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RESEARCH PAPER

Optimization of Biodegradable Sponges as Controlled Release Drug Matrices. I. Effect of Moisture Level on Chitosan Sponge Mechanical Properties

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ABSTRACT

Cross-linked chitosan sponges as controlled release drug carrier systems were developed. Tramadol hydrochloride, a centrally acting analgesic, was used as a model drug. The sponges were prepared by freeze-drying 1.25% and 2.5% (w/w) high and low M.wt. chitosan solutions, respectively, using glutaraldehyde as a cross-linking agent. The hardness of the prepared sponges was a function of glutaraldehyde concentration and volume where the optimum concentration that offered accepted sponge consistency was 5%. Below or above 5%, very soft or very hard and brittle sponges were obtained, respectively. The determined drug content in the prepared sponges was uniform and did not deviate markedly from the calculated amount. Scanning electron microscopy (SEM) was used to characterize the internal structures of the sponges. The SEM photos revealed that cross-linked high M.wt. chitosan sponges have larger size surface pores that form connections (channels) with the interior of the sponge than cross-linked low M.wt. ones. Moreover, crystals of the incorporated Tramadol hydrochloride were detected on the lamellae and within pores in both chitosan sponges. Differences in pore size and dissolution medium uptake capacity were crucial factors for the more delayed drug release from cross-linked low M.wt. chitosan sponges over high M.wt. ones at pH 7.4. Kinetic analysis of the release data using linear regression followed the Higuchi diffusion model over 12 hours. Setting storage conditions at room temperature under 80–92% relative humidity resulted in soft, elastic, and compressible sponges.

Key Words: Chitosan; Sponges; Cross-linking; Controlled release; Tramadol hydrochloride.

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INTRODUCTION

Chitin is one of the most abundant polysaccharides in nature. This biopolymer is a structural component of the exoskeleton of crustacea i.e., crabs, shrimps, and lobsters, as well as insects and in some fungi as yeasts.[1]

Chitosan (the principal derivative of chitin) is a hydrophilic biopolymer obtained industrially by hydrolyzing the amino acetyl groups of chitin by an alkaline deacetylation. Its molecular formula is (1,4)2-amino-2-deoxy-D-glucose.[2] Chitosan is insoluble at neutral and alkaline pH values but forms salts with inorganic and organic acids such as acetic acid. Upon dissolution, the amine groups of the polymer are protonated and the resultant soluble polysaccharide is positively charged.[3] Since chitosan exhibits favorable biological properties such as nontoxicity, biocompatibility, and biodegradability, it has attracted great attention in the pharmaceutical and biomedical fields.[1] Biomedically, chitosan is reported to have pharmacological properties such as hypocholesterolemic action, wound healing properties, antacid and antiulcer activity. In addition, its polycationic character gives chitosan the ability to bind strongly to several mammalian cells, leading to many potential uses, including hemostatic activity where it could be used in the obliteration of dead space created by teeth extraction and spermicidal use. While large number of studies have been published in the past years indicated the high efficiency of cross-linked chitosan for the production of various controlled release drug carrier systems such as microspheres,[4–7] films,[8,9] membranes,[10,11] beads,[12] tablets,[13] and gels,[14–16] very few publications discussed the preparation of chitosan as sponges.[17–22] Several attempts were made to manipulate sponge consistency and elasticity including: a) cross-linking low molecular weight chitosan only,[17] b) using different acid types for preparation of chitosan solution,[18] c) mixing chitosan with gelatin to create a softer polymer and cross-linking mixture,[18] and d) addition of ionic and nonionic plasticizers to the chitosan-gelatin mixture.[19] The results obtained from these trials did not achieve a compromise between the sponge mechanical properties and their release patterns.

In this study, both low and high molecular weight chitosan sponges were prepared. The factors that reportedly affect sponge consistency viz., chitosan concentration, glutaraldehyde concentration, and quantity were studied. In a newly adopted strategy, the sponges were stored under various relative humidities in a trial to manipulate sponge consistency without the addition of any plasticizer. Tramadol hydrochloride, a centrally acting synthetic analgesic,[23] was the drug of choice. The drug plasma half-life is short and its usual doses are given every 4–6 hours. The drug undergoes significant first-pass metabolism following oral administration.[24] Therefore, the drug-chitosan sponge as an alternative delivery system is promising in wound healing after teeth extraction and/or implantation where the biodegradable nature of chitosan alleviates the need for surgical removal of the sponges after insertion. Moreover, the associated pain perception is expected to be reduced for hours due to the release of the analgesic drug in a controlled manner.

EXPERIMENTAL

Materials

Tramadol hydrochloride was kindly provided by Memphis Drug Co., Cairo, Egypt. High molecular weight chitosan [degree of deacetylation 80%, viscosity of 5400 cps (1% solution in 1% acetic acid), molecular weight distribution 500,000–5,000,000 g/mol] was kindly provided by Cognis Deutschland GmbH, Düsseldorf, Germany. Low molecular weight chitosan [degree of deacetylation 85%, viscosity of 45 cps (1% solution in 1% acetic acid), molecular weight distribution 300,000–2,000,000 g/mol] was purchased from Aldrich Chemical Co., St. Louis, MO. Glacial acetic acid, disodium hydrogen phosphate, potassium dihydrogen phosphate, and glutaraldehyde (25% solution) were purchased from Merck, Darmstadt, Germany. Other materials used in this study were of pharmaceutical or analytical grade and were used as received.

Methods

Preparation and Viscosity Measurements of Chitosan Solutions

Various concentrations of high M.wt. chitosan solutions, viz, 0.25%, 0.5%, 0.75%, 1%, and 1.25% (w/w) and low M.wt, one viz. 0.5%, 1%, 1.5%, 2%, and 2.5% (w/w) in 1% acetic acid were prepared, respectively. The viscosity of the prepared solutions was measured using a Brookfield viscometer [Model DV-III] at room temperature.

Preparation of Controlled-Release High and Low M.wt. Chitosan Sponges

Cross-linked chitosan matrices were prepared using 2.5 grams of 1.25 of high M.wt. chitosan and 2.5% (w/w) of low M.wt. one in 1% acetic acid solution, respectively. Tramadol HCl (150 mg) was

dispersed in each solution. Increasing volumes of 1%, 3%, 5%, and 7% (v/v) of glutaraldehyde solution were added separately to the prepared chitosan-drug mixtures. Stirring at 50 rpm for 15 minutes using a magnetic stirrer (Thermolyne Corporation, Dubuque, IA) was necessary to enhance the glutaraldehyde cross-linking effect. The cross-linked solutions were then poured into glass vials and freeze-dried (Labconco Corporation, Kansas City, MO) at $-40^{\circ}C$, under a vacuum of $33 \times 10-3$ mBAR. Uncross-linked lyophilized chitosan products containing tramadol HCl were obtained upon applying the same procedure without the addition of glutaraldehyde solution.

Determination of Drug Content

A sponge was extracted in 100 mL of 0.1 N HCl. After filtration through a cellulose acetate membrane (0.45 μm), the concentration of tramadol HCl in the solution was determined spectrophotometrically (Shimadzu 1601 PC double beam spectrophotometer, Kyoto, Japan) at 272 nm.[25] The drug content in the sponges was determined in triplicate.

Characterization of Chitosan Sponges

Scanning Electron Microscopy (SEM)

A thin piece of a sponge (0.5 mm) was fixed on an SEM sample holder with double-sided adhesive tape and coated with a layer of gold of 150 A° for 2 min using a sputter coater (Edwards S-150A, England) in a vacuum of 3×10^{-1} atm of argon gas. The sample was then examined using a scanning electron microscope (Jeol JSM T20, Tokyo, Japan).

In Vitro Release of Drug from Chitosan Sponges

This study was carried out using United States Pharmacopoeia (USP) Dissolution Tester Apparatus I (Hanson SR6, Chatsworth, CA). The chitosan sponge was placed in a basket, which was then dipped in a 900-mL Sorensen's phosphate buffer (pH 7.4) contained in the 900 mL vessel of the USP Dissolution Tester Apparatus. The release study was carried out at $37 \pm 0.5^{\circ}C$ and the basket was rotated at a speed of 50 rpm. Five-mL samples were withdrawn after 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, and 12 hours, filtered through cellulose acetate membrane (0.45 μm), and the concentration of tramadol HCl in the solution was determined spectrophotometrically (Shimadzu UV-1601 PC double beam spectrophotometer, Kyoto, Japan) at 272 nm. All the release experiments were run in duplicate.

The obtained release data were subjected to kinetic treatment according to zero, first, and Higuchi diffusion models.[26] The correlation coefficient (r^2), order of release pattern, and half-life were determined in each case.

Dissolution Medium Uptake Capacity

A sponge was accurately weighed and placed in a small bottle containing 30 mL of Sorensen's phosphate buffer (pH 7.4) at room temperature. The bottle was turned up and down two times to ensure complete wetting of the sponge. The sponge was removed from the buffer solution after 0.25, 3, and 6 hours by means of a small forceps, allowed to drain by careful dropping on a filter paper, and reweighed. The increase in weight represents the weight of the buffer solution taken by the sponge, which was calculated as a ratio of the weight of absorbed buffer solution to the weight of the dry sponge at each period of time as follows:

$$\text{Dissolution medium uptake capacity } (g/g) = (W_{wet} - W_{dry})/W_{dry}$$

The dissolution medium uptake of the sponges was determined in duplicate.

Moisture Sorption Capacity of the Sponges

In order to determine the optimum conditions for obtaining soft and elastic matrices, the prepared cross-linked chitosan sponges were stored at room temperature under various relative humidities as follows: the sponges were conditioned by placing in a dessicator over anhydrous calcium chloride for 2 days. The conditioned sponges were accurately weighed and then placed at room temperature in six relative humidity (RH) chambers set at 43%, 65%, 75%, 80%, 92%, and 97% (RH) using saturated solutions of potassium carbonate, sodium nitrite, sodium chloride, ammonium sulfate, potassium nitrate, and potassium sulfate, respectively.[27] The experiment was carried out for 12 weeks where the sponges were reweighed every day for 7 days and every week for the rest of the 12 weeks. Increase or decrease in weight was then determined and percent moisture sorption was calculated using the following formula:

$$\frac{\text{Weight of exposed sponge} - \text{weight of conditioned sponge}}{\text{Weight of conditioned sponge}} \times 100$$

The percentages calculated were plotted against time to establish the point of maximum sorption for each sponge. All experiments were run in duplicate.

Determination of Sponge Compressibility

The previously stored sponges at various relative humidities were subjected to compressibility testing approved by the American Society for Testing Materials (ASTM), designated as ASTM test No. D570-59T.[28] The original thickness of the sponges was determined using a micrometer. The sponges were placed between two clean, smooth, and parallel horizontal plates. Loads were added to the upper plate until the thickness of the sponges was reduced by 25 ± 5%. The loads were released after 1 hour and the thickness of the sponges was remeasured after 30 min rest at room temperature. All the experiments were run in duplicate. The compression percentage was calculated as follows:

$$\text{Compression } \% = (t_1/t_0) \times 100$$

where t_0=original thickness, t_1=thickness of sponge 30 min after removal of loads.

RESULTS AND DISCUSSION

Viscosity Measurements of the Prepared Chitosan Solutions

Viscosity is an important property of chitosan solution and a function of its concentration. A steep increase in viscosity was observed at concentrations above 0.75% and 1% (w/w) of high and low M.wt. chitosan, respectively (Fig. 1a,b). This may be explained by the increased effective volume of the polymer molecules caused by hydration and mutual electrostatic repulsion of charged functional groups in the acid medium, i.e., + NH3 on the chitosan chains and their corresponding extension.[29] Indeed, an incomplete dissolution of chitosan was observed at acetic acid concentration lower than 1%, and uniform solutions in 1% acetic acid could not be obtained from high and low chitosan concentrations above 1.25% and 2.5%, respectively. Therefore, based on the solution's homogeneity and highest viscosities obtained from 1.25% and 2.5% (w/w) high and low M.wt. chitosan respectively, these concentrations were chosen for preparation of sponge matrices due to their expected drug retardation effect.

Effect of Glutaraldehyde Concentration on Chitosan Sponges

Cross-linked chitosan has been established as sustained-release drug matrices where the amino groups of chitosan molecules are active on cross-linking by dialdehydes such as glutaraldehyde.[10,17,18,30] It was noticed that the hardness of the prepared sponges

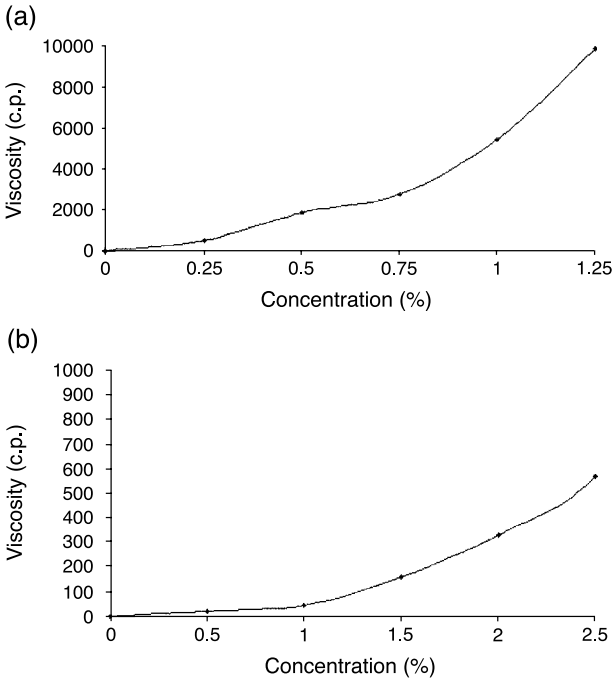


Figure 1. Effect of increasing (a) high M.wt. and (b) low M.wt. chitosan concentration on the solution viscosity using 1% acetic acid.

was a function of glutaraldehyde concentration, 7% > 5% > 3% > 1% (v/v). Sponges cross-linked with 1% or 3% glutaraldehyde solution were too soft, whereas sponges cross-linked with 7% glutaraldehyde solution were very hard and brittle. Five percent glutaraldehyde solution was the concentration of choice, as it offered relatively less hard and more suitable sponge consistency. Likewise, sponges cross-linked with volumes of 5% glutaraldehyde solution higher than 0.1 and 0.05 mL for high and low M.wt. chitosan solutions, respectively, were hard and brittle. Therefore, 0.1 and 0.05 mL of 5% glutaraldehyde solution were suggested to be the suitable concentrations for cross-linking 2.5 grams of 1.25% and 2.5% of high and low M.wt. chitosan solutions, respectively. Indeed, the cross-linked high M.wt. chitosan sponges were harder than cross-linked low M.wt. ones.

In contrast to cross-linked sponges, uncross-linked lyophilized products were fluffy, and homogenous intact matrices could not be achieved.

Drug Content

The drug content in the uncross-linked lyophilized products as well as the cross cross-linked chitosan sponges was uniform and did not deviate markedly from

Table 1. Determination of (a) drug content and (b) half-life in the prepared chitosan matrices.

Chitosan matrices	(a) % Drug recovered (Mean ± SD)	(b) Half-life (h)
High M.wt. chitosan lyophilized product	97 ± 0.4	0.551
Crosslinked high M.wt. chitosan sponge	96 ± 0.4	3.017
Low M.wt. chitosan lyophilized product	97 ± 0.5	1.853
Crosslinked low M.wt. chitosan sponge	95 ± 0.3	4.904

the calculated amount where approximately 95% up to 97% of the drug was recovered (Table 1a).

Morphology of Chitosan Sponges

Figure 2a,b show the photographs, 10x, of cross-linked high and low M.wt. chitosan sponges, respectively. Cross-linked sponges could not be easily differentiated from each other. They appear compact, yellowish brown in color, and take the shape of their glass containers.

SEM Photos

Scanning electron microscopy of cross-linked high and low M.wt. chitosan sponges revealed different types of structures within the sponge matrix as seen in Figs. 3 and 4. Freeze drying chitosan mixtures at −40°C resulted in: *sheetlike structures* that formed a flat smooth surface as indicated by area ''a'' and *open pores* characterized by two aspects, *surface pores* formed from a series of open semiellipsoids or hemispheres with walls that frequently had a sheetlike

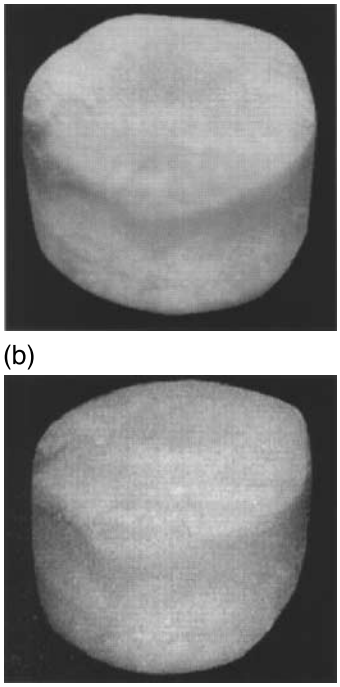


Figure 2. Photos, 10x, of (a) cross-linked high and (b) cross-linked low M.wt. chitosan sponge.

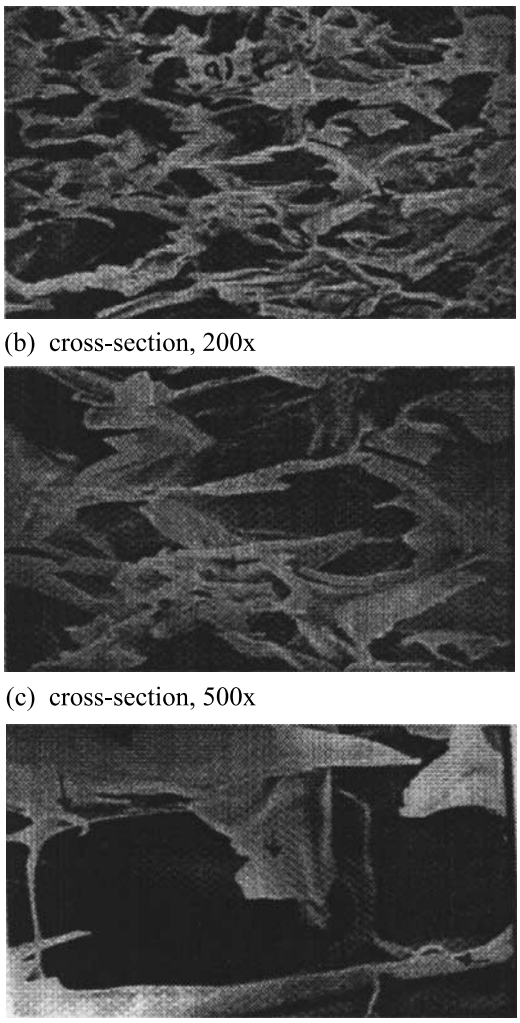
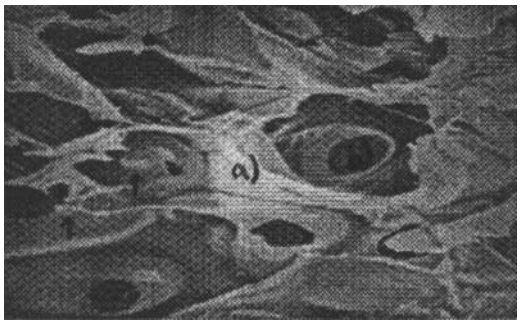
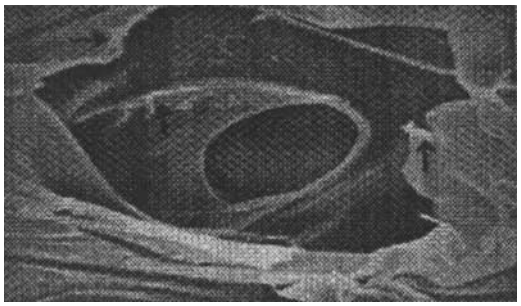


Figure 3. Scanning electron micrographs of cross-linked high M.wt. chitosan sponge.

(a) cross-section, 200x



(b) cross-section, 500x



(c) cross-section, 750x

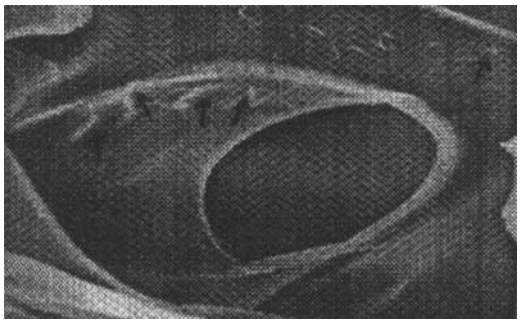


Figure 4. Scanning electron micrographs of cross-linked low M.wt. chitosan sponge.

structure, and *channels* formed from open surface pores that continued into the deeper layers of the sponges as indicated by area ''b.''

These different areas could be explained with reference to the ice crystal growth described by Doillon et al.,[31] where unorganized ice crystal growth may induce irregular sublimation with recrystallization of water vapor and secondary sublimation in the same areas during the freeze-drying procedure. This phenomenon might explain in part the partial collapse of pores during freeze-drying and formation of sheetlike structures. On the other hand, regular ice crystal growth may induce a more regular sublimation between the surface and the interior of the sponge without recrystallization of the water vapor, leaving a continuous open channel structure. The photos also showed that cross-linked high M.wt. chitosan sponges have larger pore sizes than cross-linked low M.wt. ones. Moreover, crystals of tramadol HCl were detected on the lamellae and within the pores of both chitosan sponges as indicated by arrows, which explained the immediate and the delayed drug release portions respectively.

Dissolution Medium Uptake Capacity

Dissolution medium uptake capacities at room temperature of chitosan sponges as well as its lyophilized products are graphically illustrated in Fig. 5. In general, uncross-linked lyophilized products have higher dissolution medium uptake capacity than cross-linked sponges of the same M.wt. They absorbed water very fast and disintegrated completely within 5–6 hours. In contrast to untreated products, the cross-linking process decreased the solubility of chitosan, and cross-linked sponges were more resistant to dissolution medium where they disintegrated into small fractions after 7–8 hours. In fact, the dissolution medium uptake capacity of high M.wt. chitosan sponges was higher than that of low M.wt. ones.

Drug Release

The release characteristics of tramadol HCl from chitosan sponges and its uncross-linked lyophilized products are graphically illustrated in Fig. 6. The release of tramadol HCl was relevantly sustained from cross-linked sponges, which is expected to be due to the decrease in the solubility and permeability of chitosan by the cross-linking process.[32,33] It is widely accepted that a volume of the dissolution medium is required to dissolve the drug incorporated in chitosan sponges. Both absorption of dissolution medium and diffusion of the dissolved drug out of the sponges are limited by the cross-linked chitosan matrices that have low permeability to either dissolved drug or dissolution medium, and this may be the rate limiting step of drug release. On the other hand, in uncross-linked lyophilized matrices, the drug release was no longer extended for more than 6 hours when the chitosan matrix was completely disintegrated.

It is worthwhile commenting that all dissolution profiles were characterized by a large percentage of drug release in the first hour for both cross-linked (35.6% and 18.7%) and uncross-linked (60.4% and 37.5%) matrices. This result could be attributed to surface drug that is detected on the lamellae and not entrapped in the inner matrix of the sponges as substantiated from SEM photos (Figs. 3 and 4).

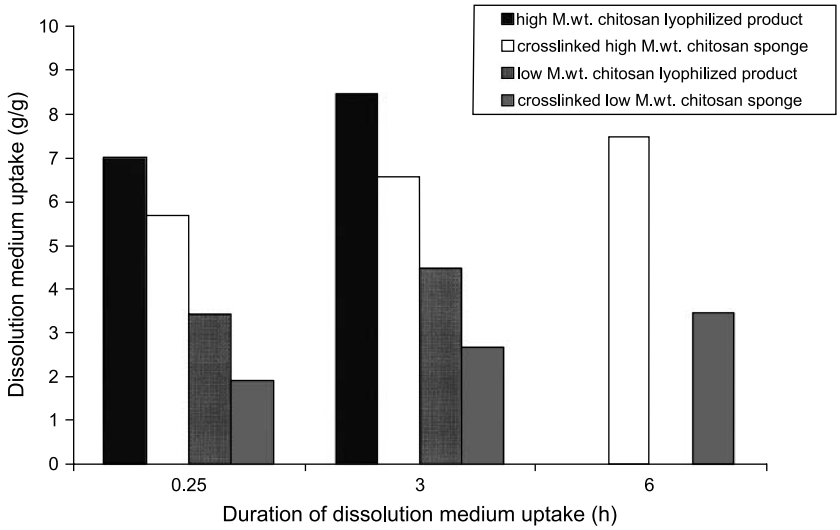


Figure 5. Dissolution medium uptake capacity of different chitosan matrices.

Interestingly, the release of tramadol HCl from the low M.wt. chitosan sponge was slower than that from the high M.wt. sponges. This unexpected result was explained with reference to pore size structure where much smaller pore sizes were evident in low M.wt. chitosan sponge (Figs. 3 and 4).

The amount of tramadol HCl released from all chitosan sponges and its lyophilized products showed a linear relationship with the square root of time (correlation coefficient > 0.97); therefore, the release rate over 12 hours followed the theoretical model by Higuchi. Moreover, the largest half life value of the dissolution profiles was achieved from cross-linked low M.wt. chitosan sponge (Table 1b).

Moisture Sorption Capacity of the Sponges

Our prepared sponges had poor mechanical properties (poor elasticity and compressibility), and it was more or less difficult to verify their uses after teeth extraction and/or implantation. For this reason, the moisture sorption capacity of the prepared sponges

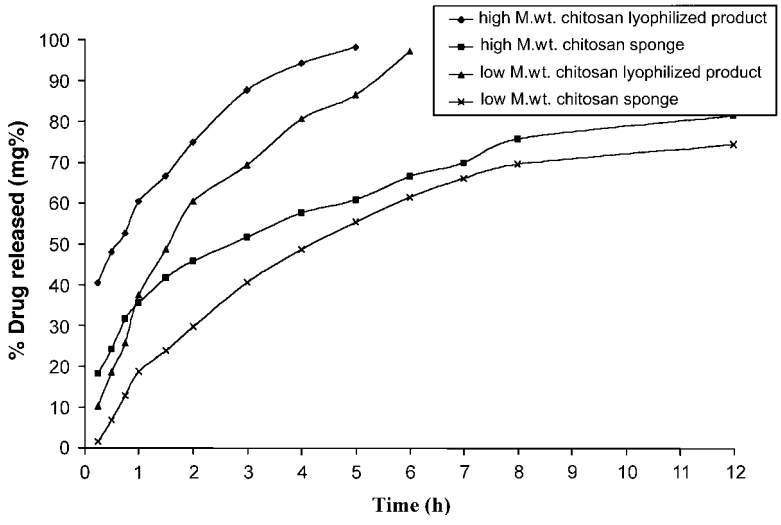


Figure 6. Release of tramadol HCL from different chitosan matrices.

stored under different relative humidities was studied in a trial to manipulate its consistency (Fig. 7). Storage of sponges at 43% and 65% RH resulted in relatively low amounts of moisture sorption where they reached a point of equilibrium at the end of 3 and 4 days, respectively. These relative humidities did not produce any apparent physical changes in the sponges by the end of the 12 weeks, and the sponges, especially high M.wt. ones, were still hard and brittle. Unfortunately, storage under 97% RH, led to high moisture sorption (up to 152.4% and 123.5% for low and high M.wt. sponges, respectively) where it reached a point of equilibrium at the end of 6 days. Undesirable physical changes were observed in low M.wt. sponges by the end of the storage period where they became so soft and darker in color than high Mwt. ones.

Adaptation of moisture levels at 75% and 80% RH, for low M.wt sponges and 92% RH for high M.wt ones resulted in moderate amounts of moisture sorption (up to 33.5, 45.1 and 48%, respectively). This was considered to be the optimum recommended level that rendered the sponges soft with excellent accepted consistency.

Compressibility of Sponges

The percentage compressibility of the stored sponges under various relative humidities is graphically illustrated in Fig. 8. It was found that there is a direct proportional relationship between moisture sorption capacity of the sponges and their percentage compressibility.

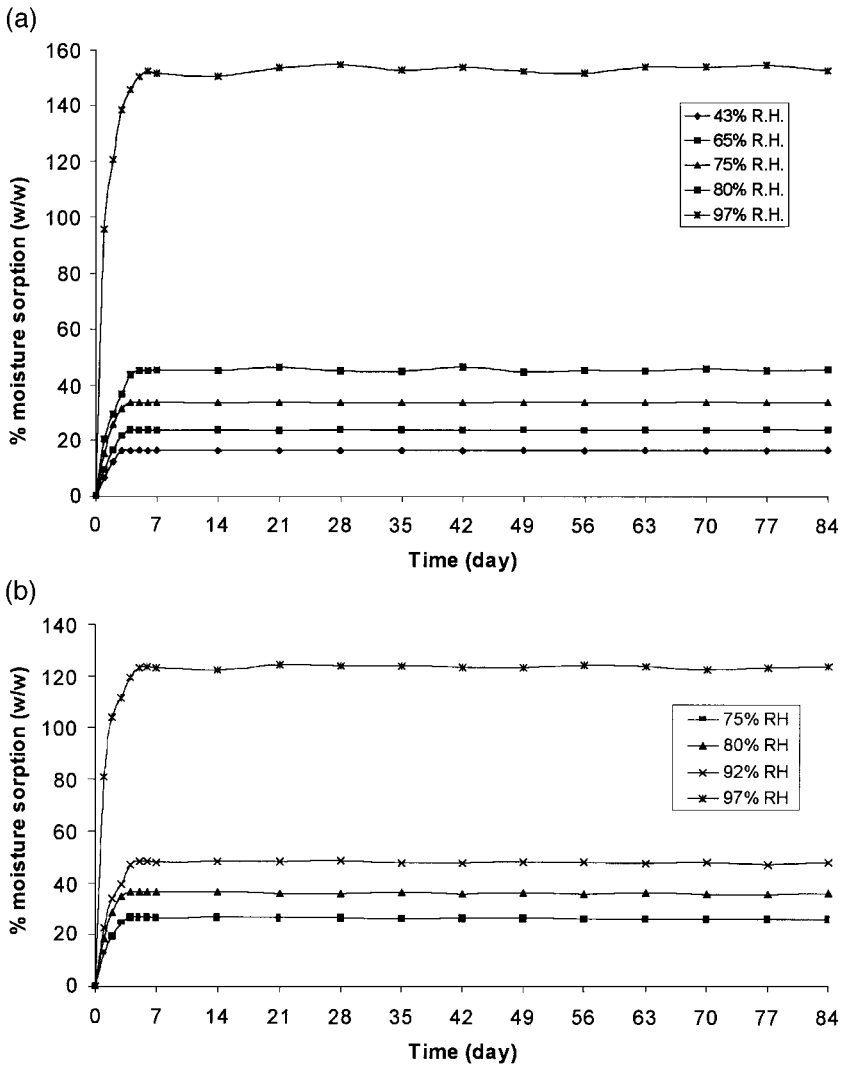


Figure 7. Moisture sorption capacity of (a) cross-linked low M.wt. and (b) cross-linked high M.wt. chitosan sponges stored at different relative humidities.

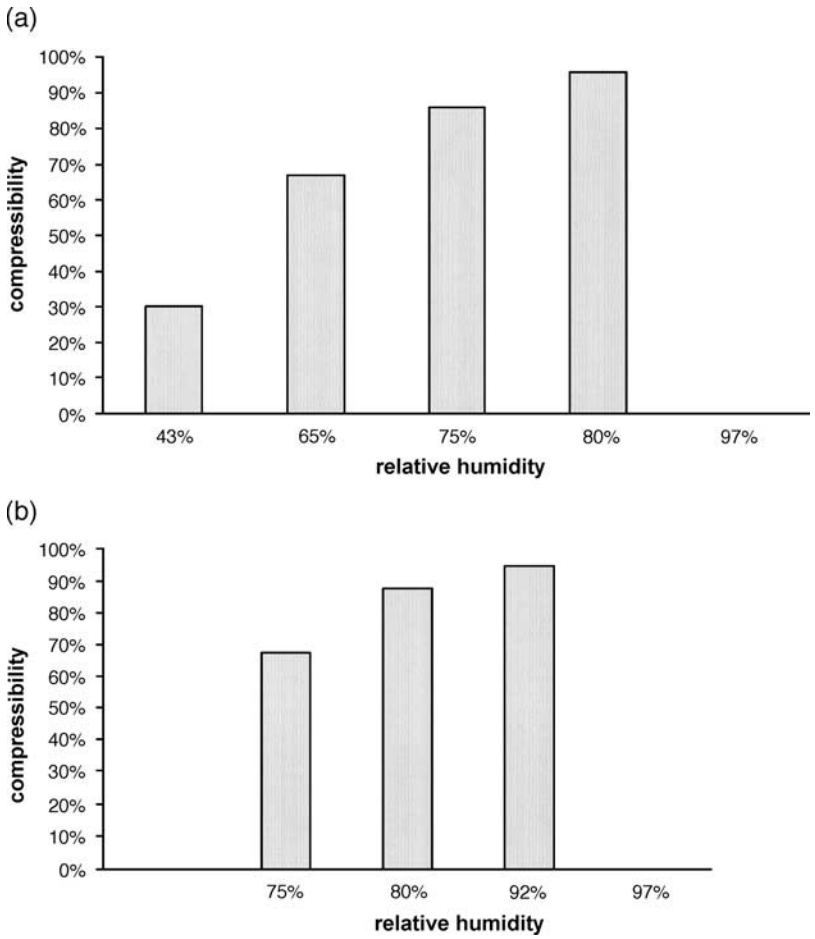


Figure 8. Compressibility of (a) cross-linked low M.wt. and (b) cross-linked high M.wt. chitosan sponges stored at different relative humidities.

For an ideal elastic sponge, the percentage compression theoretically should be 100%, corresponding to an elasticity quotient of 1.[18] In practice, maximum percentage of 96% and 95% was achieved after storing the sponges at 80% and 92% RH for low and high M.wt. chitosan, respectively. Unfortunately, sponges stored at 97% RH, especially low M.wt. ones, adhered to the plates and could not be compressed because they were too soft.

CONCLUSIONS

The controlled release of Tramadol hydrochloride, the model drug, from chitosan sponge matrices was successfully achieved by cross-linking low molecular weight chitosan with glutaraldehyde. A compromise between sponge mechanical properties and its delayed pattern was realized. The hardness of the prepared sponges was a function of glutaraldehyde concentration and volume. Optimization of relative humidity under 80–92% effectively resulted in soft, elastic chitosan sponges without any additives or plasticizer, therefore providing the soft and elastic material required for patients' comfort and optimal healing.

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