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Implantable Biodegradable Sponges: Effect of Interpolymer Complex Formation of Chitosan With Gelatin on the Release Behavior of Tramadol Hydrochloride

Nagwa H. Foda, Hanan M. El-laithy, and Mina I. Tadros
Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ABSTRACT The effect of interpolymer complex formation between positively charged chitosan and negatively charged gelatin (Type B) on the release behavior of tramadol hydrochloride from biodegradable chitosan-gelatin sponges was studied. Mixed sponges were prepared by freeze-drying the cross-linked homogenous stable foams produced from chitosan and gelatin solutions where gelatin acts as a foam builder. Generation of stable foams was optimized where concentration, pH of gelatin solution, temperature, speed and duration of whipping process, and, chitosan-gelatin ratio drastically affect the properties and the stability of the produced foams. The prepared sponges were evaluated for their morphology, drug content, and microstructure using scanning electron microscopy, mechanical properties, uptake capacity, drug release profile, and their pharmacodynamic activity in terms of the analgesic effect after implantation in Wistar rats.

It was revealed that whipping 7% (w/w) gelatin solution, of pH 5.5, for 15 min at 25°C with a stirring speed of 1000 rpm was the optimum conditions for stable gelatin foam generation. Moreover, homogenous, uniform chitosan-gelatin foam with small air bubbles were produced by mixing 2.5% w/w chitosan solution with 7% w/w gelatinsolution in 1:5 ratio. Indeed, polyionic complexation between chitosan and gelatin overcame the drawbacks of chitosan sponge mechanical properties where, pliable, soft, and compressible sponge with high fluid uptake capacity was produced at 25°Cand 65% relative humidity without any added plasticizer. Drugreleasestudies showed a successful retardation of the incorporated drug where the $t_{50\%}$ values of the dissolution profiles were 0.55, 3.03, and 4.73 hr for cross-linked gelatin, un-cross-linked chitosan-gelatin, and cross-linked chitosan-gelatin sponges, respectively. All the release experiments followed Higuchi's diffusion mechanism over 12 hr. The achieved drug prolongation was a result of a combined effect of both cross-linking and polyelectrolyte complexation between chitosan and gelatin. The analgesic activity of the

Address correspondence to Hanan M. El-laithy, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo, Egypt; Tel: +2 012 312 4034; Fax: +202 761 2764; E-mail: hmellaithy@soficom.com.eg

implanted tramadol hydrochloride mixed chitosan-gelatin sponge showed reasonable analgesic effect that was maintained for more than 8 hr. Therefore, the use of chitosan and gelatin together appears to allow the formulator to manipulate both the drug release profiles and the mechanical properties of the sponge that could be effectively implanted.

KEYWORDS Sponges, Cross-linking, Stable foams, Controlled release, Tramadol hydrochloride

INTRODUCTION

In recent years, sponges based on natural biodegradable polymers have attracted much attention for drug implantation as taking out the implant after treatment always causes new damage to the wound. The patient comfort and optimum healing require soft and elastic material (Kristl et al., 1993). Chitosan, the principal derivative of naturally occurring chitin, is a hydrophilic cationic copolymer obtained industrially by hydrolyzing the amino acetyl groups of chitin by an alkaline deacetylation (Kas, 1997). Since chitosan exhibits favorable biological properties, such as nontoxicity, biocompatibility, and biodegradability, it has attracted great attention in the pharmaceutical and biomedical field, especially for controlled release drug delivery (Thakkar et al., 2004; Dastan & Turan, 2004; Han & Stevens, 2004; Nunthanid et al., 2004). However, few publications were developed on the preparation of chitosan sponges (Denkbas & Obadasy, 2000; Oungbho & Müller, 1997; Leffler & Müller, 2000; Park et al., 2000; Lai et al., 2003). In our previous work (Foda et al., 2004), a new strategy was adopted to compromise between the sponges' mechanical properties and their drug release pattern by storing them under various relative humidities without the addition of any plasticizer. However, a relatively high humidity (80%) was required to maintain soft, elastic, and compressible sponge.

On the other hand, absorbable gelatin sponge is an official monograph in USP (The United States Pharmacopeia 24 & National Formulary 19, 2000), used as haemostatic and coagulant in surgical procedures because of its capability of absorbing and holding within its meshes several times its weight of whole blood. Although gelatin sponges have high elasticity and can be well hydrated because of their high porosity and large surface area, the drugs implanted in or onto sponge lamellae could only be retarded for a short period of time (Simamora et al., 1996).

On this basis it seems promising to combine chitosan and gelatin in a trial to overcome the disadvantages of chitosan and gelatin sponges. Improved sponge mechanical properties, better drug release profile, and enhanced wound fluid absorption capacity could be expected as a result of this polyelectrolyte complex formation.

Thus, the objectives of this study were three-fold. In the first instance, the suitable conditions for the preparation of gelatin sponge, namely, concentration of gelatin solution, its pH, temperature, and whipping conditions were adapted. Secondly, chitosan–gelatin sponges were prepared and evaluated as controlled release carrier for tramadol hydrochloride, a centrally acting synthetic analgesic drug that is characterized by a short plasma half life and undergoes significant first-pass metabolism following oral administration (Physicians' Desk Reference, 2002). Finally, the pharmacodynamic activity of the implanted sponge into Wistar rats in terms of analgesic effect was evaluated by applying the hot plate method (Woolfe & Mac Donald, 1944).

EXPERIMENTAL

Materials

Tramadol hydrochloride was kindly provided by Memphis Drug Co., Cairo, Egypt. Low molecular weight chitosan (degree of deacetylation 85%, viscosity of 45 cps (1% solution in 1% acetic acid) was purchased from Aldrich Chemical Co., St. Louis, MO. Gelatin Type B (225 bloom) was obtained from Sigma 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

Optimization of Manufacturing Conditions of Gelatin Foams

Density and mechanical propertiesof gelatin sponges were controlledbyadjusting various conditions expected

to affect a generation of stable foams which could be obtained upon lyophilization stable sponges.

Optimization of Gelatin Solutions

The effect of gelatin concentration and its pH on foam formation were studied. 100 g of various concentrations of freshly prepared gelatin solutions 1, 3, 5, 7, 9, and 11% (w/w) adjusted to pH values of 4.5, 5.5, and 6.5 (before, at, and after its isoelectric point, respectively) using diluted solutions of acetic acid and ammonia were prepared for foam generation.

Optimization of Whipping Conditions

Gelatin foams were produced by the whipping method. The used homogenizer (VirTis, Gardiner, NY) consists of double-jacketed thermostatic stainless steel tank and a speed controllable stirrer with a special blade that enhances air bubbles incorporation and foam stability. Different gelatin solutions were transfered into the foam apparatus tank and generated into foams under different conditions. The effect of foam operation temperature (25, 35, 40, 50°C), whipping duration (5, 10, 15, and 30 min), and speed of stirring (500, 1000 rpm) were studied.

Characterization of Gelatin Foams

The resulting foams were evaluated with respect to foam uniformity (defined as the homogeneity of air bubbles examined under an electron microscope), foam stability at 25°C (defined as separation of the liquid part from derived foams in a cylinder along 60 min after finishing foam generation), and apparent foam density (estimated by dividing the foam weight (mg) in a cylinder over the foam volume (mL) in the same cylinder). Finally, the foam volume which is the reciprocal of the apparent foam density was also calculated and the optimum conditions for generation of stable foams were determined.

Generation of Chitosan-Gelatin Foams

Gelatin was used as a foam builder for the preparation of chitosan-gelatin foams. 7% (w/w) aqueous gelatin solutions and 2.5% (w/w) low molecular weight (MW) chitosan solution in 1% acetic acid were used. Different chitosan-gelatin mixtures of 1:1, 1:5, and 1:10 were first subjected to viscosity measurements using Brookfield digital viscometer (DV-III, Brookfield Engineering Laboratories, Inc., Stoughton, MA) at 25°C andthen whipped into foams using a homogenizer with a stirring speed of 1000 rpm for 15 min. The apparent foam density and foam stability were compared.

Preparation of Chitosan-Gelatin Sponges

150 mg of tramadol HCl was incorporated into 2.5 g of 1:5 chitosan:gelatin mixture. The mixture was generated into stable foam at 25°C with a stirring rate of 1000 rpm for 15 min. The resulting foams were then cross-linked with 5% glutaraldehyde solution. Continuous stirring at 50 rpm for 15 min using a magnetic stirrer, (Thermolyne Corporation, Dubuque, Iowa) was necessary to the enhance glutaraldehyde cross-linking effect. The cross-linked foams were poured into glass vials and freeze dried (Freeze dryer, Labconco Corporation, Missouri, Kansas city) at –40°C, under a vacuum of 33 × 10–3 mbar (Formula A). Un-cross-linked chitosan-gelatin foams (Formula B) and cross-linked gelatin foams (Formula C) containing the drug were also prepared and freeze dried.

Determination of Drug Content

The sponges were soaked overnight at 25°Cin 100 mL of 0.1 N HCl. After filtration through a cellulose acetate membrane (0.45 μm), the concentration of drug in the solution was determined spectrophotometrically (1601-PC double beam spectrophotometer, Shimadzu, Kyoto, Japan) at 272 nm (Moffat et al., 1986). The assay was run in triplicate.

Characterization of the Sponges

Photography

Both cross-linked gelatin and chitosan-gelatin sponges were photographed (Camera, Hi-matic GF, Minolta Camera Co., Osaka, Japan) to examine their morphological characteristics, particularly size and shape.

Scanning Electron Microscopy (SEM)

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

In vitro Drug Release from the Sponges

This study was performed using USP Dissolution Tester Apparatus I (Dissolution tester, Hanson SR6, Chatsworth, CA). Each 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 performed at 37 + 0.5°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 hr, filtered through cellulose acetate membrane (0.45 μm). The drug in the solution was determined spectrophotometrically at 272 nm. All the release experiments were run in triplicate. The obtained release data were subjected to kinetic treatment according to zero, first, and Higuchi diffusion models (Higuchi, 1963). The correlation coefficient (r), the order of release pattern, and $t_{50\%}$ value was determined in each case. The obtained $t_{50\%}$ values were subjected to statistical evaluation using ANOVA at a 5% level of significance.

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 25°C. The bottle was turned up and down twice to ensure complete wetting of the sponge. The sponge was removed from the buffer solution after 0.25, 4, and 8 hr 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 capacity was determined in duplicate and the results were subjected to statistical evaluation using ANOVA at a 5% level of significance.

FT-IR Spectroscopy

FT-IR spectra between 4000 and 500 cm^{-1} of tramadol HCl, low MW chitosan, gelatin, and cross-linked chitosan-gelatin sponge were determined using KBr disc (FT-IR, vector 22, Bruker Optics, Billerica, MA, England). The smoothing of spectra and the base line correlation procedures were used.

Mechanical Properties of Sponges

Our goal was the estimation of the mechanical properties of the sponges following the addition of gelatin. For this reason, the sponges were first conditioned by placing in a desiccator over anhydrous calcium chloride for 2 days. The conditioned sponges were accurately weighed and then placed at 25°Cin 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 (O'Neil et al., 1989). The experiment was performed 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. Moreover, these sponges kept 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 (1959 supplement to book of ASTM Standards, 1959). 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 hr and the thickness of the sponges was remeasured after 30 min rest at 25°C. All the experiments were run in duplicate. The compression percentagewas 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. For an ideal

elastic sponge this percentage would theoretically have a value of 100%, corresponding to elasticity quotient of 1 (Leffler & Müller, 2000).

Evaluation of Tramadol HCl Analgesic Activity

Pharmacodynamic Evaluation of Tramadol HCl Analgesic Activity

The analgesic activities (E) of tramadol HCl solution in normal saline (Formula D), the immediate release tramadol HCl capsule (Tramax®, Hi Pharm drug company, Cairo, Egypt), and the selected chitosan-gelatin sponge (Formula A) were evaluated applying the hot plate method in rats described by Woolfe and Mac Donald (Woolfe & Mac Donald, 1944). All the experiments were performed with adult male healthy Wistar rats (National Research Center, Dokki, Giza, Egypt) weighing between 180–220 g. Before using them in this study, the animals were housed for 7 days under constant environmental and nutritional conditions according to "the principles of laboratory animals care", (NIH publication 85–23, revised, 1985). Prior to the experiment, the rats were fasted overnight and were uniformly hydrated each with 3 mL water before the administration of the test formulae to reduce variation. The rats were randomly allocated to four groups of six rats each. Formula A was implanted subcutaneously into the back of rats in group 1 just lateral to the midline under general anesthesia with ether (Schmidt et al., 1995). The cutaneous wound was then closed with one wound clip (Weinhold et al., 1998).

Animals in the other three groups were subjected to sham operation. Tramax® capsules were dispersed for peroral application by a gavage where one capsule (containing 50 mg of tramadol HCl) was put into a graduated glass flask, dispersed by adding isotonic saline solution up to 12.5 mL, and mixed on a magnetic stirrer for 15 min at 25°C. Each rat in group 2 received the equivalent dose of the prepared dispersion (4 mg/mL) by oral gavage. The animals of the third group were injected subcutaneously with Formula D and finally, the last group was acting as a control and treated with normal saline only. Each group received the specified formula in a dose equivalent to 20 mg/Kg (Gercek et al., 2004; Tsai & Won, 2001)

The rats were placed individually in a beaker immersed in a thermostatic controlled water bath (Julabo Labortechnik GmbH, Seelbach, Germany) maintained at 55 ± 0.5°C. The response latency was evaluated on the basis of either hind paw lick or jump reaction, following contact with the beaker. The latency period was recorded after the following time intervals 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 12 hr for each animal following administration of the formulae. The mean and the standard deviation (SD) were calculated for each group. Statistical evaluation of the obtained latency period values after 2 and 12 hr was performed using ANOVA at a 5% level of significance.

RESULTS AND DISCUSSION

Optimization of Manufacturing Conditions of Gelatin Foams

The properties and the stability of gelatin foams are strongly affected by gelatin concentration, its pH, and conditions of whipping process. There is a linear, gradual increase in gelatin solution viscosity upon increasing gelatin concentration. Abrupt viscosity increase was observed with gelatin concentration above 5% (w/w). Stable homogenous gelatin foams with small air bubbles could only be achieved from gelatin concentrations of 7% at 25°C. Drainage of liquid with foam shrinkage and collapse of foam column were observed at lower gelatin concentrations. Higher gelatin concentrations than 7% were not suitable for foam generation where the foams were very viscous and nonhomogenous. It was also found that gelatin foams could not be prepared at 35°C or higher because of the disruption of the foam lamellae. Moreover, larger foam volume and lower density were produced at pH 5.5 near gelatin isoelectric point (pI = 5.2) where maximum stability and rigidity of protein films is achieved (Halling, 1981). Lower or higher pH values from pI resulted in the formation of surface charge on the protein molecules which, in turn, leads to a decreased film rigidity and hence to foam lamella rupture.

The homogenizer stirring speed of 500 rpm was not able to produce stable foams. Stirring of 1000 rpm with whipping duration of more than 10 min was required. It is obvious from Table 1 that increasing whipping duration resulted in a decrease in the apparent foam density. Longer whipping for 30 min did not significantly affect foam properties because of the uncontrollable and inconstant stirring speed. Therefore, whipping 7% gelatin solution at pH 5.5 for

TABLE 1 **Effect of Whipping Duration on Gelatin Foam Properties**

Gelatin concentration (% w/w)	Whipping duration (min)	Apparent density (mg/ml)	Foam volume (ml/g)	Foam uniformity	Foam stability
7	5	396	2.52	Nonhomogenous, large air bubbles	unstable
	10	376	2.65	Nonhomogenous, large air bubbles	unstable
	15	360	2.77	Homogenous, small air bubbles	stable
	30	355	2.81	Homogenous, small air bubbles	stable

15 min at 25°Cwith stirring speed of 1000 rpm was found to be optimum for stable gelatin foam generation.

Generation of Chitosan-Gelatin Foams

The data in Table 2 show that increasing chitosan ratio resulted in an increase in the viscosity of chitosan-gelatin mixtures suggesting that a strong interaction took place between the positively charged chitosan and the negatively charged gelatin-B solutions. Such polyionic complex was expected to diminish the disadvantages of chitosan and gelatin sponges alone. Coacervation of gelatin and chitosan could reduce the solubility of gelatin on one hand, thus providing effective prolongation of drug release. On other hand, using gelatin as a foam builder in the preparation of chitosan-gelatin sponge would improve the mechanical properties of chitosan. In contrast to viscosity, the foam stability showed an inverse proportion relationship with increasing chitosan concentration where nonhomogenous unstable foam was produced even after extending the whipping duration for more than 45 min when the ratio reached 1:1. The separation of fluid phase from the foam was concomitant a few minutes only after preparation. Therefore, 1:5 chitosan:gelatin ratio was chosen because of the stability of its foam mixture where homogenous, uniform foam with small air bubbles were produced as shown in Fig. 1.

Drug Content

The drug content in the un-cross-linked lyophilized products as well as the cross-linked chitosan-gelatin sponges was uniform and did not deviate markedly from the calculated amount where approximately 96 to 98% of the drug was recovered (Table 3a).

Morphology and SEM Photos

Figs. 2a,b show the photographs, 10×, of cross-linked gelatin, and chitosan-gelatin sponges, respectively. Unlike cross-linked gelatin sponges which were yellow in color and very soft, cross-linked chitosan-gelatin ones appeared white and more dense. Their SEM photos (Fig. 3) revealed that gelatin sponges were more porous than chitosan-gelatin ones. This high porosity would provide a large surface area available for water uptake and dissolution. Tramadol crystals were detected on the lamellae and within the pores of both sponges.

Dissolution Medium Uptake Capacity

Dissolution medium uptake capacities at 25°C of cross-linked gelatin sponges, as well as both cross-linked and un-cross-linked chitosan-gelatin sponges are graphically illustrated in Fig. 4. It is clear that gelatin sponges have significantly higher ($p < 0.0001$) dissolution medium uptake capacity where they absorbed water very fast and dissolved completely within 4 hr.

TABLE 2 **Effect of Chitosan-Gelatin Ratio on Foam Properties**

Chitosan: Gelatin mixture	Viscosity (cp)	Apparent density (mg/mL)	Foam volume (mL/g)	Foam uniformity	Foam stability
1:10	120	440	2.27	Homogenous, small air bubbles	Stable
1:5	170	500	2	Homogenous, small air bubbles	Stable
1:1	380	610	1.63	Nonhomogenous, large air bubbles	Unstable

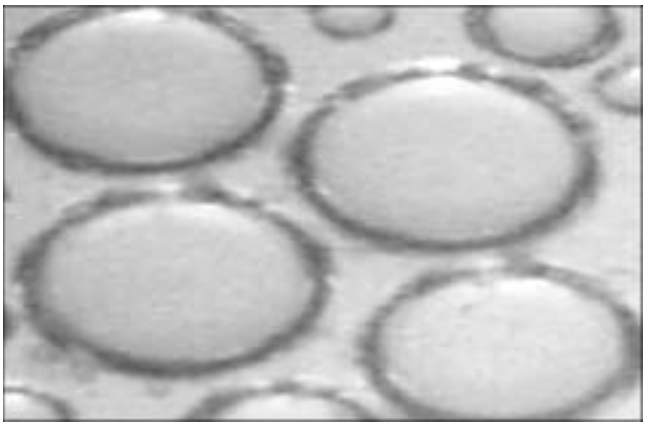

FIGURE 1 A Micrograph, 200×, of 1:5 Chitosan-Gelatin Stable Foam.

TABLE 3 Determination of (a) Drug Content and (b) $t_{50\%}$ in the Prepared Matrices

Matrices	(a) % Drug recovered (Mean ± SD)	(b) $t_{50\%}$ (hr)
Cross-linked gelatin sponge	97.24 ± 0.22	0.55
Un-cross-linked chitosan-gelatin matrix	96.29 ± 0.51	3.07
Cross-linked chitosan-gelatin sponge	98.05 ± 0.28	4.73

Indeed, the addition of chitosan significantly decreased ($p < 0.0001$) water uptake capacity of gelatin sponges ($p < 0.0001$) which was further reduced by cross-linking. The insolubility of chitosan-gelatin matrices was assumed to be caused by the polyionic complexation between the two polymers (Remunan & Bodmeier, 1996). The cross-linking process greatly modified chitosan-gelatin matrices where cross-linked sponge was more resilient to dissolution medium than un-cross-linked ones. They deformed and disintegrated into small fractions after 10 hr. It is remarkable that there was no significant difference between uptake capacity at 4 and 8 hr for un-cross-linked or cross-linked chitosan -gelatin sponges.

Drug Release

Fig. 5 illustrates the in vitro release of tramadol hydrochloride from different sponges. It is clear that cross-linking of gelatin did not succeed in sustained tramadol hydrochloride release where rapid drug liberation was observed ($t_{50\%} = 0.55$ hr). This could be attributed to complete disintegration of the sponges leading to such rapid release behavior. Addition of chitosan succeeded in prolonging the drug release rate even without cross-linking ($t_{50\%} = 3.03$ hr) for un-cross-linked chitosan-gelatin sponges). This supports the efficiency of the formed polyelectrolyte complex between positively charged chitosan and negatively charged gelatin B in sustainment drug release. The effect of cross-linking process on chitosan-gelatin matrix was very evident in slowing drug release rate ($t_{50\%} = 4.73$ hr). This is thought to be attributed to the formation of a higher dense matrix which is characterized by smaller pore sizes structure and reduced permeability to either dissolved drug or dissolution medium the rate limiting step in drug release (Kanke et al., 1989; Akbuga & Durmaz, 1994). A marked significant difference ($p < 0.01$)

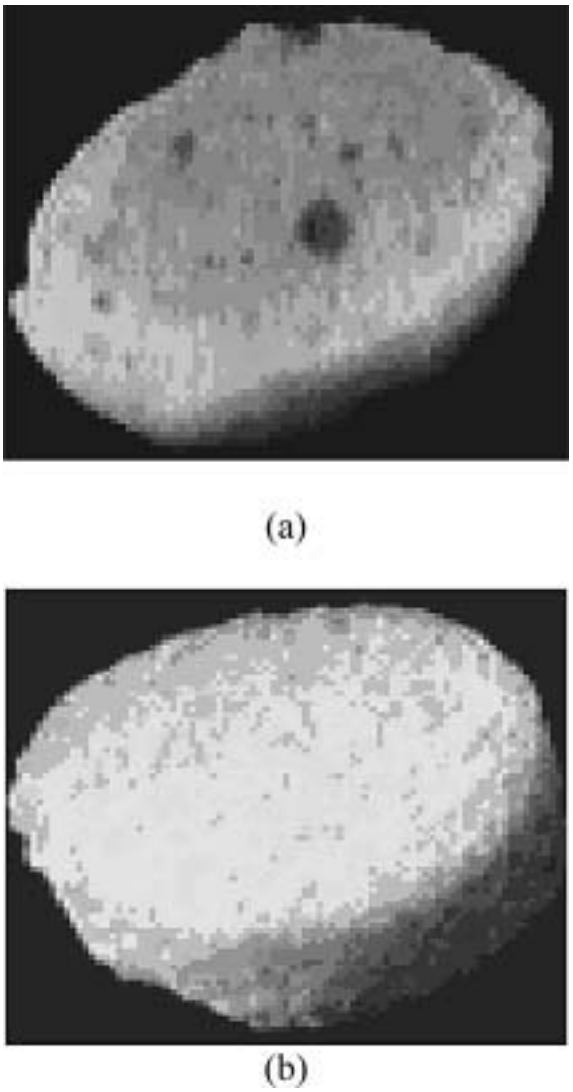

FIGURE 2 Photos, 10×, of (a) Cross-linked Gelatin and (b) Cross-linked Chitosan-Gelatin Sponge.

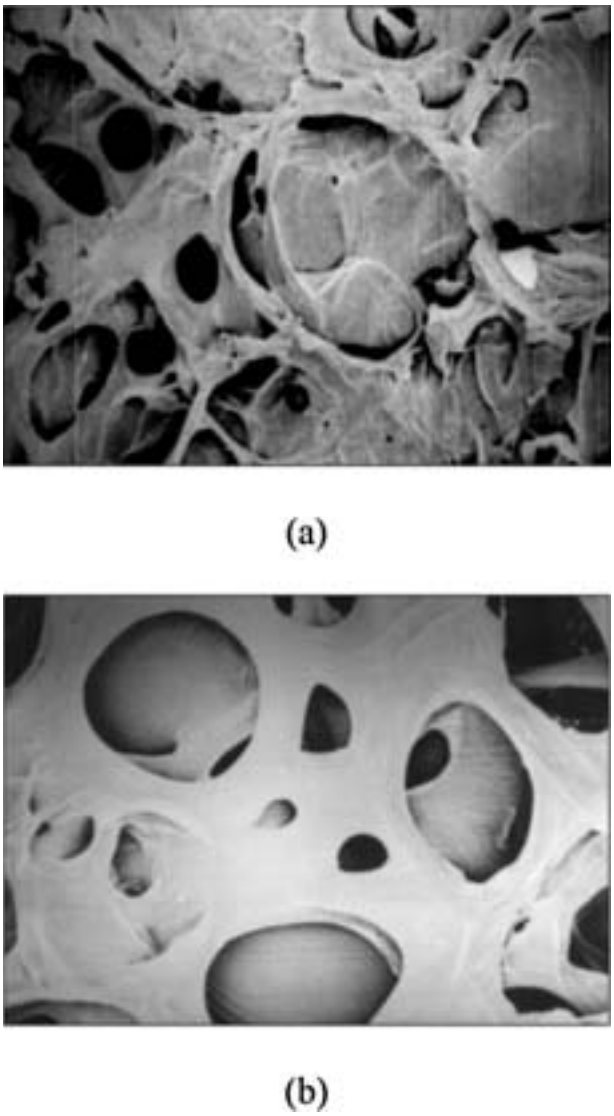

FIGURE 3 **Scanning Electron Micrographs of (a) Cross-linked Gelatin and (b) Cross-linked Chitosan-Gelatin Sponge.**

was observed between the obtained $t_{50\%}$ values of different formulae, suggesting that the complexation between chitosan and gelatin and cross-linking of the resulting complex have pronounced significant effects on drug liberation from sponges.

The kinetic analysis of the drug release data followed diffusion controlled mechanism from all prepared sponges, where a linear relationship exists between the amount of drug liberated and the square root of time (r = 0.994, 0.972 and 0.876 for Formulae A, B, and C, respectively).

FT-IR Spectroscopy

The results of FT-IR spectra are shown in Fig. 6. The IR spectrum of gelatin showed the characteristic amide I, amide II, and OH peaks at 1650, 1557, and 3400 cm^{-1} respectively. In the IR spectrum of tramadol hydrochloride, the characteristics OH shoulders at 3300 cm^{-1}, aromatic CH stretching at 3050 cm^{-1}, aliphatic CH stretching at 2900 cm^{-1}, and aromatic ring stretching at 1600 cm^{-1} can be seen. For chitosan, peaks at 3350, 2900, and 1650–1690 represent OH, aliphatic CH stretching, and C = O shoulders. It is evident that only slight shift in some of the groups characteristic of drug, chitosan, and gelatin took place with overlapping and broadening of similar peaks. No new bands were detected in the spectra of chitosan-gelatin sponge indicating no interaction between drug and chitosan-gelatin matrix. Prolongation of drug release has been reported when a polymer-drug conjugate occurred (Narayani & Reo, 1993; Narayani & Reo, 1996).

Mechanical Properties of the Sponges

The effect of gelatin addition on mechanical properties of the prepared sponges stored under various relative humidities was assessed quantitatively using ASTM test as described above. Storing chitosan-gelatin sponge at 43 and 65% RH were considered to be promising since soft sponges were obtained. They absorbed relatively moderate amounts of moisture (30.4 and 43.5%, respectively) and reached a point of equilibrium at the end of 4 days. Satisfactory compressibility percentage of 97% that indicates pliable and elastic sponge matrix was only achieved at 65% R.H. Adaptation at higher moisture levels rendered the sponges either very soft (at 75% and 80% RH) or gummy and dark in color (at 97% RH) that adhered to the plates and could not be compressed. This was a result of large moisture sorption capacity of the sponges at higher moisture level (up to 55.1%, 123.4%, and 180.0% respectively)

Evaluation of Tramadol HCl Analgesic Activity

As shown in Fig. 7, the maximum analgesic activities of the tramadol HCl solution (Formula D) and the immediate release Tramax® capsules, expressed as latency period in the hot plate test, (E = 29.0 ± 2.4 and 24.5 ± 1.9 s after 2 hr, respectively) were significantly higher ($p < 0.05$) than that of Formula A. This is in agreement with the reported data showing that the mean peak serum concentration of tramadol HCl

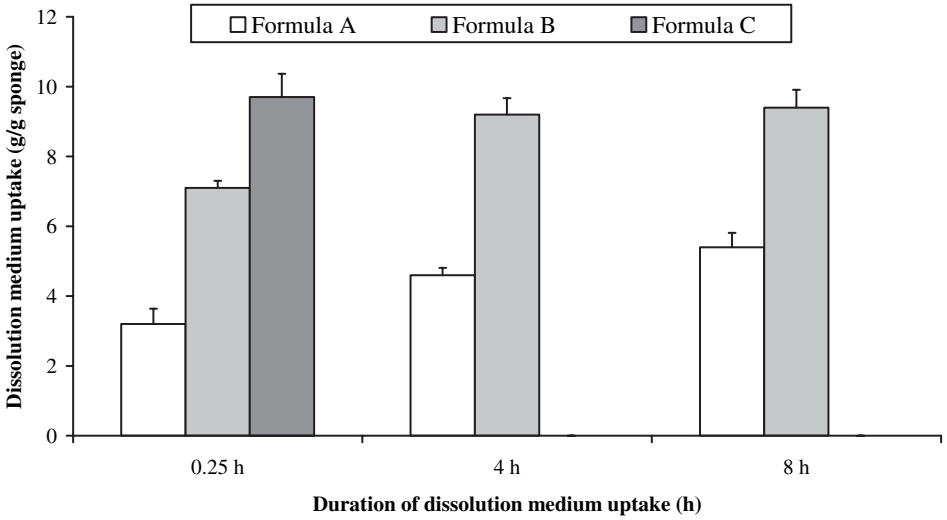

FIGURE 4 **Dissolution Medium Uptake Capacity of Different Sponges (mean ± SD, *n* = 2).**

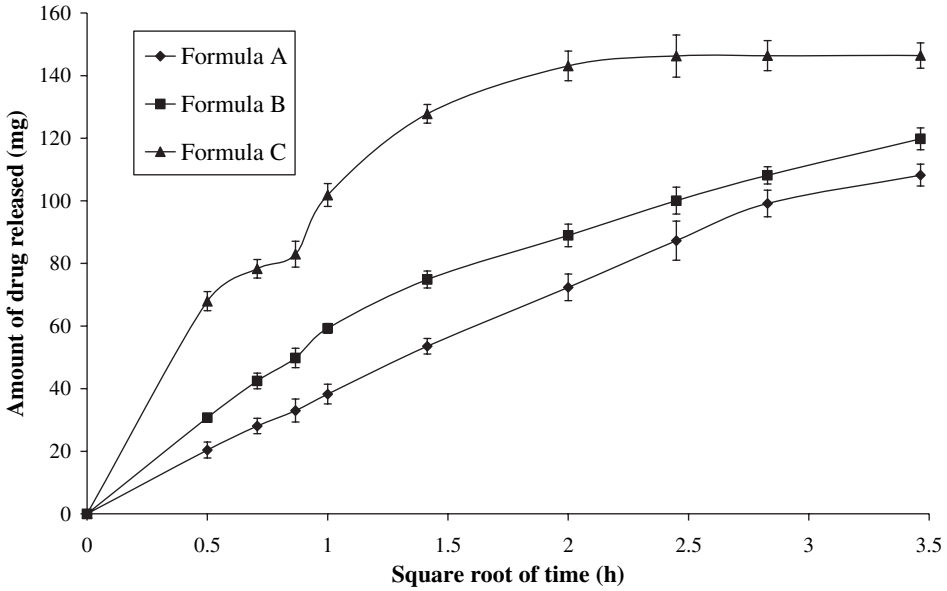

FIGURE 5 **In Vitro Release of The Drug From Different Sponges (mean ± SD, *n* = 3).**

is achieved within 2 hr following administration (O'Neil et al., 1989). The analgesic activity of the former formulae decreased and diminished after 6 hr (E = 5.1 ± 0.8 and 4.2 ± 0.9 s, respectively). On the other hand, the analgesic activity of tramadol HCl chitosan-gelatin sponge (Formula A) was maintained for a longer period as indicated by achieving approximately a constant latency period (E~19–20 s) from the second to the fifth hour. After 12 hr, the analgesic activity of Formula A (E = 6.5 ± 0.9 s.) was still maintained and was found to be significantly higher ($p < 0.01$) than that of both Formula D and Tramax® capsules (E = 2.5 ± 0.4 and 2.0 ± 0.7 s. respectively). Indeed, the analgesic activity of (Formula A) was observed for more than 8 hr. This could indicate that the entrapment of the drug in the sponge matrix significantly controlled the rate of drug release over a considerable period of time, suggesting that the chitosan-gelatin sponge is considered a suitable controlled release drug carrier system that could be effectively implanted.

CONCLUSION

The study has indicated that Gelatin Type B was suitable as a foam builder for the production of chitosan-gelatin sponges to overcome the drawbacks of chitosan sponge mechanical properties and the disadvantage of gelatin sponge in retarding the drug release. The

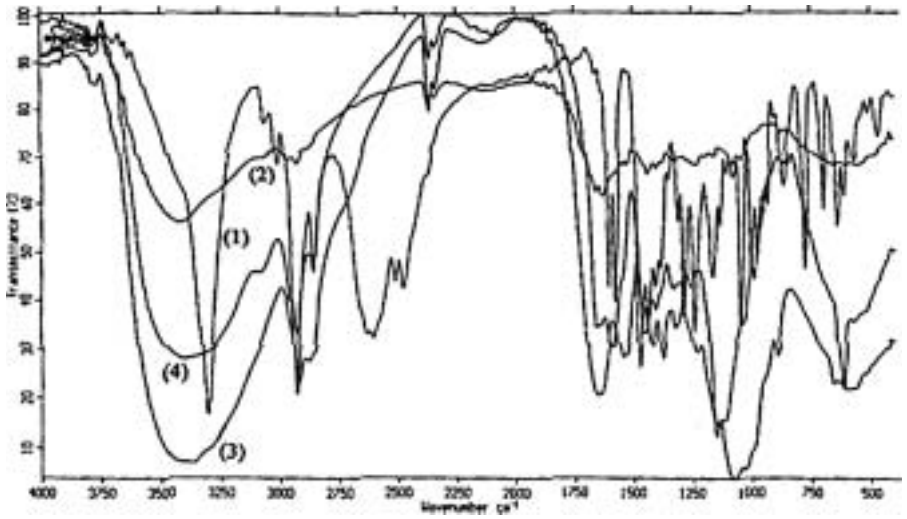

FIGURE 6 **IR spectra of (1) Drug, (2) Gelatin-B, (3) Low MW Chitosan and (4) Cross-linked Chitosan-Gelatin-B Sponges.**

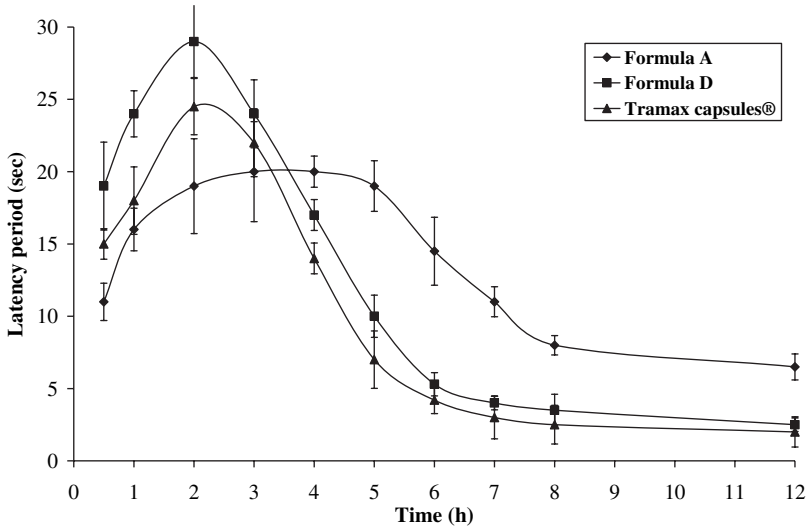

FIGURE 7 **Analgesic Activity of Tramadol HCl From The Selected Formulations Determined by Hot Plate Method (mean ± SD, *n*=6).**

polyionic complexation between chitosan and gelatin was considered as a potential role in prolonging the drug release from mixed sponge system. Clearly, it is possible to manipulate the mechanical, release, and morphological properties of chitosan-gelatin sponges via judicious choice of composition and optimizing the relative humidity under 65% without plasticizer addition. Thus, pliable soft matrix that is inevitably required for implantation was achieved.

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