# Chitosan–Polyelectrolyte Complexation for the Preparation of Gel Beads and Controlled Release of Anticancer Drug. I. Effect of Phosphorous Polyelectrolyte Complex and Enzymatic Hydrolysis of Polymer

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**ABSTRACT:** Enzymic hydrolyzed chitosan was employed to prepare chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel beads using a polyelectrolyte complexation method for the sustained-release of anticancer agent, 6-mercaptopurine (6-MP). pH responsive swelling ability, drug-release characteristics, and morphology of the chitosan gel bead depends on polyelectrolyte complexation mechanism and molecular weight of the enzymic hydrolyzed chitosan. The complexation mechanism of chitosan beads gelled in pentasodium tripolyphosphate or polyphosphoric acid solution was ionotropic crosslinking or interpolymer complex, respectively. The drug-release patterns of all chitosan gel beads in pH 6.8 seemed to be diffusional based, which might be in accordance with the Higuchi model, whereas release profiles of the chitosan–tripolyphosphate gel beads in pH 1.2 medium seemed to be non-Fickian diffusion controlled due to the swelling or matrix erosion of the beads. The rate of 6-MP releasing from chitosan–tripolyphosphate or chitosan–polyphosphoric acid gel matrix were significantly increased with the decreased molecular weight of enzymic hydrolyzed chitosan. However, the dissolution rates of 6-MP entraped in chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel matrix were significantly slower than the dissolution rate of the original drug. These results indicate that the chitosan–polyphosphoric acid gel bead is a better polymer carrier for the sustained release of anticancer drugs in simulated intestinal and gastric juice medium than the chitosan–tripolyphosphate gel beads. © 1999 John Wiley & Sons, Inc. J Appl Polym Sci 74: 1868–1879, 1999

**Key words:** enzymic hydrolyzed chitosan; chitosan–tripolyphosphate; chitosan–polyphosphoric acid gel beads; 6-MP

## INTRODUCTION

An anticancer agent with an elimination half-life may result in a decrease of the therapeutic potential and present such side effects as severe bone marrow depression and gastrointestinal damage. One of the possible approaches for overcoming these disadvantages and improving chemotherapeutic activity is the method using macromoleculars as drug carriers. During the last decade, the incorporation of anticancer drugs into a variety of polymeric drug delivery systems has become popular for the preparation of sustained release dosage forms.[1–5]

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Chitosan, the deacetylated derivative of biopolymer "chitin," is a natural polycationic polymer that has received much attention in the pharmaceutical and biomedical fields, owing to its good biocompatibility and low toxicity.[6,7] Chitosan is a copolymer of D-glucosamine and *N*-acetyl-D-glucosamine, which could be hydrolyzed gradually by glycosidases such as lysozyme.[8–12] Also, chitosan is one of the biodegradable polymers. The glucosamine units of chitosan could be transformed into the uncoiled and positively charged water-soluble form in acid medium. Due to its acid nonresistance, chitosan dissolved quickly after swollen in gastric juice and could not achieve sustained release ability in the GI tract expect for crosslinking using chemical reagents or complexes using counterpolyions. Recently, chitosan has been developed to be employed as an agent for the preparation of microspheres or gel beads. Chitosan microspheres crosslinked by glutaraldehyde was prepared for the sustained release of cisplatin and 5-fluorouracil.[13–16] However, the use of glutaraldehyde is undesirable because of its toxicity. Chitosan has also been used for the preparation of nontoxic polyelectrolyte–complex products with natural polyanions such as carboxymethylcellulose, alginic acid, dextran sulfate, heparin, xanthan etc., for the immobilization and release of drugs or proteins.[17–24] The polyelectrolyte complex was prepared by the formation of complexes from the interaction of oppositely charged polyelectrolytes. The mechanism of polyelectrolyte complexes can be varied by changing the chemical structure of component polymers, such as molecular weight, flexibility, functional group structure, charge density, stereoregularity, and compatibility, as well as reaction conditions: pH, ionic strength, concentration, mixing ratio, and temperature.[25–28]

The primary purpose of the present study was to develop a nontoxic, interpolymer complex or ionic-crosslinked chitosan gel bead by polyelectrolyte complex methods for the improvement of drug release properties. Swelling ability, morphology, and drug release characteristics of chitosan–polyelectrolyte gel beads all depended on the molecular weights of enzymic hydrolyzed chitosan and the mechanism of polyelectrolyte complexation using pentasodium tripolyphosphate or polyphosphoric acid. Ionotropic network formation due to crosslinking of polyionic chains of chitosan with multivalent counter ions, tripolyphosphate, or an interpolymer complex of chitosan with polyphosphoric acid could be used to obtain a spherical matrix by the method of an in-liquid curing process. By modifying the chitosan–polyelectrolyte complex, prolonged release of 6-MP from chitosan–polyphosphoric acid or chitosan–tripolyphosphate gel beads without being further crosslinked by glutaraldehyde could be achieved both in simulated intestinal and gastric medium. This study presents the result of application of chitosan–tripolyphosphate or chitosan–polyphosphoric acid beads as swelling-controlled or diffusional-controlled release of 6-MP in simulated gastric (pH 1.2) or intestinal fluids (pH 6.8) solution. The effects of molecular weight of enzymic hydrolyzed chitosan on drug release were also investigated.

## EXPERIMENTAL

### Materials

Chitosan, with various weight-average molecular weights (70,000, 750,000, and 2,000,000), was obtained from Fluka (Switzweland), which are marked as M70,000, M750,000, and M2,000,000, respectively. Hen egg-white lysozyme (Sigma, St. Louis, MO), 50,000 unit/mg, was a commerical product and used without further purification 6-MP, buffer saline tablets, polyphosphoric acid, and pentasodium tripolyphosphate were all purchased from Sigma. The degree of deacetylation were determined by elemental analysis and the titration method, which were about 86% on a molar basis for chitin. All other materials were of reagent-grade purity.

### Enzymic Hydrolysis of Chitosan

The rate of lysozyme hydrolysis at 37°C was measured by viscometric procedures. A Ubbelohde type viscometer was used for the viscosity measurement of chitosan at 37°C according to the method of Funatsu. A lysozyme solution (1.0 mL, 10,000 units/mL) was added to chitosan solution (19 mL) for digestion, and the mixture (with 500 units/mL of lysozyme) was incubated at 37°C with mechanical shaking. Then the relative viscosities were measured at various time intervals. The enzymic hydrolyzed chitosan was heated at 60°C for 60 min to denature the lysozyme for future use.

### Synthesis of Chitosan–Tripolyphosphate and Chitosan–Polyphosphoric Acid Gel Beads

Chitosan (15 g) was dissolved in 500 mL dilute acetic acid (1% v/v) to prepare the chitosan solution. The chitosan solution was enzymic hydro-

lyzed using lysozyme, as described previously, to decrease the molecular weight. Chitosan or enzymic hydrolyzed chitosan were dropping into a gently agitated pentasodium tripolyphosphate solution (10% w/v) or polyphosphoric acid solution (10 wt %) and stood in the solution for 2–30 min. The pH value of tripolyphosphate and polyphosphoric acid solutions were pH 8.6 and pH 4.0, respectively. The solidified beads were filtered and washed with 100 mL of deionized water repeatly, then dried in a vacuum oven at 40°C for 4 h. Drug incorporated beads were prepared by dispersed 6-MP (0.2 g) in 10 mL of chitosan solution before dropping into the tripolyphosphate or polyphosphoric acid solution. The final products were stored in a desiccator for future analysis.

## IR Spectra Analysis

The chitosan–tripolyphosphate and chitosan–polyphosphoric acid beads were analyzed using Bio-rad model FTS-155 spectraphotometer. The peak viration of P═O or P—O adsorption at 1100–1200 $cm^{-1}$ was detected to monitor the reaction of intermolecular complex between chitosan and pentasodium tripolyphosphate or polyphosphoric acid.

## Electron Scanning Microscopy

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

## EDS Analysis of Phosphorous Distribution

The phosphorous distribution in chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel beads were analyzed by EDS analysis. The chitosan–TPP gel beads were gold coated as described previously, and then phosphorous distribution was examined using Hitachi S-2300 electron scanning microscopy with an attachment of EDS (Delta Class Analyzer, Level I).

## Equilibrium Swelling Studies

The water sorption capacity of chitosan–tripolyphosphate or chitosan–polyphosphoric acid gel beads was determined by swelling the gel beads in media of pH 1.0–7.0 at room temperature, respectively. A known weight (200 mg) of various chitosan gel beads without the drug were placed in the media for the required period of time. The wet weight of the swollen beads were determined by first blotting the beads with filter paper to remove adsorbed water on the surface and then weighed immediately on an electronic balance. The percent of swelling of chitosan gel beads in the media was then calculated from the formula:

$$E_{SW} = [(W_e - W_o)/W_o] \times 100$$

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

## 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 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 6-MP was assayed by using a double-beam UV spectrophotometer (Milton roy spectronic 3000) at 360 nm.

## Dissolution Studies

The release of 6-MP from chitosan gel beads was measured by using the dissolution (Hanson research, Dissoette II) and autosampling (Hanson research, SR6) systems. The dissolution medium was 500 mL phosphate buffer saline solution (pH 6.8) and hydrochloric acid solution (pH 1.2) to simulate the intestinal and gastric juice. The medium placed in a 1-liter round flask fitted with a pump for autosampler to remove the medium, and stirred with a mechanical stirred at a rate of 100 rpm. The dissolution medium temperature was maintained at 37°C. An equivalent quantity of 100-mg 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 spectrophotomerically at 360 nm. To maintain the original volume, each time 5 mL of the medium was replaced with fresh water.

# RESULTS AND DISCUSSION

## Enzymic Hydrolysis of Chitosan

The enzymic hydrolysis of chitosan was studied by plotting viscosity vs. time. Figure 1 shows the

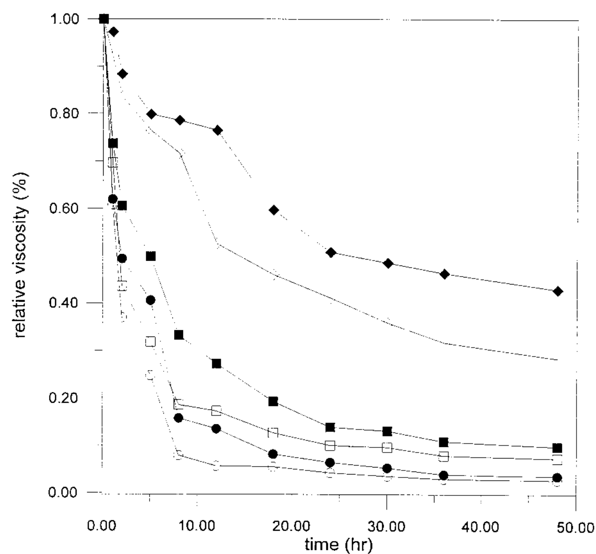



**Figure 1** Time-dependent decrease of relative viscosities (the viscosity of hydrolyzed chitosan/original viscosity of chitosan) of the enzymic hydrolyzed chitosan. ■ M2,000,000 (500 U/mL), □ M2,000,000 (1000 U/mL), ● M750,000 (500 U/mL), ○ M750,000 (1000 U/mL), ◆ M70,000 (500 U/mL), ◇ M70,000 (1000 U/mL).

digestibility of various chitosan. Chitosan were hydrolyzed by lysozyme, and the relative viscosities (the viscosity of hydrolyzed chitosan/original viscosity of chitosan) of the lysozymic hydrolysis decreased significantly. Viscosity-average molecular weight of enzymic hydrolyzed chitosan were determined by the Mark-Houwink equation as follows:[29]

$$[\eta] = K[M_v]^{\alpha}$$

$$K = 1.64 \times 10^{-30}[D]^{14.0}$$

$$\alpha = -1.02 \times 10^{-2}[D] + 1.82$$

where $[\eta]$ is the intrinsic viscosity, $M_v$ is the viscosity-average molecular weight, and $D$ is the deacetylation degree of chitosan. As shown in Table I, the viscosity-average molecular weight of chitosans were successfully reduced by digestion using lysozyme within 48 h. The lysozyme had less effect on M70,000 chitosan, which has the same deacetylation degree as M750,000 and M2,000,000 chitosan. Chitosan is a random type copolymer of *N*-acetyl-D-glucosamine and D-glucosamine. The lower digestibility of M70,000 may be due to the deficiency in block types of the *N*-acetyl-D-glucosamine sequences in chitosan. It was proven that lysozyme could only act on *N*-acetyl-D-glucosamine sequences with more than three residues.[10,11] Lysozyme could not act on the D-glucosamine sequence and segments in which relatively small fractions of *N*-acetyl-D-glucosamine residues are randomly distributed. It was also reported that the active site of lysozyme consists of six subsites, A–F, which bind the *N*-acetylglucosamine residues of chitin.[8] Alternate sites interact with the acetamide side chains, and the *N*-acetyl group of the residues located at subsites C and E must be intact for enzymic hydrolysis to occur. Thus, if the deacetylation of chitin occurs at random, the probability of finding six contiguous residues with the necessary acetamido side groups is dependent on the conformation of deacetylated chitin. Accordingly, if the *N*-acetyl-D-glucosamine residues in M70,000 chitosan are randomly distributed but not in sequence, the enzymic hydrolysis rate would be reduced significantly.

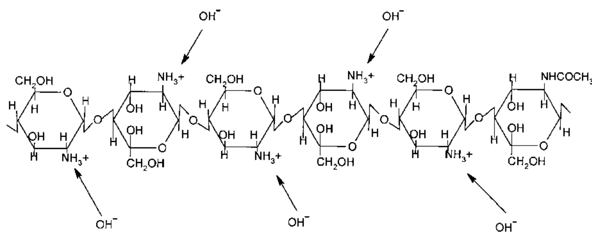
## Synthesis of Chitosan–Tripolyphosphate and Chitosan–Polyphosphoric Acid Gel Beads

Chitosan, the polycationic polysaccharide, forms gel with multivalent counterions through the for-

**Table I Time-Dependent Decrease of Viscosity-Average Molecular Weight ($M_v$) of the Enzymic Hydrolyzed Chitosan**

| Hydrolysis Time (h) | Lysozyme Concentration | | | | | |
|---|---|---|---|---|---|---|
| | 500 (U/mL) | | | 1000 (U/mL) | | |
| 0 | 6.3[a] | 64.7[b] | 185.6[c] | 6.3[a] | 64.7[b] | 185.6[c] |
| 6 | 6.0 | 21.4 | 67.8 | 5.7 | 20.6 | 32.1 |
| 12 | 5.4 | 12.7 | 43.4 | 4.6 | 6.6 | 15.7 |
| 18 | 4.8 | 0.9 | 11.0 | 3.9 | 0.6 | 3.9 |

[a] M70,000, b: M750,000, c: M2,000,000
[b] $M_v$: ×10,000
[c] $[\eta] = K[M_v]^n$; $K_a = 2.261 \times 10^{-3}$, $K_b = 2.153 \times 10^{-3}$, $K_c = 1.860 \times 10^{-3}$; $n_a = 0.9346$, $n_b = 0.9377$, $n_c = 0.9469$.



(a) Deprotonation

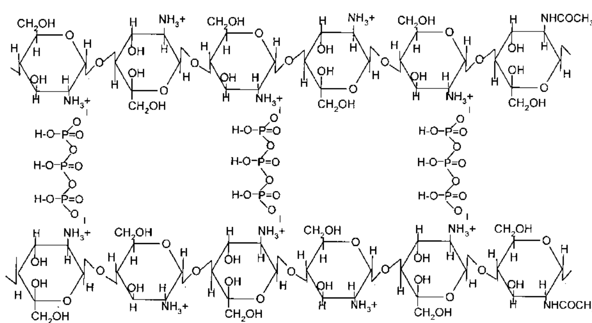


**Figure 2** Ionic interaction of chitosan in pentasodium tripolyphosphate aqueous (a) deprotonation, (b) ionic crosslinking.

mation of intermolecular or intramolecular linkages by ionic interaction. Chitosan gel beads were prepared by the ionic interaction between a positively charged amino group and negatively charged counterion, tripolyphosphate, and polyphosphoric acid. The small molecule polyelectrolyte, penatsodium tripolyphosphate, dissociated in water and release out $OH^-$ ions, so, both $OH^-$ and $P_3O_{10}^{5-}$ ions coexisted in the tripolyphosphate solution. The $OH^-$ or $P_3O_{10}^{5-}$ ions could ionicly react with the binding site —$NH_3^+$ in chitosan by deprotonation or ionic crosslinking, respectively (Fig. 2). Chitosan solution was dropped into tripolyphosphate solution and gelled spheres formed instantaneously by ionotropic gelation. The pH values of chitosan solution (1.5 w/w % in 0.5 v/v % acetic acid) and of 10% tripolyphosphate solution were 4.2 and 8.6, respectively. When the gelation of chitosan beads occurred, the acetic acid within the chitosan droplets were neutralized rapidly by $OH^-$, which diffused out to the droplets. Accompained with gelling through the beads was the diffusion of acetic acid into the external phase. The pH indicators methyl red or bromothymol

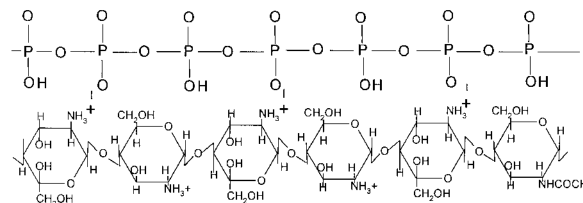


**Figure 3** Ionic interaction of chitosan in polyphosphoric acid aqueous interpolymer complex.

blue were added to the chitosan solution prior to bead formation to detect time-dependent pH changes within the beads during the preparation. The pH value inside the beads gradually changed from pH 4.2 through neutral pH values to basic pH values for complete gelling across the beads. These results show that the precipitations of complexes in this state were formed both by deprotonated and ionic crosslinking. Both dissociated $OH^-$ and $P_3O_{10}^{5-}$ ions in tripolyphosphate solution could diffuse into chitosan droplets during gelling in tripolyphosphate solution. The macromolecule polyelectrolyte, polyphosphoric acid, reacted with chitosan through an interpolymer complex between —$[P_2O_5^{4-}]$— and —$NH_3^+$ groups to form three-dimensional interpenetrating interpolymer complex networks (Fig. 3). The pH values of the phosphoric acid solution were 4.0, so deprotonation would not occur in this complexation reaction. Figure 4 showd the IR spectra of chitosan gel

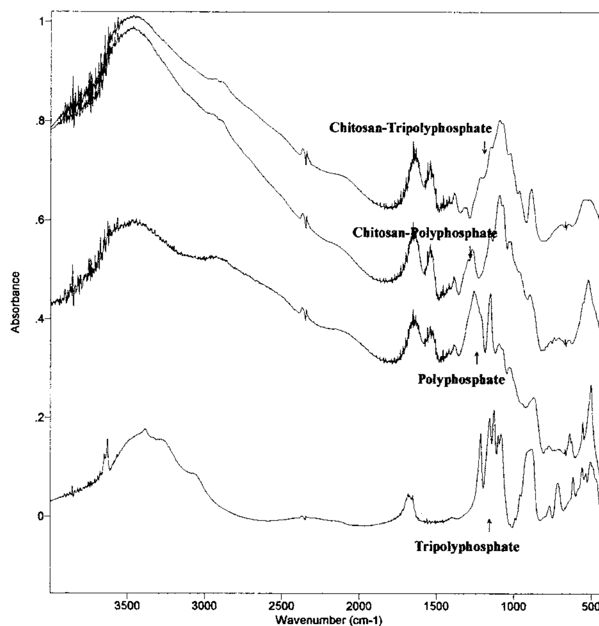



**Figure 4** IR spectra of chitosan–tripolyphosphate and chitosan–polyphosphoric acid beads.

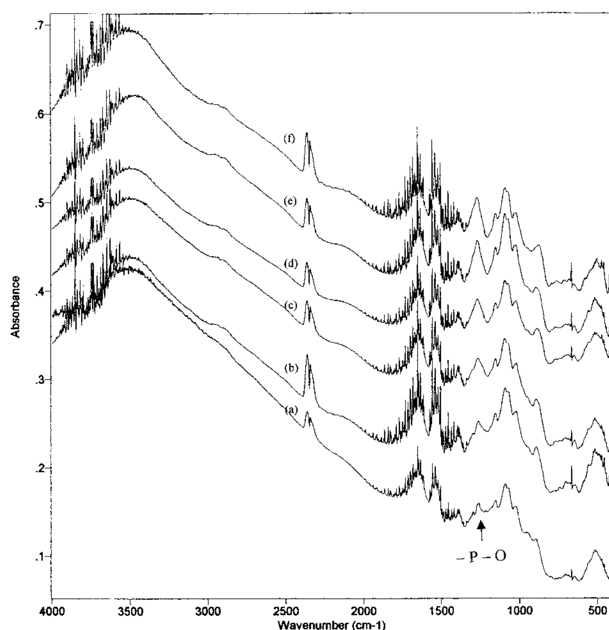



**Figure 5** IR spectra of chitosan–polyphosphoric acid beads; gelling time: (a) 1 min, (b) 3 min, (c) 5 min, (d) 7 min, (e) 10 min, and (f) 15 min.

beads prepared in pentasodium tripolyphosphate and polyphosphoric acid solutions. The presence of a P═O or P—O group is indicated by the peak at the frequence of 1180 or 1250 $cm^{-1}$, respectively. By keeping the gelling time within 30 min, the intensity of the P—O absorbance in the chitosan–polyphosphoric acid complex significantly increased along with increasing the gelling time (Fig. 5), whereas the intensity of the P═O absorbance in the chitosan–tripolyphosphate complex only slightly increased with the increasing in gelling time (Fig. 6). The increase of the intensity of P—O or P═O absorbance indicated the increase of the interchain linkage of $—NH_3^+$ groups in chitosan by polyphosphoric or tripolyphosphate ions. This demonstrated that the complex reaction of the chitosan bead gelled in polyphosphoric acid solution was significantly influenced by gelling time, whereas the complex reaction of the chitosan bead gelled in pentasodium tripolyphosphate solution was less time dependent. Figure 7 shows the EDS analysis of the line profiles of phosphorous distribution on the cross-section of various chitosan gel beads that were prepared in pentasodium tripolyphosphate or polyphosphoric acid solution, respectively. The results appear as a higher phosphorus signal on the outer layer but lower in the inside matrix when chitosan beads were gelled in polyphosphoric acid solution. This result suggested that the ionic interaction between chitosan and polyphosphoric acid was dependent on the diffusion of macromolecular polyphosphoric ions through the chitosan–polyphosphoric acid complex membrane. After the formation of the chitosan–polyphosphoric acid complex outer layer, there was increased diffusion resistance for the macromolecular polyphosphoric ions to diffuse through the gelled film into the inside matrix [Fig. 8(b)]. So, the chitosan–polyphosphoric acid complexation in the gel beads was time dependent. The gelling mechanism of chitosan–tripolyphosphate was different from that of chitosan–polyphosphoric acid. Initially, the $OH^-$ ions competed with $P_3O_{10}^{5-}$ ions to react with the protonated amino group of chitosan on the surface of the beads as soon as the chitosan droplets contact with the tripolyphosphate solution. After the formation of the gelled outer layer, the resistant for the larger $P_3O_{10}^{5-}$ ions to diffuse through the gelled film into the inside matrix was higher than the resistance of $OH^-$ ions to diffuse into the beads, due to the smaller molecular size of the $OH^-$ ions. Then, the gelation of chitosan beads was gradually transferred to be coacervation phase inversion mechanism, which mainly resulted from the neutalization of a protonated amino group of chitosan by $OH^-$ ions [Fig. 8(a)]. So, the line profile of phosphrus signal in the inner matrix of chitosan gel beads have less variation in the radial direction. The chitosan–tri-

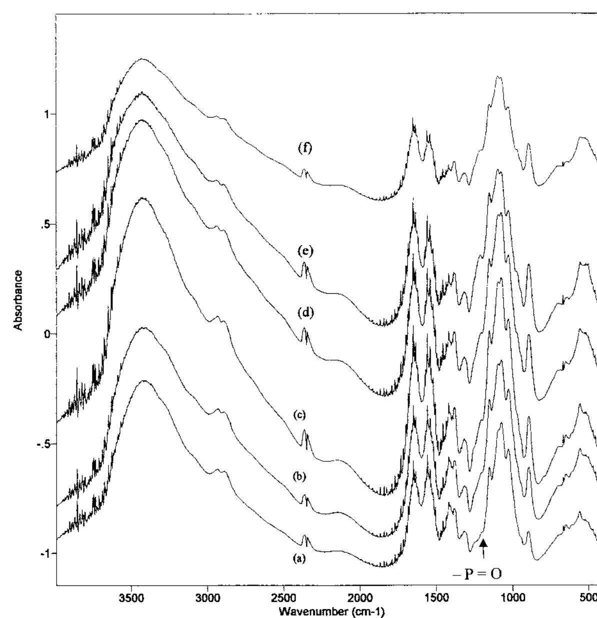
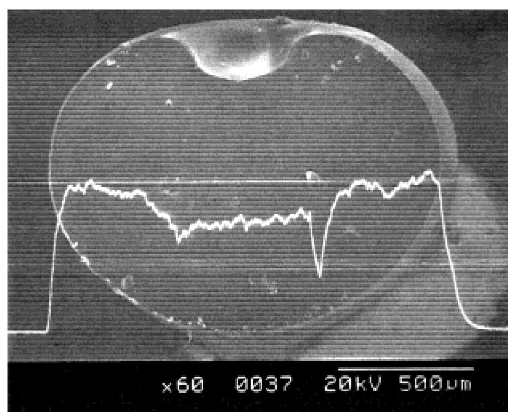
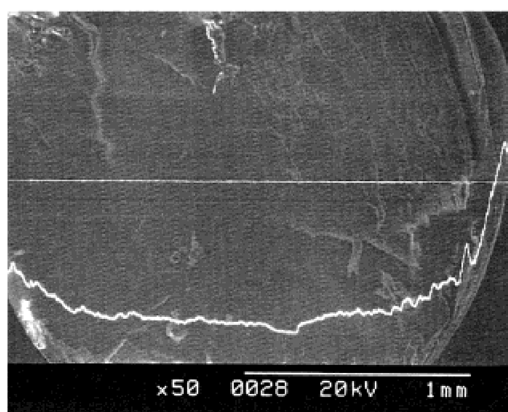



**Figure 6** IR spectra of chitosan–tripolyphosphate beads; gelling time: (a) 1 min, (b) 3 min, (c) 5 min, (d) 7 min, (e) 10 min, and (f) 15 min.




(a)




(b)

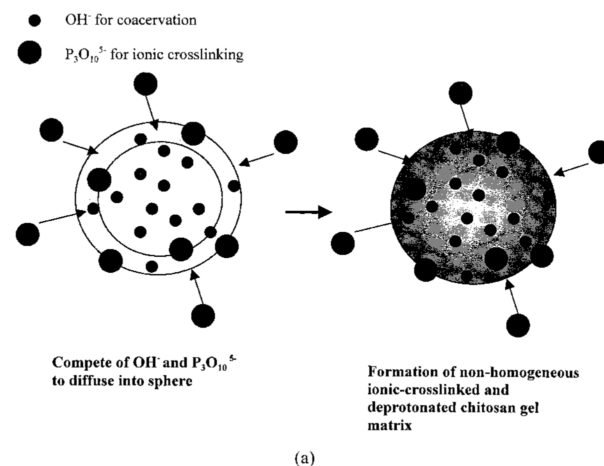
**Figure 7** EDS analysis of the line profiles of phosphorous distribution in chitosan–gel beads (a) chitosan–tripolyphosphate beads, (b) chitosan–polyphosphoric acid beads.

polyphosphate complexation in the gel beads was less time dependent when the outer layer was formed.

Figure 9 shows the SEM pictures of morphology for the chitosan gel beads. The chitosan–tripolyphosphate was spherical, whereas the enzymic hydrolyzed chitosan–tripolyphosphate gel beads became shrinkage. The intermolecular and intramolecular linkages, which are responsible for the successful gelation, increase with the increase of molecular weight. Enzymic hydrolysis of chitosan resulted of the poorly gelled beads due to the decrease of molecular weight.

## Swelling of Chitosan–Tripolyphosphate and Chitosan–Polyphosphate Gel Beads

A convenient proof of crosslinking is the swelling behavior of the chitosan–polyphosphoric acid or chitosan–tripolyphosphate beads. Swelling characteristics of various chitosan beads were carried out in dissolution media of pH 1–7. Figure 10 shows the equilibrium swelling behavior of two kinds of chitosan gel beads synthesized by the complex with polyphosphoric acid or pentasodium tripolyphosphate. It was indicated that pH responsive swelling ability of the chitosan gel beads were quite different according to the different types of the chitosan–polyelectrolyte complex. In a pH 1–7 range, the chitosan–polyphosphoric acid gel beads were only slightly swollen. This is due to higher stability of the chitosan–polyphosphoric acid complex by the formation of interpenetrating interpolymer complex networks, whereas, the chitosan–tripolyphosphate gel beads displayed pH responsive swelling behavior. In a pH 3–7 media, the ionic-crosslinked chain of chitosan–tripolyphosphate gel did not dissociate, so the slight swelling of chitosan–tripolyphosphate beads in



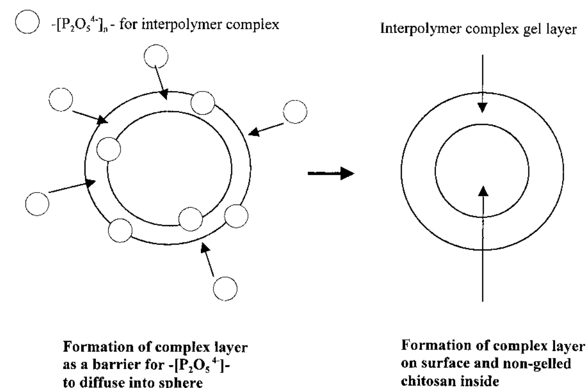
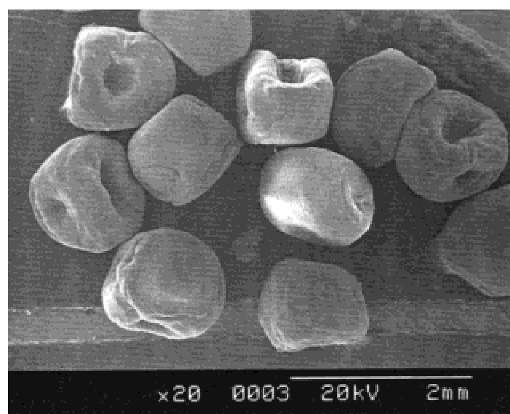
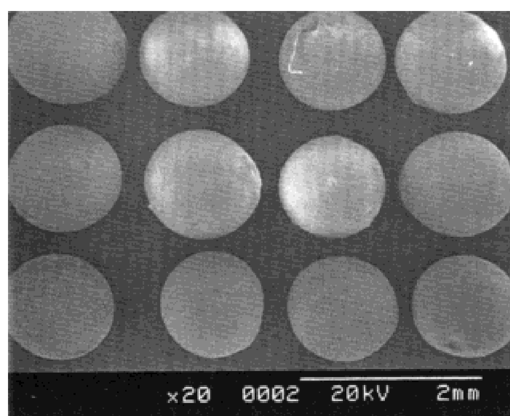



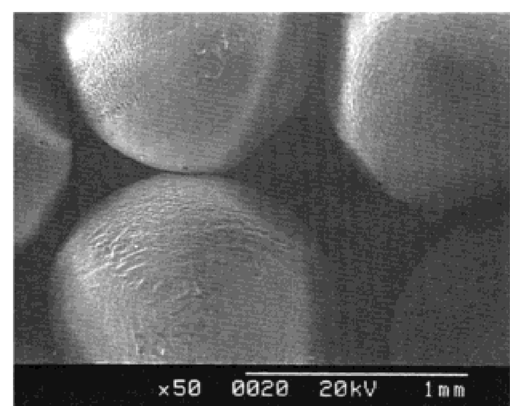

**Figure 8** In-liquid curing mechanism of chitosan gel beads (a) chitosan–tripolyphosphate beads formation by ionic crosslinking, (b) chitosan–polyphosphoric acid beads formation by an interpolymer complex.




(a)




(b)




(c)

**Figure 9** SEM pictures of chitosan–tripolyphosphate gel beads: (a) ×20, $M_v$: 9000 of enzymically hydrolyzed chitosan, (b) ×20, $M_v$: 63,000 of chitosan, (c) ×50, $M_v$: 63,000 of chitosan.

this medium would be attributed to the hydration or partial ionization of uncrosslinked —$NH_2$ sites in chitosan–tripolyphosphate complex. The swelling percent of chitosan–tripolyphosphate beads in this media were lower than 60%. When the pH value of dissolution medium was decreased from pH 3 to pH 1–2, the swelling degree of chitosan–tripolyphosphate beads significantly depended on the swelling time. In this state, swelling was dominated by the dissociation of the interchain linkage of —$NH_3^+$–$P_3O_{10}^{5-}$. Table II shows the phosphorus elution of chitosan–tripolyphosphate beads and chitosan–polyphosphoric acid beads in a pH 1–7 media. This result indicated that the chitosan–polyphosphoric acid beads had a lower degree of chain-scission ratio than that of the chitosan–tripolyphosphate beads in acidic media. In pH 1–2 of the dissolution media, the chitosan–tripolyphosphate beads swelled within 24 h, then gradually disintegration due to the scission of interchain linkage, whereas, the chitosan–polyphosphoric acid beads only slightly swelled but did not dissolve in the pH 1–2 media. This would be attributed to the formation of undegradable interpenetrated networks by the interpolymer complexation of chitosan and polyphosphoric acid. These results indicated that the chitosan–polyphosphoric acid beads have more antiacid ability and less pH responsive characteristic than that of the chitosan–tripolyphosphate beads. The swelling ability of chitosan–tripolyphosphate beads prepared by different molecular weights of

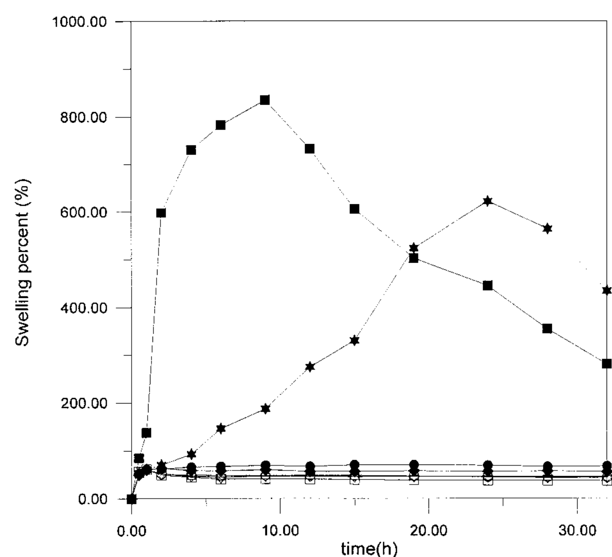



**Figure 10** pH-dependent swelling of chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel beads ($M_v$: 63,000). Chitosan–tripolyphosphate beads: ■, pH 1.0; ★, pH 2.0; ●, pH 3.0; ◆, pH 6.0. Chitosan–polyphosphoric acid beads: □, pH 1.0; ☆, pH 2.0; ○, pH 3.0; ◇, pH 6.0.

**Table II pH-Dependent Phosphorus Elution (within 12 h) from Chitosan–Tripolyphosphate and Chitosan–Polyphosphate Gel Beads**

| | Phosphorus Elution | |
|---|---|---|
| pH Value | Chitosan–Tripolyphosphate | Chitosan–Polyphosphate |
| pH 1.0 | 82% | 8% |
| pH 2.0 | 76% | 6% |
| pH 3.0 | 13% | 5% |
| pH 4.0 | ~ 6% | lower than 3% |
| pH 5.0 | ~ 5% | lower than 3% |
| pH 6.0 | ~ 5% | lower than 3% |
| pH 7.0 | ~ 3% | lower than 3% |

enzymatically hydrolyzed chitosan were shown in Figure 11. Chitosan–tripolyphosphate gel beads prepared by enzymic hydrolyzed chitosan disintegrated quickly in acidic medium after swelling. The swelling of chitosan–tripolyphosphate beads decreased with the decreased molecular weight of enzymatically hydrolyzed chitosan, whereas the disintegration rate of chitosan–tripolyphosphate beads increased with the decreased molecular weight of enzymicly hydrolyzed chitosan. This is due to less of a chain relaxation ability from entanglment because of the shorter polymer chain of the enzymically hydrolyzed chitosan. These differences in swelling and disintegration characteristics may result in the variation in drug release behavior.

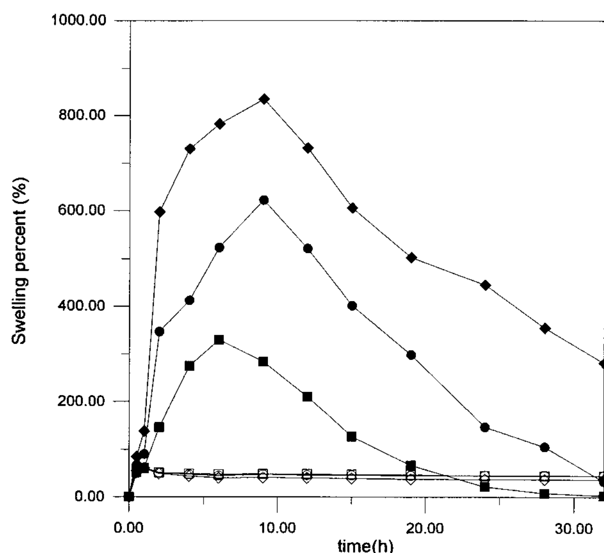



**Figure 11** Molecular weight-dependent swelling of chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel beads in pH 1.0 medium. Chitosan–tripolyphosphate beads: ■, $M_v$: 9000 of enzymically hydrolyzed chitosan; ●, $M_v$: 25,000 of enzymically hydrolyzed chitosan; ◆, $M_v$: 63,000 of chitosan. Chitosan–polyphosphoric acid beads: □, $M_v$: 9000 of enzymically hydrolyzed chitosan; ○, $M_v$: 25000 of enzymically hydrolyzed chitosan; ◇, $M_v$: 63,000 of chitosan.

## Kinetics of Drug Release Form Chitosan–Tripolyphosphate and Chitosan–Polyphosphate Gel Beads

The chitosan–tripolyphosphate and chitosan–polyphosphoric acid beads demonstrated significantly different drug-release behavior. In the pH 6.8 medium, the chitosan gel is considered to be an inert matrix, because the chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel was not swollen and not dissolved in this medium during the experiment. Figure 12 shows the release profiles of 6-MP from the chitosan–tripolyphosphate and chitosan–polyphosphoric acid beads in a pH 6.8 medium. The results of drug release from chitosan–tripolyphosphate beads are consistent with the prediction of a theoretical model developed by Higuchi, which has been used to describe the diffusional-based drug release from polymer matrices. The drug release kinetics may be expressed by the following equation:[30]

$$Q = S \cdot [D_s \cdot \varepsilon(2A - \varepsilon \cdot C_s)C_s \cdot t/\tau]^{1/2}$$

where $Q$ is the amount of drug released at time $t$, $\epsilon$ and $\tau$ are the porosity and tortuosity of the polymer matrix describeing a combined effect of drug solubility in the release medium ($C_s$), drug diffusivity ($D_s$), and the exposed surface area ($S$). Figure 13 shows the release percentage of 6-MP from the chitosan beads against the square root of time. The released amount of 6-MP from the beads increased linearly with the square root of

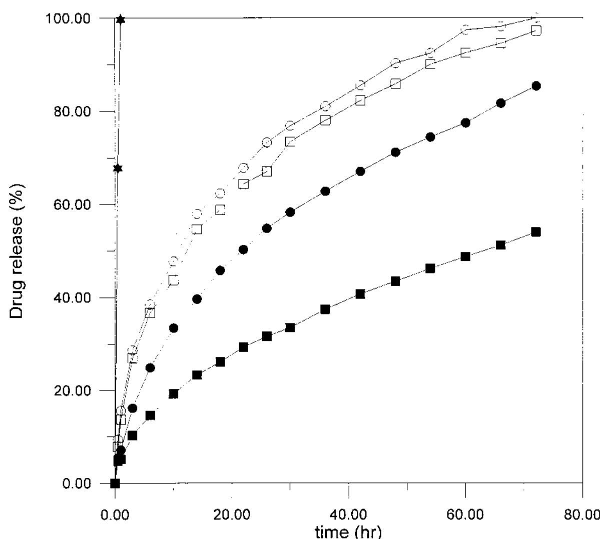



**Figure 12** Effect of molecular weight on release profiles of 6-MP in pH 6.8 medium from chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel beads. Chitosan–tripolyphosphate beads: ■, $M_v$: 63,000 of enzymically hydrolyzed chitosan; ●, $M_v$: 25,000 of enzymically hydrolyzed chitosan. Chitosan–polyphosphoric acid beads:□, $M_v$: 63,000 of enzymically hydrolyzed chitosan; ○, $M_v$: 25,000 of enzymic hydrolyzed chitosan; ★, pure 6-MP.

time, indicating that the diffusion of 6-MP from the beads could be fitted with the Higuchi model, whereas the profiles of 6-MP released from chitosan–polyphosphoric acid beads deviated from the Higuchi model due to the different polyelectrolyte complex mechanism. Chitosan–tripolyphosphate beads prepared by enzymically hydrolyzed chitosan led to increases of tortuosity and a decrease of porosity of the gel matrix due to fewer polymer chain entanglements (Fig. 12). This effect increases the rate of 6-MP release from chitosan–tripolyphosphate or chitosan–polyphosphoric acid gel beads in simulated intestinal fluid (pH 6.8). The decreased molecular weight of enzymically hydrolyzed chitosan has less of an effect on 6-MP release from chitosan–polyphosphoric acid gel beads due to the formation of a interpolymer complex network.

In the pH 1.2 medium, the chitosan–tripolyphosphate gel significantly swelled, and might be gradually dissolved in this medium during the experiment, but chitosan–polyphosphoric acid gel did not swell. Figure 14 shows the release profile of 6-MP from the chitosan–tripolyphosphate or chitosan–polyphosphoric acid beads in THE pH 1.2 medium. In general, the drug release increased with the decrease of the swelling degree of the gel matrix. In the simulated gastric fluid (pH 1.2), the release profiles of 6-MP from the chitosan–polyphosphoric acid beads were much slower than that of chitosan–tripolyphosphate beads. This result indicated that chitosan–polyphosphoric acid gel beads prepared under an interpolymer complex mechanism have better drug-release retardation ability than chitosan–tripolyphosphate gel beads prepared under an ionic crosslinking mechanism, when the beads dissolved in simulated gastric fluid (pH 1.2). It could be attributed to the good acid-resistant ability of the chitosan–polyphosphoric acid beads, and would not result from quickly swollen or disintegration in simulated gastric fluid that has been described in the swelling studies. Chitosan–tripolyphosphate beads prepared by enzymiccally hydrolyzed chitosan led to increased disintegration of the gel matrix. This effect significantly increased the rate of 6-MP release from chitosan–tripolyphosphate gel beads in simulated gastric fluid (Fig. 14). As described previously, the chitosan–polyphosphoric acid gel beads has lower swelling ability, and will not disintegrate in acid medium. Accordingly, the decreased molecular weight of enzymically hydrolyzed chitosan has less effect on the drug release rate of chitosan–polyphosphoric acid gel beads due to the formation of an interpolymer complex network.

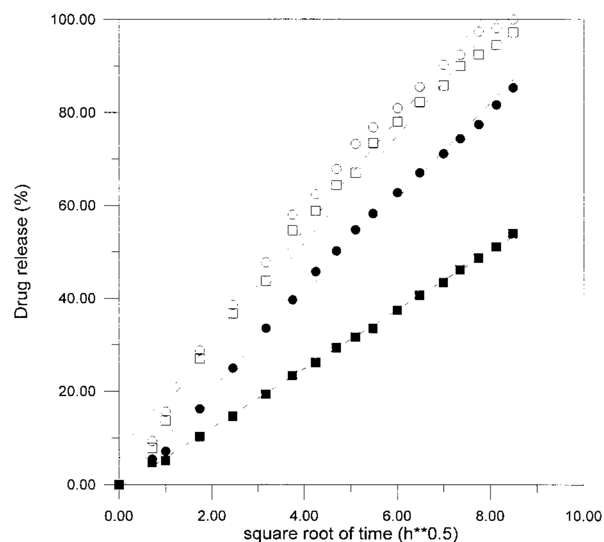



**Figure 13** Amount of 6-MP release in pH 6.8 medium from chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel beads vs. the square root of time (Higuchi model). Chitosan–tripolyphosphate beads: ■, $M_v$: 63,000 of enzymically hydrolyzed chitosan; ●, $M_v$: 25,000 of enzymically hydrolyzed chitosan. Chitosan–polyphosphoric acid beads: □, $M_v$: 63,000 of enzymically hydrolyzed chitosan; ○, $M_v$: 25,000 of enzymically hydrolyzed chitosan.

To examine the sustained release properties of the drug delivery of chitosan–tripolyphosphate or chitosan–polyphosphoric acid beads during GI tract transition, the drug release profile from chitosan beads containing 6-MP was simulated by the pH shift dissolution test method (Fig. 15). The release of 6-MP from the chitosan–tripolyphosphate beads was rapid at the acidic medium (pH 1.2) and slow at the neutral medium (pH 6.8). However, the release characteristics of chitosan–polyphosphoric acid beads were kept sustained throughout the pH shift dissolution test. These results suggest that chitosan–polyphosphoric acid beads containing 6-MP might have potential as a dosage form for the oral delivery of the anticancer drug 6-MP.

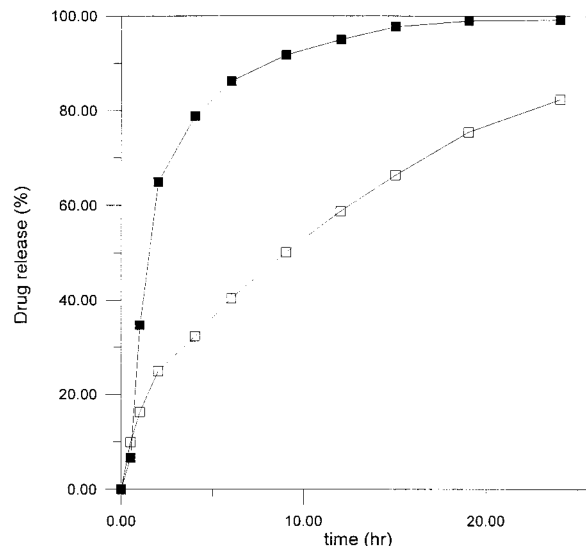



**Figure 15** Release profiles of 6-MP from chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel beads in pH 1.2 dissolution medium for 3 h followed by pH 6.8 medium. Preparative conditions; ■, chitosan–tripolyphosphate gel beads, $M_v$: 63,000 of chitosan; □, chitosan–polyphosphoric acid gel beads, $M_v$: 63,000 of chitosan.

## CONCLUSION

The polyelectrolyte complexation method to prepare chitosan gel beads was developed in this study. The study describes the mechanism of chitosan droplets gelled in polyphosphoric acid or pentasodium tripolyphosphate solution, and the complexation effects on 6-MP releasing from the chitosan–polyphosphoric acid or chitosan–tripolyphosphate gel matrix in acid and netural medium, respectively. The mechanism of chitosan gelled in pentasodium tripolyphosphate solution were deprotonation, accompanied with ionic crosslinking, whereas the mechanisms of chitosan gelled in polyphosphoric acid solution was the interpolymer complex.The releasing behavior of the chitosan–tripolyphosphate or chitosan–polyphosphoric acid gel beads in pH 6.8 medium seem to be diffusion control, whereas in pH 1.2 medium the release behavior of the chitosan–tripolyphosphate exhibit chain relaxation swollen control. Nevertheless, another major factor controlling release rate in both media are, therefore, considered to be the molecular weight of enzymically hydrolyzed chitosan. From the results in this study, it is indicated that the chitosan–polyphosphoric acid gel beads might prove useful as a polymer carrier for the sustained release of the anticancer drug, 6-mercaptopurine, in simulated intestinal and gastric juice medium.

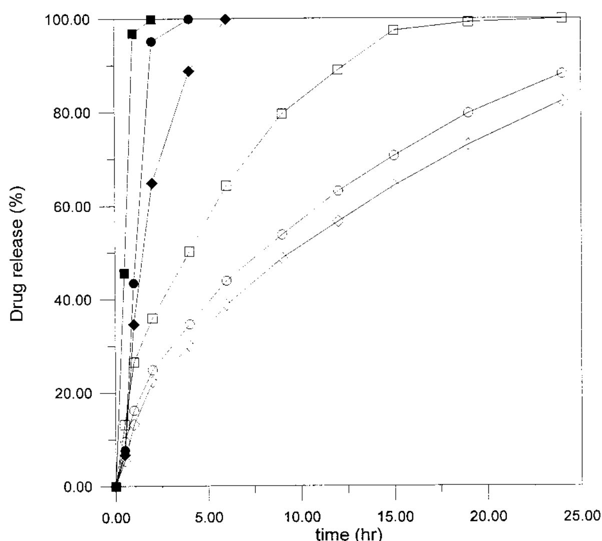



**Figure 14** Effect of curing mechanism on release profiles of 6-MP in pH 1.2 medium from chitosan–tripolyphosphate and chitosan–polyphosphoric acid gel beads. Chitosan–tripolyphosphate beads: ■, $M_v$: 9000 of enzymically hydrolyzed chitosan; ●, $M_v$: 25,000 of enzymically hydrolyzed chitosan; ◆, $M_v$: 63,000 of chitosan. Chitosan–polyphosphoric acid beads: □, $M_v$: 9000 of enzymically hydrolyzed chitosan; ○, $M_v$: 25,000 of enzymically hydrolyzed chitosan; ◇, $M_v$: 63,000 of chitosan.

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