Studies on Semi-Interpenetrating Polymer Network Beads of Chitosan–Poly(ethylene glycol) for the Controlled Release of Drugs

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ABSTRACT: Semi-interpenetrating polymer network beads of chitosan and poly(ethylene glycol) were prepared and characterized for controlled release of drugs. A viscous solution of chitosan and poly(ethylene glycol) in 2% acetic acid was extruded as droplets with the help of a syringe and crosslinked using glutaraldehyde. The structural studies of the beads were performed by using a Fourier transform infrared spectrophotometer and scanning electron microscope. The swelling behavior, solubility, hydrolytic degradation, and loading capacity of the beads for isoniazid were investigated. The structural changes of the beads at pH 2.0 and 7.4 were put forward using the data obtained by infrared and ultraviolet spectroscopy. The prepared beads showed 82% drug-loading capacity, which suggested that these semi-interpenetrating polymer network beads are suitable for controlled release of drugs in an oral sustained delivery system. © 2001 John Wiley & Sons, Inc. J Appl Polym Sci 80: 639–649, 2001

Key words: beads; chitosan; controlled release; crosslinking; IPN; isoniazid; swelling

INTRODUCTION

Biodegradable polymers add new dimensions to drug-delivery devices. A variety of degradable polymers are potentially useful for this purpose.[1] Currently, the interest in chitin, chitosan (CHI), and their derivatives as biodegradable drug carriers has increased substantially.[2–5] Using biodegradable polymers, functional microcapsules have been developed[2] in which permeability is controlled by temperature, pH, light, and electric fields. One method to obtain such microspheres is to first produce a kind of pre-microspheres and then to coat or graft them with environment-sensitive materials.[2,6]

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Chitin is a high molecular weight linear polymer of *N*-acetyl-D-glucosamine (*N*-acetyl-2-amino-2-deoxy-D-glucopyranose) units linked by β-(1→4) bonds. It is a highly insoluble material resembling cellulose in its solubility and low chemical reactivity. It may be regarded as cellulose with hydroxyl at position C-2 replaced by an acetamido group. Like cellulose, it naturally functions as a structural polysaccharide. It is most abundant in crustaceans, insects, and fungi. Deacetylated chitin is an amino polymer with many potential uses such as a textile sizer, adhesive, emulsifier, and pharmaceutical component.[7,8] CHI is nontoxic and easily bioabsorbable,[9] and has been explored for the release of several drugs.[10,11] The use of CHI in the development of oral sustained release preparations was indicated based on the intragastric floating tablets of CHI.[12,13] CHI with its antacid and antiulcer characteristics may prevent or weaken drug

Table I Composition of Chitosan Beads

Code	Chitosan (g)	PEG (g)	Glutaraldehyde (10 mL)	2% Acetic acid (mL)
CHI	0.5	—	—	20
CHI–PEG	0.5	0.5	—	20
B1	0.5	0.5	25.00%	20
B2	0.5	0.5	12.00%	20
B3	0.5	0.5	6.25%	20
B4	0.5	0.5	3.13%	20
B5	0.5	0.5	1.57%	20

irritation in the stomach.[14,15] Yao et al.[2] have reviewed microcapsules and microspheres related to CHI.

Biocompatibility of poly(ethylene glycol) (PEG) makes it a choice material favoring biomedical applications.[16] Graham and McNeill[17–19] pioneered crosslinked PEG-diisocyanates network for drug-delivery devices. They studied the release behavior of PEG hydrogels loaded with low molecular weight compounds. In the present investigation, PEG was used to enhance the swelling, solubility, and release properties by forming intermolecular crosslinks with CHI hydroxyl groups. The CHI–PEG system has shown better results than the previously studied CHI–glycine system,[20–22] which may be attributed to the water diffusivity and pore-forming properties of PEG.

EXPERIMENTAL

Materials and Methods

CHI was obtained as a gift sample from the Central Institute of Fisheries Technology, Cochin, India. Impurities were removed by dissolving 1 g of CHI 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 CHI was repeatedly washed with hot water and dried in a vacuum oven at 20°C. The molecular weight and %*N* deacetylation CHI were found to be 2.9×10^6 and 61% respectively.[22] Isoniazid ($C_6H_5CONHNH_2$) was obtained as a gift sample from Pharmachem, Bahadurgarh, India. PEG (MW = 6000) was procured from Hi Media Laboratories, India, and used as received.

Preparation of CHI Beads

Purified CHI and PEG were dissolved in 2% acetic acid while stirring for 3 h at room temperature. The homogeneous mixture was extruded in the form of droplets using a syringe into an NaOH-methanol solution (1:20 w/w) under stirring conditions (600 rpm). The beads were washed with hot and cold water successively. The resultant beads were placed in a glutaraldehyde solution of known composition at 50°C for about 10 min for crosslinking CHI chains. Finally, the beads were washed with hot and cold water successively and vacuum dried at 30°C for 45 min. The composition of the prepared beads is given in Table I. To prepare drug-loaded beads, a known amount of isoniazid drug (85 mg/125 mg/150 mg) was suspended in the CHI–PEG solution before extruding the viscous solution into alkaline-methanol solution.

Infrared (IR) Spectra of the CHI Beads

IR spectra of the CHI beads were recorded on KBr pellets by using a Perkin-Elmer-1600 Fourier Transform spectrophotometer.

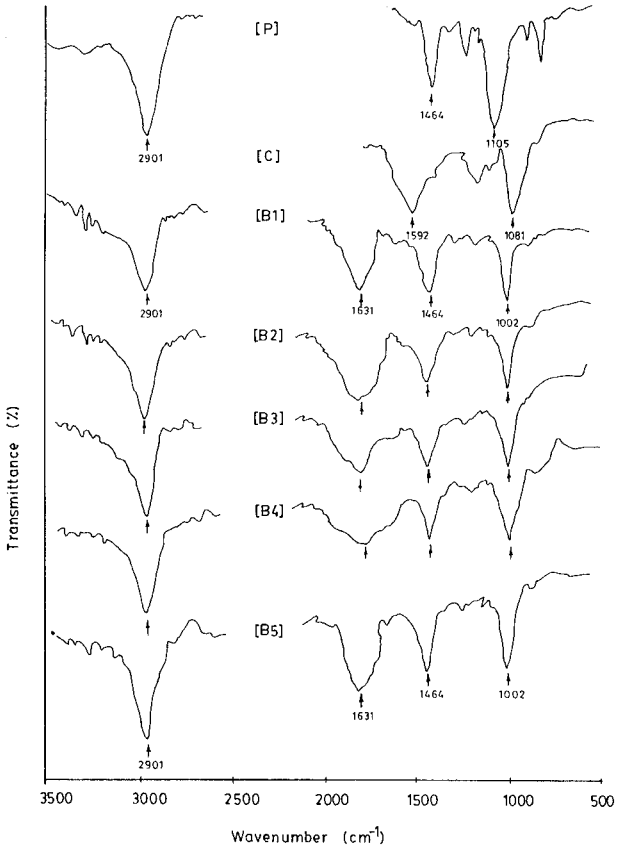

Figure 1 FTIR spectra of PEG (P), CHI (C), and crosslinked CHI beads (B1–B5).

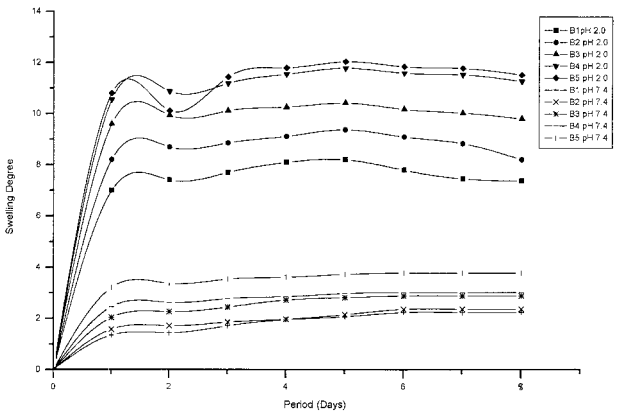

Figure 2 Swelling behavior of beads (B1–B5) in pH 2.0 and pH 7.4 at 37°C.

Swelling and Degradation Studies

Swelling behavior of CHI beads (B1–B5) at different pH was studied. The degree of swelling for each sample at a time t was calculated by using the following relationship:

$$\text{Degree of swelling} = (Wt - Wo)/Wo \qquad (1)$$

Where W_t and W_o are the weights of the beads at time t and in a dry state, respectively.

The crosslinked CHI beads were expected to undergo degradation by the hydrolysis of the amino/imine bonds; hence, hydrolytic degradation[23] of the beads (B1–B5) was studied under physiological conditions. CHI beads were placed in 100 mL solutions of pH 2.0 and pH 7.4 at 37°C under unstirred conditions and the hydrolytic degradation of the beads was observed microscopically at different time intervals.

Solubility Measurement of the Beads

A sample of beads was accurately weighed (0.1 g) and immersed in 2% aqueous acetic acid solution for 24 h and percent solubility (% S) was determined by the following equation:

$$S(\%) = (Wo - Wt)/Wo \times 100 \qquad (2)$$

Where W_o is an initial weight of the beads and W_t is a weight of the vacuum-dried beads after immersion for 24 h.

Evaluation of Drug-Loading Capacity of the Beads

A sample of drug-loaded beads (0.1 g) was kept in a 100 mL solution of acetic acid (2%) at 30°C for 48 h. After centrifugation, the drug in the supernatant and washings of the beads was assayed by recording absorbance at 193 nm with an ultraviolet spectrophotometer (Shimadzu, UV-VIS-1601 PC). The drug loading capacity (LC) of the beads and drug encapsulation efficiency (EE) of the process were calculated from eqs. (3) and (4) as shown below.

$$LC = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Weight of the Beads}} \qquad (3)$$

$$EE = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Total amount of drug}} \qquad (4)$$

Scanning Electron Microscopy (SEM)

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

IR/UV Spectra to Monitor Structural Changes of the Beads

To investigate the structural changes of the crosslinked beads (B1–B5), the above swelling experiments were repeated. At predetermined intervals of time, the swelling solution was filtered and UV spectra of the filtrate were recorded using a Shimadzu 1601 UV-Visible spectrophotometer. The beads were then dried at 35–45°C and IR spectra were recorded to note the structural changes by comparing with IR spectra of initial dry beads.

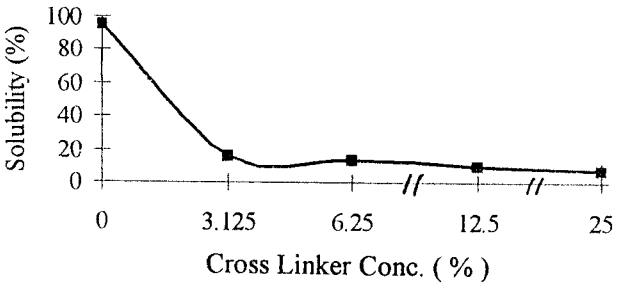

Figure 3 Dependence of solubility for crosslinked CHI beads on glutaraldehyde concentration.

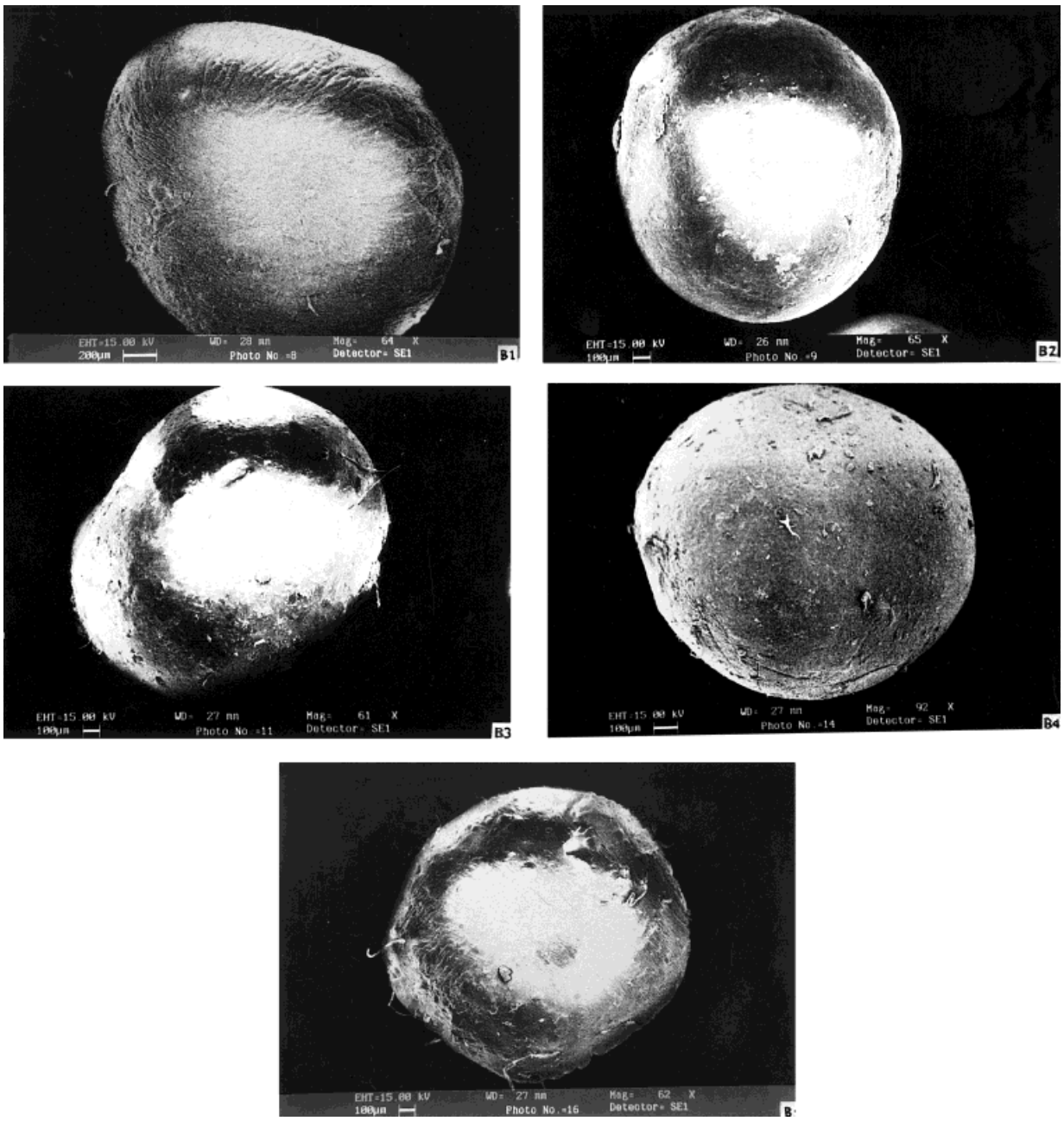

Figure 4 (a) SEMs of the crosslinked CHI beads (B1–B5), and (b) their morphology (B1*–B5*).

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 the drug were added to the release medium (100 mL). At predecided intervals, aliquots of 1 mL were withdrawn, filtered, and assayed by recording the absorbance at 193 nm.

RESULTS AND DISCUSSION

IR Spectra of CHI Beads

Figure 1 shows the IR spectra of PEG (P), CHI (C), and crosslinked beads (B1–B5). The peak at 1592 cm^{-1} in the IR spectra [Fig. 1 (C)] can be assigned to the amino group of CHI. In contrast with spectra (P) and (C), a significant new peak at 1631 cm^{-1} in the spectra (B1–B5) is attributed to

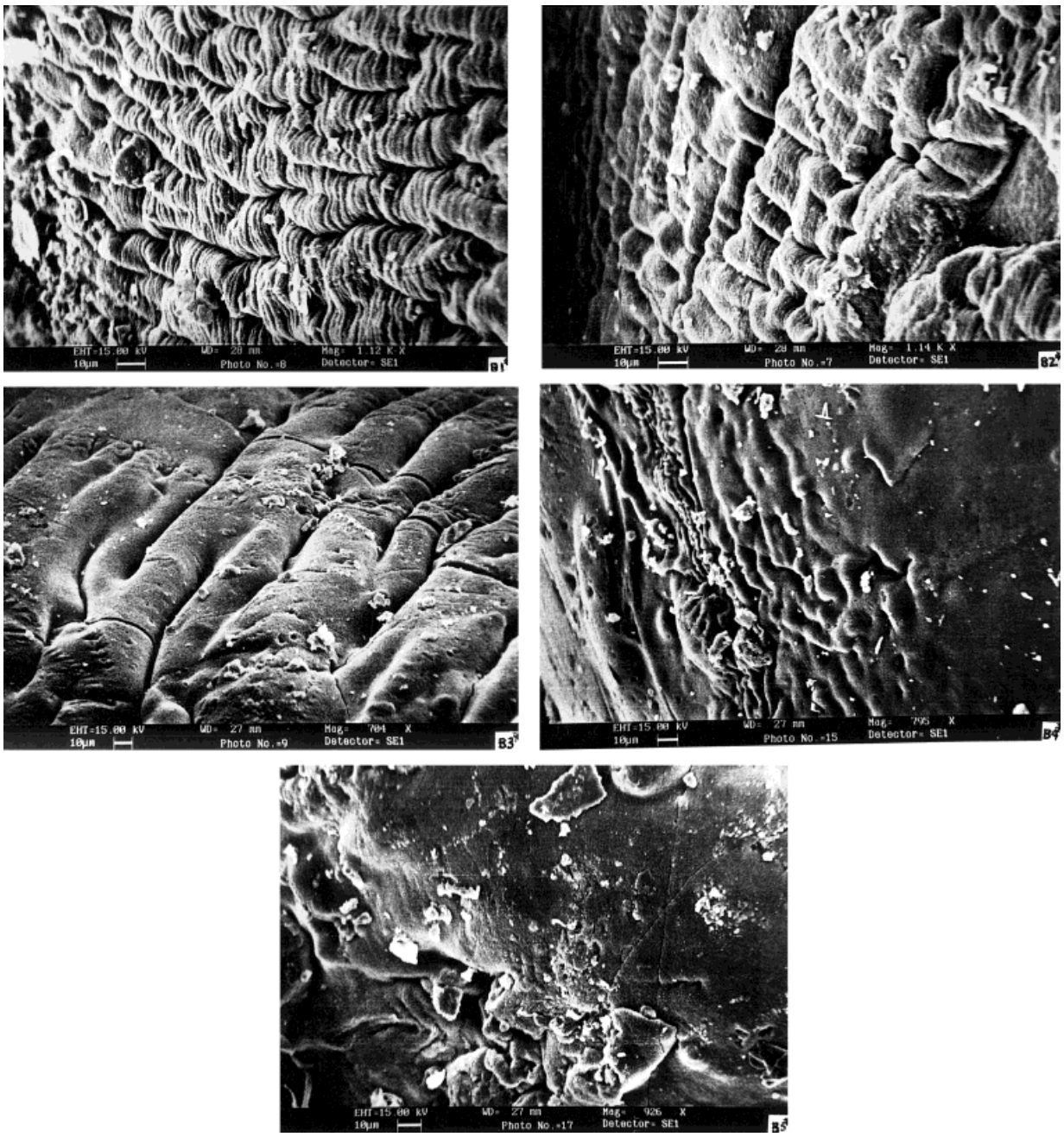

Figure 4 (*Continued from the previous page*)

the formation of the C═N group by imine reaction between amino groups of CHI with aldehydic group of glutaraldehyde. On decreasing the glutaraldehyde concentration, the peak corresponding to 1631 cm^{-1} is broadened gradually (B1–B4). The peaks at 1464 and 2901 cm^{-1} in the beads (B1–B5) are the characteristic peaks of the PEG. The peaks at 1002 (B1–B5), 1081 (C), and 1105 (P) cm^{-1} in the spectra are attributed to C—O stretching vibrations in PEG, CHI, and crosslinked beads, respectively. In this polymer system, a complexation through cooperative hydrogen bonding takes place.[24]

Swelling and Degradation Studies

The swelling response of the glutaraldehyde crosslinked CHI beads in solutions of pH 2.0 and pH 7.4 at 37°C is shown in Figure 2. Generally, the swelling process of the beads in pH < 6 involves the protonation of amino/imine groups in

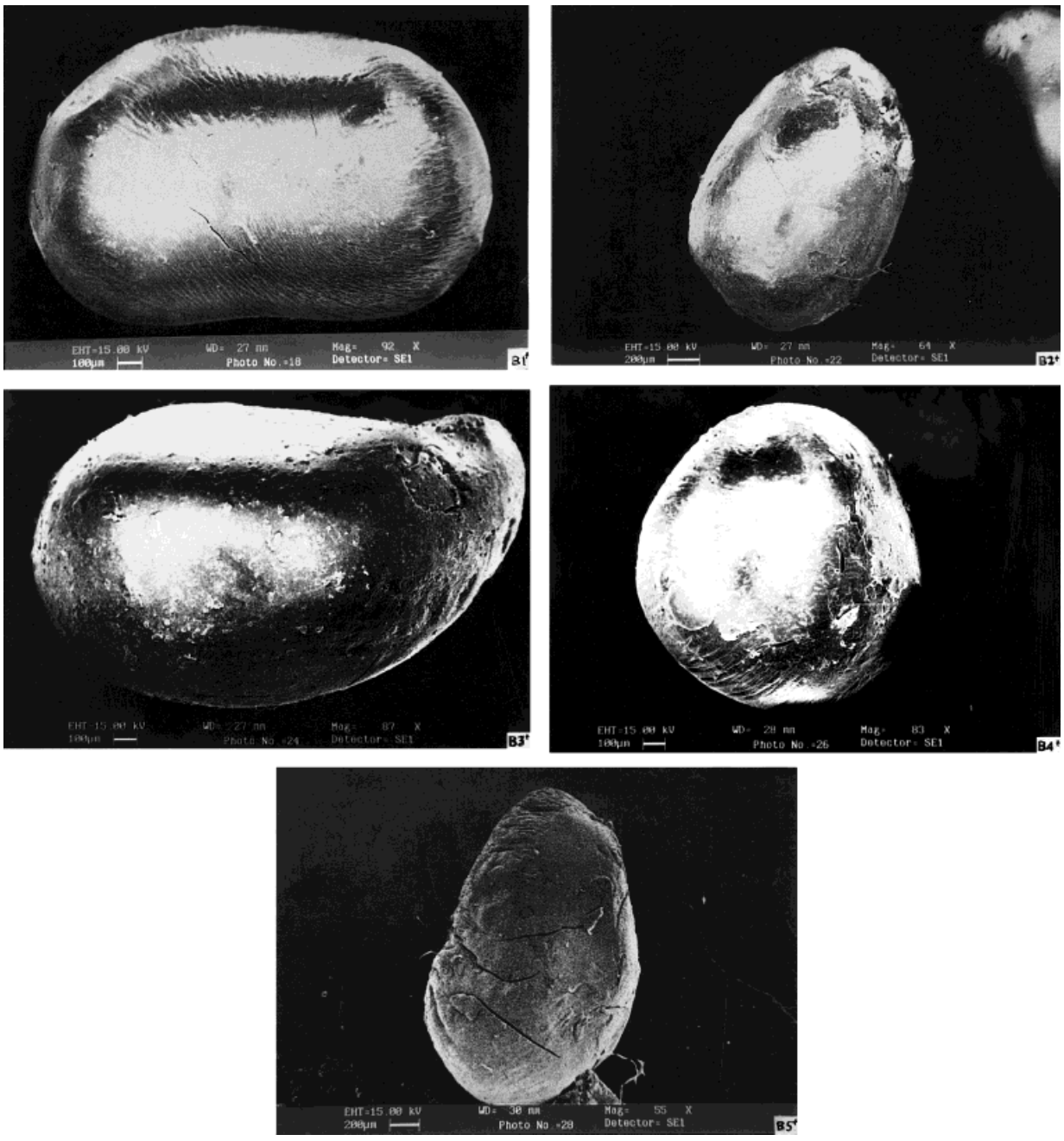

Figure 5 SEMs of the crosslinked CHI beads after swelling 4 days ($B1^+$), 4 days ($B2^+$), 3 days ($B3^+$), 2 days ($B4^+$), and 1 day ($B5^+$) (a) and their morphology ($B1^\bullet$–$B5^\bullet$) (b).

the beads and mechanical relaxation of the coiled polymeric chains. The process of swelling is expected to complete in two stages. In the first stage, amino/imine groups at the bead surface were protonized leading to dissociation of the hydrogen bonding between amino/imine groups and other groups. The protonation facilitates the solvent penetration from the sample surface forming a sharp boundary or moving front separating the insolvated polymer region with that of the swollen portion of the beads. In the second stage, protons and counterions diffuse into the bead to protonate the inner amino/imine groups, dissociating the hydrogen bonds. This process of protonation continues until the whole structure of the beads is collapsed and solvated.

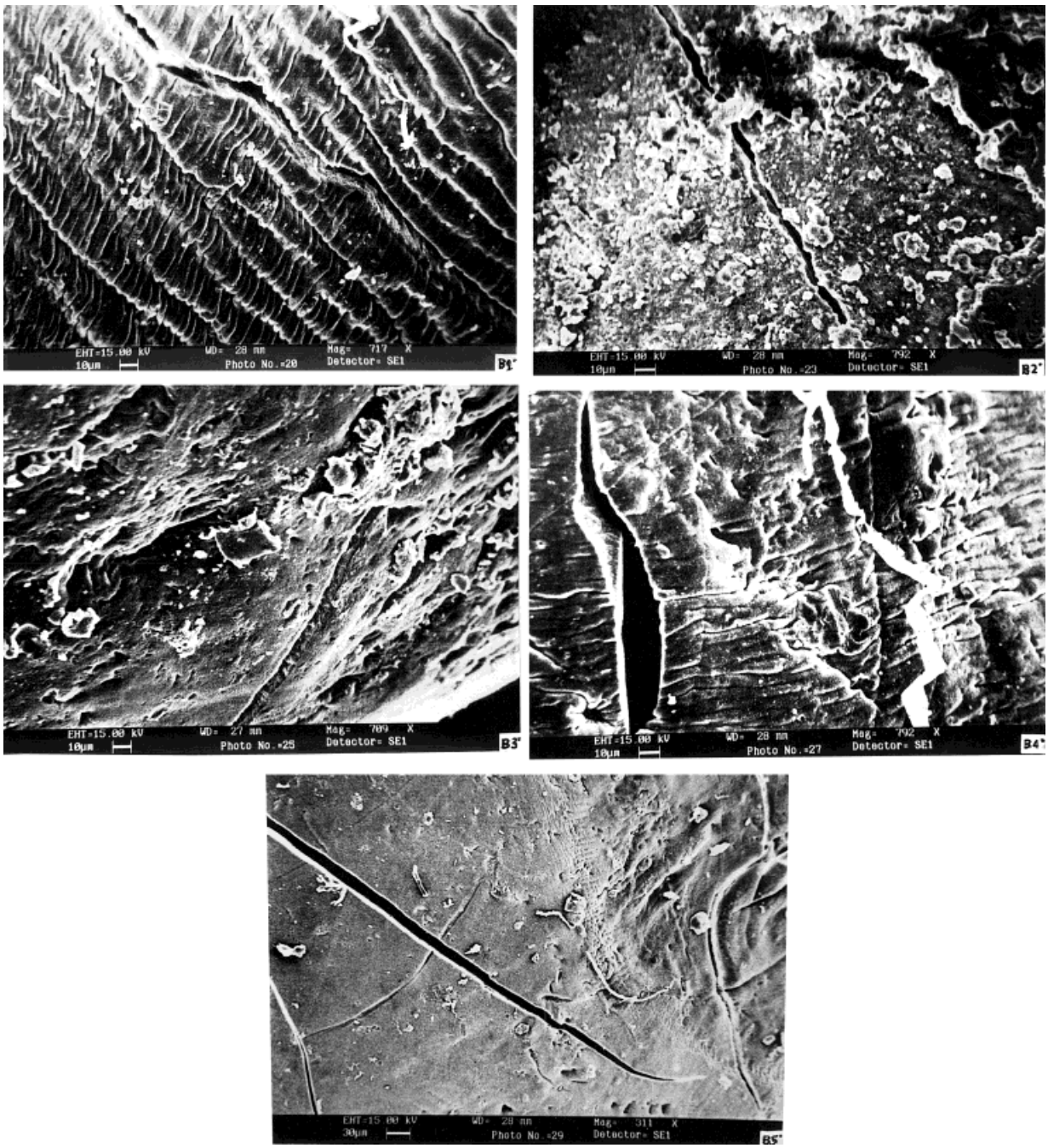

Figure 5 (*Continued from the previous page*)

From Figure 2 it is clear that the swelling of beads takes place more significantly at pH 2.0 than at pH 7.4. Moreover, the swelling equilibrium is established faster at pH 7.4 than at pH 2.0,[6] and no further swelling is observed at pH 7.4. The degree of swelling of the beads in solution of pH 2.0 begins to decline after equilibrium swelling, which is attributed to hydrolytic degradation of the beads. During this process, the cleavage of imine bond takes place. The beads of different compositions have followed the order of swelling of B5 > B4 > B3 > B2 > B1, which is directly related to the degree of crosslinking. In the present case, the degree of swelling is very high in solution of pH 2.0 to that of pH 7.4, which is attributed to dominating inherent hydrophobicity of the CHI beads at high pH, which prevents swelling of the beads in neutral and alkaline media.

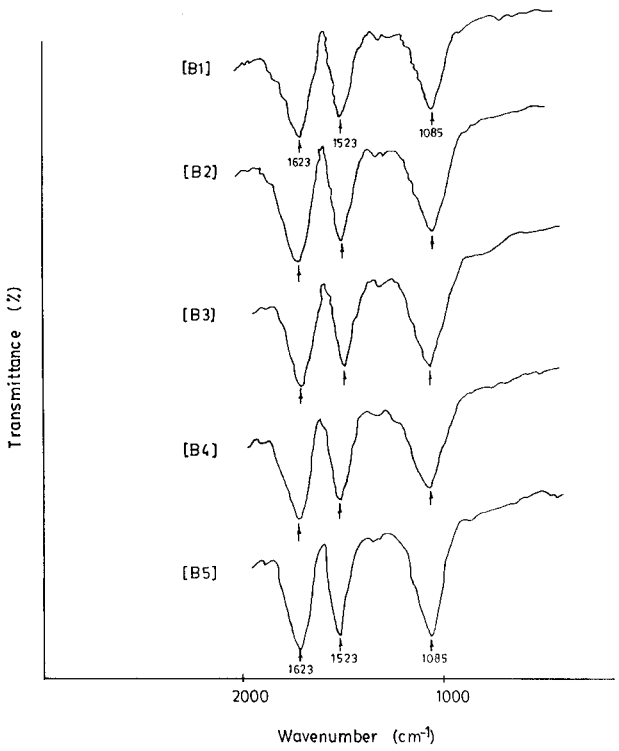

Figure 6 FTIR spectra of crosslinked CHI beads after swelling (pH 2.0) 4 days (B1), 4 days (B2), 3 days (B3), 2 days (B4), and 1day (B5).

The crosslinked CHI beads (B1–B5) and beads without crosslinker (CHI, CHI–PEG), when placed in phosphate buffer of pH 7.4 at 37°C, retained their shape and physical integrity for the studied period. This is because of the inherent hydrophobicity of the CHI beads dominating at high pH. However, when the beads were placed in a HCl solution of pH 2.0, the disintegration started slowly from the eighth day onward. The CHI–PEG beads were swollen about 15–20 times the original size and appeared to be gelled. The CHI beads were almost dissolved in 17 days, whereas the crosslinked beads (B1–B5) disintegrated to fine particles within 4 months. Complete degradation of the CHI beads to water-soluble molecules or monomers appears to be a very slow process and could not be followed until the end.

Solubility of the Beads

The solubility of the beads depends on the degree of crosslinking. Figure 3 shows the solubility of the crosslinked beads (B1–B5) exposed to 2% acetic acid solution for 24 h. The beads without PEG have maximum solubility and the beads with PEG (CHI-PEG) are less soluble because of the existence of intermolecular crosslinks, which are totally absent in pure CHI beads. The addition of crosslinker (glutaraldehyde) greatly influences the solubility behavior, as evident from the Figure 3. The extent of degradation and solubility of the polymer depends on the concentrations of the crosslinkers used.[25] In the present case, the solubility of the beads followed the order CHI > CHI–PEG > B5 > B4 > B3 > B2 > B1.

SEM Studies

Crosslinked CHI–PEG beads prepared by using different concentrations of glutaraldehyde have rough and dense surfaces. The beads are almost spherical in shape and the size varies from 1444–2008 μm [Fig. 4(a)]. From the morphology of the beads [Fig. 4(b)], one can observe rough and folded surfaces of the beads, and that decreasing the concentration of crosslinking agent contributed to an increase in swelling and a decrease in complexity of the surface folding. Scanning elec-

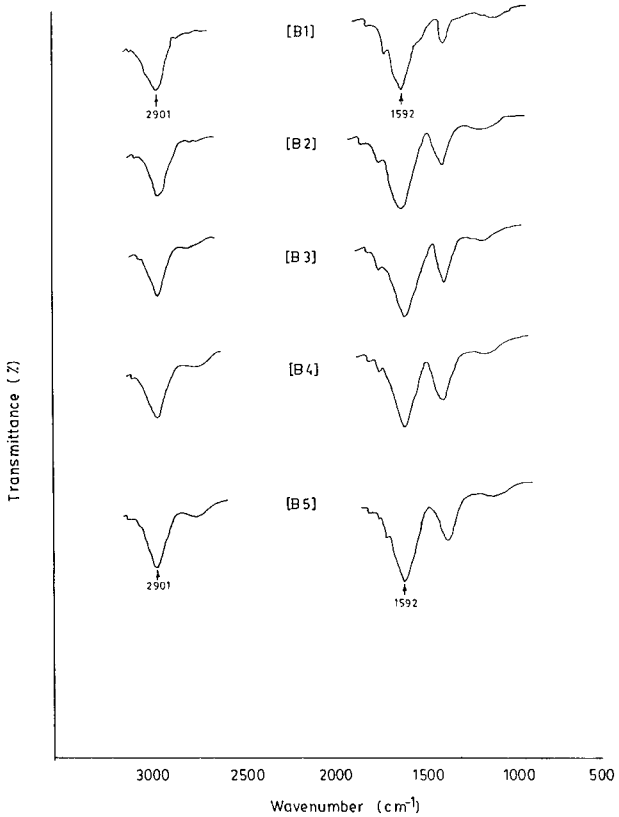

Figure 7 FTIR spectra of crosslinked CHI beads after swelling (pH 7.4) 4 days (B1), 4 days (B2), 3 days (B3), 2 days (B4), and 1day (B5).

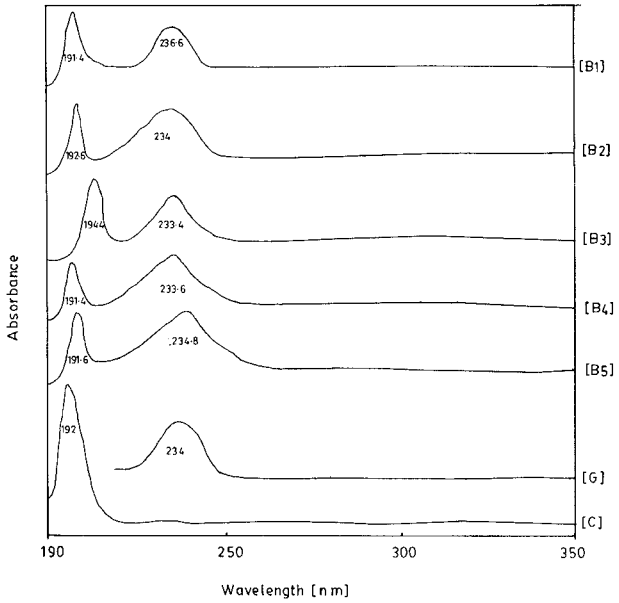

Figure 8 UV spectra of glutaraldehyde (G), CHI (C), and crosslinked CHI beads 4 days (B1), 4 days (B2), 3 days (B3), 2 days (B4), and 1day (B5) in pH 2.0.

tron micrographs of the beads after swelling for 4 days ($B1^+$), 4 days ($B2^+$), 3 days ($B3^+$), 2 days ($B4^+$), and 1 day ($B5^+$) in pH 2.0 solution are shown in Figure 5(a). The size of the beads after swelling varies from 1312–1625 μm. During the swelling process, cracks and pores appeared on the surface of the beads. From the morphological studies [Fig. 5(b)], it is evident that the complex folded structure collapsed after attaining equilibrium swelling in the solutions of pH 2.0. On decreasing the glutaraldehyde concentration, the cracks and pores appearing on swelling the beads in pH 2.0 are larger (30 μm and 10 μm), whereas the beads with maximum crosslinker have shown cracks and pores of 16 μm and 6 μm respectively [Fig. 5(b)].

IR/UV Spectra in Support of Structural Changes

FTIR spectra of CHI beads at equilibrium in swelling solution of pH 2.0 are recorded at different intervals of time (Fig. 6). The spectra B1, B2, B3, B4, and B5 are recorded at 4 days, 4 days, 3 days, 2 days, and 1 day, respectively. Imine groups in the network get protonated in acidic pH and, as a result, the hydrogen bonding dissociates promoting swelling of the beads. The IR spectra of the swollen beads B1–B5 show two new peaks at 1623 and 1523 cm^{-1} assigned to $^+NH_3$ absorption peaks[22] in the spectra (Fig. 6), which supports the formation of $^+NH_3$ within the beads when swollen in pH 2.0. The peaks corresponding to PEG (1464 cm^{-1} and 2901 cm^{-1}) disappeared in the beads (B1–B5) after attaining equilibrium swelling, which indicates the dissolution of PEG from the network.

Figure 7 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 (Fig. 7); meanwhile, the peaks assigned to PEG at 1464 cm^{-1} and 2091 cm^{-1} also weaken, but the rate is slower than in the case of pH 2.0. 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. 7), 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 C of Figure 1, it was found that the peak at 1592 cm^{-1} in (B1–B5) Figure 7 becomes similar to that of CHI. This

Table II Amount of Drug[a] Released (μg) at 37°C as a Function of Time

Period (h)	B1		B2		B3		B4		B5	
	pH 2.0	pH 7.4	pH 2.0	pH 7.4	pH 2.0	pH 7.4	pH 2.0	pH 7.4	pH 2.0	pH 7.4
1	4.36	2.83	6.28	3.16	7.99	3.67	8.34	4.54	10.34	6.04
2	6.38	4.20	9.54	4.99	11.31	5.75	11.81	7.67	13.81	9.17
3	8.25	5.37	12.28	6.76	14.20	7.58	15.00	10.1	17.00	11.67
4	9.87	6.89	14.51	8.07	16.60	8.53	18.04	11.67	20.04	13.17
5	11.30	7.00	16.27	8.80	18.87	9.31	20.38	12.71	22.38	14.21
6	12.53	7.64	17.56	9.44	20.44	9.95	22.43	13.29	24.43	14.79
24	13.55	8.27	18.51	9.95	21.68	10.53	24.11	13.58	26.11	15.08
48	14.52	8.72	19.35	10.64	22.39	11.28	24.90	13.90	26.90	15.40

[a] 45 μg INH loaded mg^{-1} bead.

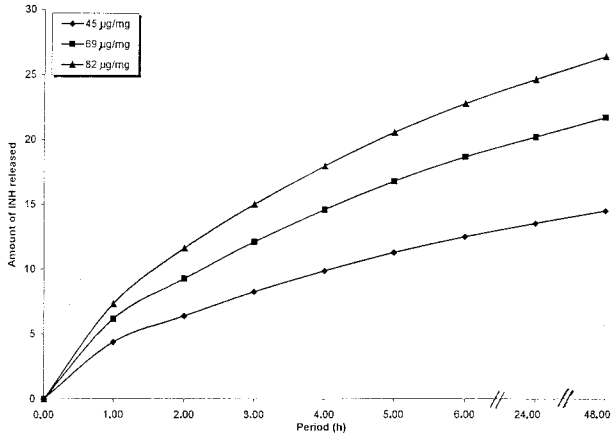

Figure 9 Release of INH from the beads (B1) vs time in solution of pH 2.0 at 37°C.

elucidates that the changes in structure of the beads may result in the transformation of C═N to C—N, except its cleavage, which makes the IR spectrum of N—H from C—N similar to that of amino groups of CHI. However, 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 4 days (B1), 4 days (B2), 3 days (B3), 2 days (B4), and 1 day (B5). But a characteristic peak of CHI around 200 nm, because of its dissolution from the beads, was observed. However, there is no peak relating to glutaraldehyde perhaps caused by the cleavage of 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 8 (B1–B5), 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 191.4, 192.6, 194.4, 191.4, 191.6 nm and 233.6, 234, 233.4, 233.6, 234.8 nm in Figure 8 (B1–B5) attribute to the dissolution of CHI and cleavage of imine bond, respectively. This may have resulted 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 CHI in the swollen beads. The changes of imine bond within the network in the present case can be expressed as follows:

$$—NH_2$$

$$+ —CHO \xleftarrow{\text{pH 2.0}} —C═N— \xrightarrow{\text{pH 7.4}} —C—N—$$

Drug-Release Studies

The release of encapsulated isoniazid (INH) is noted in HCl (pH 2.0) and phosphate buffer (pH 7.4) at 37°C. The amount of drug released as a function of time is depicted in Table II. The release of the drugs from the beads in HCl (pH 2.0) is higher than that which occurred in phosphate buffer (pH 7.4). As discussed above, the inherent hydrophobicity of the CHI beads dominate at high pH value preventing faster swelling, which might be the reason for the slow release of drugs in phosphate buffer (pH 7.4).

The release pattern of the highly drug-loaded beads has been found to be similar to that of the beads loaded with a lower amount of drug (Fig. 9). The percentage of release of drug from CHI beads was decreased with increased concentration of INH. However, the total amount of INH released was found to be more from highly loaded beads in comparison to the beads loaded with low concentrations.

CONCLUSION

Crosslinking of biodegradable polymers is potentially important to control the degradation rates. From these investigations, it is evident that the rate of swelling of the matrix and release of drugs is dependent on the degree of crosslinking and solution pH. Therefore, by varying the crosslink densities desired, drug-release rates can be achieved. The crosslinking also helps in optimizing drug-entrapping capacity and its sustained release for extended periods. Because of the bioactivities of CHI itself, its formulations with drugs may have dual therapeutic effects.

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