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Chitosan Formulations for Steroid Delivery: Effect of Formulation Variables on In Vitro Characteristics

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ABSTRACT Chitosan film formulations for steroid delivery after craniomaxillofacial surgery were formulated by using three different types of chitosan with respect to their molecular weight as low, medium and high. Film formulations were prepared by casting/solvent evaporation technique. In vitro characterization, film thickness, equilibrium swelling degree, in vitro release profiles and surface morphologies were investigated. For two different types of crosslinkings, the release of dexamethasone sodium phosphate (DSP) can be extended as the molecular weight increases. As a result, chitosan film formulations should be beneficial for steroid delivery for a certain time after craniomaxillofacial surgery.

KEYWORDS Chitosan, Film, Molecular weight, Crosslinking, In vitro release

INTRODUCTION

Chitosan is a natural polysaccharide that is obtained from the outer shell of crabs and shrimps as a result of the N-deacetylation process of the chitin, a natural component of the outer shell (Pangburn et al., 1982; Hirano et al., 1989). The biocompatibility and biodegradability of chitosan, makes it one of the most advantageous alternative for drug delivery. In numerous studies, different types of drug delivery systems have been investigated for the controlled release of the active ingredients at the site of action (Lim et al., 1997; Denkbas & Odabasi, 2000; Senel et al., 2000; Shu & Zhu, 2000; Arica et al., 2002; Martins et al., 2004; Freitas et al., 2005).

Chitosan film formulations can be prepared by using its solubility in diluted acidic solutions. The release of the drug substance can be modified by changing the formulation parameters as molecular weight of the polymer, type of the crosslinking agent and duration of the crosslinking. Film formulations are especially suitable for application because of their elastic property. They can be easily shaped for the anatomic structure of the human body. This provides a complete duration of the drug substance at the site of action.

In this study, chitosan film formulations for the steroid delivery after cranio-maxillofacial surgery have been investigated. Edema is one of the major

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problems that patients meet after surgery. The degree of edema determines the relief conditions of the patients in the postoperative phase. There is still no such regular treatment for the edema since it is a varying complication with respect to each patient. Griffes et al (1989) have investigated the effect of single dose dexamethasone, which is the commonly used steroid for edema, administered preoperatively. Only in the first day after the operation, systemic dexamethasone was reported as beneficial in decreasing the edema. The duration of the drug substance can be increased by using chitosan film formulations that will also provide the bypass of the many side effects of the systemic steroid administration.

MATERIALS AND METHODS

Materials

Three different types of chitosans with respect to their molecular weights have been used as low, medium and high molecular weight chitosans purchased from Aldrich Chemicals Co. Inc, (Milwaukee, WI, USA). The drug substance dexamethasone sodium phosphate (DSP) was a generous gift from Deva Holding A.Ş., Turkey. For the crosslinking process, tripolyphosphate (TPP) and sodium hydroxide (NaOH) solution (25% w/v) was purchased from Sigma (Steinheim, Germany). All other chemicals were chemical grade and used without further purification.

Chitosan Film Preparation

Chitosan film formulations were prepared by casting/solvent evaporation technique, i.e. dissolving the polymer in 1.5% (v/v) solution of acetic acid in distilled water. The concentration of steroid was determined as the 20% of the polymer used in the formulation. The steroid, DSP, was completely dissolved in 1.5% (v/v) solution of acetic acid and after necessary amount of chitosan was added into the solution and mixed for nearly 1 hr on a magnetic stirrer until a clear viscous gel was formed free from bubbles. The resulting chitosan gel was poured in a Petri dish and left to dry at room temperature until a constant weight was obtained. The film formulations were crosslinked by two different methods described by Chandy & Sharma (1991) and Dureja et al. (2001) by using NaOH and TPP respectively. After the film formulations reach a constant weight, they were soaked with the crosslinking solutions for 30 min. The film formulations were washed with distilled water to remove the excess of the crosslinking solution from the surface.

In order to evaluate the effects of a hydrophilic excipient of release of DSP from film formulations, glycerol was incorporated into the film formulation at a concentration of 10% with respect to the total volume of the gel as described by Brown et al. (2001). For the evaluation of effect of chitosan molecular weight on film properties and in vitro release, six different formulations were prepared. The formulation codes of the chitosan films are briefly shown in Table 1.

Film Thickness

The thickness of the film formulations were measured by using a Somet-Inox micrometer. The measurements were performed with six different points in order to evaluate the statistical difference, if any. The homogeneity of the film formulations in thickness is evaluated by these measurements.

Equilibrium Swelling Studies

The water absorption capacities of the chitosan film formulations were determined by weighing the film pieces (1 x 1 cm^2) before and after placing in pH 7.4 phosphate buffer solution. Initially, dry film pieces were accurately weighed and placed in buffer solution. At certain time intervals (5, 10, 20, 40, 60 and 90 minutes) film pieces were removed from the medium and weighed again after removal of the excess water. The water absorption capacities (WAC) were determined by using Eq. (1) where w_1 is the maximum swollen weight and w_2 is the dry weight of the chitosan film piece. The equilibrium

TABLE 1 Code of film formulations

Formulation code	Type of chitosan molecular weight	Crosslinking
A	Low	
B	Medium	
C	High	NaTPP
D	High (without glycerol)	
E	Low	
F	Medium	
G	High	NaOH
H	High (without glycerol)	

swelling degrees for all formulations were determined by three replicates and the results were expressed as the mean ± standard deviation by using Eq. (1).

$$\mathrm{WAC} = [(w_1 - w_2)/w_2] \times 100 \quad (1)$$

In Vitro Release Studies

In vitro release of DSP from chitosan film formulations were evaluated in a horizontal laboratory shaker with three replicates. Chitosan film pieces (1 x 1 cm^2) were accurately weighed after they were placed briefly in flasks containing 25 mL phosphate buffer solution (pH 7.4). These flasks were immersed in a constant temperature water bath at 37 ± 0.5°C shaking the flasks at 50 rpm. At various time intervals, 2 mL samples were withdrawn and immediately replaced with the equal volume of the fresh medium at the same temperature. The drug content was then calculated after determining the UV absorbencies of the samples at 242 nm.

Surface Morphology

The morphological properties of the chitosan film formulations were investigated by using Jeol SEM ASID-10 device at 80 KV. Chitosan film pieces were mounted on metal stubs with a conductive silver paint and then sputtered with a 150 Å thick layer of gold in a Biorad apparatus.

RESULTS

Film Thickness

After measurements at six different points, the results were expressed in terms of mean ± standard deviation. The thickness of film formulations were 350 ± 23.21 μm, 382 ± 41.18 μm and 377 ± 37.36 μm respectively for low, medium and high molecular weight chitosan film formulations. There was no significant difference found in thickness between formulations with respect to their molecular weights analyzed by Mann Whitney U-test. ($p < 0.05$)

Equilibrium Swelling Studies

The chitosan film formulations prepared by using medium molecular weight polymer and neutralized by 0.2*M* NaOH solution absorbed water up to 209.75 ± 2.23% of its initial weight. On the other hand, film formulations of medium molecular weight chitosan crosslinked with 5% NaTPP solution absorbed only 132.01 ± 1.19% of its initial weight. The t_{max} for water absorption rates observed was 4 hr and 0.5 hr for NaOH and TPP, respectively. The total profile for water absorption rates was similar for both types of crosslinkings. It was maximum for medium molecular weight and minimum for high molecular weight polymer prepared without glycerol, respectively (Figs. 1a and 1b).

In Vitro Release Studies

In vitro release profiles are used to evaluate the effect of molecular weight, crosslinking process and mechanism of crosslinking on release of DSP from film formulations. In the in vitro release experiments no film floating or folding, that would possibly affect the release rate of DSP, was observed. Molecular weight of the polymer clearly affects the release characteristics of DSP for both types of crosslinking (Figs. 2a and 2b). As the molecular weight increases, the amount of DSP released decreases depending upon the entrapment of the active ingredient in network like structure of the polymer chains. High capacity for retaining water could be advantageous in relation to the development of slow release formulations because it might facilitate the formation of gels that would control drug release. The formulations prepared by using TPP as the crosslinking agent provide a higher release than those of the formulations of NaOH neutralized, which are nearly twice for all molecular weight types. Another interesting result was that the film formulations prepared without using glycerol as the plasticizer, significantly lowered the amount of DSP released depending on the decreased hydrophilicity of the formulations for both two crosslinking types. All of the release experiments were continued until an asymptote was observed between the last two experimental points.

Surface Morphology

The scanning electron microscopy photographs clearly show that no crystal like structures exist on the surface of the chitosan film formulations. The surfaces of all formulations are regular and uniform in structure (Fig. 3).

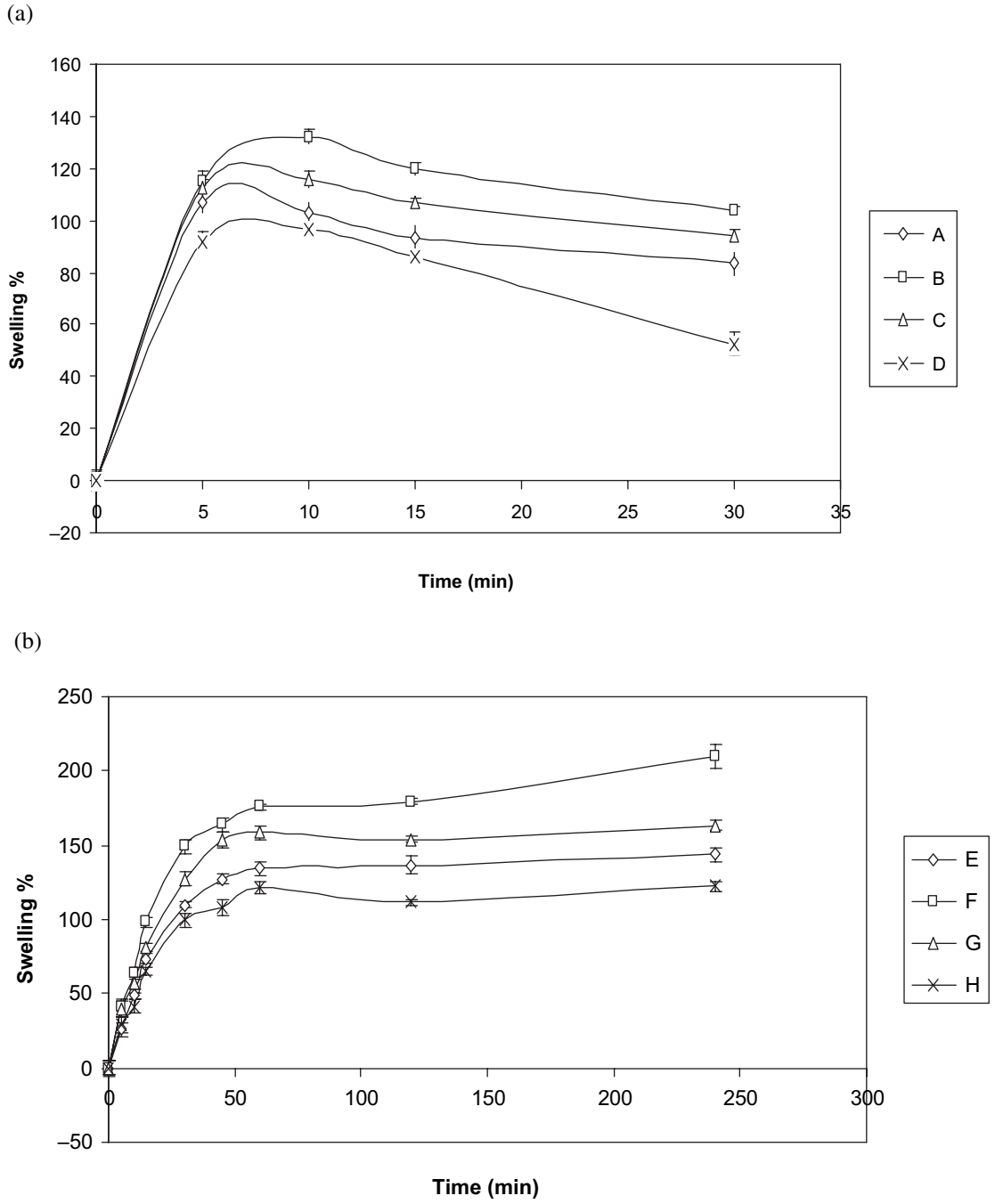
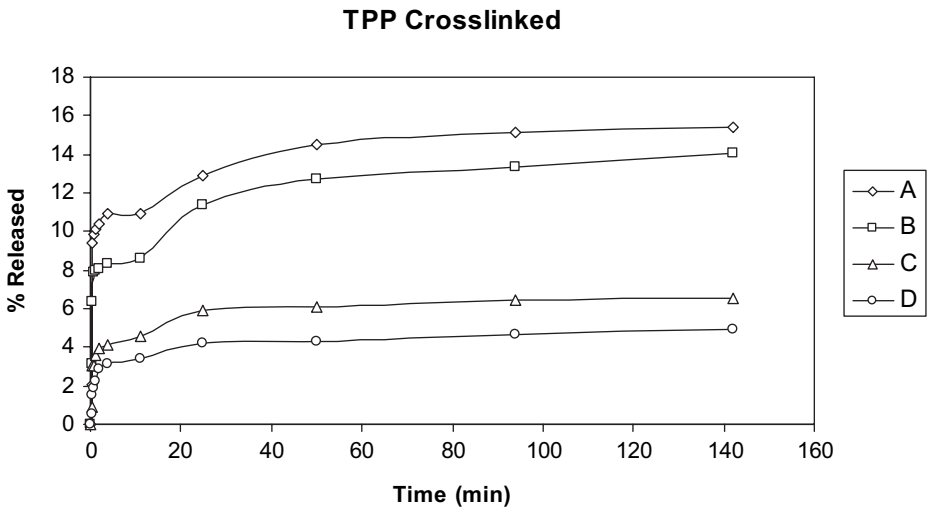


FIGURE 1 **The Equilibrium Swelling Profiles of the Film Formulations: (a) NaTPP Crosslinked, (b) NaOH Neutralized. A to H denotes formulation codes given in Table 1.**

DISCUSSION

The preparation by solvent casting method is a very easy and simple way to obtain homogenous chitosan films without any precipitation from the active ingredient DSP. The main effect of incorporating plasticizer in film formulations is to provide a suitable shape for the focused region of application on the body. In this study, the possible change in drug release depending on the use of plasticizer was investigated and from the in vitro release data, it was found that for both crosslinking types, a significant decrease in released amount of DSP occurs with the removal of glycerol from the formulations. The main mechanism of the plasticizer in the formulation is to reduce the attractive force between the polymer chains. Because of this reduction, the penetration of release medium into the gel would be facilitated. In addition, reduction in attractive

(a)



(b)

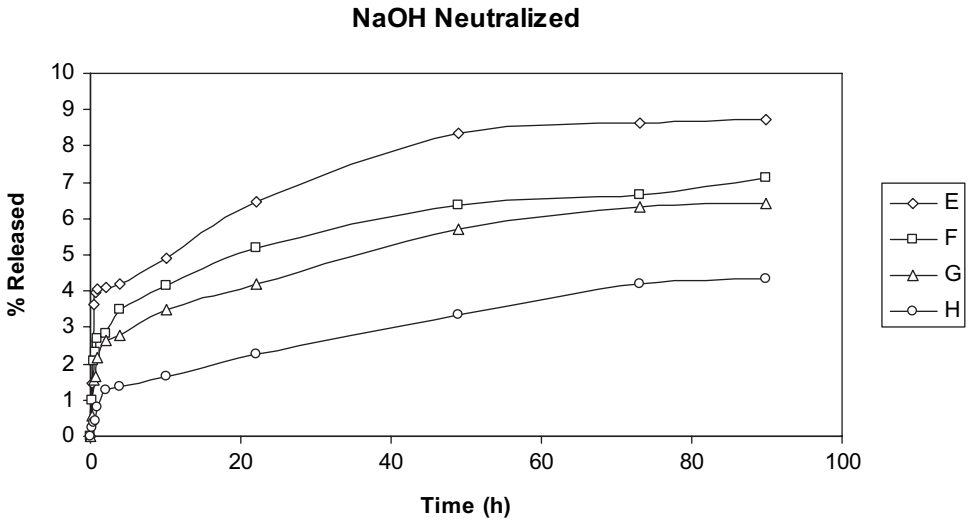


FIGURE 2 **In Vitro Release Profiles of DSP From Film Formulations Prepared With Two Different Crosslinking Agents: (a) TPP (b)NaOH. A to H denotes formulation codes given in Table 1.**

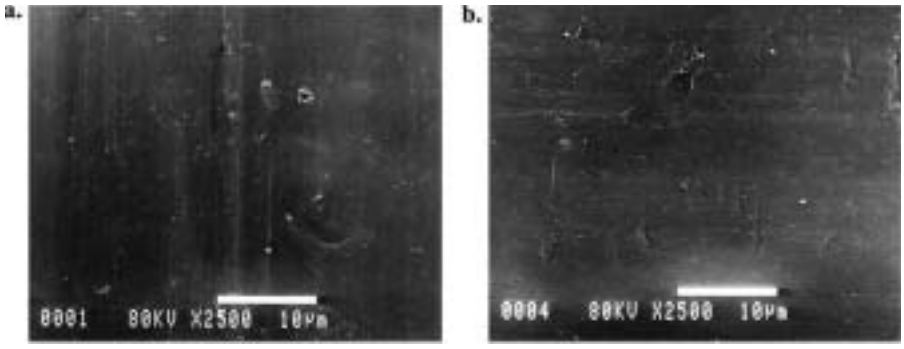


FIGURE 3 **SEM photographs of the film formulations. (a. NaTPP crosslinked; b. NaOH neutralized).**

forces causes an increase in the mobility of macromolecules and high mobility rates provide high diffusion rates for macromolecules resulting in higher release rate of the drug incorporated in the film formulation (Fan & Singh, 1989; Siepmann et al., 1999).

A similar profile for drug release rate was observed with the varying molecular weight of chitosan. As the molecular weight of the polymer increases, the amount of DSP released from film formulations decreases in both of the crosslinking processes. Chitosan gels increase in viscosity as the molecular weight of polymer increases. Viscosities also increase as the degree of deacetylation increases, because the poly electrolytic characteristics of the chitosan become more marked. The Brookfield viscosities of the three polymer types were 20,000, 200,000 and 800,000 cps for low, medium and high molecular weights, respectively. Molecular weight and viscosity were found to be the most important variables for controlling the release rate of DSP from the formulations. Regarding this situation, the possible reason for the decrease in released amount of DSP as the molecular weight increases is the interactions between the polymer chains and the more unique structure formed by the high molecular weight chitosan. The increase viscosity of the gel, a parameter related with the molecular weight, formed in the preparation process of the film formulations leads to a decrease in penetration of solvent molecules into the film structure. These observations are similar to Entwistle and Rowe (1979) where polymer plasticizer interaction and high intrinsic viscosity result in minimum mechanical changes .

TPP is one of the agents used for the crosslinking process for chitosan polymer. It has the ability to form weak linkages between the positively charged amino groups of the chitosan (Bodmeier et al., 1989). The positively charged amino groups of chitosan interact with the negatively charged groups of the TPP and subsequently intermolecular and intramolecular linkages between the groups are formed. In this study, the aim was to evaluate the difference in drug release between TPP and NaOH crosslinking (Sawayanagi et al., 1982; Chandy & Sharma, 1991). As a result, TPP crosslinked film formulations provided a higher release for a period of time approximately two-fold than that of the NaOH neutralized formulations.

CONCLUSION

Chitosan film formulations would be beneficial for the steroid delivery for a certain time after the craniomaxillofial surgery. The film formulations would be easily located under the skin during the surgery without need of any other extra invasion. By the controlled delivery of DSP at the site of interest, the by pass of the side effects of systemic steroid administration in the pre or post operative phase would be maintained.

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