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STRUCTURAL CHANGES AND RELEASE CHARACTERISTICS OF CROSSLINKED CHITOSAN BEADS IN RESPONSE TO SOLUTION pH

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Key Words: Beads, Biodegradable, Chitosan, Controlled Release, Swelling Degree

ABSTRACT

The present investigation describes a novel method for preparing beads based on crosslinked chitosan with glutaraldehyde interpenetrating glycine polymer network. Four type of beads, viz., CHI1 (composed of chitosan, glycine and glutaraldehyde); CHI2 (composed of chitosan and glutaraldehyde); CHI3 (composed of chitosan and glycine) and CHI4 (only chitosan) were prepared and their release characteristics were studied using thyamine hydrochloride (Thy-HCl) as a model drug. Structural changes during swelling of CHI1 beads in solutions of different pH were studied using IR and UV spectroscopy.

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INTRODUCTION

Biodegradable polymers have been used extensively in biomedical areas in the form of sutures, wound-covering materials, artificial skin, and for the controlled release of drugs [1]. To improve therapeutic efficiency and reduce or eliminate side effects of oral controlled drugs, it is found to be reasonable to deliver drugs to specific regions of the gastrointestinal (GI) tract. Various compounds have been targeted to the colon in the form of prodrug [2]. However, a more universal drug delivery system, which is not drug specific, is more desirable. Several methods of targeting have been reported out of these pH variations within the GI tract [3] and the exploitation of bacterial enzymes localized within the colon [4] are of current interest in controlled drug delivery systems.

Micro bead carriers have important potential applications for the administration of therapeutic molecules. Recent advances have shown that polymeric devices are useful for high molecular weight drugs [5], for the drugs, which should be delivered in a minute quantity [6] with zero-order kinetics [7].

Chitosan, a natural polysaccharide, having structural characteristics similar to glycosamino glycans, is non-toxic and easily bioabsorbable [8], and has been currently in use for the release of several drugs [9, 10]. The use of chitosan in the development of oral sustained release preparations is based on the intragastric floating tablets of chitosan [11, 12]. Chitosan, due to its antacid and antiulcer characteristics, prevents or weakens drug irritation in the stomach [13, 14]. Therefore, chitosan has a great potential for its use as a suitable carrier in sustained delivery systems.

In the past, workers have tried several methods and systems to prolong the retention of the dosage form in the stomach [15-17]. However, a reservoir system of chitosan beads or microgranules for oral use has rarely been reported in the literature [11, 12, 18]. Yao *et al.* reviewed microcapsules/microspheres related to chitosan [19]. From the physicochemical point of view, chitosan has the special property of gelling when in contact with anions, thus allowing the formation of beads under very mild conditions. The degradation rate of the beads depends upon the degree of crosslinking and the pH of the medium.

Recently, procedures for preparing semi-interpenetrating polymer network hydrogels of crosslinked chitosan/polyether [20, 21] and crosslinked chitosan/poly (ethylene glycol) macromer [22] were reported. Semi-inter-penetrating polymer network beads of crosslinked chitosan-glycine for controlled release of drugs is a novel approach reported to date. In this communication, the prepa-

ration techniques of bead with/without crosslinking and their release characteristics were discussed. Efforts have also been made to study the structural changes of crosslinked beads in solutions of different pH and the related mechanism was put forward with the help of the spectroscopic data obtained from IR and UV spectral analyses.

EXPERIMENTAL

Materials and Methods

Chitosan was obtained as a gift sample from the Central Institute of Fisheries Technology, Cochin, India. Impurities were removed by dissolving 1 g of chitosan in 75 ml of 2% acetic acid and passing through a filter. This transparent viscous solution was precipitated in 100 ml of 1M NaOH. The precipitate was repeatedly washed with hot water and dried in a vacuum oven at 20˚C. The molecular weight of purified chitosan was determined by a viscometric method using the Mark-Houwink equation [23]: $[\eta] = K_m M^{\alpha}$, where $K_m = 1.81 \times 10^{-3}$, $\alpha = 0.93$. The average molecular weight ($\bar{M}v$) of the chitosan was 2.9×10^6. The % N deactylation of chitosan was determined using the following relationship [24] which was found to be 61%.

$$\% \text{ N-deactylation} = (1\text{-}A_{1655}/A_{3340} \times 1/1.33) \times 100$$

Where A is the logarithmic ratio of the absorbance and transmittance at the given wave number. All other chemicals used were of analytical grade.

Preparation of Thyamine Hydrochloride Loaded CHI1 Beads

0.5 g of purified chitosan was dissolved in 35 ml of 2% acetic acid under stirring for 3 hours at room temperature. In this solution, approximately 0.1 g of glycine and a known amount of drug were added and again stirred for 1 hour. The homogeneous mixture was extruded in the form of droplets using a syringe into NaOH-methanol solution (1:20 w/w) under stirring conditions. The resultant beads were then placed in a water jacket containing approximately 10 ml of glutaraldehyde solution (12.5% v/v) maintained at 60˚C. Finally, the beads were washed with hot and cold water successively and vacuum dried at 30˚C. Beads without the drug were also prepared using similar method for studying swelling properties.

Preparation of Thyamine Hydrochloride Loaded CHI2 Beads

0.5 g of purified chitosan was dissolved in 35 ml of 2% acetic acid under stirring for 3 hours at room temperature. In this solution, a known amount of drug was added and again stirred for 1 hour. The homogeneous solution was extruded in the form of droplets using a syringe in NaOH-methanol solution (1:20 w/w) under stirring conditions. The resultant beads were then placed in a water jacket containing approximately 10 ml of glutaraldehyde solution (12.5% v/v) maintained at 60˚C. Finally, the beads were washed with hot and cold water successively and then vacuum dried at 30˚C.

Preparation of Thyamine Hydrochloride Loaded CHI3 Beads

0.5 g of purified chitosan was dissolved in 35 ml of 2% acetic acid under stirring for 3 hours at room temperature. In this solution, approximately 0.1 g of glycine and a known amount of drug were added and again stirred for 1 hour. The homogeneous mixture was extruded in the form of droplets using a syringe into NaOH-methanol solution (1:20 w/w) under stirring conditions. The resultant beads were washed with hot and cold water successively and then vacuum dried at 30˚C.

Preparation of Thyamine Hydrochloride Loaded CHI4 Beads

0.5 g of purified chitosan was dissolved in 35 ml of 2% acetic acid under stirring for 3 hours at room temperature. In this solution, a known amount of drug was added and again stirred for 1 hour. The homogeneous solution was extruded in the form of droplets using a syringe in NaOH-methanol solution (1:20 w/w) under stirring conditions. The resultant beads were washed with hot and cold water successively and then vacuum dried at 30˚C.

Swelling Studies

Swelling behavior of the chitosan beads (CHI1-CHI4) has been studied in solutions of different pH. The degree of swelling for each sample at time t was calculated by using the following expression:

$$(Wt-Wo)/Wo$$

Where Wt and Wo are the weights of the beads at time t and in the dry state, respectively.

Preparation of the Samples for IR/UV Spectra

CHI1 beads have shown significant controlled release rates of thyamine hydrochloride in both acidic and basic solutions. Therefore, to understand the swelling behavior and release mechanism of the beads, the CHI1 samples were swollen in solutions of pH 2.0 and pH 7.4, which were taken out at desired time intervals and dried completely in an oven at 35-45˚C for about 2.5 hours. The IR spectra of the dried beads were recorded with a double beam Perkin-Elmer FTIR-1600 spectrophotometer using KBr pellets. In an attempt to investigate the changes related to solution pH, the above swelling experiments were repeated. At certain intervals, the swelling solution was filtered and the UV spectra of the filtrate were recorded with the help of Shimadzu 1601 UV-VIS spectrophotometer.

Drug Assay

A sample of beads was accurately weighed (100 mg), and kept in 100 ml of 2% acetic acid at 30˚C for 48 hours. After centrifugation, the Thy-HCl in the supernatant was assayed by spectrophotometer at 230 nm.

Drug Release Studies

The release rate experiments were performed in a glass apparatus at 37˚C under unstirred conditions, in acidic (pH 2.0) and basic (pH 7.4) solutions. Beads (100 mg) containing a known amount of Thy-HCl were added to the release medium. At appropriate intervals, 1-ml samples were with drawn, filtered and assayed. The absorbance was measured at 230 nm. The results were plotted as the amount of cumulative Thy-HCl release into the dissolution medium from beads *versus* time.

RESULTS AND DISCUSSION

Swelling Studies

Figure 1 shows the swelling behavior of beads in solutions of pH 2.0 and pH 7.4 at 37˚C. It was observed that no significant swelling of the beads occurred in a solution of pH 7.4. In a solution of pH 2.0, a substantial swelling in beads was observed in the beginning, but after attaining a maxima, a decreasing trend was observed which indicated that the dissolution of the beads has exceeded the

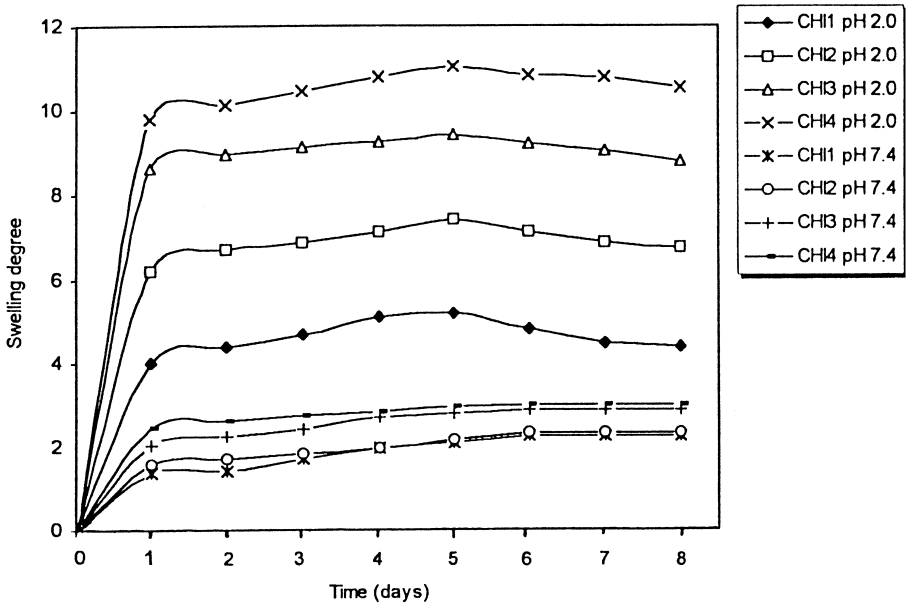

Figure 1. Swelling behavior of the beads measured as a function of time in pH 2.0 and in pH 7.4 at 37°C.

degree of swelling. The structural changes took place during swelling of the crosslinked beads in solutions of different pH were supported by the IR and UV spectra (Figures 3-4) recorded at different stages of swelling.

Figure 2 shows the IR spectra of glycine (A), initial dry beads CHI1 (B) and chitosan (C). The peak at 1592 cm^{-1} in the IR spectrum of chitosan [Figure 2 (C)] can be assigned to the amino group. In contrast with spectra (A) and (C), there is a significant new peak at 1631 cm^{-1} in spectrum (B), which can be attributed to the formation of C(N due to imine reaction between amino groups from chitosan and aldehyde groups in glutaraldehyde. The peaks at 1485 and 2671 cm^{-1} are characteristic peaks [Figure 2 (A) & (B)] from glycine within the beads. The peaks at 1038, 1045 and 1081 cm^{-1} in spectra (A)-(C) are due to C(O stretching vibration in glycine, CHI1 beads and chitosan respectively [25, 26]. The glycine is amphoteric in nature and expected to interact with chitosan through intermolecular physical crosslinks.

FTIR spectra of chitosan beads at different stages of swelling in pH 2.0 were shown in Figure 3. Figure 3 (A) shows the IR spectrum of initial dry beads, whereas spectra B, C, and D are recorded at 1 hour, 2 days and 5 days of swelling in acidic medium. The imine groups of the crosslinked beads in acidic solution

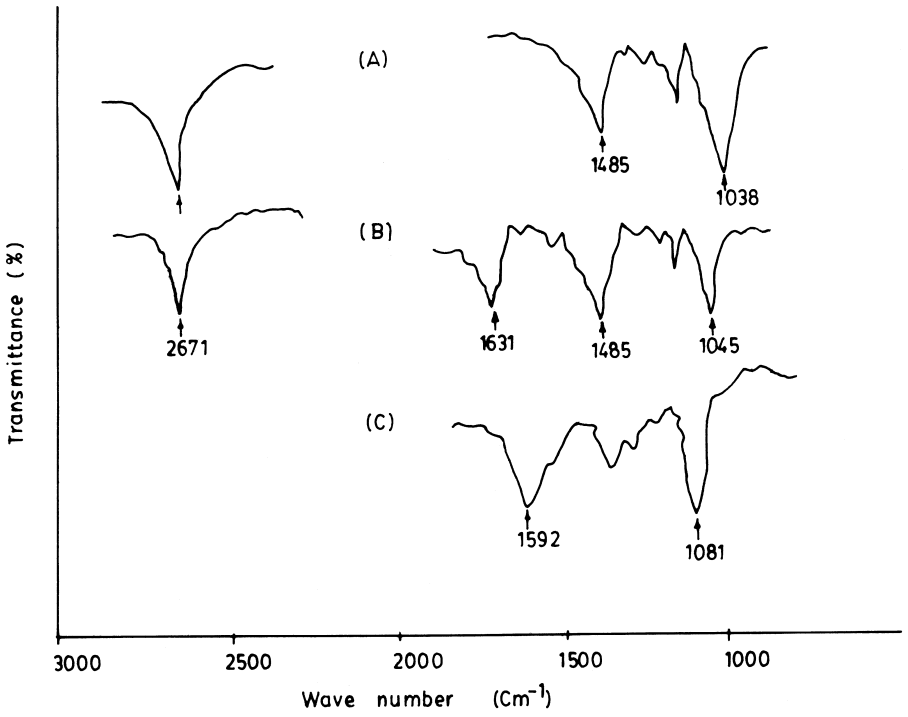

Figure 2. FTIR spectra of (A) glycine, (B) CHI1 beads, and (C) chitosan.

get protonated, and as a result, the hydrogen bonding dissociates promoting swelling of the beads. By contrast with spectrum 3(A), there are two new peaks at 1623, 1523 cm^{-1} assigned to NH_3^+ absorption peaks [23, 24] in the spectra 3 (B), (C) and (D), respectively. These peaks have supported the formation of NH_3^+ within the beads when swollen in acidic pH. The change in the characteristic peak heights of glycine at 1485 cm^{-1} and 2671 cm^{-1} in beads at different stages of swelling has clearly indicated the dissolution of glycine from the network. The comparison of the spectra [Figure 3 (A-D)], has clearly indicated that the characteristic C(O stretching vibration peaks in glycine and stretching peak of the hydroxyl groups in chitosan [25-27] are shifted from 1045 cm^{-1} to 1100 cm^{-1} on swelling the samples in solution of pH 2.0.

Figure 4 shows the IR spectra of the initial dry beads and the beads after swelling for definite time intervals in solution of pH 7.4. From the spectra, it is clear that, the peak at 1631 cm^{-1} assigned to C=N group changes with swelling times and almost disappears [Figure 4 (D)]. Meanwhile, the peaks assigned to glycine at 1485 and 2671 cm^{-1} also weaken, but the rate is slower than that in the

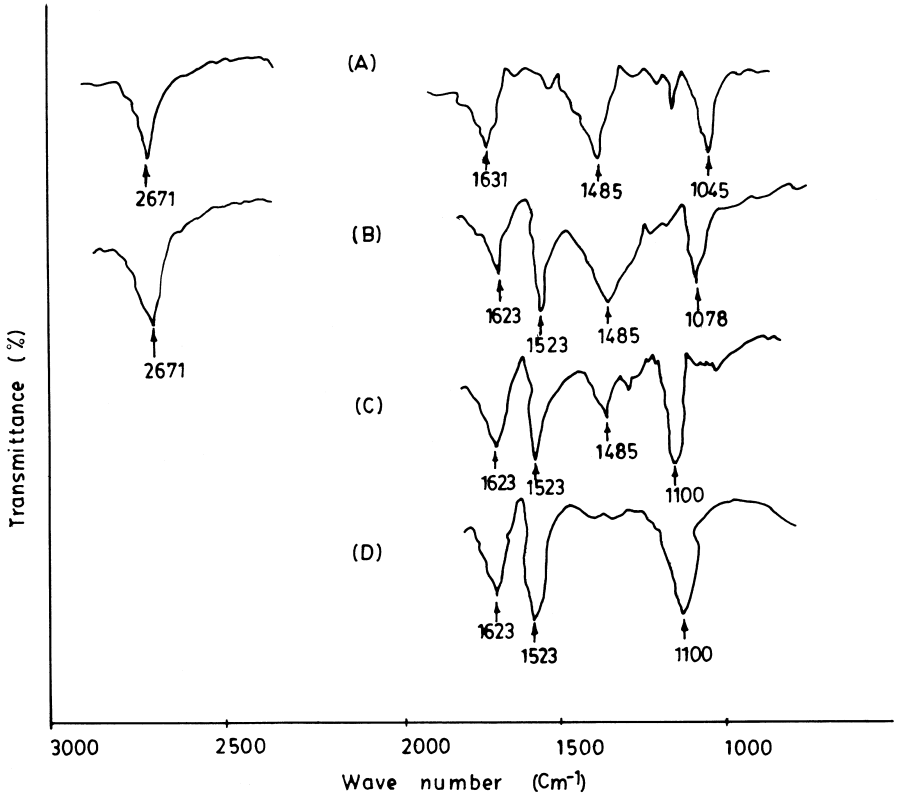

Figure 3. FTIR spectra of (A) initial dry bead CHI1 and (B) swollen beads in pH 2.0 at 37°C for 1 hour, (C) 2 days, (D) 5 days.

case of pH 2.0. In addition, it was noticed that there was no peak related to NH_3^+ in the IR spectrum of swollen bead in pH 7.4 [Figure 4 (B-D)], which have confirmed that the imine groups within the beads were not protonized in pH 7.4, leading to a lower swelling degree of the beads in pH 7.4.

In comparison to the spectrum of chitosan [Figure 2 (C)], it was found that the peak at 1592 cm^{-1} [Figure 4 (D)] becomes similar to that of chitosan. This elucidate that the changes in structure of the beads may result from the transformation of C=N to C(N other than its cleavage, which makes the IR spectrum of N—H from C—N similar to that from amino groups of chitosan. On the other hand, it was confirmed from the UV spectrum [Figure 5] that the imine bonds within the bead did not break on swelling in pH 7.4 for 2 days. In contrast with the UV spectrum of chitosan solution in pH 7.4, it was observed that the peak at 202 nm [Figure 5(C)] could be assigned to chitosan due to the dissolution of chitosan from the beads. However, there is no peak relating to glu-

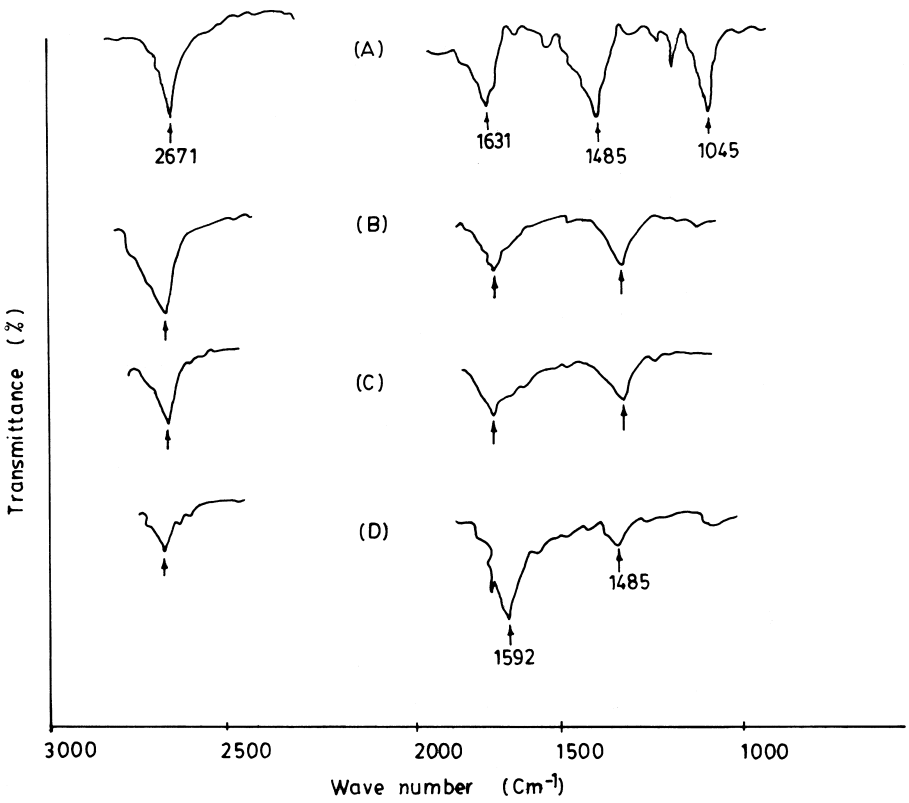

Figure 4. FTIR spectra of (A) initial dry bead CHI1 and (B) swollen beads in pH 7.4 at 37°C for 1 hour, (C) 2 days, (D) 5 days.

taraldehyde perhaps caused by the cleavage of C=N. Therefore, it may be reasonable to assume that the imine bond change may be attributable to the conversion of C=N to C—N in pH 7.4.

In Figure 6, we can confirm the cleavage of imine bond as the bead was swollen in pH 2.0 at 37˚C. It was shown that the peaks at 202 and 233.5 nm [Figure 6 (C)] attributed to the dissolution of chitosan and cleavage of imine bond respectively. This may result from the hydrolysis of the imine bond to amino and aldehyde groups after the beads were swollen continuously for a long time and the further dissolution of chitosan in the swollen beads.

Release Studies

Figure 7 shows the release profile of Thy-HCl from chitosan beads (28 μg/mg bead loaded) for various time intervals in acidic (pH 2.0) or basic solutions (pH 7.4) at 37˚C. There is a burst release initially for the first hour in both

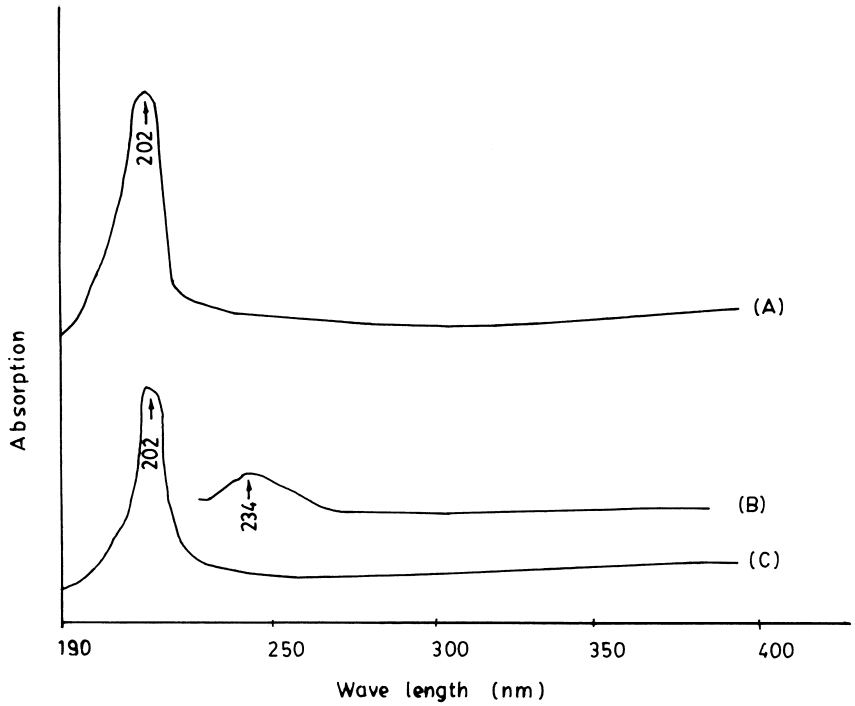

Figure 5. UV spectra of (A) chitosan, (B) glutaraldehyde solution in pH 7.4 and (C) solution left after the initial dry beads CHI1 were swollen for 2 days at 37°C.

acidic and basic media followed by an almost constant release of Thy-HCl from the matrix for the studied period of 72 hours. The amount and percentage release of the loaded drugs were much higher in acidic solution than in basic solution.

Similarly, the dissolution profiles of Thy-HCl from the chitosan beads, loaded with higher amounts of drug (43 μg/mg and 69 μg/mg bead loaded) has also been studied in acidic and basic media as a function of release time interval and shown in Figures 8 and 9. The release pattern of the highly drug loaded beads has been found to be similar with that of the beads loaded with lower amounts of the drug. These observations have also suggested that the percentage of drug released from chitosan beads decreased with increase in concentration of Thy-HCl. However, the total amount of Thy-HCl released from the highly drug loaded beads was found to be higher in comparison to the beads loaded with a lower amount of the drug.

Therefore, from the Figures 7-9 it is evident that the amount and percentage of Thy-HCl released was much higher in acidic and basic solutions for all the three studied concentrations of the drug loaded. It is understood that the

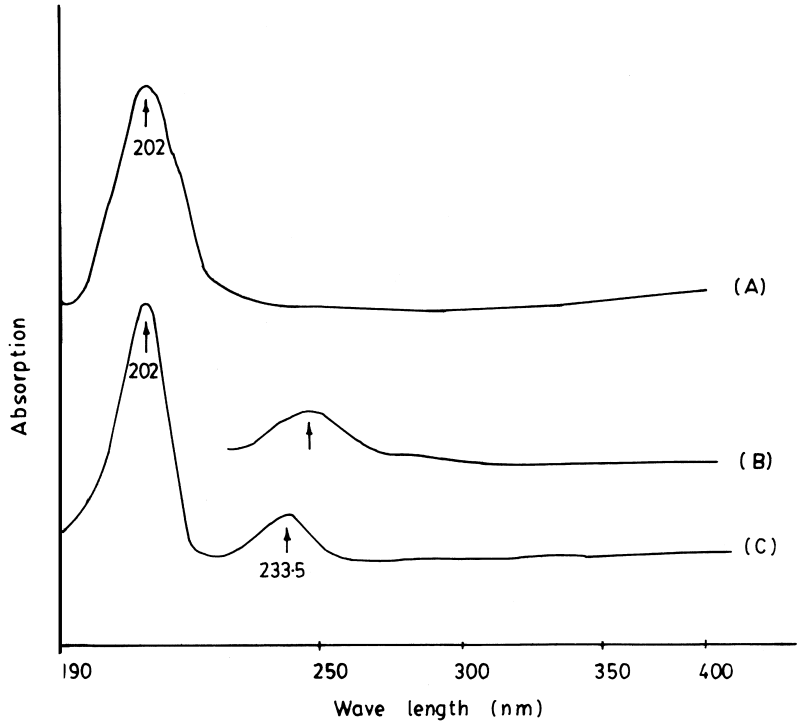

Figure 6. UV spectra of (A) chitosan, (B) glutaraldehyde solution in pH 2.0 and (C) the solution left after the initial dry beads CHI1 were swollen in pH 2.0 for 2 days at 37°C.

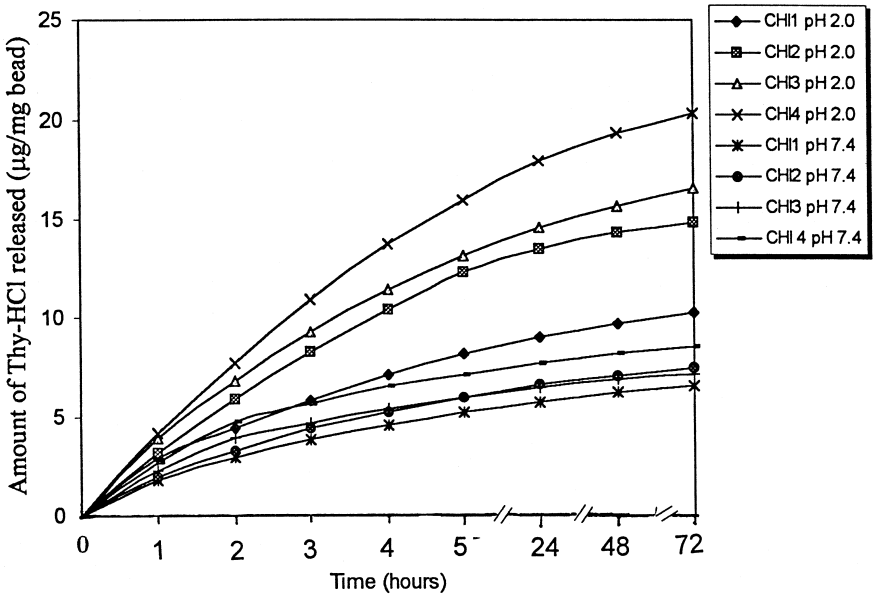

Figure 7. Release of thyamine hydrochloride from chitosan beads (28 µg loaded/mg beads) vs. time in pH 2.0 and in pH 7.4 solution at 37°C.

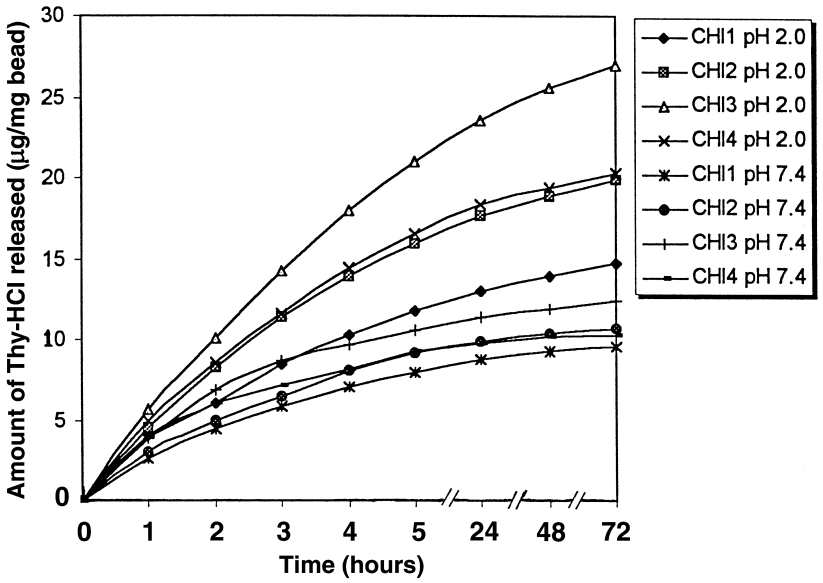

Figure 8. Release of thyamine hydrochloride from chitosan beads (43 µg loaded/mg beads) vs. time in pH 2.0 and in pH 7.4 solution at 37°C.

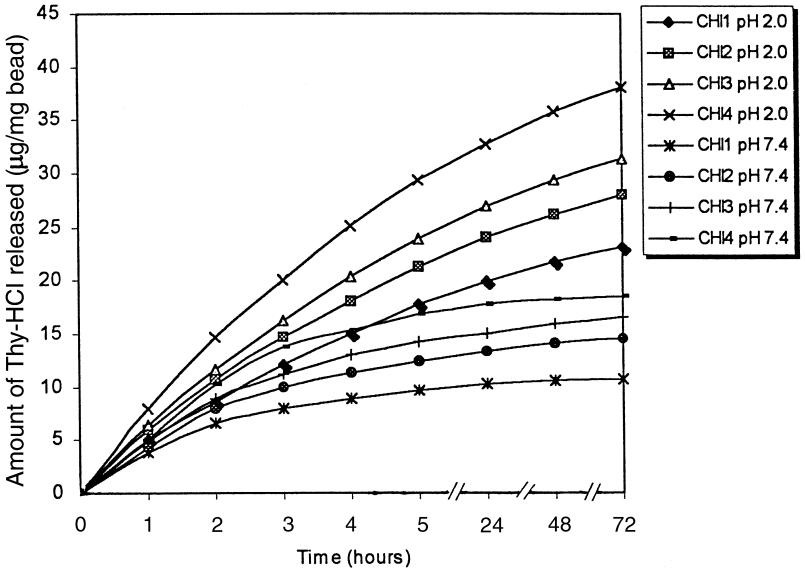

Figure 9. Release of thyamine hydrochloride from chitosan beads (69 µg loaded/mg beads) vs. time in pH 2.0 and in pH 7.4 solution at 37°C.

mechanism of drug release is due to the diffusion through swollen beads in pH 2.0 solution, whereas, the swelling in pH 7.4 solution is less and the drug release is less.

On the basis of the experimental observations, the swelling behavior and the mechanism for dissolution of Thy-HCl from the CHI1 beads was well discussed. It is clearly evident from these discussions that the drug release depends on solution pH and swelling behavior of the matrix. The more swelling, the more is the drug release and vice versa.

CONCLUSION

These chitosan beads showed a pH-dependent swelling behavior which made them appropriate for the delivery of drugs at a controlled rate. On the other hand, chitosan has some interesting properties such as mucoadhesivity, biocompatibility, and non-toxicity, which render it as an interesting material.

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