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ABSTRACT: Indomethacin chitosan beads were prepared by ionotropic gelation with multivalent tripolyphosphate (TPP) ions. Various process variables such as amount of drug loading, chitosan gel concentration, TPP concentration and gelation time were investigated for their effects on drug release. In vitro release characteristics were studied in two kinds of dissolution medium; I) phosphate buffer pH 6.2, 900 ml and II) 0.1 N HCl 750 ml for the first 2 h and then added 250 ml of 0.2 M tribasic sodium phosphate, final pH 6.8. The release of indomethacin from chitosan beads in phosphate buffer pH 6.2 did not depend on gelation time and TPP concentration, but decreased as amount of drug loading was increased. Furthermore, the release of indomethacin from chitosan beads in dissolution medium II decreased with the increases in gelation time, TPP concentration and amount of drug loading. The chitosan beads swelled in acidic medium and formed a hydrogel matrix whereas they did not swell or dissolve in phosphate buffer pH 6.2. Most of indomethacin chitosan beads swelled in 0.1 N HCl and then eroded after adding 0.2 M tribasic sodium phosphate, except those prepared by using 1% drug loading, 3% chitosan gel and 2.5% TPP at any gelation time which still remained in spherical swollen beads. It was suggested that the drug release from the chitosan beads depended on the penetration of the dissolution medium into the beads, the eventual swelling and/or dissolution of the chitosan matrix, and the dissolution and subsequent diffusion of the drug through the swollen or unswollen chitosan matrix.

KEY WORDS : indomethacin, chitosan beads, release characteristics

INTRODUCTION

Chitosan is highly versatile polysaccharide which is becoming a potential pharmaceutical excipient because of its biodegradable and biocompatible (1). In Thailand, we could obtain chitosan from shrimp shell in marine industry. It was of interest to investigate our local chitosan as a carrier for drug delivery system. Preparation of chitosan bead by ionotropic gelation using tripolyphosphate (TPP) anion has been demonstrated in several reports (2-9). The release rate of drug from chitosan bead depended on molecular weight of chitosan, amount of drug loading, gelation time and counterion. In this study, effects of some process variables such as amount of drug loading, chitosan gel concentration, TPP concentration and gelation time on in vitro drug release characteristics from chitosan beads were investigated.

MATERIALS AND METHODS

Materials

Indomethacin (Vertex Chemicals, Hong Kong) and tripolyphosphate pentasodium salt anhydrous (Sigma Chemical, USA) were used as received. Chitosan (viscosity 240 cps) was a gift from Unicord, Thailand. Other chemicals were of reagent grade.

Methods

Preparation of indomethacin chitosan beads

The chitosan solution (2 and 3%) in diluted acetic acid (2% v/v) was prepared. Indomethacin powder (less than 80 mesh) was dispersed in the chitosan solution at the concentrations of 1, 3 and 6% w/w. These suspensions were soni-

cated to get rid of air bubble for 30 minutes and then dropped through a glass syringe with a size 21 gauge needle into a gently agitated tripolyphosphate solution (1.0, 1.5 and 2.5% w/w). The chitosan beads obtained were removed from the counterion solution at the predetermined interval of 0.5, 3 and 6 h. The beads were washed three times with distilled water and then air-dried at room temperature overnight.

Microscopic study

Surface and texture characteristics of chitosan beads were photographed using stereomicroscope and scanning electron microscope (Hitachi, Japan).

Swelling study

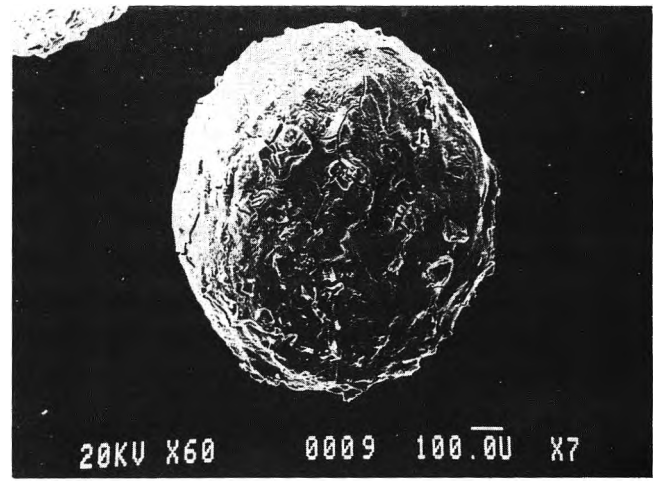
The dried chitosan beads were stirred in 0.1 N HCl for 2 h with subsequent adding of 0.2 M tribasic sodium phosphate (3:1), final pH 6.8 at 37°C and 75 rpm in the same manner as the condition used in in vitro release study. For each batch, twenty swollen beads were taken out periodically and the diameters of the beads were measured using the projection method (Nikon®, Nippon Kogaku K.K., Japan). The magnitude of swelling was presented by the ratio of the average of the diameters of swollen beads to that of the dried beads.

In vitro drug release study

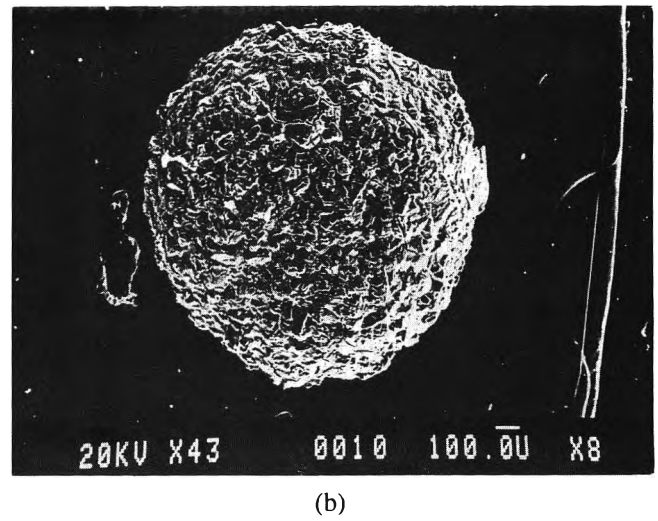
In vitro drug release studies were conducted using dissolution test apparatus I (basket method), USP XXIII, at 75 rpm and 37°C. An appropriate amount of indomethacin chitosan beads containing 75 mg of indomethacin was used. The drug release tests were performed following the USP XXIII drug release test method for indomethacin extended-release capsules, test 2 and that for delayed-release (enteric-coated) articles, method A. The dissolution medium used for indomethacin extended-release capsules, test 2 was phosphate buffer pH 6.2, 900 ml. For the delayed-release (enteric-coated) articles, method A, the dissolution medium used was 0.1 N HCl, 750 ml for the first 2 h which was then added with 250 ml of 0.2 M tribasic sodium phosphate in order to obtain a final pH of 6.8 in a total volume of 1000 ml. Samples were collected at suitable time intervals and assayed spectrophotometrically at 318 nm(Beckman Instruments, USA).

RESULTS AND DISCUSSION

The chitosan bead formation was critically dependent on the viscosity of the chitosan gel, gelation time and TPP concentration. In this study, we used chitosan made from shrimp shell which was abundant waste in marine industry in Thailand. In preliminary study, it was found that the 2% to 3% of chitosan gel in acetic acid gave a suitable viscosity for preparation of the chitosan beads. The result showed that 30 min was the minimum period to obtain uniform bead. Bodmeier et al. (2) also reported that the period necessary to obtain complete cross-linking was 20-35 min. In this study, gelation time was varied as 30 min, 3 h and 6 h. It was found that the spherical and sufficiently strong beads could not be formed with TPP of lower than 1% after gelation for 3 h. When the TPP of higher than 2.5% was used, the beads were eroded and not stable in shape after stirring for a long period. The indomethacin chitosan beads obtained were rounded shape as shown in Figure 1. It was indicated that a higher



(a)



(b)

Figure 1 Scanning electron photomicrographs of indomethacin chitosan beads prepared by using 3% chitosan gel, 1.5% TPP and 3 h gelation time with (a) 1% and (b) 6% drug loading (magnification of 60X)

drug loading (6%) imparted a higher degree of surface roughness to the beads. This resulted from the increasing amount of drug crystals at the surface of the beads (2,10).

Swelling characteristics

In the preliminary study, it was observed that indomethacin chitosan beads did not swell in phosphate buffer pH 6.2, but swelled in 0.1 N HCl. This result agreed well with the previous report that chitosan did not swell at pH > 6. Hence, the swelling study of chitosan beads in phosphate buffer pH 6.2 as a function of time was not carried out. The swelling of chitosan beads were studied in dissolution medium II (0.1 N HCl for 2 h with subsequent adding of 0.2 M tribasic sodium phosphate, final pH 6.8).

Most of the chitosan beads obtained eroded after soaking in 0.1 N HCl for 2 h. Exceptionally, the indomethacin chitosan beads which were prepared by using 1% drug loading, 3% chitosan gel and 2.5% TPP with gelation time of 0.5, 3 and 6 h still remained in spherical shape after immersing in dissolution medium II for 12 h. In 0.1 N HCl, the chitosan beads swelled and their diameters increased with increasing time (Table 1). After swelling in 0.1 N HCl for 2 h, the swelling ratio of chitosan beads was about 3. When 0.2 M tribasic sodium phosphate was subsequently added, the bead size decreased with increasing time and then became constant. It was suggested that the phosphate ion from tribasic sodium phosphate solution might diffuse into the chitosan beads, thereby encouraging the formation of cross-links between chitosan molecules. Therefore, the resultant network caused densification of the beads and a decrease in bead size.

Table 1. Swelling ratio of indomethacin chitosan beads prepared by using 3% chitosan gel, 2.5% TPP, 1% drug loading with various gelation times

Time (min)	Swelling ratio		
	0.5 h	3 h	6 h
20	2.41	2.41	2.22
40	2.63	2.69	2.41
60	2.62	2.83	2.53
80	2.78	2.98	2.79
100	2.90	3.08	2.83
120	2.92	3.02	2.89
150	3.08	3.01	1.69
180	2.71	1.83	1.60
210	1.82	1.65	1.63
240	1.76	1.68	1.69
360	1.77	1.69	1.65

Release characteristics in phosphate buffer pH 6.2

Phosphate buffer pH 6.2 was the dissolution medium stated in the USP monograph of extended release indomethacin capsule (11). It was observed that the chitosan beads remained intact throughout the period of dissolution study in phosphate buffer pH 6.2. It resulted from the fact that chitosan could not form gel at pH > 6. In this study the effects of gelation time, TPP concentration, concentration of chitosan gel and amount of drug loading on drug release from indomethacin chitosan beads were investigated.

Figure 2 shows the effect of gelation time on drug release from indomethacin chitosan beads prepared by using 3% drug loading, 3% chitosan gel and 1.5% TPP. It was indicated that the gelation time did not affect drug release from chitosan beads. This suggested that cross-linking between chitosan molecules and TPP was completely formed after gelation for 30 min. This optimum time was close to that reported by Bodmeier et al. (2).

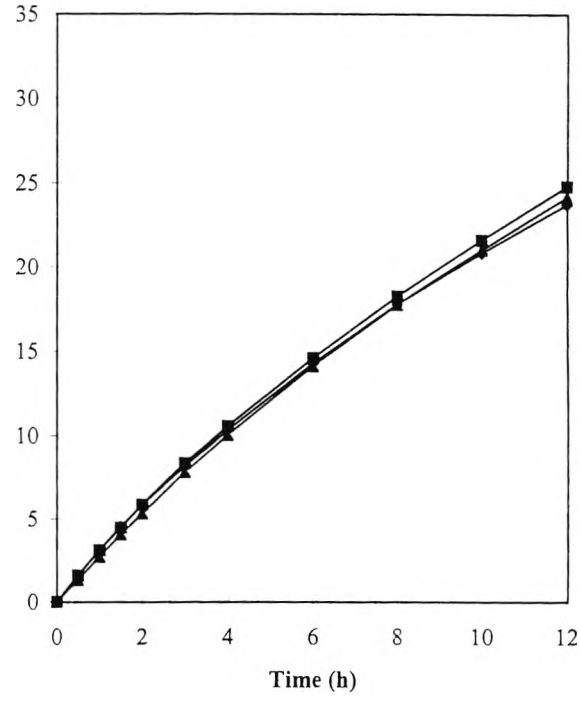


Figure 2 Dissolution profiles of indomethacin chitosan beads prepared by using 3% drug loading, 3% chitosan gel and 1.5% TPP at (◆) 0.5, (■) 3 and (▲) 6 h gelation time in phosphate buffer pH 6.2.

Effect of TPP concentration on drug release from indomethacin chitosan beads prepared by using 3% drug loading, 3% chitosan gel and 3 h gelation time is shown in Figure 3. It was shown that drug release did not significantly depend on the TPP concentration used. It was suggested that using TPP in the range of 1% to 2.5% could form comparable cross-linking with chitosan. Bodmeier et al. (2) also reported that the release of drug from chitosan beads varied insignificantly with increased TPP concentration.

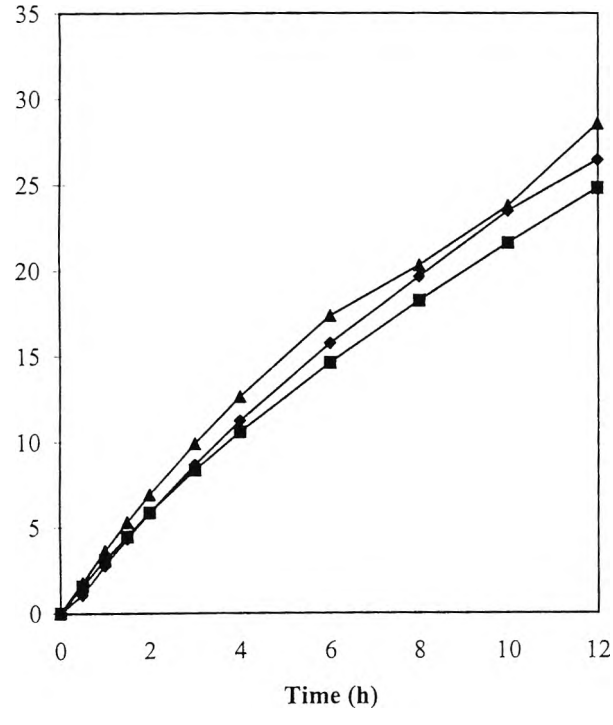


Figure 3 Dissolution profiles of indomethacin chitosan beads prepared by using 3% drug loading, 3% chitosan gel and 3 h gelation time with (◆) 1.0, (■)1.5 and (▲) 2.5% TPP in phosphate buffer pH 6.2.

The dissolution profiles showing the effect of amount of drug loading on drug release from indomethacin chitosan beads are shown in Figure 4. At any chitosan gel concentration used, the drug release from chitosan beads decreased as the amount of drug loading increased. It was possible that a higher proportion of the hydrophobic drug such as indomethacin inside the beads hindered penetration of the dissolution medium into the matrix, thereby reducing the solubility of the drug and hence the release rate. The effect of chitosan gel concentration on drug release from indomethacin chitosan beads is also shown in Figure 4. The concentration of chitosan gel was varied at 2% and 3%. At 1% drug loading, it was shown that the drug releases from both chitosan beads prepared by using 2% and 3% chitosan gel were comparable. At 3% and 6% drug loading, the chitosan beads prepared by using 3% chitosan gel had a higher release rate than those prepared by using 2% chitosan gel. It was suggested that the more amount of chitosan in matrix made the chitosan bead more hydrophilic and therefore released drug at a higher release rate.

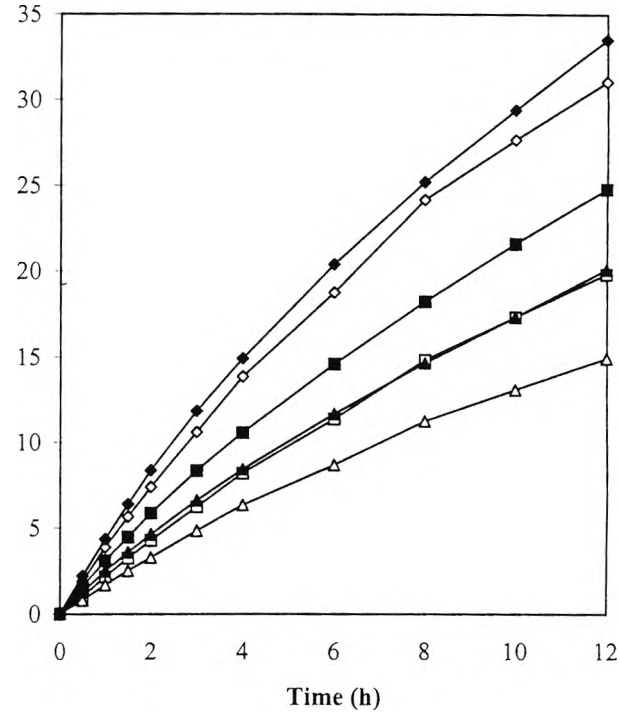


Figure 4 Dissolution profiles of indomethacin chitosan beads prepared by using 2% (open symbol) and 3% (closed symbol) chitosan gel, 1.5% TPP and 3 h gelation time with (◆) 1, (■) 3 and (▲) 6% drug loading in phosphate buffer pH 6.2.

Release characteristics in dissolution medium used for enteric coated dosage form

The method of drug release study and the dissolution medium used were adopted from that used for delayed-release (enteric coated) articles, method A in USP XXIII (11). It was thought that when sustained release pharmaceuticals were orally administered, they would pass through gastric fluid and then intestinal fluid. Therefore, the chitosan beads prepared in this study were studied for their drug releases in such condition.

It was observed that the chitosan beads swelled and formed gel in 0.1 N HCl in similar manner as shown in the results of swelling study. The ionization of free amino groups of chitosan in 0.1 N HCl caused hydration and swelling of the beads prior to the dissolution of chitosan. As indomethacin was very slightly soluble in acidic medium, it was expected that very little amount of indomethacin was released from chitosan beads in 0.1 N HCl and thus the released amounts of indomethacin were not analyzed. A 0.2 M tribasic sodium phosphate was added after chitosan beads were exposed with 0.1 N HCl for 2 h. As mentioned in swelling study, it was observed that the chitosan beads of most preparations swelled and then eroded after soaking in 0.1 N HCl for 2 h, except the chitosan beads prepared by using 1% drug loading, 2.5% TPP, 3% chitosan gel with various gelation time still remained in spherical swollen beads. Some preparations of chitosan beads were selected for drug release study in order to study the effects of some process variables such as gelation time, TPP concentration, amount of drug loading and chitosan gel concentration on drug release from indomethacin chitosan beads.

Figure 5 shows the dissolution profiles of indomethacin beads prepared by using 3% drug loading, 3% chitosan gel, 1.5% TPP at various gelation times. It was shown that the release rate of indomethacin from chitosan beads prepared with 30 min gelation time was higher than those from chitosan beads prepared with 3 h and 6 h gelation time. In addition, the chitosan beads prepared with 3 h and 6 h showed a comparable initial release rate. It was suggested that the cross-linked matrix between chitosan molecules produced at 30 min gelation time might be looser than those produced at longer gelation time and thus resulted in higher release rate.

Effect of TPP concentration on drug release from indomethacin chitosan beads prepared by using 3% drug loading, 3% chitosan gel and 3 h gelation time is shown in Figure 6. The release of indomethacin from chitosan beads decreased with an increase in TPP concentration. The release rate of indomethacin from chitosan beads prepared by using 1% TPP was higher than those from chitosan beads prepared by using 1.5% and 2.5% TPP. However, using TPP at 1.5% and 2.5% gave the chitosan beads of comparable initial release rate. This result was similar to that reported by Bodmeier et al. (2) that release of sulfadiazine from chitosan beads in 0.1 N HCl decreased with increased TPP concentrations. It was suggested that higher TPP concentration gave

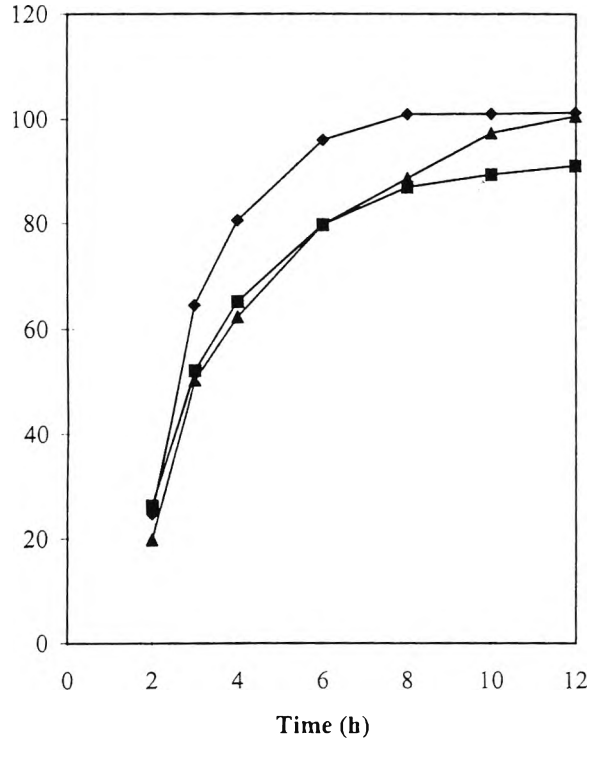


Figure 5 Dissolution profiles of indomethacin chitosan beads prepared by using 3% drug loading, 3% chitosan gel and 1.5% TPP at (◆) 0.5, (■)3 and (▲) 6 h gelation time in dissolution medium II.

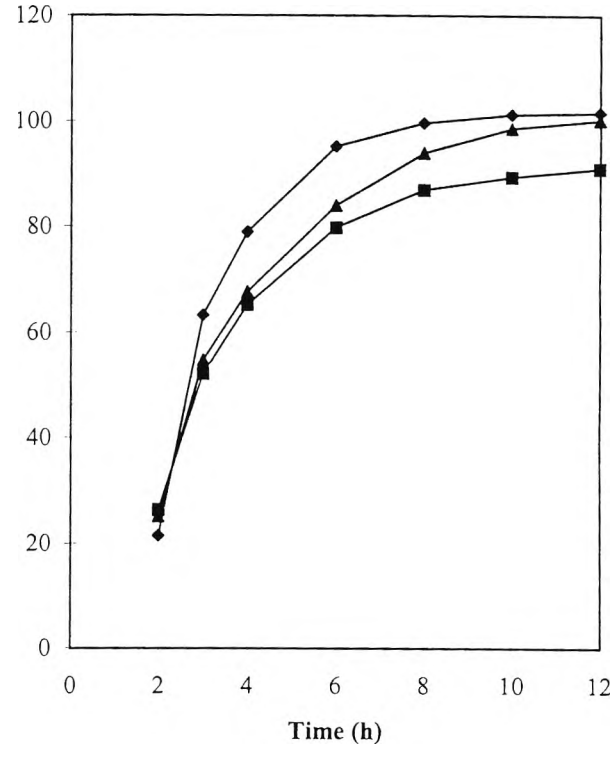


Figure 6 Dissolution profiles of indomethacin chitosan beads prepared by using 3% drug loading, 3% chitosan gel and 3 h gelation time with (◆) 1.0, (■)1.5 and (▲) 2.5% TPP in dissolution medium II.

stronger chitosan matrix, resulting in slower release rate of drug from chitosan beads.

Figure 7 shows the dissolution profiles of indomethacin chitosan beads prepared by using 2% and 3% chitosan gel, 1.5% TPP and 3 h gelation time with various amounts of drug loading. Similar to the result of drug release in phosphate buffer pH 6.2, the release rate of indomethacin from chitosan beads decreased as the amount of drug loading increased. As shown in scanning electron photomicrographs, the high amount of drug crystal was visible at the surface of the chitosan beads prepared by using higher drug loading (Figure 1). It was suggested that the higher amount of indomethacin loading gave the chitosan beads of more hydrophobic, which might retard the penetration of dissolution medium into the matrix and thus decreased drug release rate. In addition, at lower amount of drug loading, indomethacin would be dispersed well in chitosan matrix and the smaller chitosan bead was obtained. Therefore, the well dispersion of indomethacin in chitosan matrix and the higher surface area might be responsible for the higher release of indomethacin from the chitosan beads prepared by using lower drug loading. In addition, the concentration of chitosan gel did not significantly affect the release of indomethacin from chitosan beads.

The dissolution profiles of indomethacin chitosan beads prepared by using 2% and 3% chitosan gel, 2.5% TPP and 3 h gelation time with various amounts of drug loading are shown in Figure 8. With using 2% chitosan gel, the release of indomethacin from chitosan beads decreased with increased amount of drug loading. Surprisingly, when 3% chitosan gel was used, the release rate of indomethacin from chitosan beads prepared by using 1% drug loading was slower than that prepared by higher drug loading. It was of interest to observe that the chitosan beads prepared by using 1% drug loading and 3% chitosan gel were still in spherical shape whereas the others eroded and then dissolved. It was suggested that the different result of drug loading effect on release of indomethacin from chitosan beads showed the difference in mechanism of drug release. As most of indomethacin chitosan beads swelled in 0.1 N HCl and subsequently eroded, the

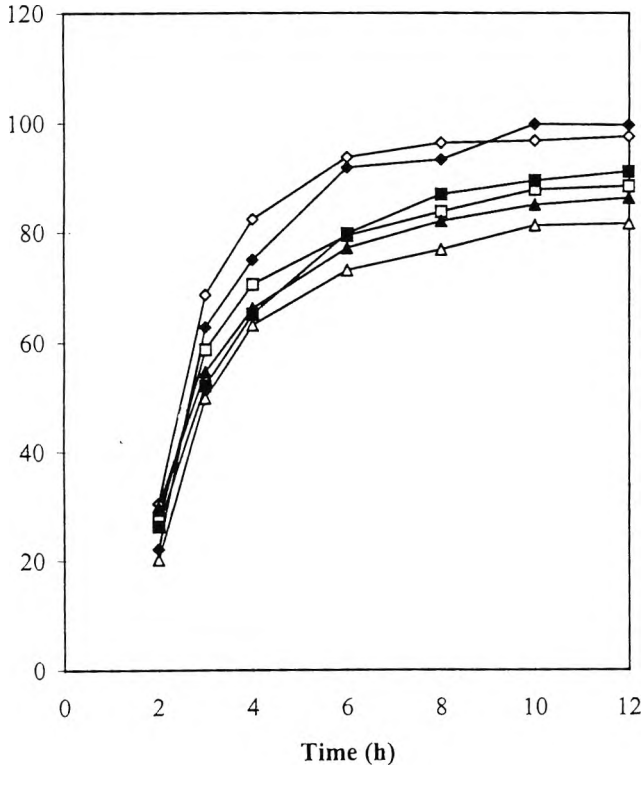


Figure 7 Dissolution profiles of indomethacin chitosan beads prepared by using 2% (open symbol) and 3% (closed symbol) chitosan gel, 1.5% TPP and 3 h gelation time with (◇, ◆) 1, (□, ■) 3 and (△, ▲) 6% drug loading in dissolution medium II.

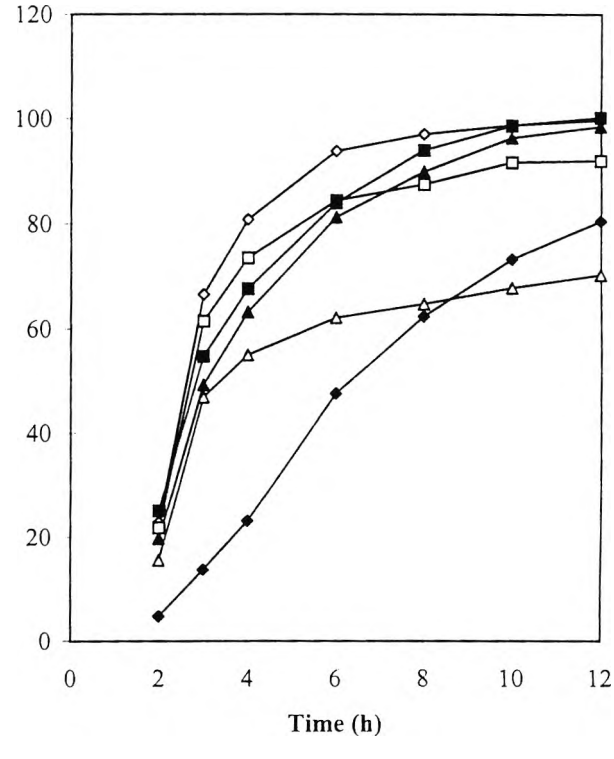


Figure 8 Dissolution profiles of indomethacin chitosan beads prepared by using 2% (open symbol) and 3% (closed symbol) chitosan gel, 2.5% TPP and 3 h gelation time with (◇, ◆) 1, (□, ■) 3 and (△, ▲) 6% drug loading in dissolution medium II.

mechanism of drug release from such chitosan beads might be attributable to polymer erosion and diffusion. On the other hand, the drug release from chitosan beads which were still in spherical swollen beads throughout dissolution test might be mostly controlled by diffusion. It was suggested that the cross-linking between chitosan molecules in the chitosan beads which were still in spherical shape after dissolution test was stronger than those in other chitosan beads. The indomethacin chitosan beads which could be seen as spherical swollen bead might be worthy for development of sustained release dosage form.

CONCLUSION

It was indicated that the release rate of indomethacin from chitosan beads in phosphate buffer pH 6.2 was lower than that in the mixture of 0.1 N HCl and 0.2 M tribasic sodium phosphate (3:1). This resulted from the fact that the chitosan beads swelled in an acidic medium and formed a hydrogel matrix whereas they did not swell or dissolve in phosphate buffer pH 6.2. It was suggested that the drug release from the chitosan beads depended on the penetration of the dissolution medium into the beads, the eventual swelling and/or dissolution of the chitosan matrix, and the dissolution and subsequent diffusion of the drug through the swollen or unswollen chitosan matrix. In addition, The process variables such as amount of drug loading, chitosan gel concentration, TPP concentration and gelation time should be optimized to obtain the indomethacin chitosan beads of desired release characteristics.

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