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Chitosan sponges as sustained release drug carriers

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Abstract

Chitosan sponges as sustained release drug carriers were prepared by freeze-drying partially *N*-acetylated chitosan gels and crosslinked chitosan solutions. Micronized triamcinolone acetonide was used as a model drug. *N*-acetylchitosan sponges were prepared from 2.5% chitosan solutions acetylated with 10.58 mmol of acetic anhydride per gram of chitosan. Crosslinked chitosan sponges were prepared from 2.5% chitosan solution crosslinked with 1.33% glutaraldehyde in respect to chitosan mass. Drug content in both *N*-acetylated and crosslinked chitosan sponges were uniform. Scanning electron microscopy (SEM) photos show a leaflet- or a platelet-like structure of both chitosan sponges. The incorporated drug was found in a crystalline form. The water uptake ability of both chitosan sponges was more than 20 times of their weight. The pH of dissolution media and the drug content of the sponges affected the release rate of the drug. The drug release at pH 1.2 was faster than at pH 7.4. The drug release at pH 7.4 was a function of square root of time over 52 h from the *N*-acetylchitosan sponges and over 36 h from the crosslinked chitosan sponges. With increasing the drug content a slower drug release was found. The delayed drug release was due to the decreased chitosan solubility by either *N*-acetylation or crosslinking. © 1997 Elsevier Science B.V.

Keywords: Chitosan; Crosslinking; Acetylation; Sustained release; Drug carrier

1. Introduction

Chitosan is a linear amino polysaccharide of β-D-glucosamine (2-amino-2-deoxy-β-D-glucan) units joined by $(1 \rightarrow 4)$-linkages. It is obtained by alkaline deacetylation of chitin manufactured from shrimp or crab shells (Sanford, 1989). Chitosan is usually characterized by an average degree of acetylation. It is insoluble in water but soluble at pH values under 6.5 in most acidic media. Acetic acid has been mostly used as a standard solvent for chitosan solutions.

The utilization of chitosan as sustained drug carrier systems is very interesting. Chitosan has the following advantages:

- Chitosan is inexpensive. It is a product of naturally abundant crustacean shells which are

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the waste of fishering manufactures in many countries.
- Chitosan is non-toxic, biocompatible and biodegradable.
- Organic solvents are not required in solubilization of chitosan.
- Compared with other polymers commonly used in controlled release technology, chitosan possesses more bioactivities.
- The polymeric cationic character along with the potentially reactive functional groups supply many possibilities on modifications of chitosan molecules.
- A variety of drug carrier systems can be produced from chitosan and its derivatives.

A large number of studies published in the last years indicate the high efficiency of chitosan on production of various sustained release drug carrier systems such as microparticles (Lin and Lin, 1992), tablets (Knapczyk, 1992), gels (Patel and Amiji, 1996) and beads (Murata et al., 1996). Floating ability and pH-dependent solubility of modified chitosan matrices are very useful in the development of oral buoyant sustained release preparations (Hou et al., 1985). Chitosan has mucoadhesive properties and can be utilized as an effective nasal drug delivery system (Illum et al., 1994).

In this study sponges of chitosan were prepared as sustained release drug carriers to be used in wound healing after tooth extraction, implantation and oral administration. The sponges were prepared by two methods; partially *N*-acetylation and crosslinking with glutaraldehyde. Triamcinolone acetonide (TA) was used as a model drug. Slower drug release from the modified chitosan was expected.

2. Materials and methods

2.1. Materials

All materials used in this study were of pharmaceutical or analytical grade and were purchased as follows; Chitopure® (degree of deacetylation 92%, viscosity of 14 mPas [1% solution in 1% acetic acid], Fish contact Bremerhaven GmbH, Bremerhaven, Germany), acetic anhydride and glacial acetic acid (Merck, Darmstadt, Germany), glutaraldehyde (25% solution, Fluka, Buchs, Switzerland), micronized triamcinolone acetonide (Diosynth, Oss, Holland). Acetonitrile and methanol in HPLC grade were purchased from Merck, Darmstadt, Germany.

2.2. Partial N-acetylation of chitosan

A total of 2.5% chitosan solution in 1% acetic acid was prepared by simple solution without heating. Insoluble impurities in the solution were separated by filtration. The acetylation was performed in the 2.5% chitosan solution at room temperature. A volume of 0.10–1.50 ml of acetic anhydride was added into a mixture of 10 g of chitosan solution and 5 ml of ethanol. The effect of acetic anhydride concentrations on gelation of chitosan solutions was observed after 12 h.

2.3. Drug containing N-acetylchitosan sponges

The formulations of the drug containing *N*-acetylchitosan sponges are presented in Table 1. The sponges were prepared using the process of preparation shown in Fig. 1. In the first step 2.5% chitosan was dissolved in 1% acetic acid. This solution was diluted with ethanol and the drug was then dispersed in the mixture. An amount of acetic anhydride corresponding to 10.56 mmol/g chitosan was added into the mixture. The mixture was stirred carefully to prevent the incorporation of air bubbles and poured into a polyethylene tray. After 12 h at room temperature the gels were

Table 1
Partial *N*-acetylchitosan sponges containing triamcinolone acetonide

2.5% Chitopure in 1% acetic acid (g)	200	200
Ethanol (ml)	100	100
Acetic anhydride (ml)	5	5
Triamcinolone acetonide (mg)	50	250
Drug content (% w/w)		
Theoretical	1.0	5.0
Actual	0.79 ± 0.01	3.41 ± 0.02

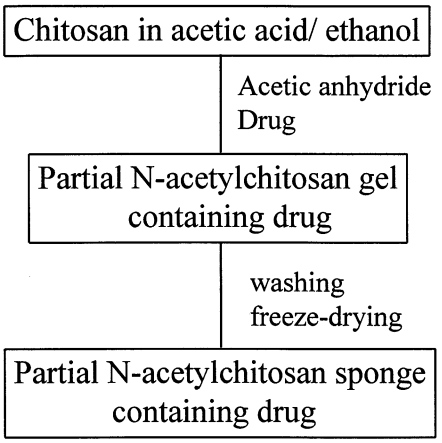

Fig. 1. Preparation process of a partial *N*-acetylchitosan sponge containing drug.

soaked with a large volume of purified water. Partailly *N*-acetylated chitosan sponges were obtained by freeze-drying of the gels.

2.4. Drug containing crosslinked chitosan sponges

Glutaraldehyde was used as the crosslinking agent in the concentration of 1.33% by weight in respect to the chitosan mass. The crosslinked chitosan matrices in Table 2 were prepared from 2.5% chitosan in 1% acetic acid solution. The crosslinking of the 300 g of chitosan solution by 2 ml of 5% glutaraldehyde was performed at pH 4.5 at room temperature for 24 h. Then the micronized drug dispersed in 0.5 ml ethanol was added and mixed thoroughly with the crosslinked solution. A crosslinked chitosan sponge was received after lyophilisation.

Table 2
Crosslinked chitosan sponges containing triamcinolone acetonide

2.5% Chitopure® solution (g)	300	300
5% Glutaraldehyde (ml)	0	2.0
Triamcinolone acetonide (g)	0.1	0.1
Ethanol (ml)	0.5	0.5
Drug content (w/w)		
Theorital	1.0	1.0
Actual	1.18 ± 0.01	1.03 ± 0.01

2.5. Characterization of chitosan sponges

2.5.1. HPLC for determination of triamcinolone acetonide (TA)

A RP-18 column (LiChrospher 100, 5 μm, Merck, Darmstadt, Germany) was used as a stationary phase. The mixture of acetonitrile, water and glacial acetic acid (43:57:0.2 by volume) was used as a mobile phase. A HPLC system (Gynkotek, Model 300, München, Germany) equipped with an injector (HPLC 360 Autosampler, Kontron, Munich, Germany), a multiple wavelength detector (Type SPDGA, Technolab, Frankfurt, Germany) and an integrator (Shimadzu, Kyoto, Japan) was used. The flow rate of the mobile phase was adjusted at 1.0 ml/min. A volume of 15 μl of assay solution was injected and the elute was monitored at 241 nm. The injection was done in duplicate. Standard solutions were prepared by dilution of a stock solution of 2 mg/ml TA in methanol to 1–20 μg/ml with the mobile phase. The peaks of chitosan and other ingredients of buffer solutions did not interfere with the peak of TA.

2.5.2. Drug content

The drug content in the chitosan sponges was determined in duplicate. A piece of $1 \times 1 \times 1$ cm of a sponge was extracted in 10 ml of 1:1 mixture of methanol: 0.1 N HCl. After filtration through a cellulose acetate membrane (0.45 μm) the concentration of TA in the solution was determined by HPLC as described.

2.5.3. Scanning electron microscopy

A thin piece of sponge (0.5 mm) was fixed on an SEM sample holder and coated with gold. The sample was examined using a scanning electron microscope (Philips XL20, Kassel, Germany).

2.5.4. Powder X-ray diffractometry

Powder X-ray diffraction patterns of the sponge systems were determined using an X-ray diffractometer (Stoe and Cie, Darmstadt, Germany) with a rotating anode. The transmission technique was performed with copper-$K_{\alpha 1}$ radiation monochromatized by a carbon monochromator at wavelength of 1.5405 Å. All measurements

were carried out with a voltage of 40 kV and a current of about 200 mA. The samples were scanned over the region of 5–50° 2-Theta with the speed of 1° 2-Theta per 10 s. The signals were received by a position sensitive detector (PSD). The X-ray diffraction was interpreted by a computer program (Micro-Vax II, Digital Equipment, Munich, Germany).

2.5.5. Infrared spectrophotometry

FT-IR spectra between 4000 and 600 cm^{-1} of the sponge systems were determined using the KBr disc technique (Perkin Elmer Fourier FT-IR 16PC spectrophotometer). A smoothing function for spectra and a base line correlation procedure were used. The spectra were saved by using a Lotus 123 computer program.

2.5.6. Water uptake capacity

The water uptake of the sponges was determined at least in duplicate. A piece of sponge ($1 \times 1 \times 1$ cm) was accurately weighed and placed in a small bottle containing 10 ml of water at room temperature. The bottle was turned up and down twice to wet the sponge. After 6 h and 10 min the sponge was removed from the water by means of small forceps. The sponge was allowed to drain by carefully dropping on a filter paper and reweighed. The increase in weight represented the weight of water taken by the sponge. The water uptake was calculated as a ratio of the weight of absorbed water to the weight of the dry sponge at each period of time as followed:

$$\text{Water uptake capacity}[g/g] = \frac{W_{wet} - W_{dry}}{W_{dry}}$$

2.5.7. Drug liberation

The release of drug from $1 \times 1 \times 1$ cm pieces of sponges was determined using the flow-through cells (Dissotest, CE6, Sotax, Basel, Switzerland) with large cell units (diameter of 20 mm). Hydrochloric acid buffer, pH 1.2 (USP 23) and 0.011 M phosphate buffer solution pH 7.4 (DAB 1996) containing 0.1% disodium edetate (EDTA) as an antioxidant were used as dissolution media. The closed systems of each 50 ml of the dissolution medium in 100 ml glass bottles were circulated in flow-through cells at the flow rate of 8 ml/min and filtrated through cellulose acetate membranes (0.45 μm). The temperature was controlled at 37 ± 0.1°C using a water-bath. At each sampling time 1 ml of dissolution medium was kept and substituted with a fresh medium. The sample was diluted as necessary and the drug concentration was then determined by HPLC as mentioned above.

3. Results and discussion

3.1. Effect of acetic anhydride concentrations

Chitosan is more susceptible to hydrolysis by lysozyme, chitinase and chitosanase than *N*-acetylchitosan (Hirano et al., 1989). Both active amino and hydroxyl groups in chitosan molecules are reactive to the acetylation. A partially *O*-acetylated and *N*-acetylchitosan gel can be achieved after acetylation of chitosan in acetic acid solution with acetic anhydride. Kristl et al., 1993 reported that the gels prepared by this method are very acidic (pH below 2.0) and fragile. Lower concentrations of acetic anhydride are needed on producing *N*-acetylchitosan gels.

In this study the selective *N*-acetylation was done using the method of Hirano et al. (1981) and Kristl et al. (1993). This method involves the treatment of chitosan by acetic anhydride in acetic acid solution containing ethanol at room temperature. The effect of acetic anhydride concentrations on gelation of chitosan solutions is shown in Table 3. Chitosan gels were achieved with acetic anhydride concentrations not less than 8.46 mmol/g of chitosan. The semisolid gels were transparent and elastic and became more rigid with increasing acetic anhydride concentrations. The pH of the gels dropped from 4.5 to 4.1 with larger amount of acetic anhydride (from 0.2 to 1.5 ml). The gels were stable when the concentrations of acetic anhydride were adjusted lower than 12.69 mmol/g of chitosan. At higher concentrations a syneresis of the gels was observed. The *N*-acetylchitosan sponges obtained after freeze-drying the gels were elastic and colorless.

Table 3
Effect of acetic anhydride concentrations on gelation of chitosan solutions

Acetic anhydride		Gelation	Syneresis
ml	mmol/ g chitosan		
0.10	4.23	−	−
0.15	6.35	−	−
0.20	8.46	+	−
0.25	10.58	+	−
0.30	12.69	+	+
0.35	14.81	+	+
0.40	16.93	+	+
0.50	21.16	+	+
0.75	31.74	+	+
1.00	42.32	+	++
1.50	63.47	+	++

Gel formation: +gelation, −no gelation.
Degree of syneresis: + < ++.

3.2. Drug content

Drug content in the *N*-acetylated and the crosslinked chitosan sponges was uniform (Tables 1 and 2). In the *N*-acetylchitosan sponges the drug content determined was much lower than the calculated amount. Only 68–79% of the incorporated drug was found in the final sponge matrices. This might be due to the loss of the soluble drug during the process of soaking the chitosan gels with many times of a large volume of water. On the other hand the drug content in the crosslinked sponges was easily adjusted by dispersion of the desired amount of drug in the solutions crosslinked chitosan. The drug content assayed in the crosslinked chitosan gels did not deviate from the calculated amount.

3.3. Morphology of the chitosan sponges

The crosslinked chitosan sponges had a dark yellow-brown color and were nonelastic. Their SEM photos are shown in Fig. 2. The *N*-acetylchitosan sponges were more porous than the crosslinked chitosan ones. The crystals of TA were detected on the lamellae of the both chitosan sponges. The SEM photos after dissolution at pH 7.4 of the sponges show that the *N*-acetylated and crosslinked chitosan sponges were insoluble at pH 7.4. Some crystals of the drug remained on the lamellae of the sponges after dissolution.

3.4. Powder X-ray diffraction

The X-ray diffraction patterns of chitosan, partial *N*-acetylchitosan and crosslinked chitosan are shown in Fig. 3. No peak indicating crystallinity of the drug was detected from the X-ray diffraction patterns of both *N*-acetylated and crosslinked chitosan sponges. It may be due to the low concentrations of incorporated drug in the sponge systems. The X-ray diffraction pattern of chitosan changed after *N*-acetylation or crosslinking. The intensity at position 20°(2 Theta) typical for the X-ray diffraction pattern of chitosan (Takai et al., 1989) was decreased or absent in the partial *N*-acetylated and the crosslinked chitosan sponges respectively.

3.5. FT-IR spectroscopy

The IR-spectra of both the chitosan and its *N*-acetyl derivative are quite similar (Fig. 4). However, a higher peak of ν-C=0 at 1690–1650 cm^{-1} compared with ν-OH between 3300 and 3100 cm^{-1} found in the partial *N*-acetylchitosan can indicate a higher degree of acetylation. In addition the band of δ-NH at 1590 cm^{-1} which predominated the band of ν-C=0 at 1670 cm^{-1} detected in the spectrum of chitosan can indicate deacetylation (Takai et al., 1989).

3.6. Water uptake capacity

The water uptake of the partial *N*-acetylchitosan and the crosslinked chitosan sponges compared with the untreated chitosan sponges is shown in Table 4. The untreated chitosan sponges absorbed water very fast and disintegrated after 10 min. Dissolution of the the untreated chitosan sponges in water was complete within 6 h. The solubility of chitosan was decreased by either the crosslinking or the acetylation. A low water permeability of *N*-acetylchitosan was reported by Hirano et al., 1980. The uptake ability after 10 min of both the *N*-acetylchitosan and the

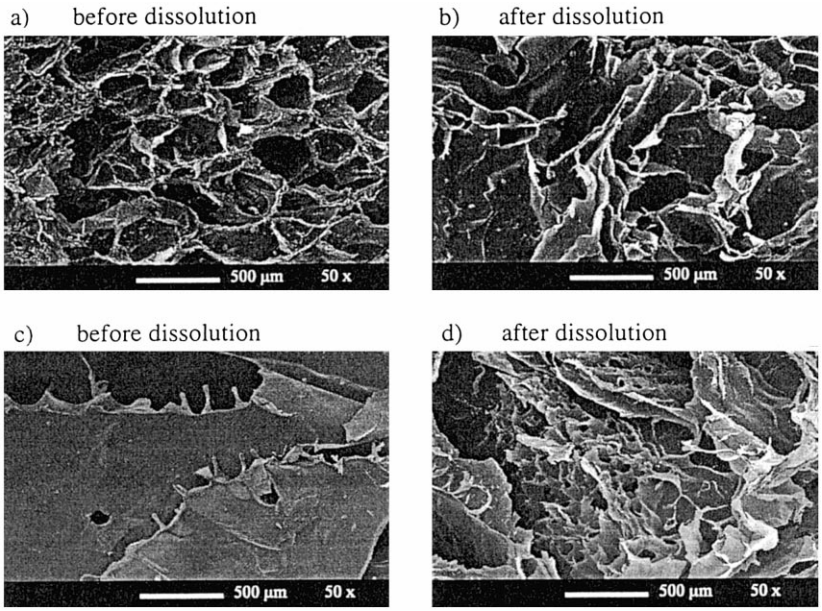

Fig. 2. Scanning electron micrographs of partially *N*-acetylated chitosan sponges (a), (b) and crosslinked chitosan sponges (c), (d) before and after dissolution at pH 7.4.

crosslinked chitosan sponges was more than 20 times of their weight. The crosslinked chitosan sponges disintegrated into small fractions after 6 h while the *N*-acetylchitosan sponges were resilient in water. The water uptake of *N*-acetylchitosan sponges was saturated within 6 h.

3.7. Drug liberation

The release of TA from the chitosan sponges is shown in Figs. 5 and 6. The drug release from all chitosan sponges depended on the pH of the dissolution media. Faster drug release from the untreated chitosan sponges was found at both pH 1.2 and 7.4. The drug release from the *N*-acetylchitosan and the crosslinked chitosan sponges was, however, sustained at both pH. It is suggested to be due to the decrease in solubility and permeability of the chitosan matrices by either the acetylation or the crosslinking of the chitosans (Kanke et al., 1989; Akbuga and Durmaz, 1994). The drug release from the *N*-acetylchitosan sponges at pH 1.2 and 7.4 followed Higuchi's mechanism as reported by Kristl et al. (1993). As seen in Fig. 5(a), the faster drug release was found from the *N*-acetylchitosan sponges at pH 1.2 ($k = 13.1 \pm 0.5\ h^{-1/2}$, $r^2 = 0.999$). At pH 7.4 slower drug release was a function of the square root of time over 52 h ($k = 10.5 \pm 0.3\ h^{-1/2}$, $r^2 = 0.999$) It is assumed to be due to a lower solubility or a decreased permeability of *N*-acetylchitosan matrix at higher pH as reported by Robert et al. (1987) and Hutchings and Sakr (1994).

The drug release from the *N*-acetylchitosan sponges also depended on the drug content (Fig. 5(b)). The release of drug was faster from the *N*-acetylchitosan sponges containing a lower drug content. The release rate constant at pH 7.4 of the sponges containing 0.8% drug was $23.3 \pm 4.3\ h^{-1/2}$ ($r^2 = 0.989$) and followed Higuchi's mechanism up to 4 h. The drug release was more prolonged from the sponges containing 3.4% TA ($k = 10.5 \pm 0.3\ h^{-1/2}$, $r^2 = 0\ 999$) These phenomena have been reported by Benita et al. (1990), Chi and Jun (1991) and Bote et al. (1993). Unfortunately no discussion was established. It can be assumed that a certain volume of the dissolution medium is required for dissolving the drug incorporated in

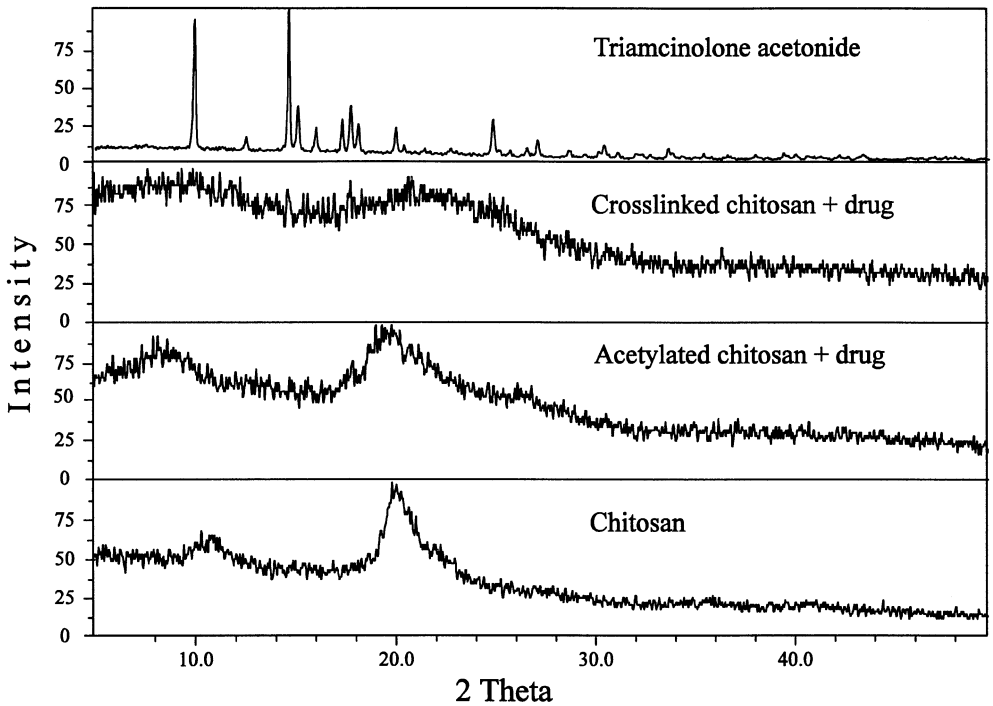

Fig. 3. X-ray diffraction patterns of chitosan systems.

chitosan matrices. Both the penetration of the dissolution medium and the diffusion of the dissolved drug through the chitosan matrices are limited. Kanke et al. (1989) stated that the pore sizes of the *N*-acetylchitosan films were much smaller than those of untreated chitosan films. A lower permeability of the *N*-acetylchitosan compared with the untreated chitosan is suggested (Hirano et al., 1980). Thus a longer period of time is needed for a complete drug release from a chitosan matrixes containing a higher drug content.

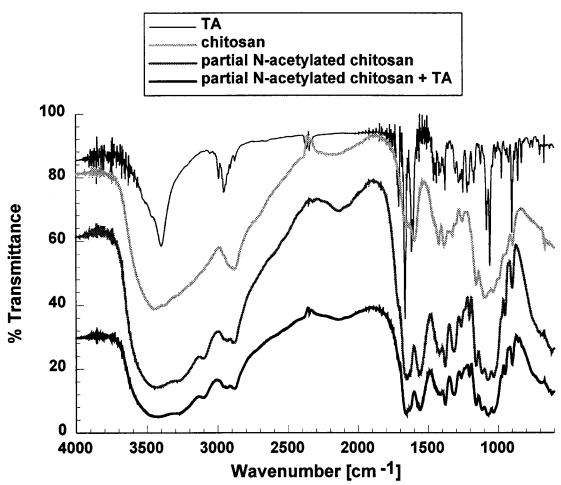

Fig. 4. IR spectra of chitosan and partial *N*-acetylchitosan sponges.

Crosslinked chitosans have been established as sustained drug release matrices such as in preparation of chitosan microparticles (Akbuga and

Table 4
Water uptake of chitosan sponges at room temperature

Chitosan sponge	Water uptake (g water/g sponge)	
	After 10 min	After 6 h
Chitosan	Disintegrated	Completely soluble
Partial *N*-acetylchitosan	27.4 ± 0.7	27.0 ± 0.7
Crosslinked chitosan	26.8 ± 0.8	Disintegrated, insoluble

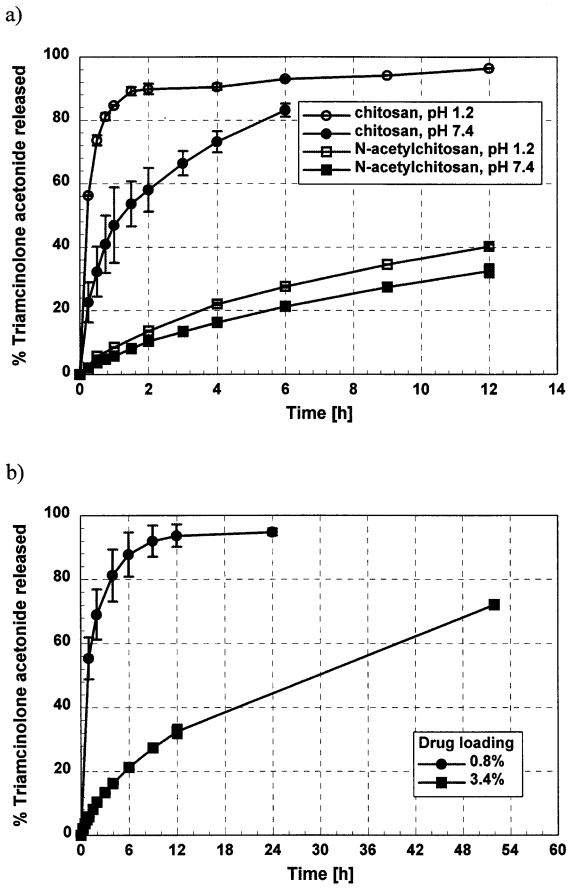

Fig. 5. Drug release from partially *N*-acetylated chitosan sponges ($n = 3$); (a) effect of pH of dissolution media and (b) effect of drug loading (pH 7.4).

Durmaz, 1994). The crosslinking delayed the drug release from the chitosan matrices at both pH 1.2 and 7.4. As found in the *N*-acetylchitosan sponges the drug release from the crosslinked chitosan sponges was also faster at pH 1.2 (Fig. 6(a)). This is suggested to be due to a higher solubility of the crosslinked chitosan at acidic pH. In this case the drug release was almost complete after 4 h. At pH 7.4 the drug release was more sustained (Fig. 6(b)). The release rate at pH 7.4 of the uncrosslinked and the crosslinked sponges were found to be $38.7 \pm 12.3\ h^{-1/2}$ ($r^2 = 0.993$) and $15.8 \pm 5.3\ h^{-1/2}$ ($r^2 = 0.928$) respectively. The release kinetic of the crosslinked chitosan sponges followed Higuchi's mechanism over 36 h.

4. Conclusion

The modified chitosan sponges were effective as sustained release drug carriers. The drug release from the *N*-acetylchitosan and the crosslinked chitosan sponges was pH dependent. The release of drug can be controlled by varying the drug content, the acetylation and the crosslinking. The kinetics of drug release from both the *N*-acetylchitosan and the crosslinked chitosan sponges followed Higuchi's mechanism.

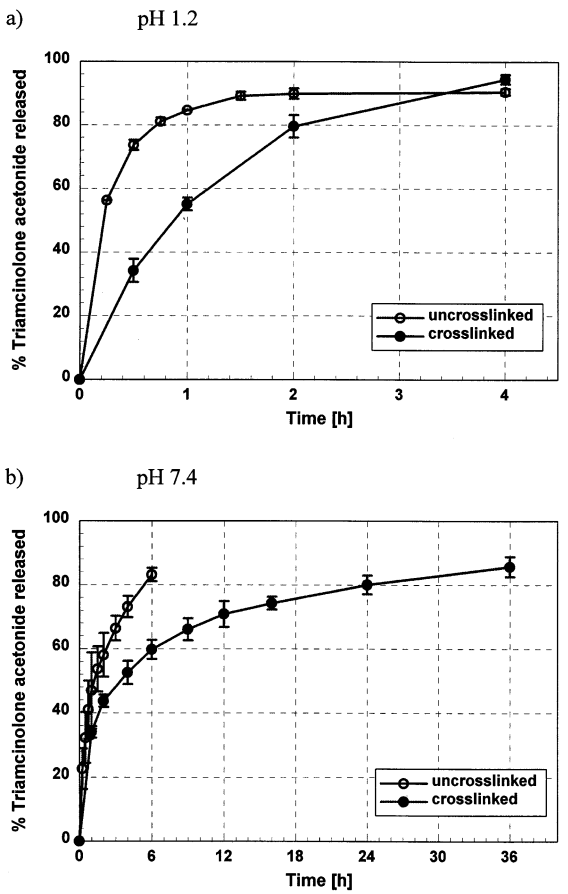

Fig. 6. Effect of crosslinking on drug release from chitosan sponges ($n = 3$), (a) at pH and (b) at pH 7.4.

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