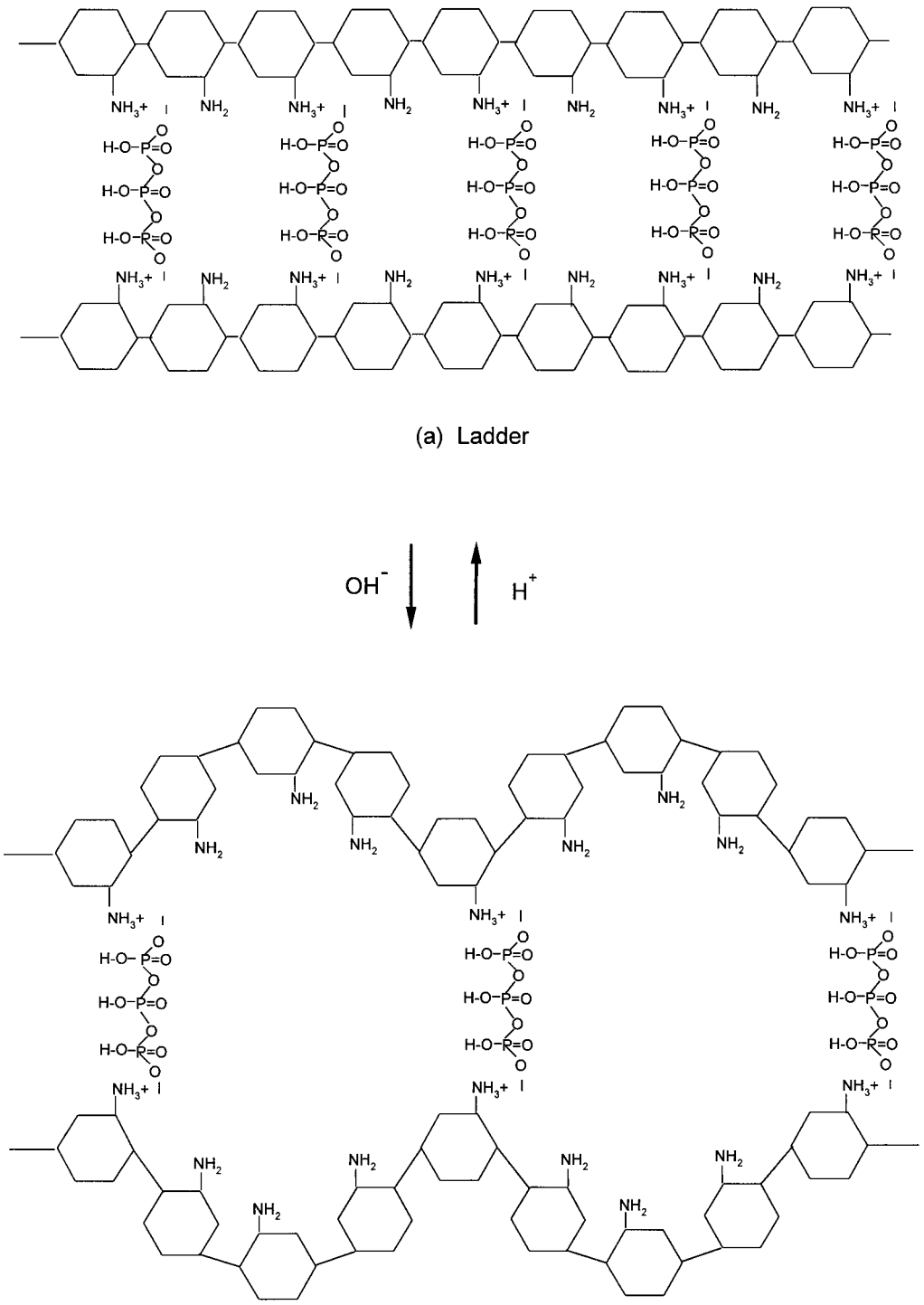


Figure 3 (*Continued from the previous page*)

pH-Responsive Swelling of Chitosan–TPP or Chitosan–PP Beads

The water-sorption capacity of the chitosan gel beads was determined by swelling the gel beads in media of pH 1–7 at room temperature. A known weight (200 mg) of various chitosan gel beads was placed in the media for the required period of time. The wet weight of the swollen beads was determined by first blotting the beads with filter paper to remove the adsorbed water on the surface and then weighing them immediately on an electronic balance. The percentage swelling

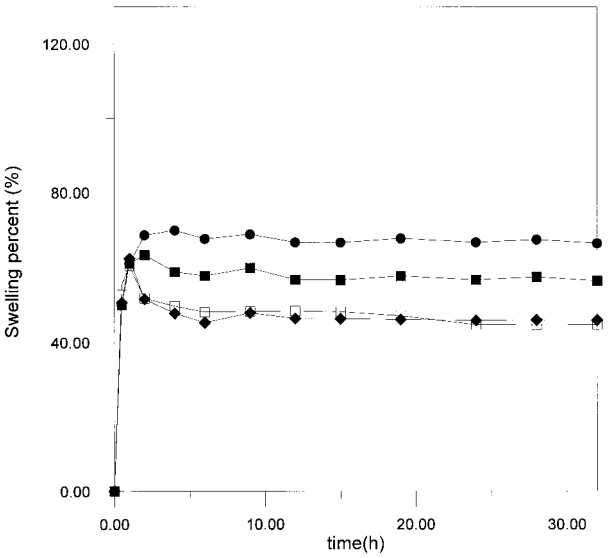


Figure 4 Swelling of chitosan–TPP gel beads in pH 6.8 medium. Chitosan–TPP beads cured in different pH TPP solutions: (●) pH 8.6 (original); (■) pH 6.0; (□) pH 4.0; (♦) pH 2.0.

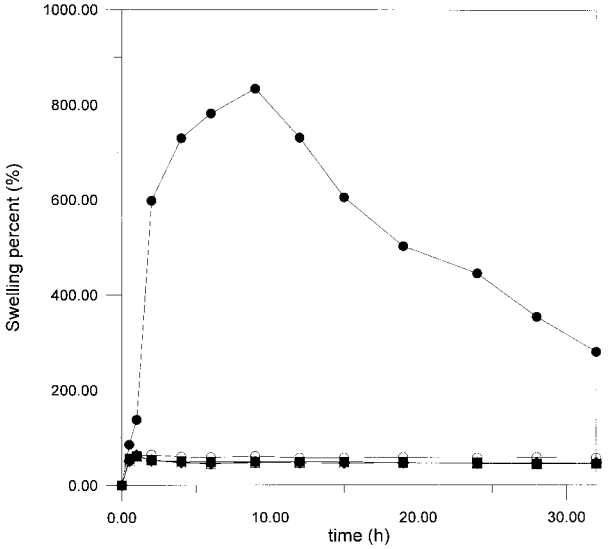


Figure 6 Swelling of chitosan–TPP gel beads in pH 1.2 medium. Chitosan–TPP beads cured in different pH TPP solutions: (●) pH 8.6 (original); (○) pH 6.0; (■) pH 4.0; (★) pH 2.0; (□) pH 1.0.

of chitosan gel beads in the media was then calculated from the formula

$$E_{sw} = [(W_e - W_0)/W_0] \times 100$$

where E_{sw} is the percent swelling of gel beads. W_e denotes the weight of the gel beads at equilibrium swelling and W_0 is the initial weight of the gel beads. Each swelling experiment was repeated three times and the average value was taken as the percentage swelling value.

IR Spectra Analysis

All the chitosan beads cured in the TPP solution (various pH values) were analyzed using a Bio-

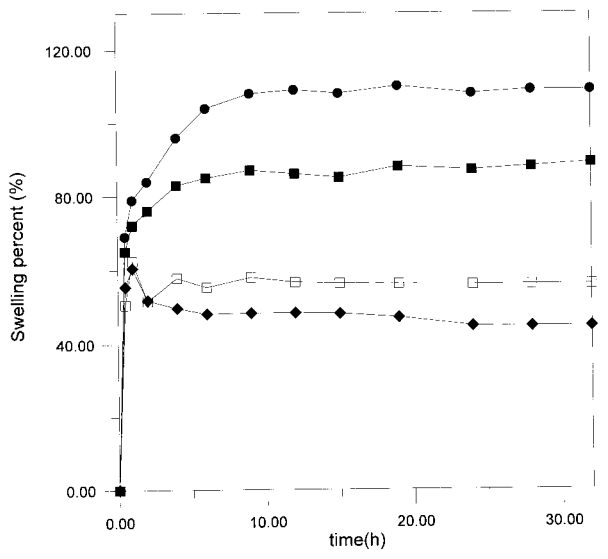


Figure 5 Swelling of chitosan–PP gel beads in pH 6.8 medium. Chitosan–PP beads cured in different pH PP solutions: (●) pH 14.0; (■) pH 10.0; (□) pH 6.0; (♦) pH 4.0.

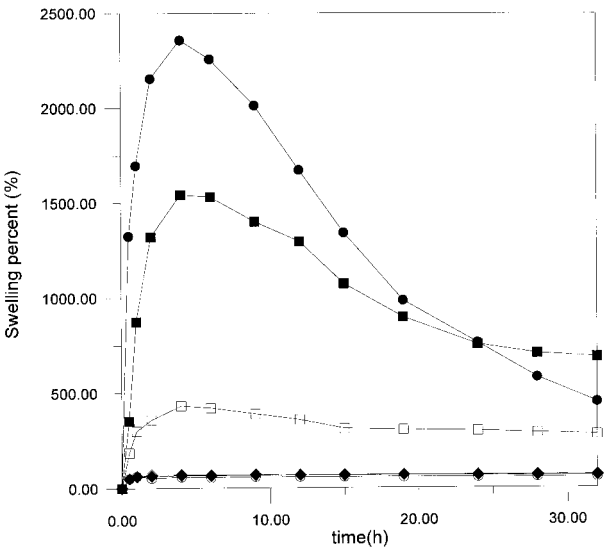


Figure 7 Swelling of chitosan–PP gel beads in pH 1.2 medium. Chitosan–PP beads cured in different pH PP solutions: (●) pH 14.0; (○) pH 12.0; (■) pH 9.0; (★) pH 6.0; (□) pH 4.0 (original).

Rad Model FTS-155 spectrophometer. The peak vibration of the P═O or P—O adsorption at 1100–1200 cm^{-1} was detected to monitor the reaction of the intermolecular complex between chitosan and TPP or PP.

Electron Scanning Microscopy

The chitosan–TPP or chitosan–PP gel beads were gold-coated to about a 500 $\times$ 10^{-8}-cm thickness using a Hitachi coating unit IB-2 coater under a high vacuum, 0.1 Torr, at high voltage, 1.2 kV and 50 mA. Coated samples were examined using a Hitachi S-2300 electron scanning microscope.

EDS Analysis of Phosphorous Distribution

The phosphorous distribution within various chitosan–TPP or chitosan–PP gel beads was estimated by EDS analysis. The chitosan–TPP gel

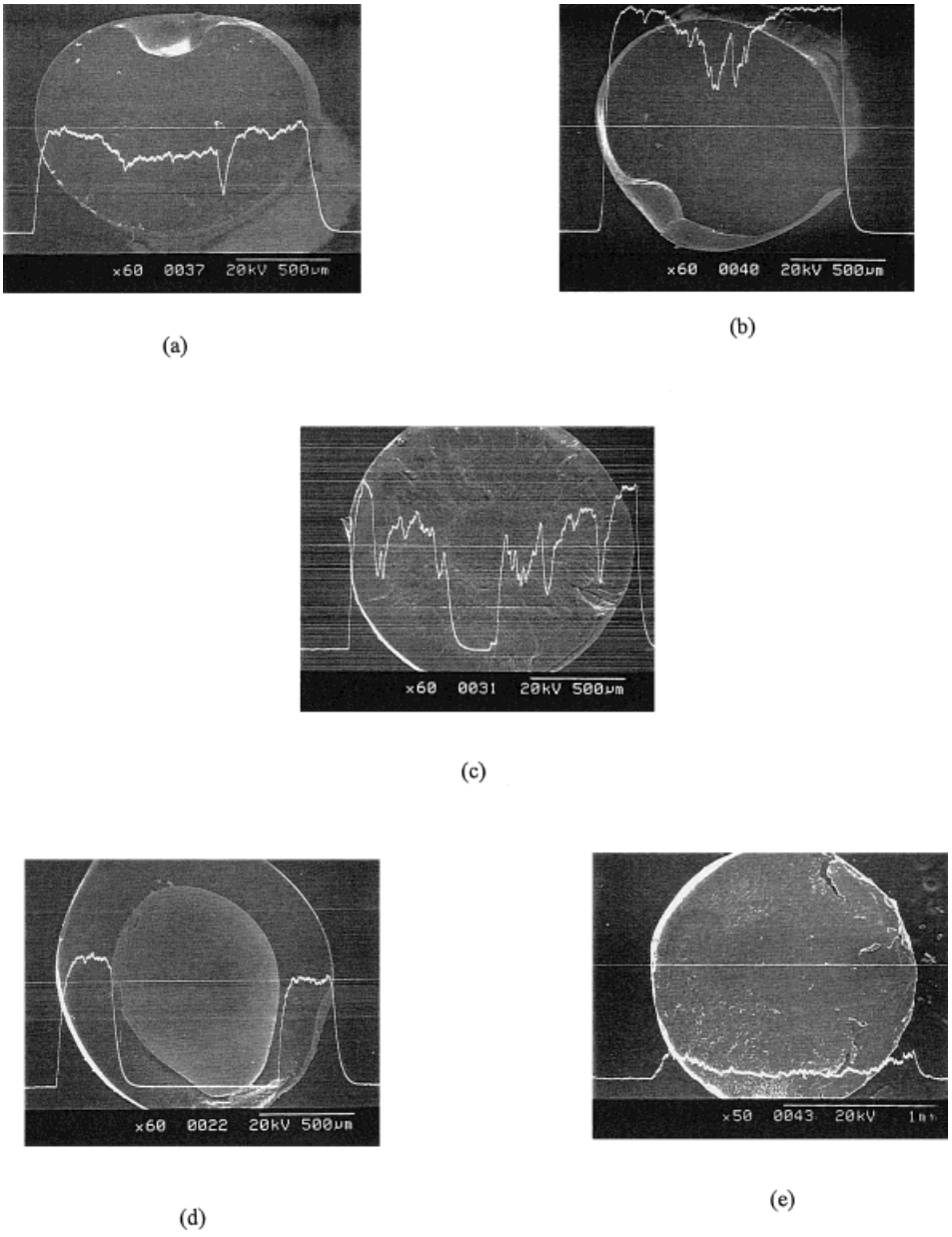


Figure 8 EDS analysis of the line profiles of phosphorous distribution in chitosan–TPP or chitosan–PP beads: (a) cured in pH 8.6 TPP for 10 min; (b) cured in pH 2.0 TPP for 10 min; (c) cured in pH 2.0 TPP for 5 min; (d) cured in pH 2.0 TPP for 2 min; (e) cured in pH 10.0 PP for 10 min.

beads were gold-coated as described previously and then the phosphorous distribution was examined using a Hitachi S-2300 electron scanning microscope with an EDS attachment (Delta Class Analyzer, Level I).

Assay of the Drug Content

Triplicate samples of 0.1 g of chitosan gel beads were placed in a mortar and triturated thoroughly. 6-MP was extracted into 150 mL of 0.1*N* HCl or the phosphate solution. After thoroughly rinsing all equipment, the whole mixture was filtered through a sintered glass suction funnel and made up to volume in a 250-mL volumetric flask. The drug was assayed using a double-beam UV spectrophotometer (Milton Roy Spectronic 3000) at 360 nm.

Dissolution Studies

The release of 6-MP from the chitosan gel beads was measured using dissolution (Hanson Research, Dissoette) and autosampling (Hanson Research, SR6) systems. The dissolution medium was a 500-mL phosphate buffer saline solution (pH 6.8) and a hydrochloric acid solution (pH 1.2) to simulate the intestinal and gastric juices. The medium was placed in a 1-L round flask fitted with a pump and an autosampler to remove the medium and stirred with a mechanical stirrer at a rate of 100 rpm. The dissolution medium tem-

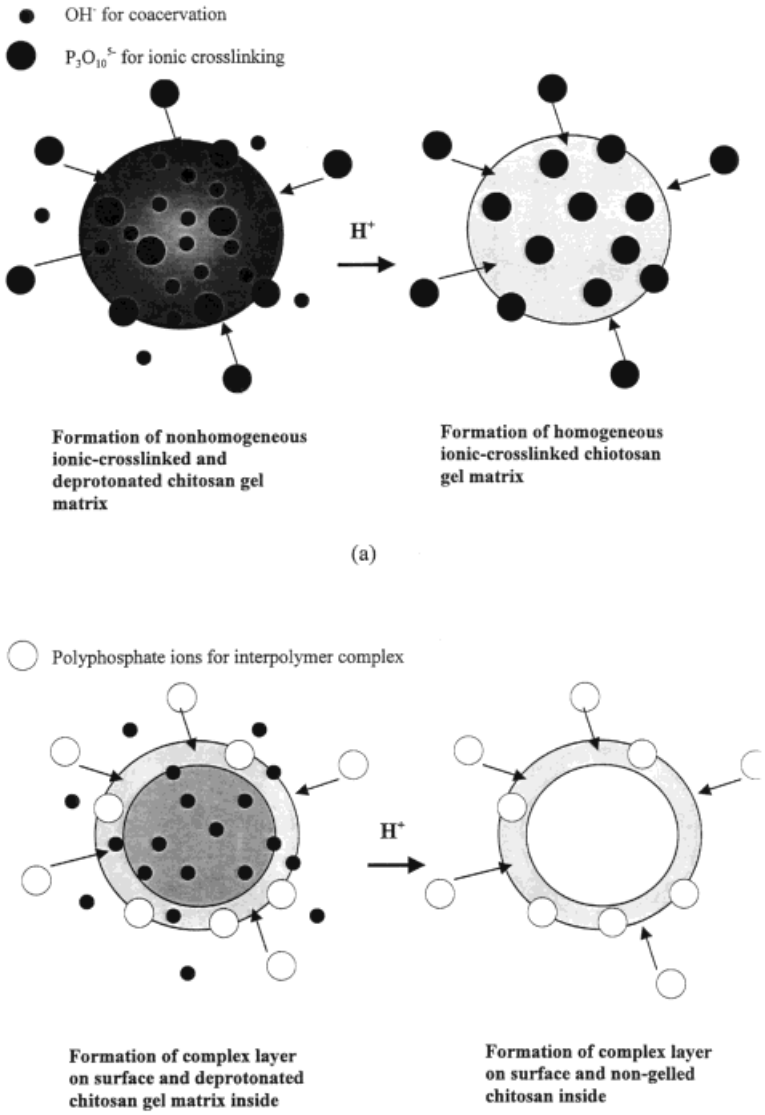


Figure 9 Transition of the in-liquid curing mechanism of (a) chitosan–TPP ionic crosslinking and (b) chitosan–PP interpolymer complex.

perature was maintained at 37°C. An equivalent quantity of 100 mg of the gel beads was dispersed in the dissolution medium. After a predetermined period, 5 mL of the medium was removed and the amount of 6-MP was analyzed spectrophotometrically at 360 nm. To keep the original volume, each time, 5 mL of the medium was replaced with fresh water.

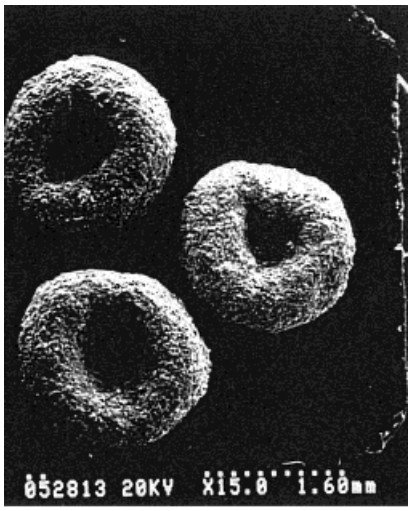
RESULTS AND DISCUSSION

Ionic Interaction Between Chitosan and TPP or PP

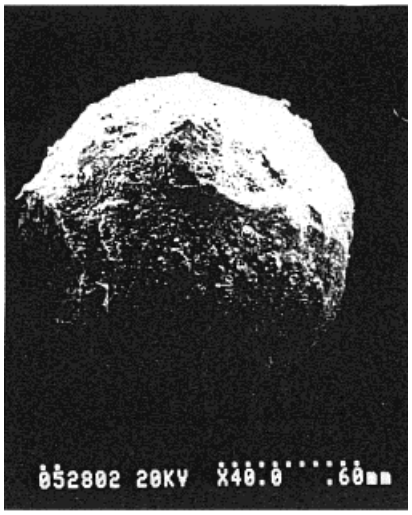
The interaction of chitosan with TPP or PP is pH-dependent. Depending on this parameter, different types of the chitosan–TPP or chitosan–PP complexes could be prepared. As described previously, the sodium TPP dissociated in water or acidic solution and released OH^- ions, so both OH^- and $P_3O_{10}^{5-}$ ions coexisted in the TPP solution. The small molecular OH^- or $P_3O_{10}^{5-}$ ions could ionically react with the bind site $—NH_3^+$ in chitosan by deprotonation or ionic crosslinking, respectively, as described previously. PP is a macromolecular polyelectrolyte which dissolves in water to form a polyanion. The anionic PP ion could ionically react with chitosan to form variant PECs depending on its pH value. The pH's of the original PP (10 wt %) and TPP solutions (10 wt %) were 4.0 and 8.6, respectively.

The chitosan solution was dropped into the PP or TPP solution and gelled spheres formed instantaneously by ionotropic gelation. In the original TPP solution (pH 8.6) or the basic PP solution, both OH^- and $P_3O_{10}^{5-}$ or PP ions in this TPP or PP solution could diffuse into chitosan droplets to react with the protonated amino group. In the original PP solution (pH 4.0) or by adjusting the pH value of the TPP solution from 8.6 (original) to lower than 7 (pH 1–6), only $P_3O_{10}^{5-}$ or PP anion ions existed in the TPP or PP solution and could diffuse into chitosan droplets to react with the protonated amine group in chitosan.

The chitosan–TPP or chitosan–PP beads prepared by curing in a different TPP or PP solution were spectrally analyzed by FTIR. As described previously, the extent of the ionic-crosslinking density or interpolymer linkage was significantly dependent on the pH value of the TPP or PP solution. Figures 1 and 2 show the IR spectra of the chitosan–TPP beads or the chitosan–PP beads prepared with different pH values of the TPP or PP solution. The presence of the P═O group of TPP or the P—O group of PP is indicated by the peak at the frequency of 1180 or 1250 cm^{-1}, respectively. By keeping the curing time at 30 min, the intensity of the P═O or P—O absorbance was enhanced along with a decreasing in pH value of the curing agent (TPP or PP solution). The increase of the intensity of the P═O or P—O absorbance indicated an increase of the bound TPP or PP ions. This result could be attributed to the increment of ionic crosslinking or interpolymer linkage with $—NH_3^+$ groups of the chitosan by TPP or PP ions. It demonstrated that the ionic reaction of the chitosan–TPP or chitosan–PP beads was significantly influenced by the pH



(a)



(b)

Figure 10 SEM pictures of chitosan–PP gel beads (a) prepared in original PP solution and (b) prepared in pH 10 PP solution.

value of the TPP or PP solution, and the ionic-crosslinking density or interpolymer linkage could be improved by the variation of the pH value of the curing agent, the TPP or PP solution.

As shown in Figure 3, it seems that a chitosan–TPP or chitosan–PP structural change occurs by varing the pH value of the curing agent, PP or TPP. In the higher pH region, chitosan may take a randomly coiled conformation because of the decrease of the ionized amino group and of a weak charge repulsion. However, in the lower pH region, the chitosan used here possibly takes a more extended form both by hydration of the protonated amino group and by a strong positive charge replusion between the the $—NH_3^+$ groups. Thus, in the higher pH range region, the chitosan–PP or chitosan–TPP complexes contain several times more chitosan repeating units than does PP or TPP, expressed as the mol ratio, and may accept the looped shape ("loop" means, in this case, PP combined with chitosan in a "slackened state"). In this state, the ionic reaction of the chitosan–PP or chitosan–TPP complex should be a pH-dependent deprotonation accompanied by a interpolymer complex or partially ionic crosslinking, although, according to the facilitation of ionizing chitosan in the lower pH range region, the chains of chitosan accept a more extended conformation and they form a complex with PP or TPP in a ladder-shaped structure. In this state, chitosan forms a complex with PP or TPP, through the formation of intermolecular or intramolecular linkages at a higher binding ratio. The gelation mechanism of the chitosan–PP or chitosan–TPP complex should be pH-dependent.

pH-Responsible Swelling of Chitosan–TPP or Chitosan–PP Beads

The intensity of the ionic crosslinking or the interpolymer complex was examined by the swelling behavior of the chitosan–TPP or chitosan–PP beads in various pH values of the aqueous media. The swelling ability of the chitosan gel beads was carried out in the media of pH 1–7. Figures 4–7 show the equilibrium swelling behavior of various kinds of chitosan–TPP or chitosan–PP beads synthesized from different binding ratios of TPP or PP to chitosan. It was indicated that the swelling ability of the chitosan–TPP or chitosan–PP beads was quite distinct according to the variant pH value of the coagulating agent, the TPP or PP solution, used. In pH 3–7 media, the ionic-crosslinked chain or interpolymer linkage of the

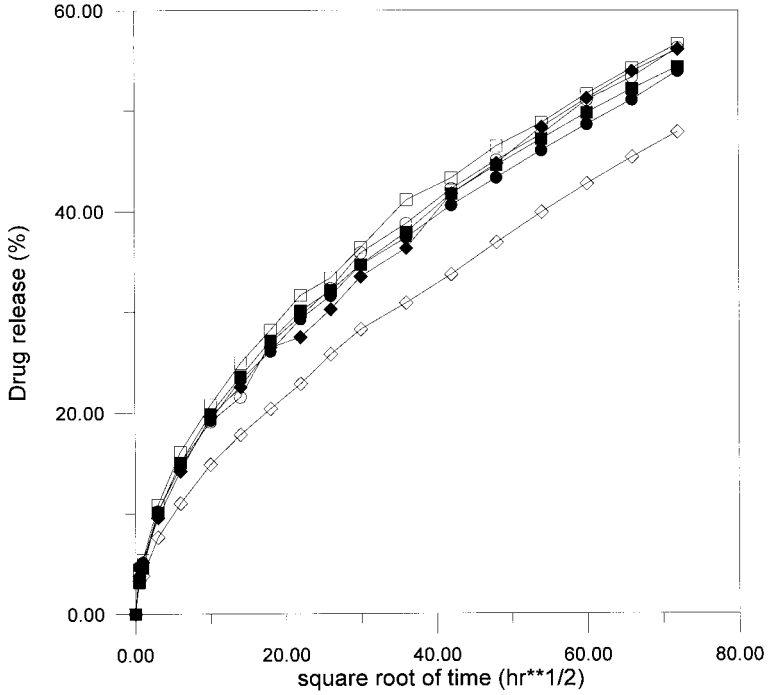


Figure 11 Effect of curing time on release profiles of 6-MP in pH 6.8 medium from chitosan–TPP gel beads. Preparative conditions: (■) cured in pH 1.0 TPP solution; (□) cured in pH 2.0 TPP solution; (●) cured in pH 3.0 TPP solution; (○) cured in pH 4.0 TPP solution; (♦) cured in pH 6.0 TPP solution; (◇) cured in pH 8.6 TPP solution.

chitosan–TPP or chitosan–PP beads did not dissociate, so the swelling of the chitosan–TPP or chitosan–PP beads in this medium would be attributed to the hydration or ionization of unreacted —NH_2 sites in the chitosan. The increase of ionic crosslinking or the interpolymer complex of chitosan–TPP or chitosan–PP reduced the unreacted —NH_2 sites in chitosan and resulted in decrease of the swelling degree of the chitosan gel beads. The swelling percents of the higher ionic-crosslinked chitosan–TPP beads (chitosan beads prepared in pH lower than 6 of the TPP solution) and the lower ionic-crosslinked ones (chitosan beads prepared in pH 8.6 of the original TPP solution) were 50 and 60%, respectively (Fig. 4). The swelling percent of the chitosan–PP beads is slightly higher than that of the chitosan–TPP beads (Fig. 5). When the pH value of the dissolution medium was decreased from pH 3 to pH 1–2, the swelling degree of the chitosan beads significantly decreased with increase of the ionic-crosslinking density or the interpolymer linkage of the beads.

By reducing the pH value of the coagulating agent, the TPP solution, from the original pH (8.6) to lower than 6 increased the ionic crosslinking density, resulting in a reducing effect of the swelling of the chitosan beads (Fig. 6). Moreover, increasing the pH value of the coagulating agent, the PP solution, from pristine (pH 4.0) to higher than 9 decreased the degree of interpolymer linkage and led to the enhancing effect of the swelling of the chitosan beads (Fig. 7). In pH 1–2 medium, the swelling of the chitosan–TPP beads was dominated by the dissociation of the interchain linkage of —NH_3^+—$P_3O_{10}^{5-}$. The chitosan gel beads prepared in the original TPP solution (pH 8.6) swelled quickly and gradually dissolved within 12 h due to the scission of interchain linkage, whereas the chitosan gel beads obtained in the acidic TPP solution only slightly swelled but did not dissolve in the pH 1–2 media because only slight chain-scission occurred in the higher crosslinked chitosan–TPP complex. It could be attributed to the more stable structure of this chitosan–TPP complex due to its high degree of interchain linkages. The chitosan–PP gel beads were swollen but less disintegrated in the acidic medium because of the very high stability of the interpolymer complex network. This result indicated that the higher ionic crosslinking or interpolymer linkage of the chitosan–TPP or chitosan–PP beads had a lower degree of chain scission than that of the slightly ionic-crosslinked

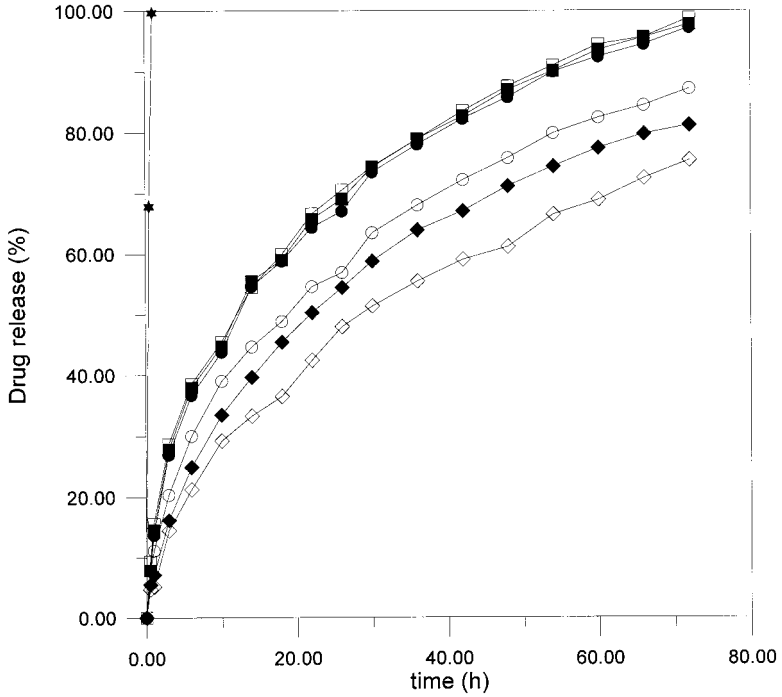


Figure 12 Effect of curing time on release profiles of 6-MP in pH 6.8 medium from chitosan–PP gel beads. Preparative conditions: (■) cured in pH 4.0 PP solution; (□) cured in pH 6.0 PP solution; (●) cured in pH 8.0 PP solution; (○) cured in pH 10.0 PP solution; (♦) cured in pH 12.0 PP solution; (◇) cured in pH 14.0 PP solution.

chitosan–TPP beads in acidic media. This result confirms our idea that the ionic-crosslinking density or the interpolymer complex characteristic of the chitosan–TPP or chitosan–PP beads could be improved by being modified by the in-liquid curing mechanism of the chitosan gel beads.

EDS Analysis of Phosphorous Distribution

Figure 8 shows an EDS analysis of the line profiles of the phosphorous distribution on the cross section of various chitosan–TPP or chitosan–PP beads which were prepared in different pH values of the TPP or PP solution. The line profile of phosphorus on the cross section of the chitosan beads that gelled in the original TPP solution (pH 8.6) has no significant variation with the radial direction, whereas the signal of phosphorus decreased with decreasing of the radial distance away from core for the chitosan beads gelled in the acidic TPP solution (pH 2.0). Besides, the signal significantly increased with increasing of the gelling time of the chitosan–TPP beads prepared in this acidic TPP solution. This result, as well as the FTIR studies, suggested that the ionic interaction between chitosan and the negatively charged counterion, TPP, was obviously dependent on the pH value of the TPP solution. According to the the line profiles of the phosphorous distribution in the chitosan–TPP beads, the in-liquid curing mechanism of the chitosan–TPP beads could approach the heterogeneous fluid–solid reaction of the unreacted core model. In the raw TPP solution, the OH^- ions competed with the $P_3O_{10}^{5-}$ ions to react with the protonated amino group of chitosan on the surface of the beads as soon as the chitosan droplets contacted the TPP solution. After the formation of a gelled outer layer, the resistance for the larger $P_3O_{10}^{5-}$ ions to diffuse through the gelled film into the inside matrix was higher than that of the OH^- ions to diffuse into the beads, due to the smaller molecular size of the OH^- ions. So, the gelled mechanism of the chitosan beads was mainly dependent on the coacervation-phase inversion because of the neutralization of the protonated amino group of the chitosan accompanied by slightly ionic crosslinking [Fig. 9(a)], whereas there were no OH^- ions in the TPP solution at a pH value lower than 7. The gelation of the chitosan beads was fully controlled by ionic crosslinking throughout the beads due to the formation of intermolecular or intramolecular linkages between the linear chitosan chain by the multivalent anion, TPP [Fig. 9(a)].

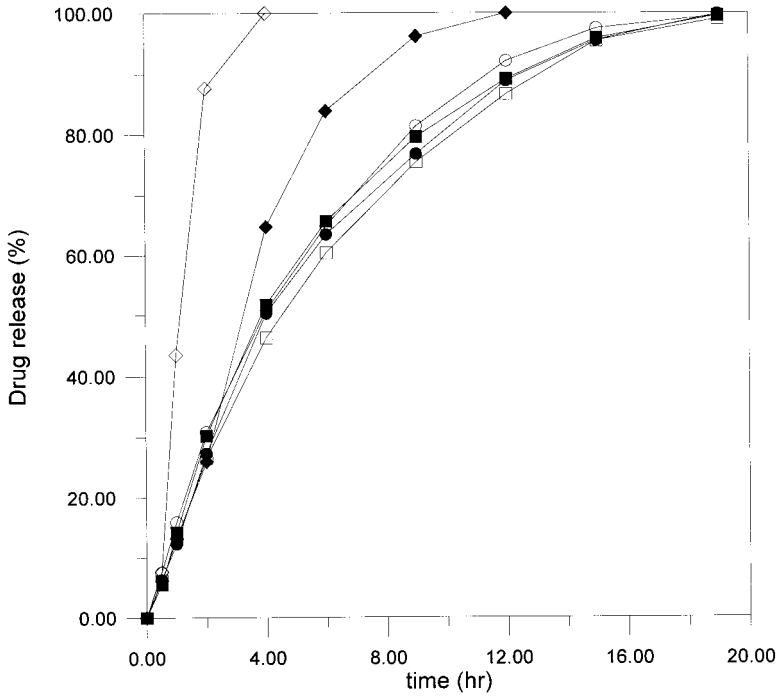


Figure 13 Effect of curing time on release profiles of 6-MP in pH 1.2 medium from chitosan–TPP gel beads. Preparative conditions: (■) cured in pH 1.0 TPP solution; (□) cured in pH 2.0 TPP solution; (●) cured in pH 3.0 TPP solution; (○) cured in pH 4.0 TPP solution; (♦) cured in pH 6.0 TPP solution; (◇) cured in pH 8.6 TPP solution.

According to this theory, in our study, the crosslinking density of the chitosan–TPP gel beads could be improved by the modification of the in-liquid curing mechanism. The chitosan–PP beads (prepared in original pH 4.0 PP solution) were disklike after being dried in air or an oven, whereas the beads prepared in higher pH of the PP solution (pH higher than 10) were spherical. The marcomolecular PP (pH 4.0) could only react with the chitosan droplet on surface but not easily diffuse into the gel matrix. The rigid chitosan–PP complex layer was formed only on the surface of the chitosan droplets [Fig. 9(b)]. After being dried in air or an oven, the sperical gel beads collapsed to form a disklike structure due to the nongelled inside matrix [Fig. 10(a)]. In the higher pH PP solution, OH^- ions could diffuse into the chitosan droplets to gel the inside matrix. The thus-formed chitosan gel bead has a chitosan–PP complex skin and a gelled chitosan matrix inside, so the chitosan–PP beads could remain spherical after drying [Fig. 10(b)]. Due to this reason, the low signal intensity of phosphorus could only be found on the outer layer of the chitosan–PP beads [Fig. 8(e)].

Kinetics of Drug Release from Chitosan Beads

The chitosan gel beads demonstrated significantly different drug-release behavior in pH 6.8 and 1.2 media. In pH 6.8 medium, the chitosan gel matrices are considered to be an inert matrix, because the chitosan gel was not swollen or dissolved in this medium during the experiment. Figures 11 and 12 show the release profiles of 6-MP from the chitosan–TPP or chitosan–PP beads in pH 6.8 medium. The release rates of 6-MP from the chitosan beads were significantly lower than those of 6-MP alone, which were dependent on the gelling mechanism of the beads. According to our description, chitosan droplets gelled in the TPP or PP solution of a pH value lower than 6 was fully controlled by ionic crosslinking or the interpolymer complex, respectively, whereas chitosan droplets gelled in the TPP or PP solution of a pH value higher than 7 only led to a pH-induced coacervation of chitosan beads accompanied with partially ionic crosslinking or an interpolymer complex. Chitosan beads prepared by this deprotonation-induced coacervation-phase separation led to increases of tortuosity and decrease of the porosity of the gel beads as compared to chitosan beads prepared by a fully ionic crosslinking mechanism. These effects decreased the release rate of 6-MP from the chitosan–TPP or chitosan–PP gel beads in the simulated intestinal fluid (pH 6.8).

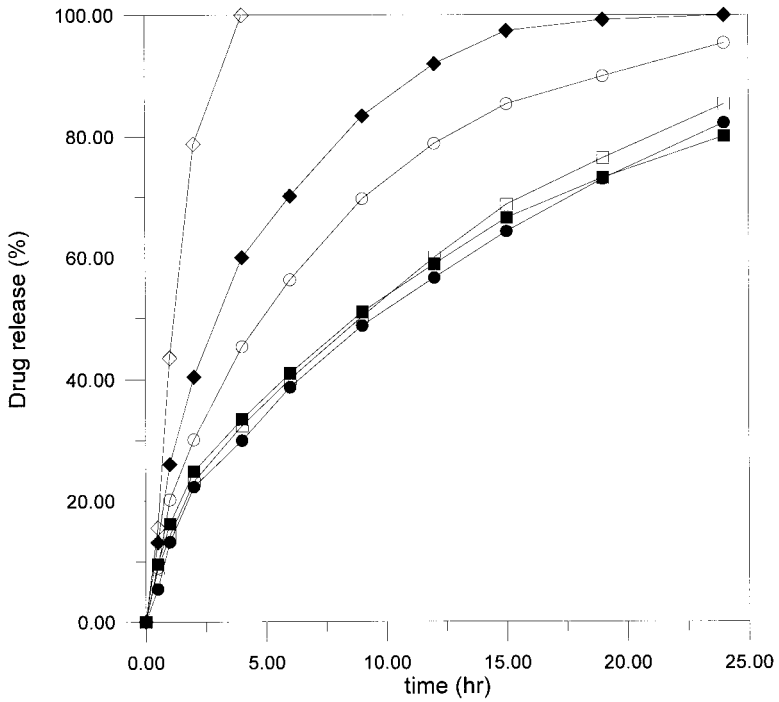


Figure 14 Effect of curing time on release profiles of 6-MP in pH 1.2 medium from chitosan–PP gel beads Preparative conditions: (■) cured in pH 4.0 PP solution; (□) cured in pH 6.0 PP solution; (●) cured in pH 8.0 PP solution; (○) cured in pH 10.0 PP solution; (♦) cured in pH 12.0 PP solution; (◇) cured in pH 14.0 PP solution.

In pH 1.2 medium, the chitosan gel could be swollen and might be gradually dissolved in this medium during experiment. Figures 13 and 14 show the release profiles of 6-MP from the chitosan–TPP or chitosan–PP beads in the pH 1.2 medium, which were much faster than the release profiles of 6-MP in the pH 6.8 medium. In general, the degree of swelling increased with decrease of the crosslinking density. In the simulated gastric fluid (pH 1.2), the release profiles of 6-MP from the chitosan beads gelled in the acidic TPP solution were much slower than those of the chitosan beads gelled in the basic TPP solution. This result indicated that the chitosan gel beads prepared under a fully ionic-crosslinking-controlled mechanism have a better drug-release retardation ability than that of the beads prepared by a partially ionic-crosslinking-controlled mechanism when the beads dissolved in the simulated gastric fluid. This could be attributed to the acid nonresistant ability of the chitosan–TPP beads prepared by a partially ionic-crosslinking-controlled mechanism and would be quickly swollen or disintegrated in the simulated gastric fluid as described in the swelling studies. The characteristics of the release of 6-MP from the chitosan–PP gel beads were also pH-dependent. The chitosan gel beads prepared in the basic PP (pH higher than 9) could be swollen in the acidic medium significantly, resulting in a quicker drug-release rate. As described previously, the variation of the ladder-loop structure of the chitosan–PP interpolymer complex was dependent on the pH value of the coagulating solution, the PP solution, used. The increased pH value of the PP solution decreased the PP/chitosan binding ratio of the chitosan–PP complex, resulting in a quicker drug-release rate that was due to the easily swelling of the chitosan–PP complex.

To examine the sustained-release properties of the drug delivery of chitosan–TPP or chitosan–PP beads during GI tract transition, the drug-release profile from the chitosan beads containing 6-MP was simulated by the shift dissolution test method (Fig. 15). The release of 6-MP from the lower binding ratio of the ionic-crosslinked chitosan–TPP beads or the interpolymer complex chitosan–PP beads was rapid with the acidic medium (pH 1.2) and slow with the neutral medium (pH 6.8). However, the sustained release ability of the higher binding ratio of the ionic-crosslinked chitosan–TPP beads or the interpolymer complex of the chitosan–PP beads was kept throughout the pH-shift dissolution test. These results suggest that the higher binding ratio of the ionic-

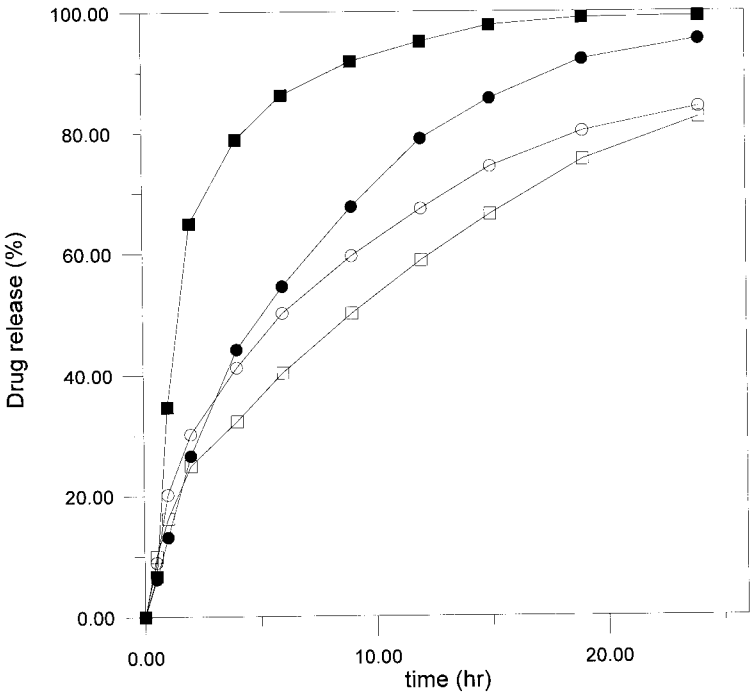


Figure 15 Release profiles of 6-MP from chitosan–TPP or chitosan–PP gel beads in pH 1.2 dissolution medium for 3 h followed by pH 6.8 medium. Preparative conditions: (■) cured in pH 8.6 (original) TPP solution; (□) cured in pH 4.0 (original) PP solution; (●) cured in pH 9.0 PP solution; (○) cured in pH 4.0 TPP solution.

crosslinked chitosan–TPP beads or the interpolymer complex chitosan–PP beads containing 6-MP might have potential as a dosage form for the oral delivery of an anticancer drug, 6-MP.

CONCLUSIONS

The in-liquid curing mechanism of chitosan beads gelling in the TPP or PP solution could be easily modified from a lower to higher binding ratio of ionic crosslinking or the interpolymer complex only by adjusting the pH value of the TPP solution. The swelling behavior of various chitosan beads in acid appeared to depend on the in-liquid curing mechanism of the beads. A higher binding ratio of ionic-crosslinked chitosan–TPP beads or the interpolymer complex chitosan–PP beads have less swelling ability due to their high stabiltiy in acid. In this study, the ionic-crosslinking density or interpolymer complex could be controlled by modifing the in-liquid curing mechanism and the material properties of the chitosan gel beads might be improved. The releasing behavior of the higher binding ratio of the ionic-crosslinked chitosan–TPP beads or the interpolymer complex chitosan–PP beads in the pH 6.8 medium seem to be diffusion-controlled, whereas in the pH 1.2 medium, the release behavior exhibited a chain-relaxation swelling control. Nevertheless, another major factor controlling the release rate in both media is therefore considered to be the gelling mechanism and the binding ratio of the ionic crosslinking or interpolymer complex of the chitosan–TPP or chitosan–PP gel beads.

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