pH dependent hydrolysis and drug release behavior of chitosan/poly(ethylene glycol) polymer network microspheres

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Semi-interpenetrating polymer network (IPN) microspheres of chitosan and poly(ethylene glycol) PEG were prepared for controlled release of drugs. A new method for the chemical crosslinking of chitosan microspheres containing isoniazid (INH) as a model drug is proposed and evaluated. The method consists of the exposure of microspheres to the vapor of crosslinking agent that act in gaseous phase under mild conditions. The structural analysis of the microspheres was carried out by FTIR-analysis. The swelling behavior, hydrolytic degradation, structural changes of the microspheres and loading capacity (LC) of the microspheres for INH were investigated. The prepared microspheres have shown 93% drug loading capacity, which suggested that these semi-IPN microspheres are suitable for controlled release of drugs in an oral sustained delivery system.

1. Introduction

Microcapsules can be used as devices for the controlled release of drugs, vaccines, antibiotics and hormones. Bioactive agents may be entrapped within cores of anionic polymers such as alginate, carrageenin, carboxy methylcellulose and poly(acrylic acid) [1–3]. Similarly, membranes can be formed using various cationic polymers susch as chitosan, poly-L-lysine, polyethylenimine and poly(allyl amine) [4–8]. The feasibility of employing chitosan microcapsules or microspheres for the oral administration of vaccines has previously been examined in our laboratory [9–12].

Chitosan has been investigated as an excipient in the pharmaceutical industry to be used in direct tablet compression [13], as a tablet disintegrent [14] for the production of controlled release solid dosage forms or for the improvement of drug dissolution [15, 16].

Chitosan as compared to traditional excipients has been shown to have superior characteristics and especially flexible in its use. Furthermore, chitosan has been used for production of controlled release implant systems for delivery of hormones over extended periods of time. Lately, the transmucol absorption promoting characteristics of chitosan has been exploited especially for nasal and oral delivery of polar drugs to include peptides and proteins and for vaccine delivery. These properties together with the very safe toxicity profile, makes chitosan an exciting and promising excipient for the pharmaceutical industry for the present and future applications [17].

This work concerns microparticulate drug delivery systems of chitosan. A new method for chemical crosslinking of chitosan microspheres containing isoniazid (INH) as a model drug is proposed and evaluated. Poly(ethyleneglycol) (PEG) is used to effect the swelling, solubility and release properties by forming intermolecular crosslinks with chitosan hydroxyl groups.

2. Experimental

2.1. 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 by 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 vacuum oven at 20 °C. The molecular weight and %N deacetylation of chitosan were found to be 2.9×10^6 g mol^{-1} and 61% respectively [10]. Isoniazid ($C_6H_5CONHNH_2$) was obtained as a gift sample from Pharmachem, Bahadurgarh, India. PEG (mol. wt. 6000 g mol^{-1}) was procured from Hi Media Laboratories, India and was used as received.

2.2. Preparation of chitosan microspheres

Purified chitosan and PEG were dissolved in 2% acetic acid under stirring for 3 h at room temperature. The

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homogeneous mixture was extruded in the form of droplets using a syringe into NaOH-methanol solution (1 : 20 w/w) under stirring conditions (600 rpm). The resultant microspheres were washed with hot and cold water respectively. The microspheres were then exposed to the vapor of glutaraldehyde for about 45 min that act in gaseous phase and under mild conditions. Finally the microspheres were washed with hot and cold water successively and vacuum dried at 30 °C. The composition of the microspheres prepared is given in Table I. To prepare the drug loaded microspheres, a known amount of INH (85 mg, 125 mg, 150 mg respectively) was added to the chitosan/PEG mixture before extruding into the alkaline-methanol solution.

2.3. IR studies

IR spectra of the chitosan microspheres were recorded on KBr pellets using Perkin-Elmer Fourier transform spectrophotometer (FTIR-1600).

2.4. Swelling studies

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

$$\alpha = (Wt - Wo)/Wo$$

where Wt and Wo are the weights of the microspheres at time t and in the dry state respectively.

2.5. Solubility measurement of the microspheres

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

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

where W_o is initial weight of the beads and Wt is weight of the vacuum dried beads after the immersion for 24 h in acetic acid solution.

2.6. Hydrolytic degradation of the microspheres

The crosslinked chitosan beads are expected to undergo degradation by the hydrolysis of the amino/imine bonds present in the beads. Hence, hydrolytic degradation [18] of the beads under physiological condition was studied. Chitosan beads were placed in 100 ml of HCI (pH 2.0) and phosphate buffer (pH 7.4) at 37 °C under unstirred conditions and the degradation of the beads was observed visually from time to time.

TABLE I Composition of chitosan microspheres

Code	Chitosan (g)	PEG (g)	Glutaraldehyde (10 ml)	2% acetic acid (ml)
M1	0.5	0.5	25.00%	20.0
M2	0.5	0.5	12.25%	20.0
M3	0.5	0.5	6.25%	20.0
M4	0.5	0.5	3.125%	20.0
M5	0.4	0.5	1.125%	20.0

2.7. IR/UV spectra to monitor structural changes of the microspheres

In an attempt to investigate the structural changes of the crosslinked beads, the 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-Vis spectrophotometer. The beads were then dried at 35–45 °C and IR spectra were recorded to notice the structural changes by comparing with IR spectra of the initial dry beads.

2.8. Evaluation of drug loading capacity of the microspheres

A sample of drug loaded beads was accurately weighed (0.1 g) and kept in 100 ml 2% acetic acid at 30 °C for 48 h. After centrifugation, the drug in the supernatant was assayed by the spectrophotometer at the λ_{max} of the drug. Similarly, the washings were assayed by spectrophotometer for the free amount of the drug. The drug loading capacity (LC) of the beads and drug encapsulation efficiency (EE) of the process were calculated from the equations as shown below:

$$LC = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Microsphere weight}}$$

$$EE = \frac{\text{Total amount of drug} - \text{Free amount of drug}}{\text{Total amount of drug}}$$

2.9. Drug assay

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

2.10. 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). Microspheres (0.1 g) containing known amount of INH were added to the release medium. At predecided time intervals, 1 ml aliquots were withdrawn, filtered and assayed by recording absorbance at the λ_{max} of drug. The cumulative INH release is measured as a function of time.

3. Results and discussions

3.1. IR spectra analysis

Fig. 1 shows the IR spectra of PEG (P), chitosan (C) and crosslinked microspheres (M1–M5). The peaks at 1592 cm^{-1} in the IR spectrum of chitosan (Fig. 1 (C)) can be assigned as amino absorption peak. In contrast with spectra (P) and (C), a significant new peak at

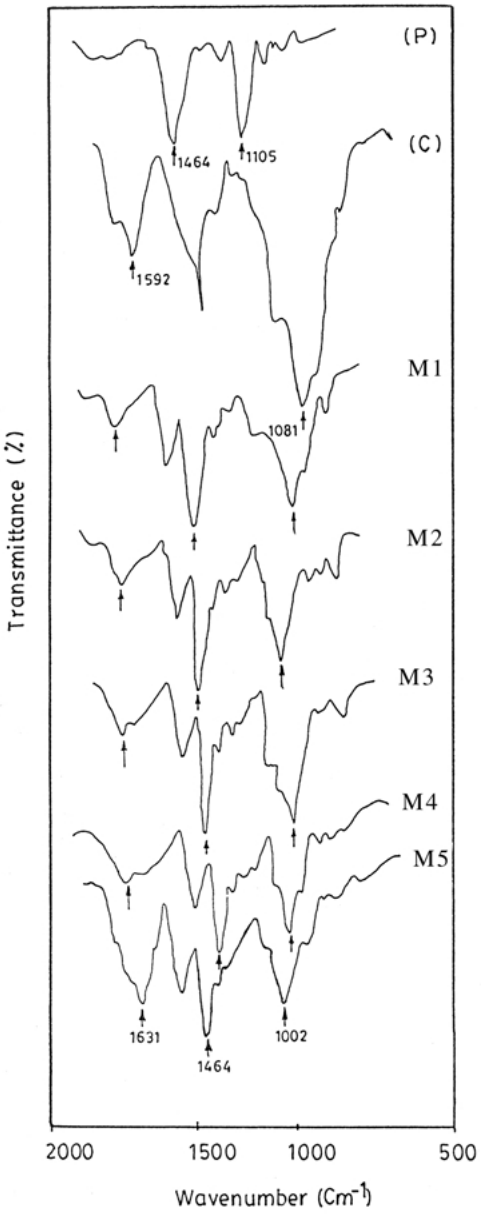

Figure 1 FTIR spectra of PEG (P), chitosan (C) and crosslinked chitosan beads (M1–M5).

1631 cm^{-1} in the spectra (M1–M5) 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 concentration, the peak corresponding to 1631 cm^{-1} is broadened gradually (B1–B4), whereas, the peak at 1464 in the microspheres (M1–M5) is the characteristic of the PEG. The peaks at 1002, 1081 and 1105 cm^{-1} in the spectra (P), (C) and (M1–M5) are due to C—O stretching vibrations in PEG, chitosan and crosslinked microspheres respectively. In this system, a complexation through co-operative hydrogen bonding takes place [19].

3.2. Swelling behavior

The swelling response of the glutaraldehyde crosslinked chitosan microspheres in solutions of pH 2.0 and pH 7.4 at 37 °C is shown in Fig. 2., from which it is clear that the character of the swelling curve changes more significantly over time in pH 2.0 than in pH 7.4. Moreover, the

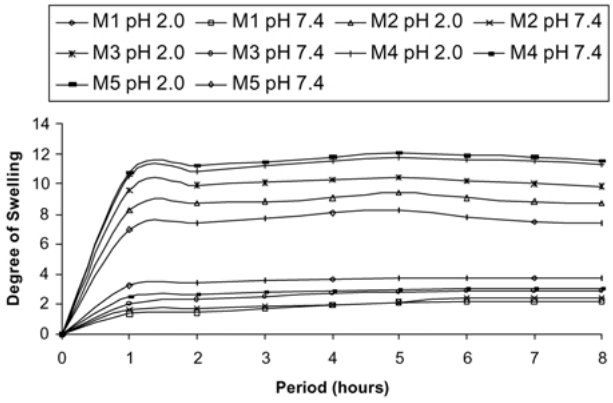

Figure 2 Swelling behavior of crosslinked chitosan beads (M1–M5) in solutions of pH 2.0 and 7.4 at 37 °C.

swelling can reach a stable equilibrium much more rapidly in pH 7.4 than in pH 2.0. It was also observed that swelling curves do not change greatly with time in pH 7.4, while in pH 2.0, the swelling degree of the microspheres begins to decline after the microspheres were swollen for some time, which may indicate the dissolution tendency of the microspheres exceeds the swelling degree. This may be due to the cleavage of imine bond in the microspheres due to the protonation.

The observed swelling rates of the crosslinked microspheres followed the order M5 > M4 > M3 > M2 > M1. It is a well known fact that the degradation of the polymer depends upon 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 due to inherent hydrophobicity of the chitosan microspheres dominating at high pH value, which prevents faster swelling in neutral and alkaline media. The rapid swelling of the microspheres with PEG as a spacer group, unlike chitosan-glycine microspheres is due to high diffusivity of water in PEG.

3.3. Solubility of the microspheres

The solubility of the microspheres depends upon the degree of crosslinking. Fig. 3 shows the solubility of the crosslinked microspheres as a function of glutaraldehyde concentration. The microspheres (C) without spacer group (PEG) have maximum solubility and the micro-

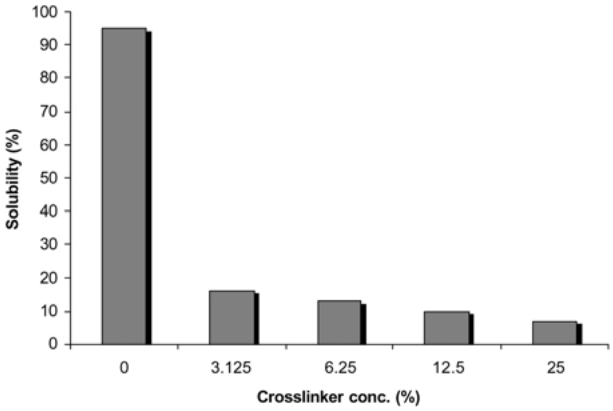

Figure 3 The dependence of solubility for crosslinked chitosan beads on glutaraldehyde concentration.

spheres (CP) with (PEG), due to the formation of physical intermolecular crosslinks are less soluble than the pure chitosan microspheres. Addition of crosslinker greatly influences the solubility behavior as evident from Fig. 3. The extent of degradation and solubility of the polymer depends upon the concentrations of the crosslinkers used [10–13]. In the present case the solubility of the microspheres followed the order C > CP > M5 > M4 > M3 > M2 > M1. Similarly, the increase in glutaraldehyde concentration reduced the swelling ratio, whereas, the swelling ratio of the microspheres without crosslinker was maximum (Fig. 4).

3.4. Degradation of the microspheres in pH 2.0 and pH 7.4

The crosslinked microspheres (M1–M5) and microspheres without crosslinker (C and CP), when placed in phosphate buffer of pH 7.4 at 37 °C were found to maintain their shape and physical integrity for the studied period. This is due to the inherent hydrophobicity of the chitosan microspheres dominating at high pH value, whereas, the microspheres when placed in HCl solution (pH 2.0), began to disintegrate very slowly from 8th day onwards. The CP microspheres were swollen about 15–20 times to the original size and appear to be gelled. It took about 17 days for C, almost soluble, whereas, 4 months in case of M1–M5 for complete disintegration of the microspheres into fine particles. Complete degradation of the polymer into water-soluble molecules or monomers appears to be very slow process and could not be followed till the end.

3.5. IR/UV spectra

FTIR spectra of chitosan microspheres at equilibrium in swelling solution of pH 2.0 are shown in Fig. 5 (M1–M5). The spectra M1, M2, M3, M4 and M5 are recorded at 4 days, 3 days, 2 days, 2 days and 1 day respectively. Imine groups of the microspheres get protonated in acidic pH and as a result the hydrogen bonding dissociates promoting the swelling of the microspheres. By contrast with Fig. 1 (M1–M5), there are two new peaks at 1623 cm^{-1} and 1523 cm^{-11} assigned to $^{+}NH_3$ absorption peaks[r] in the spectra Fig. 5 (M1–M5), which supports the formation of $^{+}NH_3$ within the microspheres when swollen in pH 2.0. The peak corresponding to PEG

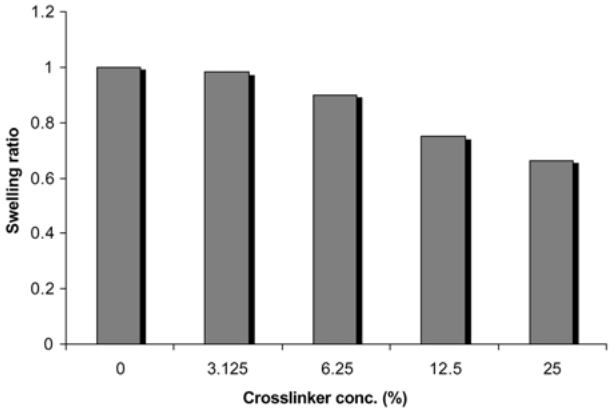

Figure 4 The dependence of swelling ratio (in pH 2.0) for crosslinked chitosan beads on glutaraldehyde concentration.

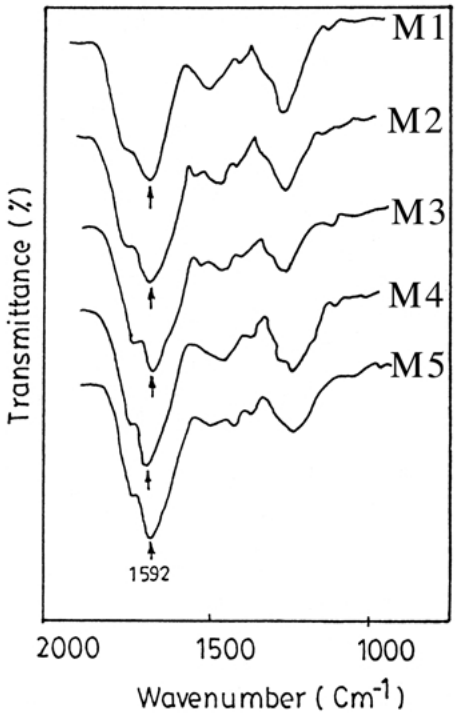

Figure 5 FTIR spectra of swollen crosslinked Chitosan beads for 4 days (B1), 3 days (B2), 2 days (B3), 2 days (B4) and 1 day (B5) at pH 2.0 in 37 °C.

(1485 cm^{-1}) disappeared in the microspheres (M1–M5) after the equilibrium swelling, which indicates the dissolution of PEG from network.

Fig. 6 shows the IR spectra of the microspheres swollen for different times in solutions of pH 7.4. The

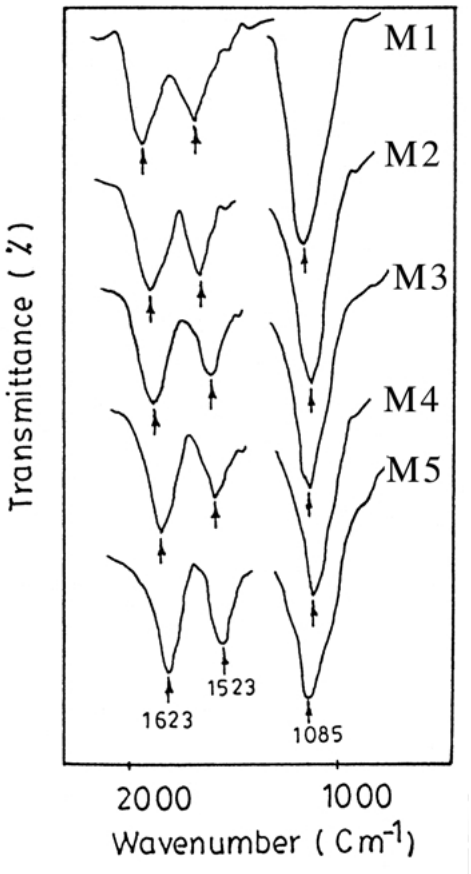

Figure 6 FTIR spectra of swollen crosslinked Chitosan beads for 4 days (B1), 3 days (B2), 2 days (B3), 2 days (B4) and 1 day (B5) at pH 7.4 in 37 °C.

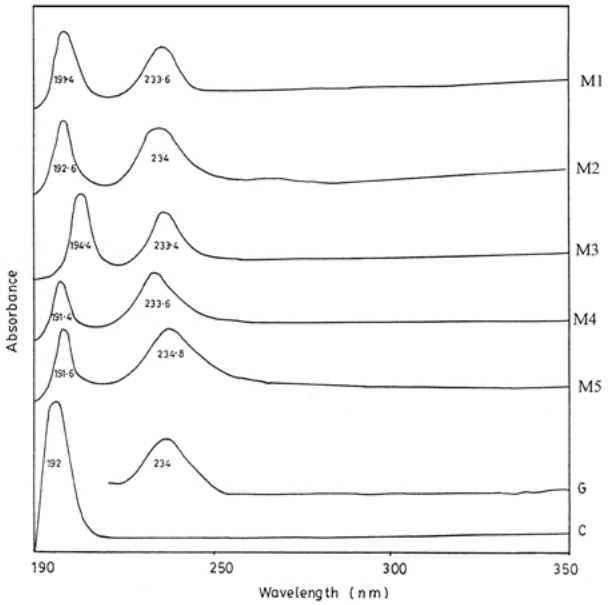

Figure 7 UV spectra of the solution left after the initial dry beads were swollen for 4 days (B1), 3 days (B2), 2 days (B3), 2 days (B4) and 1 day (B5) at pH 2.0 in 37 °C.

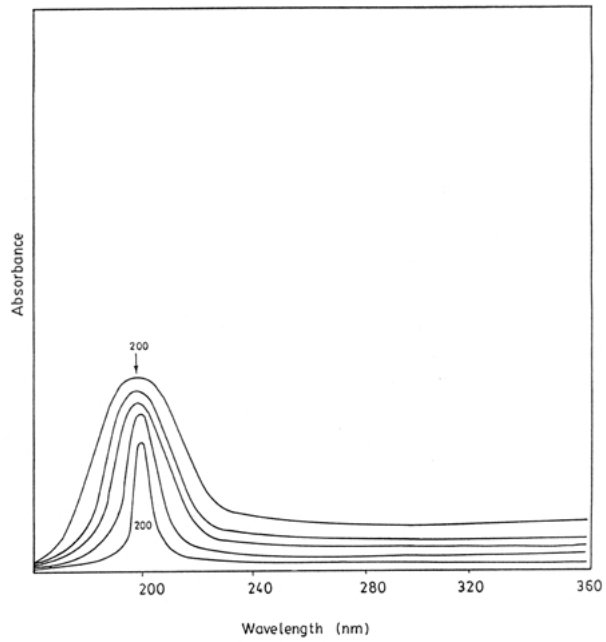

Figure 8 UV spectra of the solution left after the initial dry beads were swollen for 4 days (B1), 3 days (B2), 2 days (B3), 2 days (B4) and 1 day (B5) at pH 7.4 in 37 °C.

spectra reveal that the peak at 1631 cm^{-1} assigned to C = N absorption disappears (Fig. 6) (M1–M5), meanwhile, the peak assigned to PEG at 1485 cm^{-1} also weakens, but the rate is slower than that in 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 microspheres in pH 7.4. Fig. 6 (M1–M5), which may confirm that imine groups within the microspheres are not protonized in pH 7.4 leading to a lower swelling of the microspheres.

In comparison to the spectrum of chitosan Fig. 1 (C), it was found that the peak at 1592 cm^{-1} in Fig. 6 (M1–M5) becomes similar to that of chitosan. This elucidate that the changes in structure of the microspheres may result 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 (Fig. 7) that the imine bond within the microspheres didn't break on swelling in solution of pH 7.4 for 4 days ($M^{+}1$), 3 days ($M^{+}2$), 2 days ($M^{+}3$), 2 days ($M^{+}4$), and 1 day ($M^{+}5$). But a characteristic peak of chitosan around 200 nm, due to its dissolution from the microspheres 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.

From Fig. 8, we can confirm the cleavage of imine bonds in the swollen microspheres 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, 233.6, 234.8 nm (Fig. 8) (M1–M5) attribute 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 microspheres were swollen continuously for a long time and further dissolution of chitosan in the swolled microspheres. The changes of imine bond within the network in the present case can be expressed as follows:

$$—NH_2 + —CHO— \overset{pH\,2.0}{\longleftarrow} —C = N— \overset{pH\,7.4}{\longleftarrow} —C—N—$$

3.6. Entrapment of CPM within chitosan/PEG semi-IPN microspheres

INH is used as a model drug in order to investigate the feasibility of using chitosan/PEG semi-IPN microspheres as drug carriers. Results of the EE and drug loading of these microspheres are displayed in Fig. 9. The CPM encapsulation was affected by the initial INH concentration, lower the concentration, higher the EE. However, the drug loading was enhanced by increasing the initial drug concentration reaching a maximum of 82 μg of drug entrapped in 1 mg bead.

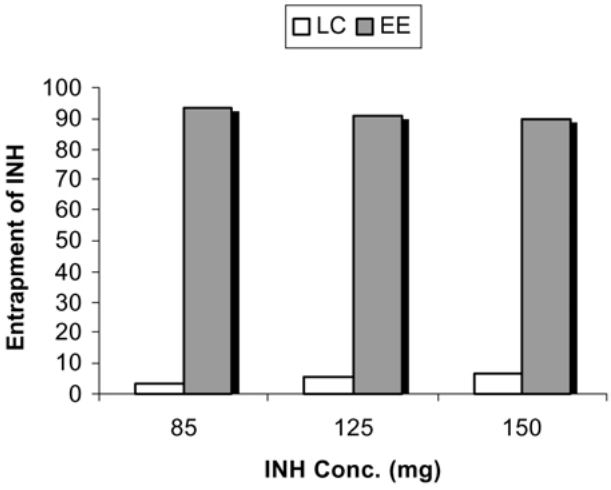

Figure 9 Effect of initial concentration on entrapment efficiency and loading capacity of the drug.

3.7. Drug release studies

Fig. 10 shows the dissolution profile of INH from crosslinked chitosan-PEG microspheres (45 μg INH loaded mg^{-1} bead) at various time intervals in acidic (pH 2.0) and basic (phosphate buffer pH 7.4) media at 37 °C. There is a burst release initially for the first hour in both acidic and basic media (pH 2.0 and 7.4) followed by an almost constant release of INH from the matrix for the 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 microspheres.

The dissolution rates of INH from the microspheres, loaded with higher amounts of the drug for various time intervals in HCl or phosphate buffer solutions were also observed (69 and 82 μg INH loaded mg^{-1} bead) as in Figs 11 and 12. The dissolution pattern of the drug was similar for all concentrations of drug studied. However, it was found that the percent of drug released from chitosan microspheres stabilized as the INH concentration increased in the microspheres, but the amount of INH released was proportional to the concentration of the drug loaded (Table II). In other words, the amount of drug released increased as a function of INH concentration in the microspheres, with no significant change in the percent release. The amount and percent release of INH were much higher in the HCl solution as compared with phosphate solution for all concentrations studied. Therefore, it is understood that the mechanism of drug release is due to the diffusion through swollen microspheres in pH 2.0, whereas, swelling in pH 7.4 is less and the drug release is less.

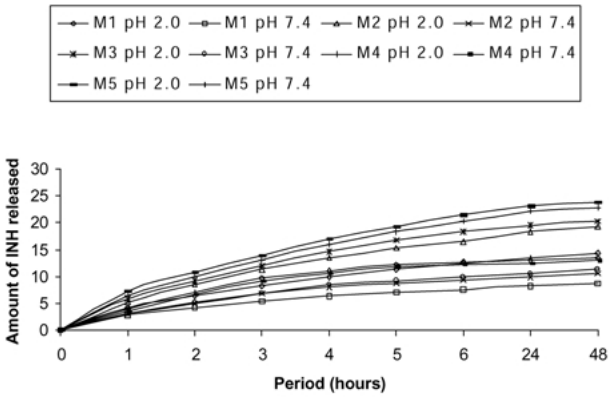

Figure 10 Release of INH from the crosslinked Chitosan beads (45 mg INH loaded mg^{-1} bead) *versus time* at pH 2.0 and 7.4 and 37 °C.

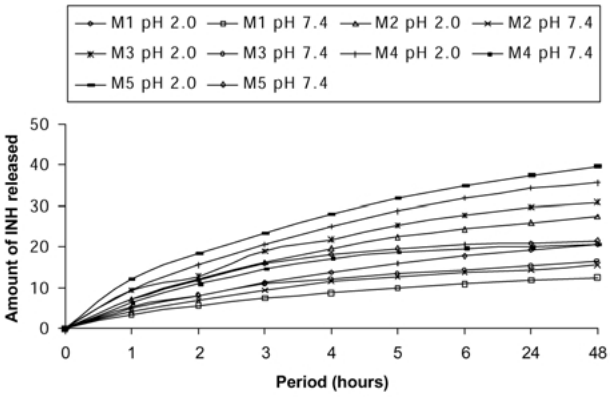

Figure 11 Release of INH from the crosslinked Chitosan beads (69 mg INH loaded mg^{-1} bead) *versus time* at pH 2.0 and 7.4 and 37 °C.

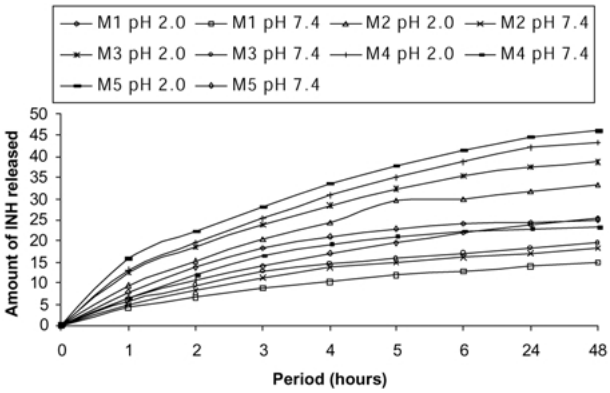

Figure 12 Release of INH from the crosslinked Chitosan beads (82 mg INH loaded mg^{-1} bead) *versus time* at pH 2.0 and 7.4 and 37 °C.

The initial phase of INH release as a function of exposure time in HCl and phosphate buffer solution is depicted in Figs 10–12. It appears that the initial burst release of INH is higher in the HCl solution as compared to the phosphate medium from the drug loaded microspheres (45, 69 and 82 μg INH loaded mg^{-1} bead respectively) for a 1 h period. However, the pattern was reversed with an increase in exposure time (Figs 10–12). The results demonstrate that with a chitosan-PEG release system, *in vitro*, a near zero-order release of INH is observed. It appears that the mechanism of drug release may be due to diffusion through the swollen microspheres in the HCl solution.

4. Conclusion

Novel chitosan microspheres that present a very interesting combination of properties, which may allow for their use as drug delivery devices were developed. These materials present a very useful transition (pH effect just in the range of physiological conditions, which may offer enormous possibilities in the field of

TABLE II INH release as a function of drug concentration from chitosan microspheres for 48 h

Concentration of INH μg mg^{-1} bead		HC1 (pH 2.0)		Phosphate buffer (pH 7.4)	
		Amount released μg mg^{-1} bead	%	Amount release μg mg^{-1} bead	%
45		14.52	32.02	8.72	19.37
69	(M1)	20.74	30.05	12.43	18.01
82		25.45	31.03	14.88	18.04
45		19.35	43.00	10.64	23.60
69	(M2)	27.49	39.80	15.55	22.53
82		33.26	40.50	18.38	22.41
45		20.39	45.30	11.28	25.06
69	(M3)	30.81	44.65	16.53	23.95
82		38.79	48.52	19.55	23.84
45		22.90	50.80	12.90	28.66
69	(M4)	35.65	51.66	20.56	29.79
82		43.37	52.89	23.32	28.43
45		23.90	53.11	13.40	29.77
69	(M5)	39.65	57.46	21.56	31.24
82		46.01	56.10	25.06	30.56

biomedical applications. The most promising result of this investigation is the maximum entrapment efficiency of the microspheres, which was found to be 93%. In our previous investigation we found 83% entrapment efficiency, by suspension crosslinking. The difference in the entrapment efficiency is attributed due to the loss of drug during suspension crosslinking, which is found to be minimum by adopting vapor phase crosslinking technique. The swelling behavior of these materials compensates for the volume shrinkage, which occurs during polymerization that can provide a mechanism for fixing prosthesis in the intramedullary cavity. We observed a near zero-order drug delivery using these microspheres and the matrix formulations can be modified further to obtain desired controlled drug delivery.

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