Research in Pharmaceutical Sciences, April 2007; 2: 43-52
Received: Sep 2006
Accepted: Feb 2007

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Development of cross-linked chitosan films for oral mucosal delivery of lidocaine

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Abstract

Lidocaine (LC) is a local anesthetic agent. The aim of this study was to prolong the anesthetic effect of this drug in the oral cavity in the treatment of oral mucositis. Films of LC were prepared with three different molecular weights (MW) of chitosan in three different concentrations (1-3%) and were then cross-linked by solvent casting evaporation method with tripolyphosphate penta sodium salt (TPP) in two concentrations (0.1 and 0.3%). Cross-linking time was 5 min and the solvent was evaporated by oven at 37 ºC. Bioadhesion, tensile strength (TS), the release and flux of drug through the films were studied. Increasing the concentration of chitosan caused decreasing the bioadhesion while increasing its MW did not change the bioadhesion significantly. The bioadhesion of cross-linked films was comparable to similar films. Cross-linking the films with TPP and increasing the chitosan MW significantly increased their TS compared to films obtained from the gels. Drug release profiles showed that increasing the concentration and MW of chitosan caused an increase in both the rate and extent of drug release. Lidocaine flux was increased by MW and concentration of chitosan but increasing TPP concentration significantly decreased it. Films prepared by 3% of high MW of chitosan and cross-linked by 0.1% of TPP (H3T1) showed a higher flux of drug (212.59 ± 26.31 $\mu g/cm^2/hr$), and relative high bioadhesion and TS. This film is expected to prolong release of LC in buccal area and is suggested for further clinical studies to evaluate its produced onset and duration of anesthesia.

Keywords: Lidocaine; Cross-linked chitosan; Film; TPP; Flux

INTRODUCTION

Patients suffering from cancer may be treated by radiation therapy which causes painful oral mucositis (1). To alleviate these adverse effects or other oromucosal complications like periodontal disease, dental analgesia, bacterial and fungal infections (oral candidiasis), aphthous ulcers and dental stomatitis (2), which may affect the patient's quality of life some local treatments including ointments, gels, buccal tablets and lozenges may be needed. However, they may be swallowed and excluded from the oral cavity by salivary clearance. To prevent the short duration of action of anaesthetic drugs and keeping the dosage form in intimate contact with the oral mucosa and especially for dental analgesia, immediate pain relief is achieved by an initial burst release and then prolonged release is advantageous in decreasing the dosing intervals. Decreasing the thickness of the adhesive tablets and increasing the flexibility of the oral dosage forms causes better comfort ability and patient compliance (3). Hot melt extrusion technology using acrylic and the cellulosic polymers like hydroxypropyl cellulose (HPMC) and hydroxypropyl methyl cellulose have been used to produce thin

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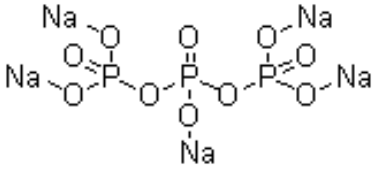
films containing LC (4). The HPC water-soluble polymer mucos-adhesive film containing topical anesthetics and antibiotics proved useful to alleviate pain due to acute radiation-induced oral mucositis, maintain good peroral feeding, and prevent secondary oral infections, without inducing adverse reactions (5). Lamination technique has also been used for production of bioadhesive transdermal/ dermal patch of LC using PVA 72000 water solution and adhesive Plastoid E 35 M® (6).

The penetration rate of LC through excised oral mucosa from hamster cheek pouch and the in vitro release rate of LC from film dosage forms with HPC as a film base containing glycyrrhizic acid (GL) as penetration enhancer was studied by Okamoto et al. (7). The *in vitro* drug release from mixed polymer films of various ratios of HPC and HPMC phthalate using LC, were also investigated (8).

Polyethylene glycol gels were used also for buccal delivery of LC that contained different ratios of Carbopol 934P (CP) and polyvinylpyrrolidone K90 (PVP). A combination of CP and PVP with complementary physical properties resulted in a prolonged buccal drug delivery (9). Oral Powders have been used to safely deliver a dose of dry powdered LC hydrochloride to the oral mucosa, producing an analgesic effect without causing tissue damage (10). A novel saliva-activated bioadhesive drug delivery system of LC hydrochloride revealed the adherence of the system to the gingival within a minute and produced a peak effect in 15 minutes. The system caused greater depth of anesthesia than the marketed topical gel with an efficacy of 81.63% (11). Another transoral delivery system of LC is a mucoadhesive DentiPatch (Noven Pharmaceuticals) containing 20% LC that produces a very low plasma concentrations of LC (12).

Chitosan is a biocompatible, biodegradable and bioactive polymer obtained from the partially alkaline deacetylation of chitin which is a glucose-based unbranched polysaccharide widely distributed in nature. Chitin is as the principal component of exoskeletons of crustaceans and insects as well as of cell walls of some bacteria and fungi. Chitosan exhibits a variety of physicochemical and biological properties resulting in numerous applications as a biomaterial in pharmaceutical and medical fields (13). tripolyphosphate penta sodium salt with molecular weight 367.86 is the most popular multivalent polyanion which can form gel with chitosan by ionotropic crosslinking interactions. Because of its non-toxic property and quick gelling ability, physical crosslinking by electrostatic interaction with TPP, instead of chemical crosslinking, can avoid possible toxicity of reagents and other undesirable side effects (14).

The purpose of this study was presenting a new dosage form with a prolonged effect for oral-mucosal delivery of LC. As mentioned above up to now many types of film forming agents have been used to prolong LC remaining time in the oral cavity but to our knowledge there is no report on the use of this drug in ionic cross-linked chitosan films. Sufficient charge numbers (or density) are necessary for anions to cross-link chitosan by electrostatic force. TPP is a multivalent anion that carries a maximum of five negative charges. On the other hand, chitosan is a weak polybase with a maximum of thousands of positive charges (Fig. 1).



TPP

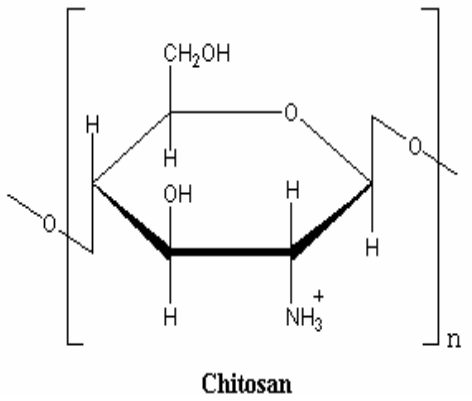


Fig. 1. The structure of TPP and chitosan.

MATERIALS AND METHODS

Materials

Lidocaine hydrochloride was a gift from Daroupakhsh Pharmaceutical Company (Iran), Chitosan with different molecular weights (low MW 150000, intermediate MW 400000, and high MW 600000, Fluka, Switzerland), sodium hydroxide, potassium dihydrogen phosphate, lactic acid, glycerine and tripolyphosphate penta sodium salt (TPP) were all purchased from Merck Chemical Company (Germany) and were used as received.

Preparation of LC films

Chitosan films were prepared by a casting/solvent evaporation technique. Chitosan solutions (1-3 w/v%) containing LC (4% w/v%) were prepared by dissolving chitosan and LC in dilute lactic acid solution (2%). Lactic acid was chosen for dissolving chitosan, as chitosan lactate can cause greater swelling and mucoadhesion compared to chitosan acetate and moreover, drug release is slower in chitosan lactate compared to its acetate salt (15). Then 0.5 g of the above solution with 10% glycerin as plasticizer (16) was poured on a glass plate (casting area 1 × 1 cm^2). The solution gels were dried for 48 h in an oven at 37 ºC, then further dried under vacuum at room temperature to constant weight. These uncross-linked films were used as control films for comparing with the cross-linked forms. The obtained dried uncross-linked films were soaked in 2 ml of aqueous solution of TPP (0.1 and 0.3%) at 4 ºC. The cross-linking time was 5 min and then the cross-linked chitosan films formed were washed with distilled water, put on a glass plate and oven-dried at 37 ºC for 48 h and then dried under vacuum at room temperature to constant weight (16). Chitosan films with a thickness of 400 μm were prepared. Twenty four formulations of uncross-linked and cross-linked films were prepared by a full factorial design using the MW of chitosan, TPP and chitosan concentration as the studied variables. The formulations of films are shown in Table 1.

Drug content measurements in films

One g of cross-linked films was soaked for 24 h in 100 ml of water and analyzed for drug content spectrophotometrically at 263.3 nm. The method of analysis was validated by measuring inter-day and intra-day variations of standard solutions of LC in water of concentrations ranging from 2.5-40 μg/ml in 3 different days and 3 times per day. The concentrations of solutions were determined from the standard curve. The CV% or Coefficient of Variation (identifying sensitivity) and percent error (identifying accuracy) were calculated as:

$$CV\% = \frac{\text{standard deviation}}{mean} \times 100$$

$$\text{Error}\% = \frac{\text{calculated concentration - actual concentration}}{\text{actual concentration}} \times 100$$

To check the specificity of the method of analysis blank cross-linked films were analysed by the same method at 263.3 nm in which no absorption was observed.

Table 1. Formulation of studied cross-linked chitosan films containing 4% lidocaine and 10% glycerin.

Formulation code	Chitosan molecular weight (Dalton)	Chitosan (%)	Tripolyphosphate penta sodium (TPP) (%)
L1T1	Low MW (150000)	1	0.1
L1T3	Low MW (150000)	1	0.3
L2T1	Low MW (150000)	2	0.1
L2T3	Low MW (150000)	2	0.3
L3T1	Low MW (150000)	3	0.1
L3T3	Low MW (150000)	3	0.3
M1T1	Medium MW (400000)	1	0.1
M1T3	Medium MW (400000)	1	0.3
M2T1	Medium MW (400000)	2	0.1
M2T3	Medium MW (400000)	2	0.3
M3T1	Medium MW (400000)	3	0.1
M3T3	Medium MW (400000)	3	0.3
H1T1	High MW (600000)	1	0.1
H1T3	High MW (600000)	1	0.3
H2T1	High MW (600000)	2	0.1
H2T3	High MW (600000)	2	0.3
H3T1	High MW (600000)	3	0.1
H3T3	High MW (600000)	3	0.3
L1	Low MW (150000)	1	-
L2	Low MW (150000)	2	-
L3	Low MW (150000)	3	-
M1	Medium MW (400000)	1	-
M2	Medium MW (400000)	2	-
M3	Medium MW (400000)	3	-
H1	High MW (600000)	1	-
H2	High MW (600000)	2	-
H3	High MW (600000)	3	-

Bioadhesion and tensile strength measurements

A TS tester device (Instron, A301, England) was used for measuring both bioadhesion and TS of the films. Bioadhesion was examined *in vitro* using freshly obtained bovine buccal mucosa without any further treatment. The bioadhesion test was carried out both on the gels or uncross-linked films and cross-linked films to see the effect of the presence of TPP on the bioadhesion of the films. Chitosan films (2.5 × 2.5 cm) were attached by cyanoacrylate adhesive on a glass disk and were fixed to the support of the TS tester using a double side adhesive. The bovine buccal mucosa was placed under the support of the instrument. The film was brought into contact with the mucosa under a very slight pressure (2 g.f) and was kept in this position for 1 min. Then the bioadhesion test was performed at a constant extension rate of 20 mm/min. The maximum force of detachment was measured as the bioadhesion force of the films. The TS test of the cross-linked films or dried uncross-linked ones were also measured as the force needed to tear the films by the same device by clamping a 3 × 2 cm part of the films between the clamps of the instrument. All bioadhesion and TS tests were performed in triplicate and the mean of the results was calculated.

In vitro release of LC

The release of LC from different chitosan films was determined by using 3.5 × 3.5 cm of films with 4.91 cm^2 diffusion area directly on a Franz (vertical) diffusion cell without using any cellulose acetate membrane. The receptor compartment was filled with 27 ml of phosphate buffered

solution (PBS, pH 7.4), which was maintained at 37 ± 0.5 ^{o}C and stirred by magnetic bar at 200 ± 5 rpm. The available diffusion area of cell was 4.91 cm^2. At predetermined time intervals until 8 h, 1 ml sample was taken from the receptor cell and immediately replaced by an equal volume of fresh receptor solution. The samples were filtered, diluted to 20 ml and assayed for LC at 263.3 nm using a UV spectrophotometer (UV mini 1240, Shimadzu, Japan) (16). To check the specificity of the analytical method in all cases the blank films (without LC) were tested by the same release method as for the loaded films and the absorbance of the samples was measured at the same wavelength.

Measuring the LC flux through the films

The *in vitro* flux was determined from the Fick's law of diffusion written as: $J_S = \frac{1}{S}\frac{dM}{dt}$ in which dM/dt is the amount of drug permeated per unit time (μg.h $^{-1}$), and S is the surface area (cm^2) of diffusion. Permeation curves were constructed by plotting the cumulative amount of drug permeated through unit area of the membrane versus time. The steady state flux was determined by regression analysis of the linear portion of the plot (17).

Statistical analysis

Statistical comparisons of the findings were made by one-way analysis of variance (ANOVA) followed by the post hoc test of Tukey. The confidence level was set at 95% (P<0.05*)*.

RESULTS

Table 2 shows drug content of the films. As this table shows all films contain about 3% drug content considering the dry weight of the films. The recovery percentage in all cases was between 88-92% of the total used drug.

Table 2. Lidocaine content and flux in studied cross-linked chitosan films (n = 3).

Formulation code	Drug content (%) ± SD	Drug recovery (%) ± SD	Flux (μg/cm^2/hr) ± SD
L1T1	3.65 ± 0.26	91.35 ± 6.57	135.30 ± 10.22
L1T3	3.60 ± 0.11	90.07 ± 2.87	109.94 ± 8.14
L2T1	3.79 ± 0.14	91.27 ± 4.14	137.68 ± 11.35
L2T3	3.63 ± 0.26	90.87 ± 7.05	109.50 ± 9.50
L3T1	3.66 ± 0.10	91.49 ± 0.56	160.36 ± 15.87
L3T3	3.53 ± 0.17	88.33 ± 4.02	109.86 ± 10.42
M1T1	3.60 ± 0.25	90.03 ± 5.39	166.74 ± 21.39
M1T3	3.59 ± 0.14	89.75 ± 0.57	109.66 ± 14.65
M2T1	3.67 ± 0.07	91.77 ± 1.87	171.15 ± 19.74
M2T3	3.57 ± 0.16	89.23 ± 4.12	113.52 ± 17.38
M3T1	3.79 ± 0.18	91.23 ± 4.40	195.37 ± 23.19
M3T3	3.56 ± 0.11	89.00 ± 2.78	111.99 ± 16.68
H1T1	3.63 ± 0.06	90.76 ± 1.50	174.34 ± 22.74
H1T3	3.70 ± 0.11	89.13 ± 2.87	124.83 ± 18.43
H2T1	3.67 ± 0.15	91.77 ± 3.76	183.53 ± 21.11
H2T3	3.57 ± 0.13	89.26 ± 4.38	112.16 ± 8.89
H3T1	3.58 ± 0.08	89.49 ± 2.06	212.59 ± 26.31
H3T3	3.52 ± 0.14	88.06 ± 2.53	117.50 ± 8.77

The results of bioadhesion and TS measurements of the films are shown in Fig. 2 and 3, respectively. As Fig. 2 shows the bioadhesion of the gels or uncross-linked films was the same as the cross-linked films.

Addition of cross-linking agents to the films of chitosan resulted in films with TS values that were higher than the control.

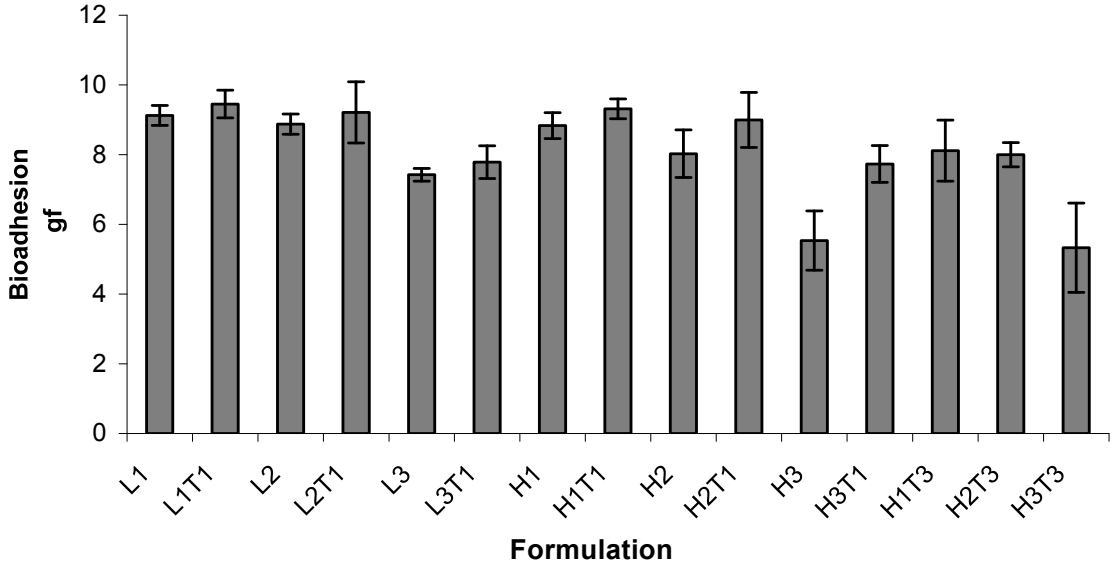


Fig. 2. Detachment force (g.f) of different TPP cross-linked films of chitosan compared with uncross-linked ones by a modified tensile strength method (n = 3).

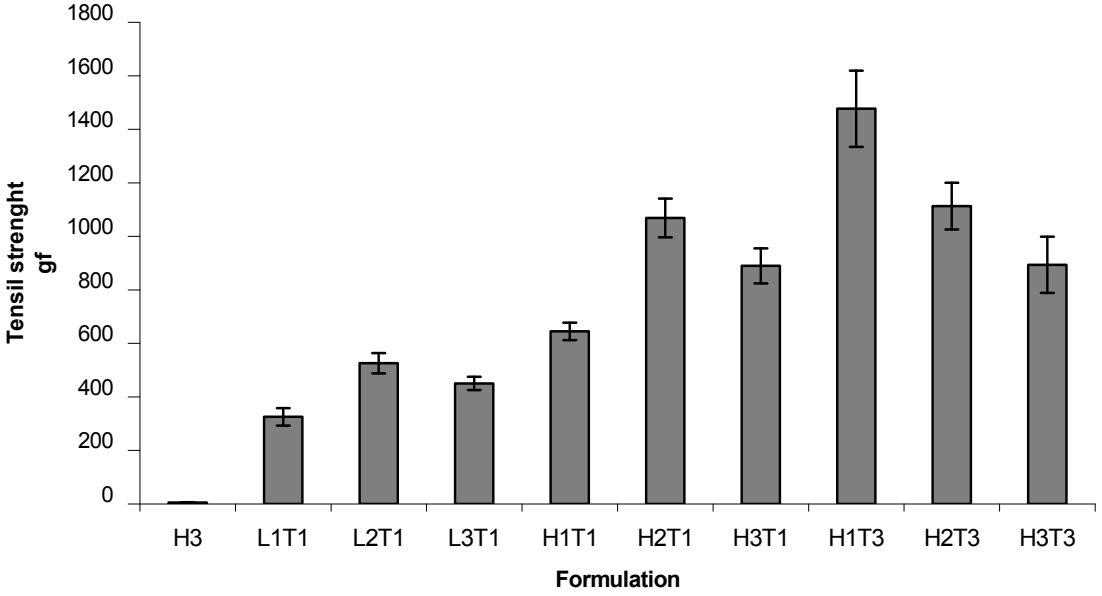


Fig. 3. Tensile strength according to gram force (g.f) of TPP cross-linked films of chitosan compared with uncross-linked ones (n = 3).

The results of drug release from cross-linked films are shown in Fig. 4 and those from uncross-linked films are shown in Fig. 5. As these figures show increasing the chitosan concentration from 1 to 3% and its MW when 0.1% of TPP was used, resulted in an increase in LC release (Fig. 4a). However, comparing Fig. 4a (in which 0.1% TPP is used) with 4b (in which 0.3 % TPP is used) indicated that a decrease in drug release happend by increasing TPP concentration. When TPP was used in 0.3% concentration, no significant difference could be seen in different concentrations of chitosan or different MW of it (Fig. 4b).

Comparing the release of LC from cross-linked films and uncross-linked forms (Fig. 5) shows more rapid drug release from uncross-linked films.

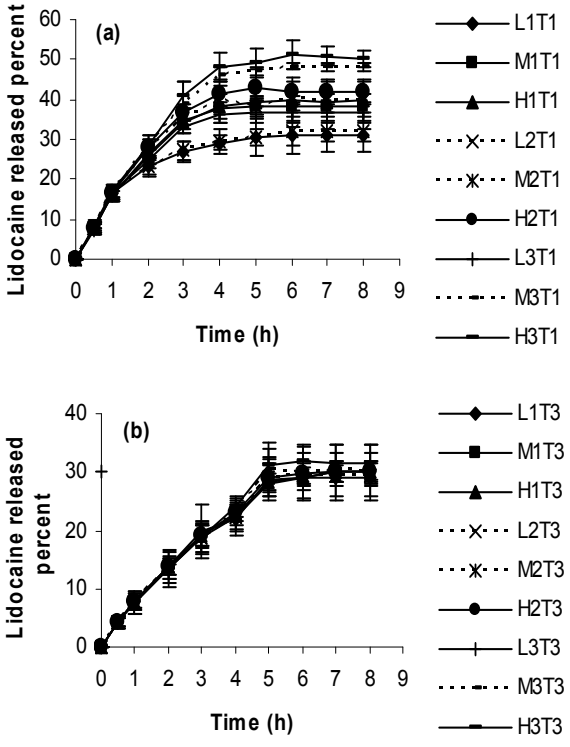


Fig. 4. Lidocaine release profiles from of cross-linked chitosan films containing different concentrations of chitosan with different molecular weights and a) 0.1% TPP or b) 0.3% TPP (n = 3).

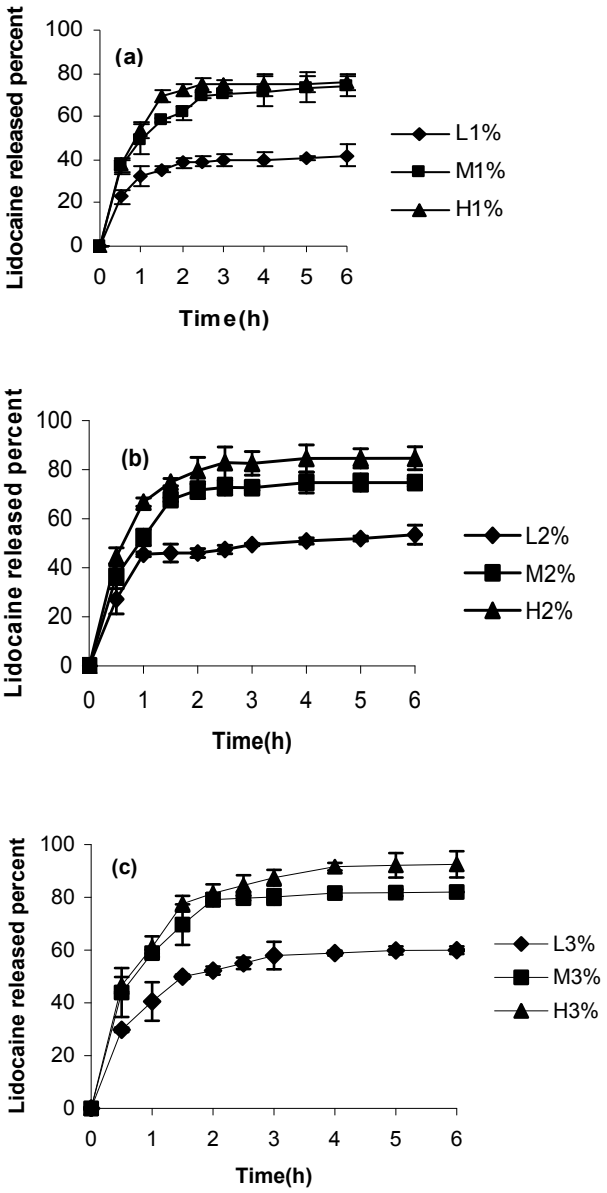


Fig. 5. Lidocaine release profiles from different uncross-linked chitosan films containing a) 1%, b) 2% and c) 3% chitosan with different molecular weights (n = 3).

DISCUSSION

In this study film vehicles were prepared using chitosan to deliver LC into the oral cavity. Due to its bioadhesive property, chitosan film is expected to remain in the oral cavity and release the

drug for a long period of time, thus enhancing the clinical effect.

As Table 2 shows all films show about 3% w/w drug content according to the dry weight of the polymer which is about 88-92% recovery of the total drug used. This mass loss of drug may be because of the high solubility of LC in aqueous medium that cause somewhat drug drainage during cross-linking in the aqueous solution of TPP. Fig. 2 shows the bioadhesion of the uncross-linked films was the same as the cross-linked ones. This showed that the presence of TPP had no effect on the bioadhesion results. Increasing the chitosan concentration (from 1% to 3%) significantly decreases the bioadhesion of the films (compare L1T1 and L3T1 or H1T3 and H3T3) while increasing the MW of chitosan does not change the bioadhesion significantly (P>0.05) (Fig. 2). It may be concluded that there is a ceiling effect or optimum concentration for the polymer above which the bioadhesion decreases. This is because of the reduction in the solvent and increased coiling of the polymer chain (19). However, cross-linking the chitosan films does not change their bioadhesion significantly (P>0.05) in comparison with uncross-linked films. This may be attributed to the decreased positive charge of chitosan after cross-linking that is compensated by contribution of the free oxygen groups of TPP in electrostatic interaction with tissues. This causes almost equal bioadhesive forces in the cross-linked and uncross-linked films. However, an exception is seen in H3 and H3T1 that bioadhesion has increased, but if high values of standard deviations are considered, the difference would be statistically insignificant (P>0.05). Like other types of chitosan, TPP at the concentration of 0.3%, as in H3T3, compared to H3 caused no significant change in bioadhesion. Yet, these results must be undertaken with caution, as the samples prepared for bioadhesion studies may not reflect the actual situation (20). TS of cross-linked chitosan films are shown in Fig. 3. Addition of cross-linking agents to the films of chitosan resulted in films with TS values that were higher than the control (H3 films prepared from H3 gels). The formation of more resistant films suggests the occurrence of ionic bonds between chitosan via chemical reaction with TPP. Increase in TS has been reported for glutenin-rich films (21) and zein films cross-linked by glutaraldehyde and formaldehyde (22). As Fig. 3 indicates increasing the chitosan MW and TPP concentration, caused increasing the values of TS or mechanical properties of the films treated with the cross-linking agent, i.e., TPP. In other words, the TS of chitosan membranes were significantly improved by introducing TPP as the cross-linking agent. Incorporation of TPP into chitosan films (H3) resulted in an approximately 160-fold increase in TS values (in H3T1). At concentration values higher than 2% chitosan a decrease in TS values was observed (like L2T1 and L3T1 or H2T1 and H3T1), which indicates that 2% could be an excess of concentration. This is also the case of films prepared by 3% high MW chitosan in which increasing the concentration of chitosan decreases the TS of the films (Compare H1T3, H2T3 and H3T3). Comparison of TS of films H3T1 and H3T3 doesn't show significant difference (P>0.05). This means the saturation of ionic bonds at 0.1% TPP, i.e. at higher concentrations of TPP (more than 0.1%) there is not any other amino groups presented in the chitosan backbone to be cross-linked with TPP. Therefore, the TS is not increased anymore and its higher concentrations can not increase the TS significantly. The higher TS values for films treated with TPP can be due to the chemical ionic bonds with respect to the different amino groups of chitosan and oxygen groups of phosphate in TPP.

The results of measuring the absorbance of the blank films under the release test showed no absorbance which confirms the specificity of the analytical method. Analysis of release data for different formulations is shown in Table 2. As this table shows LC flux increases as the concentration and MW of chitosan increased. Senel et al. (16) also reported an increase in chlorhexidine gluconate release rate by increasing the chitosan concentration. However, at specific concentrations of chitosan and TPP, increasing the MW enhances the drug flux (Table 2).

The results of drug release from cross-linked films are shown in Fig. 4 and those from uncross-linked films are shown in Fig. 5. As these figures show increasing the chitosan concentration from 1 to 3% in cross-linked films with 0.1% TPP resulted in an increase in LC release (Fig. 4a). This was seen in all MW of chitosan. On the other hand, increasing the MW of chitosan (in the case of cross-linked films with 0.1% TPP) also accelerated the drug release (Fig. 4a, 5). Increasing the TPP concentration decreases both the percentage and the rate of drug release from the films as the time required to reach the platue of the profile takes longer time (Fig. 4a compared to 4b). In all concentrations of chitosan and in the presence of 0.1% TPP, the low MW of chitosan showed significant lower release than the medium or high MW of chitosn (Fig. 4a). However, there is not any significant difference between the different MWs when 0.3% TPP is used (Fig. 4b).

Comparing the release of LC from cross-linked films and uncross-linked forms (Fig. 5) shows more rapid drug release from uncross-linked films. Considering Mucoadhesive patches releasing topical drugs in the oral cavity at a slow, predetermined rate may present distinct advantages over traditional dosage forms such as mouthwashes, oral gels and lozenges (23), these data suggest the possibility of achieving controlled drug release by the use of chitosan matrices. Rege et al. (24) used chitinosans as tableting excipients for controlling the release of salicylic acid and found at acidic pH chitinosans had protonated amines which could interact with oppositely charged drug ions and thereby modify drug release. Ramanathan and Block (25) used iontophoresis for transdermal delivery of different drugs using chitosan gel. They observed the mutual repulsion between LC hydrochloride (as the cationic drug) and the positively charged chitosan matrix caused the chitosan macromolecule to stretch and this could lead to a decrease in the total extent of electro-osmosis of LC towards the anode. These findings explain the faster release of LC from higher concentrations and MWs of chitosan compared to lower concentrations and MWs (Fig. 4a).

CONCLUSION

Cross-linking the chitosan films increases their TS while in most cases bioadhesion does not change significantly by cross-linking. High concentrations and MW of chitosan significantly increases the flux of LC through the films. Increasing the TPP concentration significantly decreases the drug release rate and flux. The film prepared from 3% high MW chitosan and cross-linked by 0.1% of TPP (H3T1) showed a high flux of drug (212.59 ± 26.31 $\mu g/cm^2/hr$), and relative high bioadhesion and TS. This film may be suggested as a suitable prolonged release dosage form of LC in buccal area. However, further clinical studies are necessary to evaluate the onset and duration of anesthesia produced by this film.

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