Semi-Interpenetrating Polymer Network Beads of Crosslinked Chitosan–Glycine for Controlled Release of Chlorphenramine Maleate

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ABSTRACT: Spherical, semi-interpenetrating polymer network beads of chitosan and glycine, crosslinked with different concentrations of glutaraldehyde were prepared for controlled release of drugs. The structural and morphological studies of the beads were carried out with FTIR and SEM techniques. The swelling behavior of the beads at different time intervals was monitored in solutions of different pH. Structural changes of the beads in response to solution pH were put forward using the data obtained from IR/UV spectral analysis. The release experiments were performed in solutions of pH 2.0 and pH 7.4 at 37°C, using chlorphenramine maleate as a model drug. The results indicate that, chitosan might be useful as a vehicle for controlled release of drugs.
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Key words: beads; chitosan; chlorphenramine maleate; controlled release; IPN; swelling degree

INTRODUCTION

Controlled-release technology emerged during the 1980s as a commercially sound methodology. Biodegradable polymers have found applications in the medical field, such as surgical sutures, surgical implants, and in drug delivery systems.[1–4] For clinical applications, it is often important to distinguish clearly between "biodegradable polymers" and "bioabsorbable polymers." Biodegradable polymers are those that decompose in the living body, but the degradation products remain in tissues for longer time. On the other hand, bioabsorbable polymers can be defined as the polymers that are eliminated from the body or metabolized therein. Although the term *bioabsorbable* is preferred in polymeric drug delivery systems, it is often used interchangeably with other terms, such as absorbable, resorbable, and biodegradable polymers.[5,6]

Chitosan [(1 → 4) 2-amino-2-deoxy, β-D-glucan] is a polyaminosaccharide, normally obtained by alkaline deacetylation of chitin,[7] a naturally occurring polymeric material. It occurs as a principal constituent of the protective cuticles of crustacea, insects, and in the cell walls of some microorganisms. Chitosan is a highly versatile polysaccharide and has been the focus of much research.[8] Chitosan is nontoxic, bioabsorbable,[9] and structurally resembles glycans. Currently, chitosan is the preferred material for controlled drug–delivery devices.[10–18] The use of chitosan in the development of oral sustained-release preparations is based on the intragastric floating tablets of chitosan.[19,20] Moreover, the antacid and antiulcer characteristics of chitosan prevent or weaken drug irritation in the stomach.[16] Therefore, chitosan has great potential for its use as a suitable

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carrier in controlled drug–delivery systems. However, a reservoir system of chitosan beads for oral use has rarely been reported in literature.[14,15,19,20] There have been no reports to date on the beads composed of chitosan and glycine as a spacer group, crosslinked with glutaraldehyde.

We have been investigating the swelling kinetics and modeling drug-release properties of chitosan beads.[21–23] The present study is an attempt to develop sustained-release crosslinked beads of chlorphenramine maleate, as a part of our studies on the pharmaceutical applications of chitosan.

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. The homogeneous transparent viscous solution was precipitated in 1*M* NaOH. The precipitated chitosan was repeatedly washed with hot water and dried in a vacuum oven at 20°C. The molecular weight of the purified chitosan was determined by viscometric method using the Mark–Houwink equation[24] $[\eta] = K_m M^a$, where $K_m = 1.81 \times 10^{-3}$, $a = 0.93$. The average molecular weight ($\bar{M}_v$) of chitosan was 2.9×10^6. The percentage N-deacetylation of the chitosan was determined using the following relationship,[25] which was found to be 61%.

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

where A is the logarithmic ratio of the absorbance and transmittance at the given wavenumber. Chlorphenramine maleate (CPM) was obtained as a gift sample from Ranbaxy Pharmaceutical Pvt. Ltd., India. All other chemicals used were of analytical grade.

Preparation of Crosslinked Chitosan–Glycine Beads

Purified chitosan and glycine were dissolved in acetic acid under stirring conditions for 3 h at room temperature. 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 beads were washed with hot and cold water, respectively. The resultant beads were placed in a water jacket containing glutaraldehyde solution at 50°C for about 10 min. Finally, the beads were successively washed with hot and cold water and vacuum-dried at 30°C. The composition of the beads prepared is given in Table I. For preparing drug-loaded beads, a known amount of drug (125, 75, and 100 mg, respectively) was added to the chitosan–glycine mixture before extruding into the alkaline–methanol solution.

Table I Composition of Chitosan Beads

Code	Chitosan (g)	Glycine (g)	Glutaraldehyde, 10 ml (%)	2% Acetic Acid (ml)
A1	0.5	0.5	25.00	20
A2	0.5	0.5	12.50	20
A3	0.5	0.5	6.25	20
A4	0.5	0.5	3.13	20
A5	0.4	0.5	12.50	20

Swelling Studies

Swelling behavior of chitosan beads (A1–A5) at different pH has been studied. The degree of swelling for each sample at time t was calculated using the following relationship:

$$\text{Degree of swelling} = (W_t - W_0)/W_0$$

where W_t and W_0 are the weights of the beads at time t and in the dry state, respectively.

IR Spectra of the Chitosan Beads

IR spectra of the chitosan beads were recorded on KBr pellets using a Perkin–Elmer Fourier Transform (FTIR-1600) spectrophotometer.

Scanning Electron Microscopy (SEM)

The shapes and surface morphology of the beads were examined using a scanning electron microscope (Leo 435 VP-England). For SEM studies, the samples were mounted on metal stubs using double-sided adhesive tape and vacuum-coated with gold.

IR/UV Spectra to Monitor Structural Changes of the Beads

In an attempt to investigate the structural changes of the crosslinked beads (A1–A5), the preceding

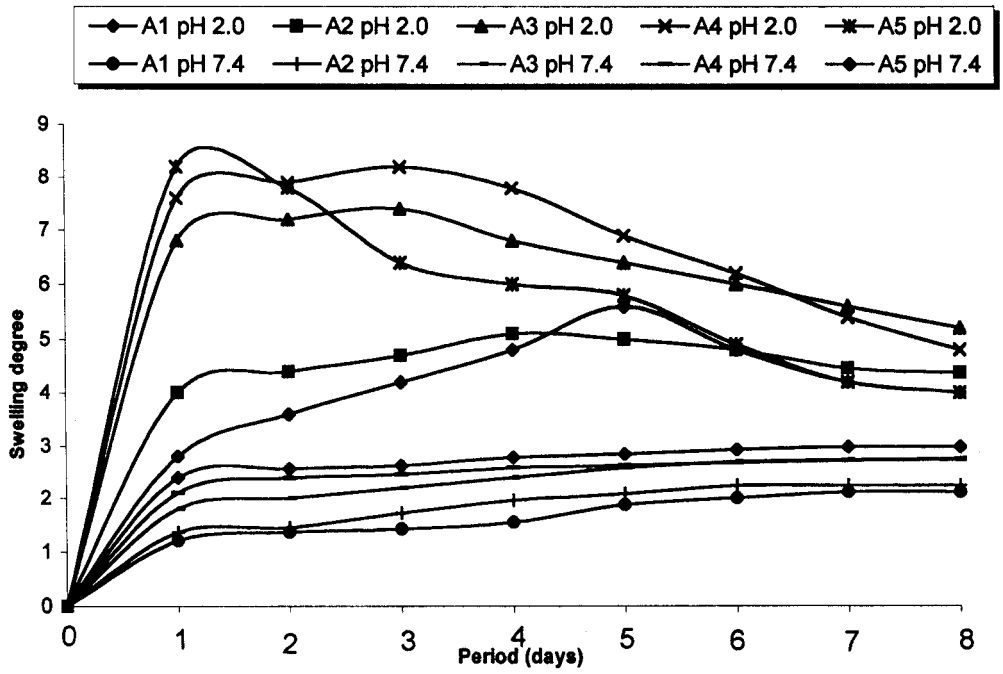


Figure 1 Swelling behavior of the crosslinked beads measured as a function of time in pH 2.0 and in pH 7.4 at 37°C.

swelling experiments were repeated. At certain intervals, the swelling solution was filtered and the UV spectra of the filtrate were recorded using a Shimadzu 1601 UV-Visible spectrophotometer. The beads were then dried at 35 to 45°C and IR spectra were recorded to notice the structural changes by comparing with the IR spectra of initial dry beads.

Drug Assay

A sample of drug-loaded beads was accurately weighed (0.1 g) and kept in 100 ml of 2% acetic acid at 30°C for 48 h. After centrifugation, the chlorphenramine maleate in the supernatant was assayed by spectrophotometer at 193.5 nm.

Drug-Release Studies

The release 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 (0.1 g) containing a known amount of chlorphenramine maleate were added to the release medium. At predecided intervals, 1 ml aliquots were withdrawn, filtered, and assayed, recording absorbance at 193.5 nm. The cumulative chlorphenramine maleate release is measured as a function of the time.

RESULTS AND DISCUSSION

Swelling Studies

The swelling response of the glutaraldehyde-crosslinked chitosan beads in solutions of pH 2.0 and pH 7.4 at 37°C is shown in Figure 1. Generally, the swelling process of the beads in pH < 6 involves the protonation of amino/imine groups in the beads and mechanical relaxation of the coiled polymeric chains. The process of protonation is expected to complete in two stages. In the first stage, amino/imine groups at the bead surface were protonized, which led to dissociation of the hydrogen bonding between amino/imine groups and other groups. The protonation resulted in solvent invading the polymer from the sample surface and forming a sharp boundary or moving front, separating the insolvated polymer region ahead of the front from the swollen bead phase behind it. In the second stage, protons and counterions diffused into the bead, to protonate the inward amino/imine groups, dissociating the hydrogen bonds. This process of protonation continued until the whole structure of the beads was collapsed and solvated.

The observed swelling rates of the crosslinked beads followed the order A5 $>$ A4 $>$ A3 $>$ A2

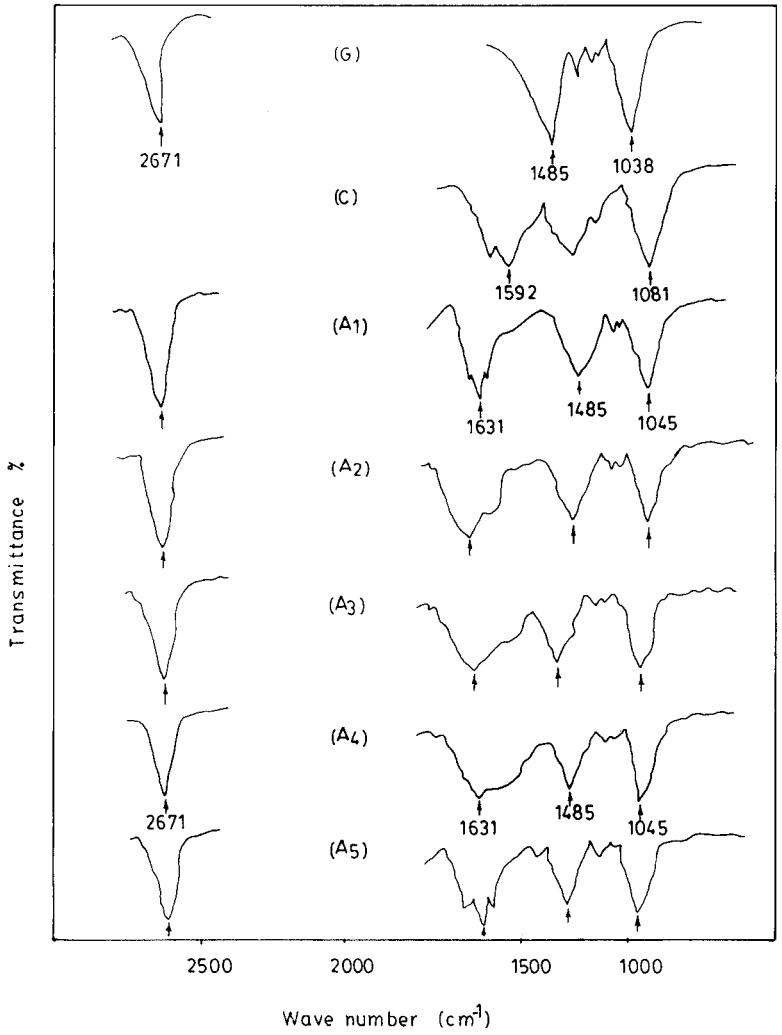


Figure 2 FTIR spectra of glycine (G), chitosan (C), and crosslinked beads A1, A2, A3, A4, and A5.

> A1. It is a well-known fact that the degradation of the polymer depends on the degree of crosslinking. In the present case, the degree of swelling is very high in solution of pH 2.0 compared to that of pH 7.4, which is due to inherent hydrophobicity of the chitosan beads dominating at high pH value, thus preventing faster swelling in neutral and alkaline media. Structural changes that take place during the process of swelling of crosslinked beads in solutions of different pH are supported by the IR and UV spectra recorded at different stages of swelling.

IR Spectra

Figure 2 shows the IR spectra of glycine (G), chitosan (C), and crosslinked beads (A1–A5). The peaks at 1592 cm^{-1} in the IR spectrum of chitosan [Fig. 2, spectrum (C)] can be assigned as amino absorption peak. In contrast with spectra (G) and (C), a significant new peak at 1631 cm^{-1} in the spectra (A1–A5) is due to the formation of C═N and this is because of the imine reaction between amino groups from chitosan and aldehydic groups in glutaraldehyde. On decreasing the glutaraldehyde concentration, the peak corresponding to 1631 cm^{-1} is broadened gradually (A1–A4), whereas, the peaks at 1485 and 2671 cm^{-1} in the beads (A1–A5) are the characteristic peaks of the glycine. The peaks at 1038, 1045, and 1081 cm^{-1} in the spectra (G), (C), and (A1–A5) are due to C—O stretching vibrations in glycine, chitosan, and crosslinked beads, respectively.[26,27] Due to

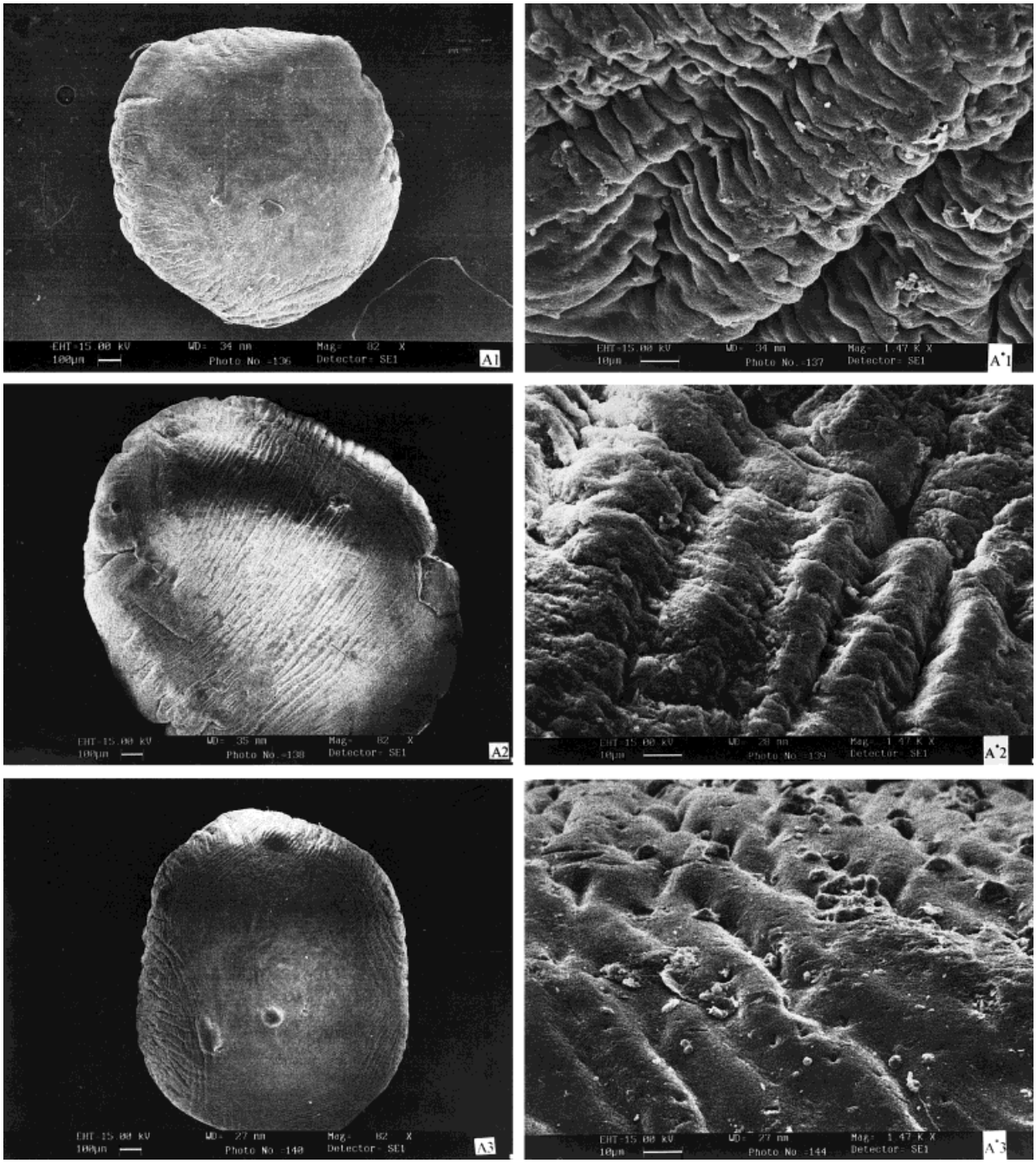


Figure 3 SEM photographs of the crosslinked beads (A1–A5) and their morphology (A*1–A*5).

the amphoteric behavior of glycine, it is expected to interact with chitosan through intermolecular physical crosslinks.

SEM Studies

Crosslinked chitosan–glycine beads prepared by using different concentrations of glutaraldehyde have rough and dense surfaces. The beads are spherical in shape and the size varies from 1270–1800 μm [Fig. 3, spectra (A1–A5)]. From the morphology of the beads as shown in Figure 3 (A1*–A5*), one can observe the rough and folded surface of the beads, and that decreasing the concentration of crosslinking agent contributed to

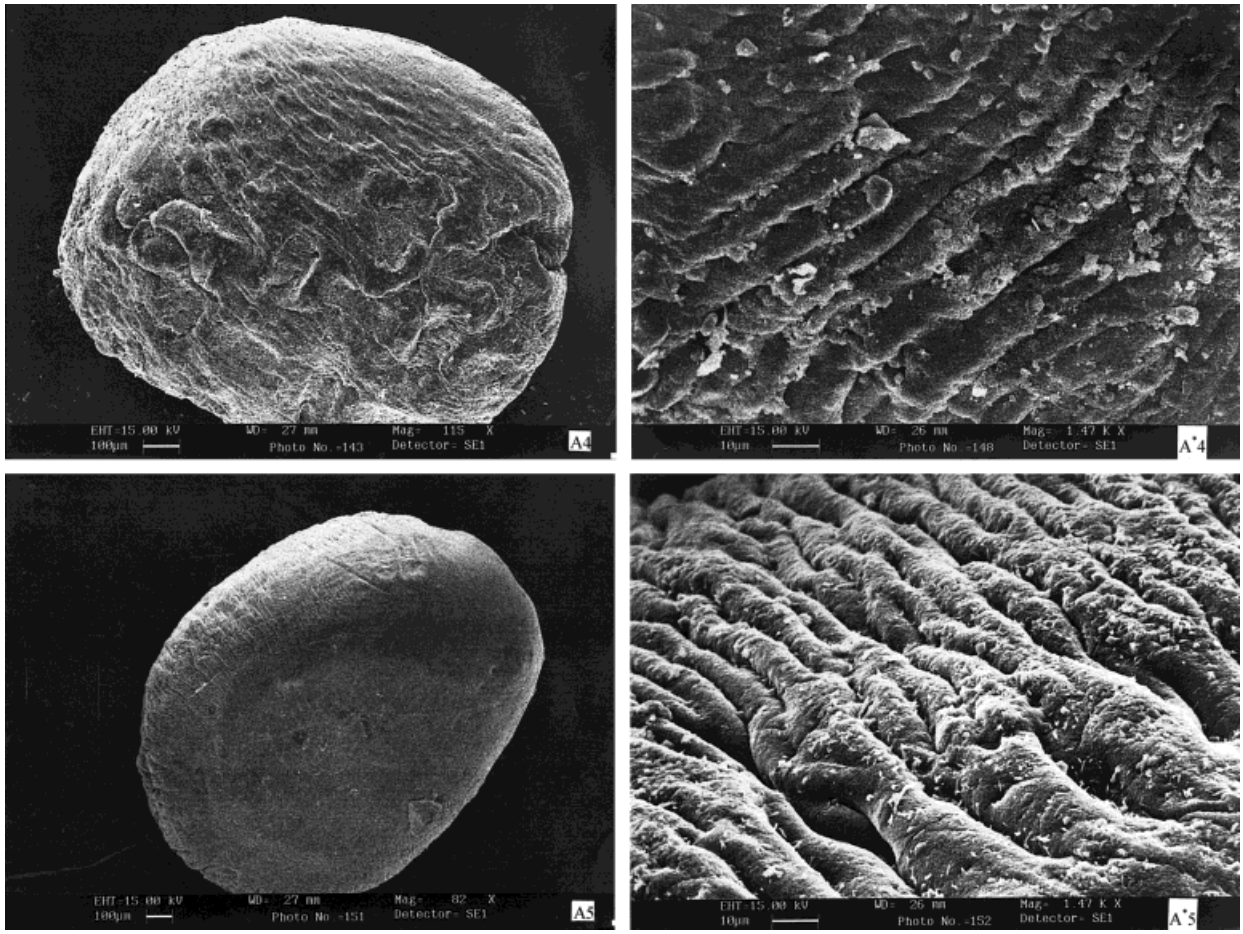


Figure 3 (*Continued from the previous page*)

an increase in swelling and a decrease in complexity of the surface folding. Huguet and coworkers[28] encapsulated hemoglobin in chitosan/alginate beads and studied the release of entrapped material. From their investigations, they concluded that increasing the concentration of chitosan results in lower swelling and slower release of encapsulated material. Therefore, in an attempt to study the effect of polymer concentration on swelling behavior and release rate, we prepared A5. Our results contradict the general proportional relationship between swelling behavior and crosslink density. Chitosan beads (A5) showed the ability to swell fast in pH 2.0 solution, irrespective of the complex surface morphology [Fig. 3, spectrum (A5)]. Scanning electron micrographs of the beads after swelling for 5 days ($A1^+$), 4 days ($A2^+$), 3 days ($A3^+$), 3 days ($A4^+$), and 2 days ($A5^+$) in pH 2.0 solution are shown in Figure 4 ($A1^+$–$A5^+$). The size of the beads after swelling varies from 1500–1800 μm. Considerable change in the shape is not observed, but cracks and pores appeared on the surface of the beads.

From the morphological studies as shown in Figure 4 ($A1^+$–$A5^+$ and $A1^\bullet$–$A5^\bullet$), it is evident that the complex folded structure of the beads starts collapsing after attaining equilibrium swelling at pH 2.0 due to protonation of the amine/imine group and breaking of the hydrogen bonds, which resulted in the formation of the cracks and the pores at the surface of the beads. The magnitude of the pores/cracks that appeared on the surface of the beads depends on the composition of the beads and the duration of the beads kept in the solution. The morphological changes that take place at the surface of the beads (Fig. 4, $A1^+$–$A5^+$ and $A1^\bullet$–$A5^\bullet$) have a direct relationship with the amount of drug released at a particular time of swelling (Fig. 8), i.e., as the extent of the cracks/pores increases, the amount of the drug released is also increased.

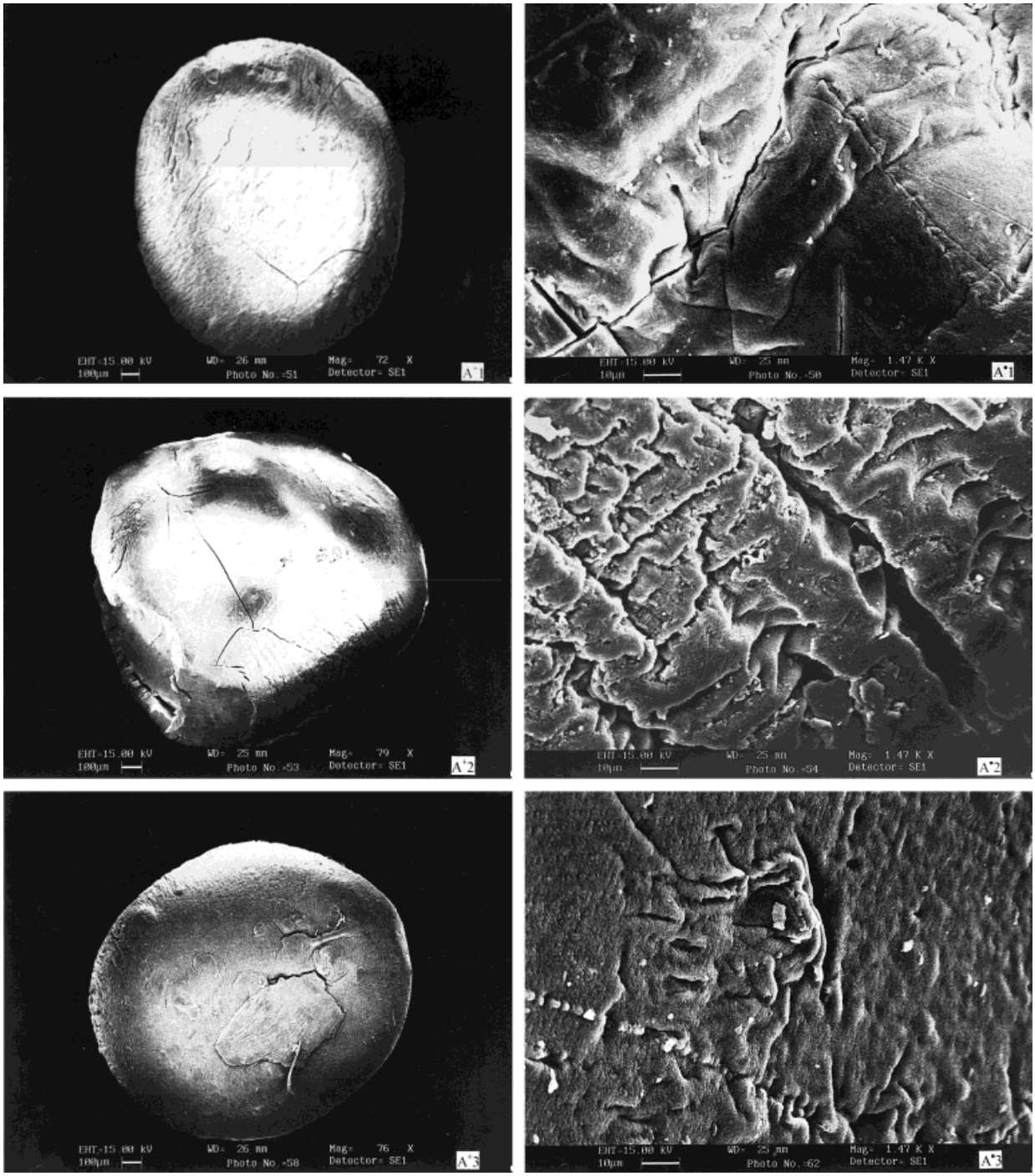


Figure 4 SEM photographs of the crosslinked beads after swelling for 5 days (A^+1); 4 days (A^+2); 3 days (A^+3); 3 days (A^+4); 2 days (A^+5) and their morphology ($A^\bullet1$–$A^\bullet5$).

IR/UV Spectra

FTIR spectra of chitosan beads at equilibrium in a swelling solution of pH 2.0 are shown in Figure 5 (A1–A5). The spectra A1, A2, A3, A4, and A5 are recorded at 5 days, 4 days, 3 days, 3 days, and 2 days, respectively. Imine groups in the network get protonated in acidic pH and the hydrogen bonding was dissociated, which promoted swelling of the beads. By contrast with Figure 2 (A1–A5), there are two new peaks at 1623 and 1523

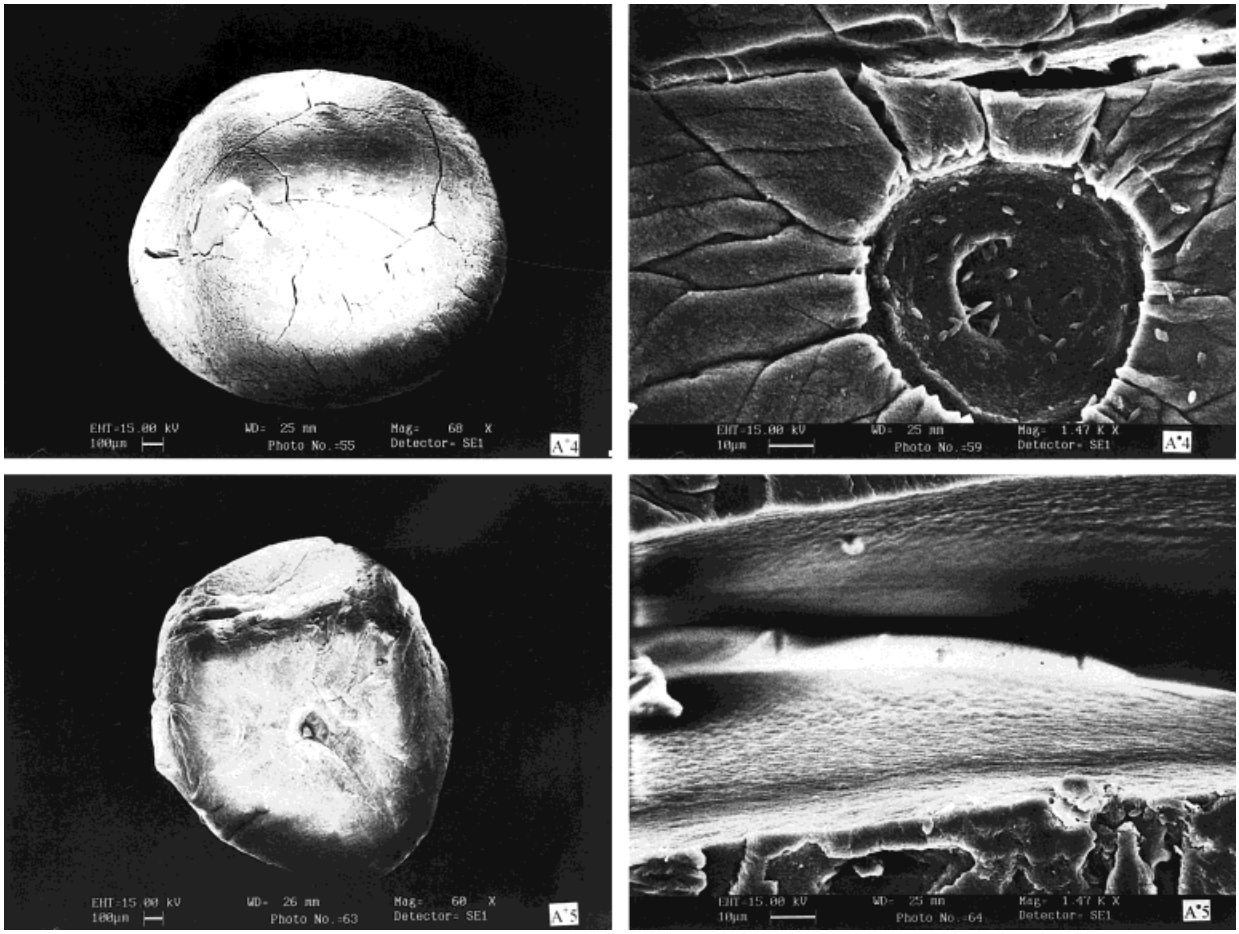


Figure 4 (*Continued from the previous page*)

cm^{-1} assigned to $^{+}NH_3$ absorption peaks[26,27] in the spectra of Figure 5 (A1–A5), which supports the formation of $^{+}NH_3$ within the beads when swollen in pH 2.0. The peaks corresponding to glycine (1485 and 2671 cm^{-1}) disappeared in the beads (A1–A5) after attaining equilibrium swelling, which indicates the dissolution of glycine from the network. On comparing the spectra of Figure 5 (A1–A5) and Figure 2 (C), the characteristic peak of chitosan[27,29] is shifted from 1045 to 1100 cm^{-1} after the sample was swollen in pH 2.0.

Figure 6 shows the IR spectra of the beads swollen for different times in solution of pH 7.4. The spectra reveal that the peak at 1631 cm^{-1} assigned to C═N absorption disappears, as shown in Figure 6 (A1–A5); meanwhile, the peaks assigned to glycine at 1485 and 2671 cm^{-1} also weaken, but the rate is slower than that in the case of pH 2.0 solution. In addition, it was noticed that there is no peak related to $^{+}NH_3$ in the IR spectra of swollen beads in pH 7.4 [Fig. 6 (A1–A5)], which may confirm that the imine groups within the beads are not protonized in pH 7.4, leading to a lower swelling of the beads.

In comparison to the spectrum of chitosan in Figure 2 (C), it was found that the peak at 1592 cm^{-1} in Figure 6 (A1–A5) becomes similar to that of chitosan. This elucidates that the changes in the structure of the beads may result in 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 of amino groups of chitosan. On the other hand, it was confirmed from the UV spectra (data not shown) that the imine bond within the beads did not break on swelling in solution of pH 7.4 for 5 days (A1), 4 days (A2), 3 days (A3), 3 days (A4), and 2 days (A5). However, a characteristic peak of chitosan around 200 nm, due to its dissolution from the beads was observed. However, there is no peak relating to glutaraldehyde perhaps caused by the cleavage of

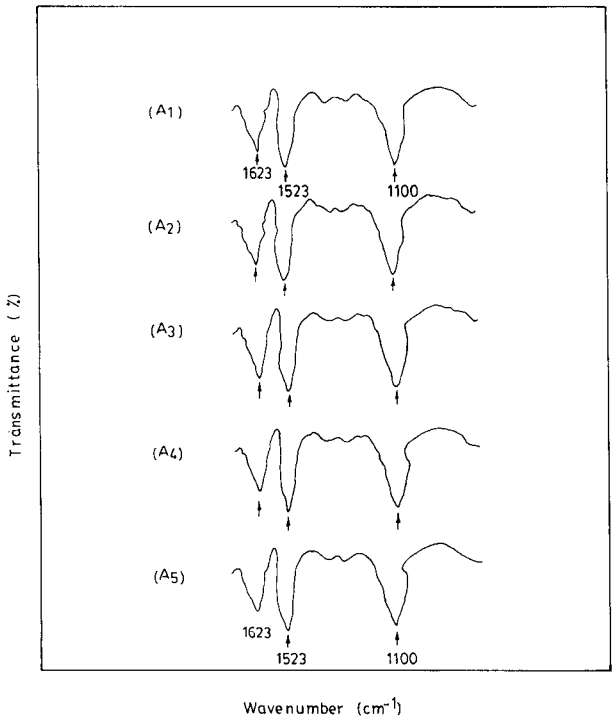
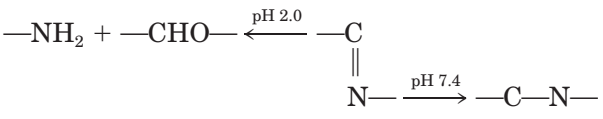


Figure 5 FTIR spectra of the crosslinked beads after swelling in pH 2.0 at 37°C for 5 days (A1), 4 days (A2), 3 days (A3), 3 days (A4), 2 days (A5).

C═N; therefore, it may be reasonable to assume that the imine bond change may be attributed to conversion of C═N to C—N in solution of pH 7.4.

In Figure 7 (A1–A5), we can confirm the cleavage of imine bonds in the swollen beads in pH 2.0 at 37°C. It was shown that the peaks at 195.4, 195.8, 197 nm and 230.2, 234, 235.4 nm in Figure 7 (A1–A5) are 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 further dissolution of chitosan in the swollen beads. The changes of imine bond within the network in the present case can be expressed as:



Drug-Release Studies

Figure 8 shows the dissolution profile of chlorphenramine maleate (CPM) from crosslinked chitosan–glycine beads (68 μg of drug loaded mg^{-1} bead) at various time intervals in acidic (pH 2.0) and basic (pH 7.4) media at 37°C. There is a burst release initially for the first hour in both media (pH 2.0 and pH 7.4) followed by an almost constant release of CPM from the matrix for the studied period of 48 h. The amount and percentage of drug release were much higher in acidic solution than in basic solution, because the release rate depends on swelling of the beads. It was already discussed in previous sections that the swelling of the beads in acidic medium is greater and faster than in basic medium.

To check the reproducibility of the results, the release profile of CPM from the chitosan beads loaded with lower amounts of drug (29 and 42 μg of drug loaded mg^{-1} bead, respectively) has also been studied in acidic (pH 2.0) and basic (pH 7.4) media, as shown in Figures 9 and 10. The release pattern of the drug-loaded beads has been found to be similar, irrespective of the amount of the drug loaded. These observations have suggested that the percentage of drug released from chitosan beads has decreased with the increase in concentration of CPM. However, the total amount

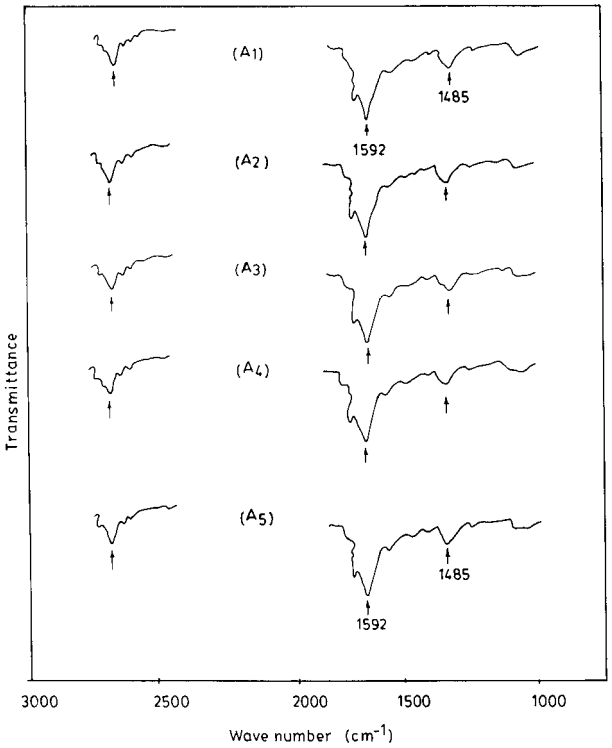


Figure 6 FTIR spectra of the crosslinked beads after swelling in pH 7.4 at 37°C for 5 days A1, 4 days A2, 3 days A3, 3 days A4, 2 days A5.

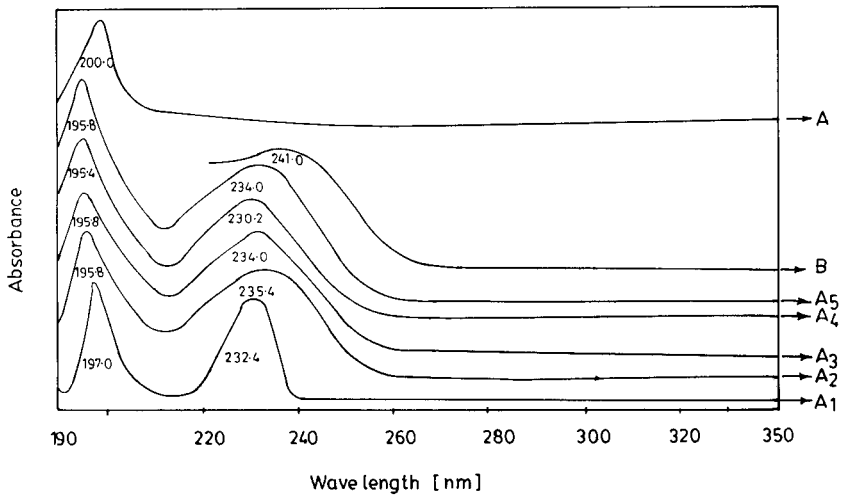


Figure 7 UV spectra of chitosan (A) glutaraldehyde (B) solution in pH 2.0 and of the solution left after initial dry beads swollen in pH 2.0 for 5 days (A1), 4 days (A2), 3 days (A3), 3 days (A4), 2 days (A5).

of CPM released from the beads loaded with a higher amount of drug was found to be higher in comparison to the beads loaded with a lower amount. It is understood that the mechanism of drug release is due to the diffusion through swollen beads in pH 2.0, whereas swelling in pH 7.4 is less and the drug release is less.

In the basic medium, there is a limited swelling of the beads, which prevents the release of drugs at a faster rate as it occurs in acidic solution. The initial fast swelling of the beads in acidic solution gives rise to significant burst effect in the beginning, but becomes almost constant as the degree of swelling of the beads attained an equilibrium. It is evident from the analysis of the initial drug-release data as reported in Figures 8–10 that the drug-release rate is first order and occurs through diffusion from the swollen beads.

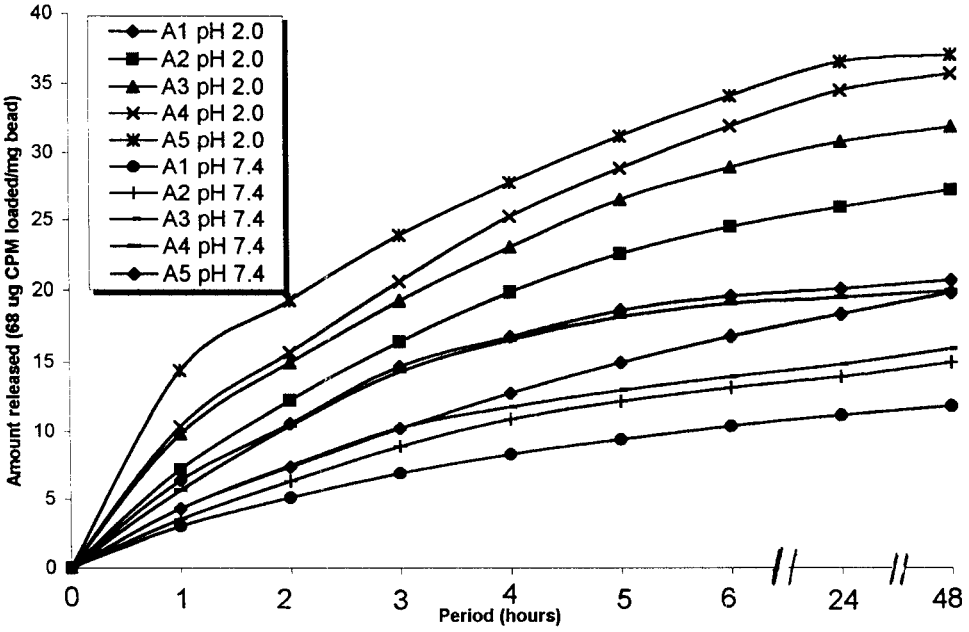


Figure 8 Release of chlorphenramine maleate for the beads (68 μg CPM loaded/mg bead) versus time in solutions of pH 2.0 and pH 7.4 at 37°C.

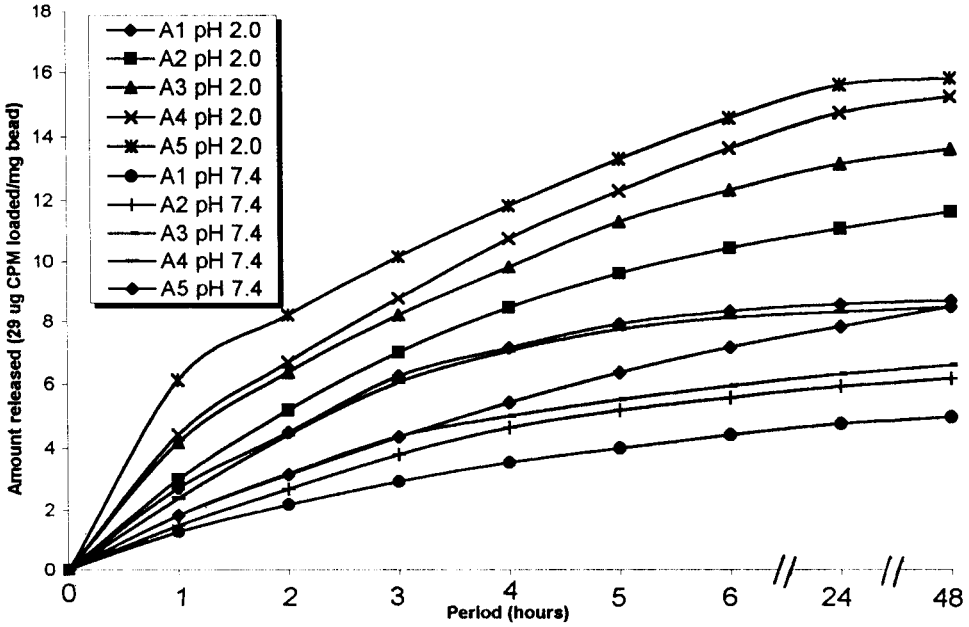


Figure 9 Release of chlorphenramine maleate for the beads (29 μg CPM loaded/mg bead) versus time in solutions of pH 2.0 and pH 7.4 at 37°C.

CONCLUSIONS

The observations of the present study have indicated that chitosan can be used successfully for the formulation of controlled drug–delivery devices. A major advantage of the chitosan beads is that they can be prepared easily under mild conditions. Furthermore, they have an optimum entrapping capacity for the studied drugs and provide a sustained release of drugs for extended periods. All these interesting features make this novel system a very promising

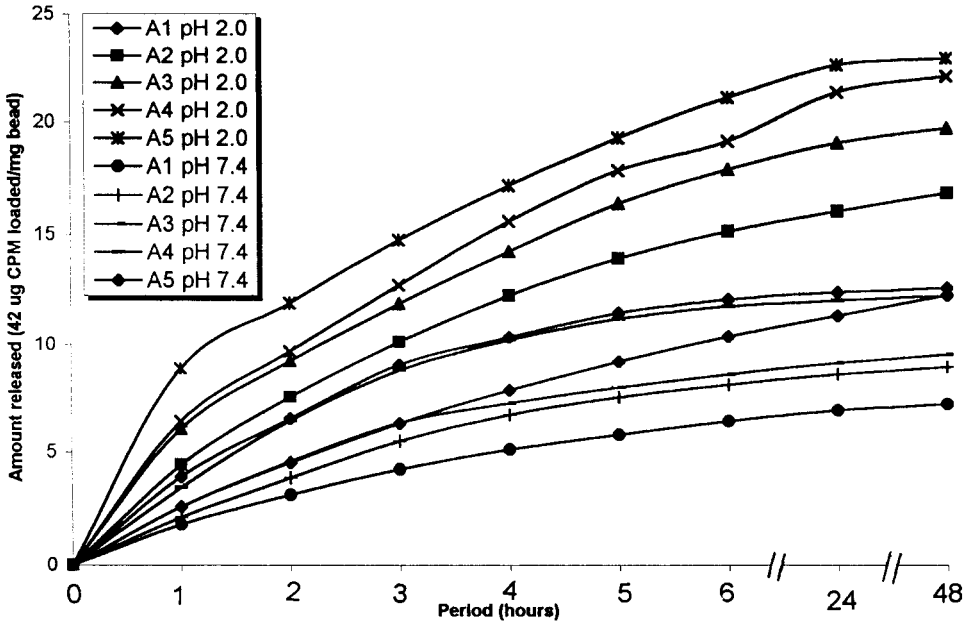


Figure 10 Release of chlorphenramine maleate for the beads (42 μg CPM loaded/mg bead) versus time in solutions of pH 2.0 and pH 7.4 at 37°C.

vehicle for the administration of therapeutics by various routes.

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