İstanbul Journal of Pharmacy
Istanbul J Pharm, 54 (1): 20–31
DOI: 10.26650/IstanbulJPharm.2024.1372602

Original Article

Preparation of lidocaine hydrochloride containing chitosan-based buccal films for mucositis: *In-vitro* evaluation and cytotoxicity assay

Esra Demirtürk[1], Tilbe Çevikelli[1], Umay Merve Güven[1] Tuğçe Boran[2], Gül Özhan[3]

[1]Çukurova University, Faculty of Pharmacy, Department of Pharmaceutical Technology, Adana, Türkiye
[2]İstanbul University-Cerrahpasa, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, İstanbul, Türkiye
[3]İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, İstanbul, Türkiye

ABSTRACT

Background and Aims: The aim of this study was to prolong the anesthetic effect of lidocaine (LC) in the oral cavity for use in the treatment of oral mucositis and to compare the *in vitro* characteristics of the film formulations prepared by using either chitosan extracted from *Metapenaeus stebbingi (M. stebbingi)* or commercial chitosan.

Methods: In this study, the *in vitro* properties of the film formulations extracted and prepared with commercial chitosan were successfully compared with the addition of different types and amounts of plasticizer and cross-linking agent. In the evaluation of the formulations, different parameters such as structure, thickness, degree of swelling, moisture content, drug content, texture profile analysis, release kinetics according to the *in vitro* drug release, and cytotoxicity evaluation were taken into consideration.

Results: Films prepared using chitosan extracted with 5% glycerol addition showed the highest strength and lowest elongation properties compared to other films (p<0.05). The thickness of the films varied between 500-1400 µm in all formulations. While it was observed that formulations prepared with medium molecular weight commercial chitosan had high surface roughness, the lowest swelling degree was observed for these formulations (77.41 ± 3.65-84.76 ± 6.34). The highest degree of swelling was calculated for the formulations prepared with extracted chitosan (137.23 ± 7.86). The *in vitro* dissolution rate results demonstrated that the increase in the molecular weight of chitosan caused a decrease in the release rate of lidocaine, while at the same time, formulations with added crosslinking agents exhibited a slower release profile. Cytotoxicity studies revealed cell viability at different polymer concentrations.

Conclusion: All the *in vitro* characterization results showed that extracted chitosan from M. stebbingi shells can be a good alternative for pharmaceutical use.

Keywords: Buccal film, chitosan, lidocaine hydrochloride, mucositis, MTT.

INTRODUCTION

Mucositis is a common complication that can cause severe ulcers and, it is characterized by ulceration and inflammation of the entire gastrointestinal tract, often leading to reduced oral intake, weight loss, decreased quality of life, unpredictable interruptions in treatment, and even a life-threatening pathological condition with the development of secondary inflammations (Pulito et al., 2020). One of the primary causes of oral mucositis is the epithelial mucosal inflammatory response to chemo and/or radiotherapy cytotoxic effects as a severe side effect of antineoplastic therapies. Approximately 40% of chemotherapy patients develop oral mucositis, and this figure rises to 90% in patients who have head and neck cancer treatment. Among these patients, around 19% need hospitalization due to experiencing a delay in high-grade mucositis treatment (Elad, Yarom, Zadik, Kuten-Shorrer, & Sonis, 2022; Pulito et al., 2020).

The current management of oral mucositis is mainly symptomatic (Hosseinjani et al., 2017). Prioritizing the prevention and/or treatment of dry mouth, infections, and pain is crucial. Topical anesthetics and mucosal coating agents can also be applied to manage symptoms. Lidocaine (LC), one of the local anesthetics, is frequently utilized as a topical agent due to its immediate onset and mild duration of action. (Brown & Gupta, 2020; Silva et al., 2017).

Mucoadhesive dosage forms such as tablets, gels and films have been studied in recent years, especially regarding their

Corresponding Author: Tilbe Çevikelli **E-mail:** tcevikelli@cu.edu.tr
Submitted: 07.10.2023 • **Revision Requested:** 03.11.2023 • **Last Revision Received:** 04.12.2023 • **Accepted:** 04.12.2023

formulation and development strategies (Cevher, Taha, Orlu, & Araman, 2008; Kottke, Majid, Breitkreutz, & Burckhardt, 2020). Mucoadhesive buccal patches may be preferred over conventional solid formulations due to their capability ability to provide an accurate drug dosage, small size, sufficient thickness, flexibility, and patient comfort (Escalona-Rayo et al., 2020; Morales & McConville, 2011). Buccal drug delivery systems are in intimate contact with the buccal mucosa to retain position in the mouth for a specified length of time. This can be achieved using mucoadhesive polymers and is an ideal feature for these systems (Kumar, Naik, Pradhan, Ghosh, & Rath, 2020; Mahdizadeh Barzoki, Emam-Djomej, Mortazavian, Moosavi-Movahedi, & Rafiee Tehrani, 2016). Although there are different synthetic polymers available, natural polymers are frequently utilized in the development of drug delivery systems because of their unique characteristics (Khade, Gadge, & Mahajan, 2020).

Chitosan is a natural polysaccharide that occurs abundantly in nature and is obtained by deacetylation of chitin. Though various natural polymers are available for the development of mucoadhesive drug delivery systems, chitosan is considered to be the most versatile natural polymer of them all. (Escalona-Rayo, Serrano-Castaneda, Lopez-Cervantes, & Escobar-Chavez, 2020; Younes & Rinaudo, 2015).

Chitosan, which is produced commercially from crustaceans, is mostly obtained from shrimp shells. *M. stebbingi*, found in the Aegean and the Mediterranean, can easily be captured and is in great demand both in Turkey and abroad. Unfortunately, there are very few detailed studies on the pharmaceutical use of the shell of this shrimp species in Turkey. One study (Küçükgülmez et al., 2011) evaluates the suitability of chitosan extracted from these shells for pharmaceutical formulations in terms of molecular weight, deacetylation degree, moisture content, water and fat binding capacity, and *in vitro* applicability as a buccal film. The study compares it with commercial chitosan.

Furthermore, the mucoadhesive nature of chitosan is another important property in providing a controlled and predictable drug release profile, making it among the first-choice drugs for controlled release in buccal administration. One reason why it is preferred above other drugs is due to its natural mucoadhesion feature obtained with a strong electrostatic adhesion force between the positively charged chitosan molecules that give chitosan a positive use feature and the negatively charged mucosal surface (Kumar, Vimal, & Kumar, 2016).

Due to its unique and attractive biological properties, including its hydrophilic nature, muco-adhesiveness, biodegradability, and nontoxicity, this cationic biopolymer is widely utilized in pharmaceutical applications and offers well-established polymeric properties (Shariatinia, 2019). Several studies have been conducted in the literature wherein chitosan was evaluated as a mucoadhesive film-forming polymer, and these studies have demonstrated the suitability of chitosan in formulating buccal films for various drugs as a carrier (Radha, Lal, & Devaky, 2022).

Using extracted and commercial chitosan as polymers, this study aimed to show the development of LC-loaded chitosan-based buccal films for the treatment of oral mucositis. *In vitro* characterization and *in vitro* cytotoxicity studies were conducted to evaluate the extracted and commercial chitosan's compatibility in mucositis treatment (Kumria, Al-Dhubiab, Shah, & Nair, 2018).

MATERIALS AND METHODS

Materials

All the pharmaceutical materials used in the study had analytical grades. Chitosan (CS, medium and low molecular weight), lidocaine hydrochloride (LC), propylene glycol (PG), glycerine (G) and tripolyphosphatepenta sodium salt (TPP) were obtained from Sigma-Aldrich (USA). Lactic acid was obtained from Merck (Germany) and pure water from a Millipore system was used for all the formulations.

Methods

Physicochemical Characterization of the Extracted Chitosan

Chitosan (CS) extraction from *M. stebbingi* shells was conducted using a modified method of that outlined by Chang, Tsai, Lee and Fu (1997). This method involves deproteinization and demineralization using sodium hydroxide (NaOH) and hydrochloric acid (HCl). Chitin residue was dried after treatment with hydrogen peroxide. Chitosan was obtained by alkali treatment of chitin with NaOH in distilled water at 120 °C, washed with deionized water to neutral pH, and then dried.

The weight measurements of the raw material and the extracted chitosan were compared to determine the chitosan extraction yield. Samples were dried at 105°C for 24 hours to determine the moisture content, and samples were heated at 530°C for 20 hours to determine the ash content (Küçükgülmez et al., 2011).

The deacetylation degree was determined using potentiometric titration and elemental analysis methods (Küçükgülmez et al., 2011; Tolaimate et al., 2000).

CS solutions in 0.2 M NaCl/0.1 M acetonitrile (AcOH) were produced at various concentrations for molecular weight determination. Using an Ubbelohde capillary viscometer in a water bath maintained at a constant temperature of 25 °C, the efflux times of the solutions were determined in triplicate, indicating the molecular weight (Küçükgülmez et al., 2011; Wang et al., 2006).

Applying a modified method of the one outlined by Wang

and Kinsela (1976), the water binding capacity (WBC) and fat binding capacity (FBC) of chitosan were determined (Wang & Kinsella, 1976).

To evaluate and compare the chemical structure of the extracted chitosan, FTIR spectra of the extracted and commercial chitosan were obtained by the Jasco FTIR-6700, at a frequency range of 4000 – 400 cm^{-1}.

Preparation of Chitosan Films Loaded with Lidocaine Hydrochloride

The films loaded with lidocaine hydrochloride (LC) were prepared by using low (L) and medium (M) molecular weight (MW) of commercial chitosan (CS) and CS extracted (E) from *M. stebbingi* shells with chemical methods. CS (2%, w/w) was dissolved by stirring in distilled water containing 2% lactic acid. Lactic acid was chosen to dissolve chitosan because chitosan lactate can cause more swelling and mucoadhesion compared to CS acetate, and it is also known that drug release is slower in chitosan lactate than in acetate salt (Cafaggi et al., 2005; Şenel et al., 2000). Plasticizers, glycerine, and propylene glycol, in different concentrations, were mixed with the solution (Table 1). After the addition of LC (4%), the solution (80 g) was spread on a glass plate (8 cm x 8 cm) and dried in an incubator at 37ºC. For the formulation of cross-linked films we used a 0.3% TPP solution.

HPLC Assay

A modified HPLC method was used for the determination of LC (Guideline, 2005; Malenovic, Medenica, Ivanovic, Jancic, & Markovic, 2005). Validation studies were performed for the data. HPLC conditions are shown in Table 2 (Demirtürk, Nemutlu, Şahin, & Öner, 2020).

Drug Content Measurements

Film sections (1 cm^2) were taken from the different areas of films (n=3) Calculations were made by measuring the weight of each film section separately. First, the films were dissolved in 10 mL of water, and the HPLC method was used to determine the LC amount of the samples. Drug content was calculated in samples examined by the validated HPLC method (Abouhussein, Nabawari, Shalaby, & Abd El-Bary, 2020).

Scanning Electron Microscopy

The surface morphology of the chitosan films was analyzed by scanning electron microscope (SEM, FEI Quanta FEG 650, US). After the samples were freeze-dried, they were placed on typical sample mounting slips and given a 20 nm thin layer of gold coating using a sputter coater unit.

Film Thickness Uniformity

The thickness uniformity of the films was determined by the digital micrometer (Showa Digimatic, China) by measuring the thickness of film samples (1 cm2) taken from the 5 different sections of the glass plates.

Swelling Degree

The swelling degree of the films was examined by causing them to swell in simulated saliva fluid (SSF) (pH 6.8) at 25 °C. This is based on the principle of leaving a known weight of the film on the media and examining it at certain intervals (0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5 h). After the swollen films were removed, they were carefully placed on filter paper to remove excess surface water and immediately weighed (n=3) (Abouhussein et al., 2020).

The following formula was used for the water uptake (Eq 1).

$$\text{Swelling Degree (\%)} = \frac{\text{weight of Fwollen Film-Initial Weight of the Film}}{\text{Initial Weight of the Film}} x100 \quad (1)$$

Moisture Content

The amount of moisture present in the films was determined by using the weighting method. A specific size of (1 cm x 1 cm) pre-weighed films was heated to 80°C until it attained a constant weight. The difference in weight gives the degree of moisture content in the films (n=3) (Anwar, Zaman, Raja, Mahmood, & Amjad, 2020).

The moisture content of the formulations was calculated by the following formula (Eq 2):

$$\text{Moisture Content (\%)} = [(\text{Initial Weight-Final Weight}) 100 / \text{Initial Weight}] \quad (2)$$

Texture Profile Analysis

Texture profile analysis was applied to characterize the mechanical properties of the films (TAXT Plus, Stable Micro Systems, United Kingdom). Tensile strength and percentage elongation of the films were evaluated. The test was carried out under conditions of pre-test, 0.1 mm/s and post-test at 0.5 mm/s speed (Alopaeus et al., 2020).

In Vitro Drug Release

In vitro drug release characteristics of the formulations were evaluated by the dialysis bag diffusion method. Simulated saliva fluid (SSF) was used as the dissolution medium (Al-Nemrawi, Alsharif, Alzoubi, & Alkhatib, 2019). Films containing LC were put in dialysis bags and then immersed in the dissolution medium containing 50 mL of SSF at 37 ± 1°C in a water bath. Samples of 1 mL were taken at specified intervals and replaced

Table 1. Codes and Compositions of Fim Formulations.

Formulation Code	Chitosan	Plasticizer	TPP
LG5	Low MW	5% glycerine	0.3%
LPG3	Low MW	3% propylene glycol	0.3%
LG10	Low MW	10% glycerine	0.3%
LPG6	Low MW	6% propylene glycol	0.3%
MG5	Medium MW	5% glycerine	0.3%
MPG3	Medium MW	3% propylene glycol	0.3%
MG10	Medium MW	10% glycerine	0.3%
MPG6	Medium MW	6% propylene glycol	0.3%
EG5	Extracted	5% glycerine	0.3%
EPG3	Extracted	3% propylene glycol	0.3%
EG10	Extracted	10% glycerine	0.3%
EPG6	Extracted	6% propylene glycol	0.3%

Table 2. HPLC conditions.

Device	Shimadzu, LC-2030C Prominence
Stationary Phase	VP-ODS C-18 column
Mobile phase	Water: Acetonitrile (50:50, v/v), pH 2.5
Oven temperature	40 ± 2°C
Flow rate	1 mL.min^{-1}
Injection volume	20 mL
Detection Wavelength	240 nm

with an equal amount of the fresh medium. LC content of the samples was analyzed by the HPLC (n=3).

Evaluation of *In Vitro* Drug Release Kinetics

Data were transferred to the DDSolver program after obtaining the LC release profiles to determine the three most important criteria: adjusted coefficient of determination (R2 adjusted), Akaike information criterion (AIC), and model selection criterion (MSC). The lowest AIC values, maximum R^2 adjusted, and MSC values were used to evaluate different kinetic models (Çevikelli et al., 2024).

Cytotoxicity Evaluation

Cell culture

L929 cell line was purchased from the American Type Culture Collection (ATCC-CCL-1, VA USA) and grown in 10% FBS and 1% penicillin-streptomycin containing Dulbecco's Modified Eagle Medium (DMEM). The cell culture medium was changed every 2-3 days, and subculturing was done when the cells reached 60-70% confluence.

Cytotoxicity assay

Cytotoxic potentials of the formulations were evaluated with MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay. The tetrazolium ring of MTT dye was metabolized to the purple-colored formazan crystals by mitochondrial active cells (Ghasemi et al., 2021). $1x10^4$/well were seeded into a 96-well plate and incubated overnight for cell attachment. The films were prepared in a cell culture medium as previously described by Yaşayan et al., 2021 for MTT assay. Briefly, both sides of the films were sterilized with UV light for 30 min. Cell culture medium was added to the films (1cm^2/mL) and incubated at 37oC, 5% CO_2. After 24 h incubation, the films were discarded, the cell culture medium was equally added to the final volume of the media, and the medium was used for the cytotoxicity assay. Then, the cells were exposed to the aforementioned cell culture medium at different dilutions (10%-30%-50%-90%-100%) for 24 h. Following 24 h exposure, 20 µL of MTT dye solution (5 mg/mL in 1xPBS) was added to each well and incubated for 3 h at 37 oC. At the end of 3 h incubation, crystals which are formed by viable cells were dissolved in 100 µL of DMSO, and optical density (OD) was read at 590 nm using a microwell plate reader (Epoch, Germany). Cell viability was calculated as a percentage relative to the control group.

Statistical Analysis

Statistical differences were analyzed using SPSS version 21.0 for Windows (SPSS Inc. Illinois, USA). The results were analyzed with a one-way analysis of variance (ANOVA) followed by Tukey's test. $p< 0.05$ values were regarded as statistically significant.

RESULTS AND DISCUSSION

Physicochemical Characterization of Extracted Chitosan

M. stebbingi is seen as an economical way to produce CS on an industrial scale due to its high availability and low-cost resources. The reason why *M. stebbingi* shells are preferred is due to their CS yield of approximately 17.5%. Similarities in moisture and ash contents were found between commercial chitosan and extracted CS. The degree of deacetylation, which is an important evaluation parameter for chitosan, was determined over 70% by elemental analysis and potentiometric titration methods

for both extracted and commercial chitosan. Since the molecular weight has a significant impact on the physicochemical and functional properties of CS, it is one of the properties that should be evaluated first. The molecular weight of extracted CS is higher than that of commercial CS (Bao et al., 2018; Sun et al., 2018). The physicochemical characteristics of extracted chitosan are summarized in Table 3.

Table 3. Physicochemical characteristics of extracted chitosan

Parameter	Extracted Chitosan
Yields (%)	17.48 ± 0.64
Moisture (%)	1.33 ± 0.08
Ash (%)	0.61 ± 0.03
Deacetylation degree (%)	95.19 ± 2.56
Molecular weight (kDa)	320-400
Water binding capacity (%)	712.99 ± 11.98
Fat binding capacity (%)	531.15 ± 12.26

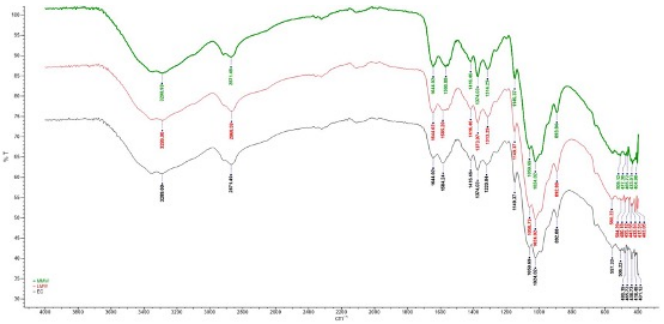

Figure 1. FTIR spectra of the commercial chitosan (HMW, MMW) and extracted chitosan (EC).

As shown in Figure 1, the chemical structure of the extracted CS contains characteristic features of the CS structure, as with the commercial CS. Stretching vibrations of primary NH2 and OH- groups are represented by a broad band around 3750 – 3000 cm^{-1} (max. at 3290 cm^{-1}), while the symmetric and asymmetric stretching vibrations of CH_2 groups of the pyranose ring in the chitosan molecules are represented by a small band between 3000 – 2800 cm-1 (max. at 2871 cm^{-1}) (Gök, Demir, Cevher, Özgümüş, & Pabuccuoğlu, 2019). The amide I bands' characteristic stretching of the CO groups is represented by the band at 1644 cm^{-1}. The small bands (maximum at 1416, 1374, and 1320 cm^{-1}) can be attributed to the bending vibrations of the free methylol groups, whereas the peaks between 1600 and 1500 cm^{-1} (maximum at 1584 cm-1) are the primary NH2 groups' bending vibrations (Gök, 2019). The symmetric and asymmetric stretching vibrations of C-O-C bonds, C-O bonds, and the skeletal vibrations of the chitosan molecule can be detected in bands between 1200 and 900 cm^{-1} (max. at 1149, 1059, and 1024 cm^{-1}), whereas the stretching vibration of the acetyl groups of the chitosan molecules is recorded at 892 cm^{-1} (Gök et al., 2019).

Surface Morphology

At the macroscopic scale, all films were homogeneous and transparent. Surface morphology provides a document for a mixture of CS and plasticizers at different ratios and types (Yeddes et al., 2020). The surfaces of each film were smooth with no apparent pores. However, the SEM micrograph of the films prepared with the extracted CS showed a more regular surface than the formulations prepared with commercial chitosan (Figure 2). Formulations prepared with commercial CS with a medium molecular weight had high surface roughness. A smoother surface was obtained from formulations prepared with low molecular weight commercial CS and extracted. Tensile characteristics may be impacted by the formulation's morphological state, for instance, by crystal formation (Preis, Knop, & Breitkreutz, 2014). Therefore, these perspectives have to be considered during the development of films for drug delivery systems (Kassem, Ismail, Naggar, & Aboulmagd, 2015; Sakloetsakun, Preechagoon, Bernkop-Schnürch, & Pongjanyakul, 2016).

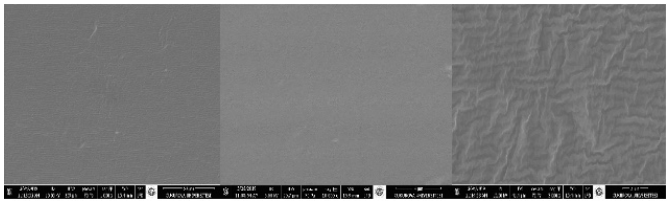

Figure 2. SEM images of films LG5, EG5, MG5, respectively.

Drug Recovery

All films were prepared with 4% w/w drug content according to the dry weight of the polymer, which is about 88.89-93.50% recovery of the total drug used (Table 4).

Thickness Uniformity

The ideal film thickness range for the oral cavity is 0.05 to 1 mm to prevent discomfort during its application. The thicknesses of the films varied between 500 ± 17.89 µm (LPG3) and 1400 ± 23.34 µm (EG10). Except for the EG10 formulation, all film formulations showed proper thickness for their application. Low standard deviations for all formulations were calculated,

Table 4. Experimental results for thickness, drug recovery and swelling degree of chitosan films ((mean±SD, n=3).

Codes of the Formulations	Thickness (µm) (±SD)	Drug Recovery (%)(±SD)	Swelling Degree (%)(±SD)
LG5	850 ± 21.46	88.89 ± 5.57	91.45 ± 4.56
LPG3	500 ± 17.89	90.50 ± 4.45	85.65 ± 5.78
LG10	1000 ± 12.87	91.68 ± 4.87	98.32 ± 4.64
LPG6	700 ± 11.56	92.51 ± 3.78	87.35 ± 2.76
MG5	750 ± 24.76	92.58 ± 4.56	80.21 ± 5.24
MPG3	500 ± 21.34	91.09 ± 4.44	77.41 ± 3.65
MG10	1000 ± 28.40	92.09 ± 2.89	84.76 ± 6.34
MPG6	650 ± 31.35	92.09 ± 5.09	80.23 ± 7.32
EG5	850 ± 23.54	93.50 ± 4.90	137.23 ± 7.86
EPG3	650 ± 31.45	88.54 ± 5.61	111.34 ± 8.35
EG10	1400 ± 23.34	91.51 ± 5.51	121.87 ± 5.98
EPG6	800 ± 13.67	91.56 ± 4.78	102.57 ± 6.67

indicating the thickness uniformity of the films (Jillani et al., 2022).

Swelling Degree

The swelling degree represents the capacity of the films to absorb the surrounding aqueous medium and affects the mucoadhesive property and the drug-release characteristics of the films (Kumria et al., 2018). Swelling of the polymer matrix initiates drug diffusion from the films, and for the hydrophilic polymers, hydration is the leading reason for the adhesion of the polymer to the mucous membrane (Mahdizadeh Barzoki et al., 2016). The high swelling of the films (Figure 3) can be attributed to the hydrophilic nature and the hydrogel formation properties of the chitosan. All of the formulations absorbed SSF medium and showed swelling rapidly after contact with the aqueous medium and maintained physical integrity by remaining intact during the study. The effect of the plasticizer on the swelling degree for chitosan films is also presented in Figure 4. In contrast to CS films with propylene glycol, the swelling degree was increased in those of prepared with glycerine. The lowest percentage swelling degrees were found with the formulations which were prepared by medium molecular weight CS. The highest percentage swelling degrees were calculated for the formulations as 137.23 ± 7.86, 111.34 ± 8.35, 121.87 ± 5.98, and 102.57 ± 6.67, respectively (p<0.05), for the films prepared with extracted CSs. The films plasticized with 5% and 10% glycerine showed higher water content than those of plasticized with 3% and 6% propylene glycol. Then, the extracted CS film obtained a higher swelling degree than films with the other types of CS (p<0.05). The significant hydrodynamic plasticizer water complex is formed by the hydrophilic and hygroscopic properties of plasticizers (Krochta, 2002). The compatibility between the polymers and plasticizers used depends on their similar chemical structures. When the chemical structures of glycerine and propylene glycol are examined, it can be seen that both have lateral hydroxide groups that can form hydrogen bonds with chitosan (Sakwanichol, Sungthongjeen, & Puttipipatkhachorn, 2019). Another factor used to estimate the compatibility of polymers is the fit between solubility parameters. This parameter is the reason why the chitosan aqueous dispersion of glycerine is more compatible than propylene glycol (Calvo et al., 2019).

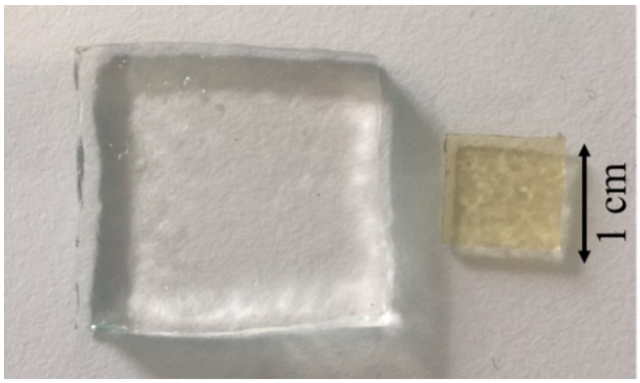

Figure 3. Photograph of the film upon swelling study.

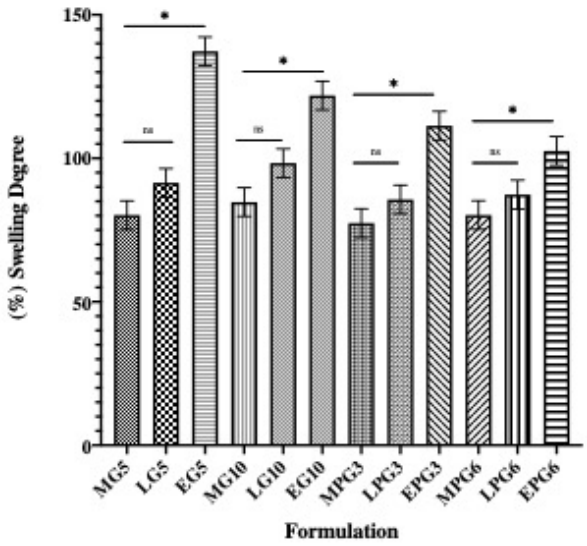

Figure 4. Swelling degrees of chitosan films. Data were shown as mean±standard deviation (SD). *; significant (p<0.05).

Moisture Content

The moisture content of thefilms was determined to investigate integrity in dry conditions. An ideal buccal film should have a moisture content below 5%, increased stability in dry conditions, and a higher moisture absorption capacity, resulting in higher adhesion in the oral cavity (Pilicheva, Uzunova, & Marudova, 2022). Formulations prepared with propylene glycol used as a plasticizer showed a high content of moisture ranging between 14.89% and 23.30%, while formulations containing glycerine showed moisture contents in an acceptable range between 0.98% and 5.47% and were found to be applicable (Figure 5).

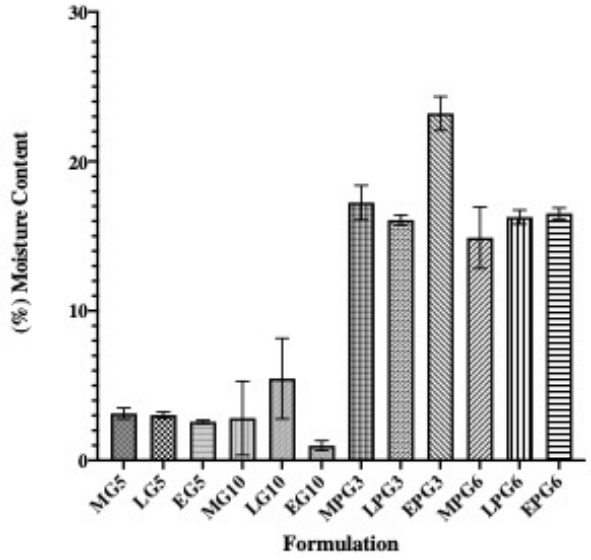

Figure 5. Moisture content of chitosan films. Data were shown as mean±SD.

Texture Profile Analysis

Since the mechanical properties of a film affect its suitability and acceptability, tensile strength and percentage elongation should be monitored for polymeric films. A buccal film should be strong enough to be easily removed from the oral cavity or to be peeled after casting while having enough elasticity to not change drug uniformity during cutting or packing (Abouhussein et al., 2020). Tensile strength determines the strength of the film under the diametric tension, and the percentage elongation represents the stretchability of the film while maintaining physical integrity (Karki et al., 2016).

Texture analyses indicated enough hardness for all films between 1.00 – 4.24 MPa, thus ensuring easy mucosal intra-pocket insertion. The values for tensile strength (TS) and film elongation of the CS film formulations are shown in Table 5. With the addition of plasticizers, films containing glycerine showed reduced tensile strength. This study concluded that films obtained using extracted chitosan with the addition of glycerine as a plasticizer are the most suitable films in terms of mechanical properties. As Figure 6 indicates, increasing the chitosan MW caused an increase in the values of TS or mechanical properties of the films. Due to the increased crosslinking density, the extracted chitosan resulted in the formation of a film with lower extensibility.

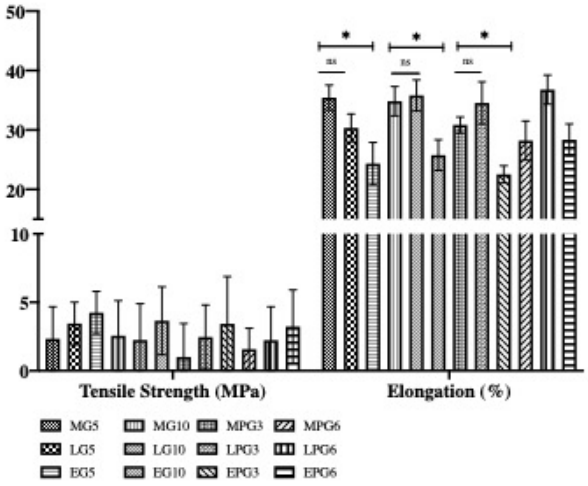

Figure 6. Tensile strength and elongation values of the chitosan film formulations data were shown as mean±SD. *; significant (p<0.05).

The percentage elongation of the films was between 22.54% and 36.78% and increased with the percentage of plasticizer content (Table 5). This finding is consistent with that previously obtained by Calvo et al. (2019), where the highest elongation for chitosan films was acquired when the highest plasticizer ratio was used. In general, we can say that when a high proportion of plasticizers are combined with a polymer such as chitosan, they increase the elongation value, causing a decrease in tensile strength.

In vitro Drug Release Studies

The in vitro drug release from uncross-linked cross-linked and films are shown in Figure 7, and Figure 8, respectively. As shown in both figureS, the increase in the MW of CS was resulted in a decrease in released amount of L. In the presence of TPP, similar drug release pattern was observed. Our aim in this study was to enhance the clinical effect of film formulations using CS. Due to its mucoadhesive properties, the films are expected to remain in the oral cavity and release the drug over an extended period. Uncross-linked films showed LC release between 60.18% and 81.27% at the end of 2 hours and reached a plateau in 6 hours by completing drug release (Figure 7). The cross-linked films released only 22.32% to 37.56% of the LC in 2 hours and maintained drug release for 12 hours. A comparison of LC release from cross-linked films indicates a slower drug release than the uncross-linked films (Figure 8).

Table 5. The values for tensile strength and film elongation of the chitosan film formulations (mean±SD, n=3).

Formulation Code	Chitosan	Plasticizer	TPP
LG5	Low MW	5% glycerine	0.3%
LPG3	Low MW	3% propylene glycol	0.3%
LG10	Low MW	10% glycerine	0.3%
LPG6	Low MW	6% propylene glycol	0.3%
MG5	Medium MW	5% glycerine	0.3%
MPG3	Medium MW	3% propylene glycol	0.3%
MG10	Medium MW	10% glycerine	0.3%
MPG6	Medium MW	6% propylene glycol	0.3%
EG5	Extracted	5% glycerine	0.3%
EPG3	Extracted	3% propylene glycol	0.3%
EG10	Extracted	10% glycerine	0.3%
EPG6	Extracted	6% propylene glycol	0.3%

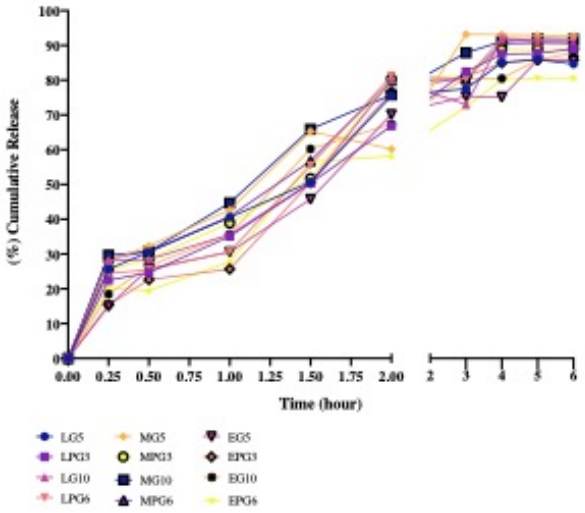

Figure 7. Lidocaine release profile from uncross-linked chitosan films. Data were shown as mean±SD.

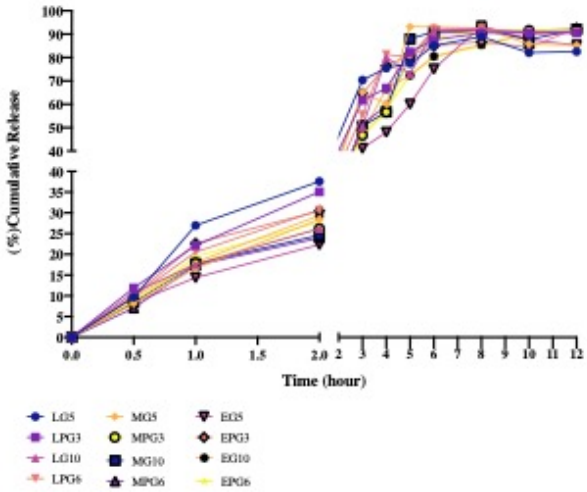

Figure 8. Lidocaine release profile from cross-linked chitosan films. Data were shown as mean±SD.

DD Solver evaluates the goodness of the model fit with statistical parameters such as adjusted coefficient of determination (R^2), Akaike Information Criterion (AIC) and Model Selection Criterion (MSC). When comparing various models, the most accurate fitting is indicated by the highest values of R^2 and MSC, and the lowest value of AIC. Based on the lowest AIC values with the highest R^2 and MSC (Table 6), the first-order model was chosen for formulations (Abdul Rasool, Mohammed, & Salem, 2021).

Cytotoxicity Assay

The cytotoxicity evaluation of the formulations was performed by MTT assay using L929 cells (Yaşayan, Karaca, Akgüner, & Bal Öztürk, 2021). The study was carried out using drug-free formulations containing LC for 24 h. The results are given in Figure 9 and Figure 10 respectively. For drug-free formulations, buccal films prepared with commercial chitosan did not reduce cell viability below 80% up to 50% dilution, while extracted chitosan (EG5) was found to show higher cytotoxic profile in comparison with commercial CH (LG5, MG5). However, extracted chitosan shows a higher cytotoxic profile, and formulation modifications should be performed. Since the therapeutic dose of LC was successfully loaded into films, LC loading to the films decreased the cell viability, and showed dose dependent cytotoxicity in comparison with drug-free formulations, as expected.

Table 6. Evaluation parameters for best fit kinetic model selection.

Formulations	Kinetic Model Parameters (R^2adj, AIC, MSC)					
	Zero-order	**First-order**	**Higuchi**	**Korsmeyer-Peppas**	**Hixson-Crowell**	**Hopfenberg**
LG5	0.1468	**0.9222**	0.8777	0.8986	0.8976	0.9110
	78.9675	**57.4136**	61.4868	60.5997	59.8835	59.4185
	-0.0635	**2.3314**	1.8788	1.9774	2.0570	2.1087
LPG3	0.1869	**0.9232**	0.8689	0.8807	0.8841	0.9122
	78.3420	**57.1023**	61.9197	61.8653	60.8069	59.1053
	-0.0153	**2.3446**	1.8093	1.8154	1.9330	2.1221
LG10	-0.0664	**0.9460**	0.8505	0.9062	0.9089	0.9383
	80.1485	**53.2929**	62.4685	59.0731	58.0114	55.3013
	-0.2865	**2.6974**	1.6779	2.0552	2.1731	2.4743
LPG6	0.0705	**0.9224**	0.8687	0.9012	0.8789	0.9113
	78.9665	**56.6143**	61.3528	59.5915	60.6274	58.6188
	-0.1491	**2.3345**	1.8080	2.0037	1.8886	2.1118
MG5	0.0514	**0.8917**	0.8647	0.8999	0.8143	0.8761
	78.0670	**58.5364**	60.5388	58.6243	63.3907	60.5492
	-0.1694	**2.0006**	1.7781	1.9909	1.4613	1.7770
MPG3	0.4371	**0.9628**	0.9294	0.9240	0.9330	0.9575
	75.5380	**51.0812**	56.8490	58.3150	56.3861	53.0852
	0.3524	**3.0698**	2.4290	2.2661	2.4804	2.8472
MG10	0.2755	**0.8982**	0.8781	0.8795	0.8628	0.8836
	77.4976	**59.8390**	61.4597	62.1497	62.5235	61.8418
	0.1000	**2.0621**	1.8820	1.8054	1.7638	1.8396
MPG6	0.3681	**0.9286**	0.8750	0.8648	0.9143	0.9184
	77.7003	**58.0732**	63.1180	64.6214	59.7155	60.0767
	0.2369	**2.4177**	1.8571	1.6901	2.2352	2.1951
EG5	0.4993	**0.9424**	0.9200	0.9097	0.8935	0.9341
	74.1437	**54.6836**	57.6363	59.5247	60.2167	56.6936
	0.4695	**2.6317**	2.3036	2.0938	2.0169	2.4084
EPG3	0.4770	**0.9301**	0.8658	0.8472	0.9040	0.9200
	76.2561	**58.1469**	64.0130	65.9809	60.9982	60.1517
	0.4259	**2.4380**	1.7862	1.5676	2.1212	2.2153
EG10	0.3143	**0.9522**	0.9127	0.9164	0.8932	0.9454
	76.5605	**52,5827**	58.0078	58.4159	59.8291	54.5873
	0.1551	**2.8193**	2.2165	2.1712	2.0142	2.5966
EPG6	0.4411	**0.9219**	0.9083	0.8987	0.8592	0.9108
	74.2172	**56.5014**	57.9493	59.6404	61.8103	58.5040
	0.3596	**2.3280**	2.1671	1.9792	1.7381	2.1055

R^2adj: R^2 adjusted, AIC: Akaike information criterion, MSC: model selection criterion.

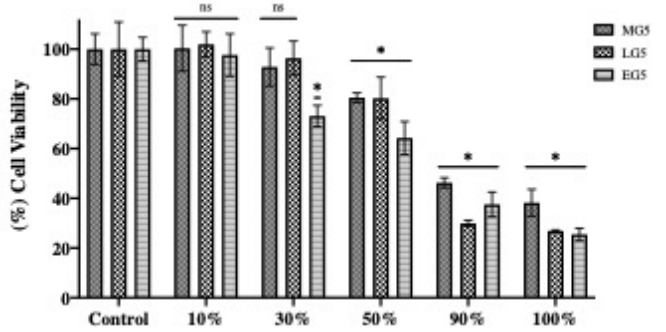

Figure 9. Effects of drug-free MG5, LG5 and EG5 formulations on cell viability. Data were shown as mean±SD. *; significant (p<0.05) versus control group.

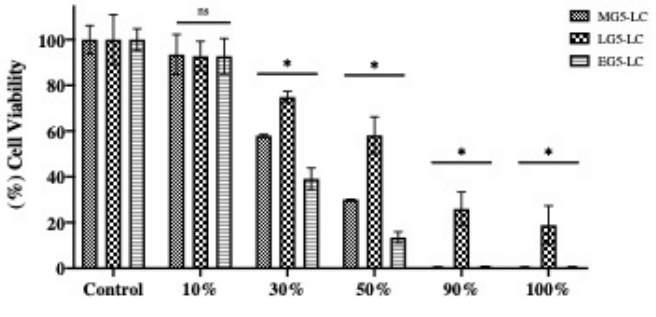

Figure 10. Effects of lidocaine containing MG5, LG5 and EG5 formulations on cell viability. Data were shown as mean±SD. *; significant (p<0.05) versus control group.

CONCLUSION

In this study, films prepared using extracted and commercial CS were investigated by differentiating CS with propylene glycol and glycerine over a wide range of composition ratios. The current work indicates that LH can be successfully loaded to CS-based films while having good mechanical and barrier properties. The addition of glycerine as a plasticizer into the CS film system changed its thermal, mechanical, and swelling properties. The in vitro characterization results show that the most promising film for drug delivery of LC is the one containing glycerine and extracted CS. Based on these observations, extracted CSwas determined to have a positive effect on the physical properties of the polymer films fabricated in this study, yet polymer concentration should be decreased according to the cytotoxicity studies. Commercial CSs showed higher cytocompatibility than the extracted chitosan, according to the MTT studies. In conclusion, lidocaine-loaded CS-based films can be applied as an alternative way to deliver LH for mucositis therapy.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.D., T.Ç., U.M.G., T.B., G.Ö.; Data Acquisition- E.D., T.Ç., U.M.G., T.B., G.Ö.; Data Analysis/Interpretation- E.D., T.Ç., U.M.G., T.B., G.Ö.; Drafting Manuscript- E.D., T.Ç., U.M.G., T.B., G.Ö.; Critical Revision of Manuscript- E.D., T.Ç., U.M.G., T.B., G.Ö.; Final Approval and Accountability- E.D., T.Ç., U.M.G., T.B., G.Ö.

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared no financial support.

ORCID IDs of the authors

Esra Demirtürk	0000-0002-8918-0073
Tilbe Çevikelli	0000-0002-0881-0644
Umay Merve Güven	0000-0003-1547-0817
Tuğçe Boran	0000-0003-4302-1947
Gül Özhan	0000-0002-6926-5723

REFERENCES

Abdul Rasool, B. K., Mohammed, A. A., & Salem, Y. Y. (2021). The optimization of a dimenhydrinate transdermal patch formulation based on the quantitative analysis of in vitro release data by DD-Solver through skin penetration studies. *Scientia Pharmaceutica, 89*(3), 33. https://doi.org/10.3390/scipharm89030033

Abouhussein, D., El Nabarawi, M. A., Shalaby, S. H., & Abd El-Bary, A. (2020). Cetylpyridinium chloride chitosan blended mucoadhesive buccal films for treatment of pediatric oral diseases. *Journal of Drug Delivery Science and Technology, 57*, 101676. https://doi.org/10.1016/j.jddst.2020.101676

Al-Nemrawi, N. K., Alsharif, S. S., Alzoubi, K. H., & Alkhatib, R. Q. (2019). Preparation and characterization of insulin chitosan-nanoparticles loaded in buccal films. *Pharmaceutical Development and Technology, 24*(8), 967-974. https://doi.org/10.1080/10837450.2019.1619183

Alopaeus, J. F., Hellfritzsch, M., Gutowski, T., Scherließ, R., Almeida, A., Sarmento, B., Škalko-Basnet, N., & Tho, I. (2020). Mucoadhesive buccal films based on a graft co-polymer–A mucin-retentive hydrogel scaffold. *European Journal of Pharmaceutical Sciences, 142*, 105142. https://doi.org/10.1016/j.ejps.2019.105142

Anwar, S., Zaman, M., Raja, M. A. G., Mahmood, A., & Amjad, M. W. (2020). Rosuvastatin, Perindopril and Ezetimibe loaded instant release buccal films: De*velopment and in vitro characterization. Journal of Applied Biomedicine*, 18(4). https://doi.org/10.32725/jab.2020.015

Bao, Y., Zhang, H., Luan, Q., Zheng, M., Tang, H., & Huang, F. (2018). Fabrication of cellulose nanowhiskers reinforced chitosan-xylan nanocomposite films with antibacterial and antioxidant activities. *Carbohydrate Polymers, 184*, 66-73. https://doi.org/10.1016/j.carbpol.2017.12.051

Brown, T. J., & Gupta, A. (2020). Management of cancer therapy–associated oral mucositis. *JCO Oncology Practice, 16*(3), 103-109. https://doi.org/10.1200/JOP.19.00652

Cafaggi, S., Leardi, R., Parodi, B., Caviglioli, G., Russo, E., & Bignardi, G. (2005). Preparation and evaluation of a chitosan salt–poloxamer 407 based matrix for buccal drug delivery. *Journal of Controlled Release, 102*(1), 159-169. https://doi.org/10.1016/j.jconrel.2004.09.019

Chang, K. L. B., Tsai, G., Lee, J., & Fu, W. R. (1997). Heterogeneous N-deacetylation of chitin in alkaline solution. *Carbohydrate Research, 303*(3), 327-332. https://doi.org/10.1016/S0008-6215(97)00179-1

Calvo, N. L., Svetaz, L. A., Alvarez, V. A., Quiroga, A. D., Lamas, M. C., & Leonardi, D. (2019). Chitosan-hydroxypropyl methylcellulose tioconazole films: A promising alternative dosage form for the treatment of vaginal candidiasis. *International Journal of Pharmaceutics, 556*, 181-191. https://doi.org/10.1016/j.ijpharm.2018.12.011

Cevher, E., Taha, M., Orlu, M., & Araman, A. (2008). Evaluation of mechanical and mucoadhesive properties of clomiphene citrate gel formulations containing carbomers and their thiolated derivatives. *Drug Delivery, 15*(1), 57-67. https://doi.org/10.1080/10717540701829234

Çevikelli, T., Güven, U. M., & Öztürk, A. A. (2024). Metronidazole Loaded Novel Microemulsion Formulation for Topical Delivery and Characterization With Validated New UPLC Method. *Fabad Journal of Pharmaceutical Sciences, 49*(1), 111-128. https://doi.org/10.55262/fabadeczacilik.1359138

Demirtürk, E., Nemutlu, E., Şahin, S., & Öner, L. (2020). Development and validation of an HPLC method for determination of rofecoxib in bovine serum albumin microspheres. *Turkish Journal of Chemistry, 44*(3), 647-655. https://doi.org/10.3906/kim-1912-45

Elad, S., Yarom, N., Zadik, Y., Kuten-Shorrer, M., & Sonis, S. T. (2022). The broadening scope of oral mucositis and oral ulcerative mucosal toxicities of anticancer therapies.*CA: A Cancer Journal for Clinicians, 72*(1), 57-77. https://doi.org/10.3322/caac.21704

Escalona-Rayo, C. F., Serrano-Castañeda, P., López-Cervantes, M., & Escobar-Chávez, J. J. (2020). Optimization of unidirectional mucoadhesive buccal patches based on chitosan and pluronic® F-127 for metoprolol controlled release: In vitro and ex vivo evaluations. *Journal of Pharmaceutical Innovation*, 15, 556-568.

https://doi.org/10.1007/s12247-019-09401-8

Gök, M. K. (2019). In vitro evaluation of synergistic effect of primary and tertiary amino groups in chitosan used as a non-viral gene carrier system. *European Polymer Journal, 115*, 375-383. https://doi.org/10.1016/j.eurpolymj.2019.03.048

Gök, M. K., Demir, K., Cevher, E., Özgümüş, S., & Pabuccuoğlu, S. (2019). Effect of the linear aliphatic amine functionalization on in vitro transfection efficiency of chitosan nanoparticles. *Carbohydrate Polymers, 207*, 580-587. https://doi.org/10.1016/j.carbpol.2018.12.013

Guideline, I. H. T. (2005). Validation of analytical procedures: text and methodology. *Q2 (R1)*, 1(20), 05.

Hosseinjani, H., Hadjibabaie, M., Gholami, K., Javadi, M., Radfar, M., Jahangard-Rafsanjani, Z., Hosseinjani, E., Shabani, N., Vaezi, M., & Ghavamzadeh, A. (2017). The efficacy of erythropoietin mouthwash in prevention of oral mucositis in patients undergoing autologous hematopoietic SCT: a double-blind, randomized, placebo-controlled trial. *Hematological Oncology, 35*(1), 106-112. https://doi.org/10.1002/hon.2250

Jillani, U., Mudassir, J., Arshad, M. S., Mehta, P., Alyassin, Y., Nazari, K., Yousef, B., Patel, M., Zaman, A., & Sayed, E. (2022). Design and evaluation of agarose based buccal films containing zolmitriptan succinate: Application of physical and chemical enhancement approaches. *Journal of Drug Delivery Science and Technology, 69*, 103041. https://doi.org/10.1016/j.jddst.2021.103041

Karki, S., Kim, H., Na, S.-J., Shin, D., Jo, K., & Lee, J. (2016). Thin films as an emerging platform for drug delivery. *Asian Journal of Pharmaceutical Sciences*, 11(5), 559-574. https://doi.org/10.1016/j.ajps.2016.05.004

Kassem, A. A., Ismail, F. A., Naggar, V. F., & Aboulmagd, E. (2015). Preparation and evaluation of periodontal films based on polyelectrolyte complex formation. *Pharmaceutical Development and Technology, 20*(3), 297-305. https://doi.org/10.3109/10837450.2013.862262

Khade, A., Gadge, G., & Mahajan, U. (2020). An overview on natural polymer based mucoadhesive buccal films for controlled drug delivery. *International Journal of Pharmacy Research & Technology (IJPRT), 10*(1), 48-57. https://doi.org/10.21276/irjps.2019.6.1.7

Kottke, D., Majid, H., Breitkreutz, J., & Burckhardt, B. B. (2020). Development and evaluation of mucoadhesive buccal dosage forms of lidocaine hydrochloride by ex-vivo permeation studies. *International Journal of Pharmaceutics*, 581, 119293. https://doi.org/10.1016/j.ijpharm.2020.119293

Krochta, J. M. (2002). Proteins as raw materials for films and coatings: definitions, current status, and opportunities. *Protein-based Films and Coatings, 1*, 1-40. https://doi.org/10.1201/9781420031980.ch1

Küçükgülmez, A., Celik, M., Yanar, Y., Sen, D., Polat, H., & Kadak, A. E. (2011). Physicochemical characterization of chitosan extracted from Metapenaeus stebbingi shells. *Food Chemistry, 126*(3), 1144-1148. https://doi.org/10.1016/j.foodchem.2010.11.148

Kumar, A., Naik, P. K., Pradhan, D., Ghosh, G., & Rath, G. (2020). Mucoadhesive formulations: Innovations, merits, drawbacks, and future outlook. *Pharmaceutical Development and Technology, 25*(7), 797-814. https://doi.org/10.1080/10837450.2020.1753771

Kumar, A., Vimal, A., & Kumar, A. (2016). Why Chitosan? From properties to perspective of mucosal drug delivery. *International Journal of Biological Macromolecules, 91*, 615-622. https://doi.org/10.1016/j.ijbiomac.2016.05.054

Kumria, R., Al-Dhubiab, B. E., Shah, J., & Nair, A. B. (2018). Formulation and evaluation of chitosan-based buccal bioadhesive films of zolmitriptan. *Journal of Pharmaceutical Innovation, 13*, 133-143. https://doi.org/10.1007/s12247-018-9312-6

Mahdizadeh Barzoki, Z., Emam-Djomeh, Z., Mortazavian, E., Akbar Moosavi-Movahedi, A., & Rafiee Tehrani, M. (2016). Formulation, in vitro evaluation and kinetic analysis of chitosan–gelatin bilayer muco-adhesive buccal patches of insulin nanoparticles. *Journal of Microencapsulation, 33*(7), 613-624. https://doi.org/10.1080/02652048.2016.1234513

Malenovic, A., Medenica, M., Ivanovic, D., Jancic, B., & Markovic, S. (2005). Development and validation of RP–HPLC method for cetrimonium bromide and lidocaine determination. *Il Farmaco, 60*(2), 157-161. https://doi.org/10.1016/j.farmac.2004.11.004

Morales, J. O., & McConville, J. T. (2011). Manufacture and characterization of mucoadhesive buccal films. *European Journal of Pharmaceutics and Biopharmaceutics, 77*(2), 187-199. https://doi.org/10.1016/j.ejpb.2010.11.023

Pilicheva, B., Uzunova, Y., & Marudova, M. (2022). Polyelectrolyte Multilayer Films as a Potential Buccal Platform for Drug Delivery. *Polymers, 14*(4), 734. https://doi.org/10.3390/polym14040734

Preis, M., Knop, K., & Breitkreutz, J. (2014). Mechanical strength test for orodispersible and buccal films. *International Journal of Pharmaceutics, 461*(1-2), 22-29. https://doi.org/10.1016/j.ijpharm.2013.11.033

Pulito, C., Cristaudo, A., Porta, C. L., Zapperi, S., Blandino, G., Morrone, A., & Strano, S. (2020). Oral mucositis: the hidden side of cancer therapy. *Journal of Experimental & Clinical Cancer Research, 39*, 1-15. https://doi.org/10.1186/s13046-020-01715-7

Radha, D., Lal, J. S., & Devaky, K. (2022). Chitosan-based films in drug delivery applications. *Starch-Stärke, 74*(7-8), 2100237. https://doi.org/10.1002/star.202100237

Sakloetsakun, D., Preechagoon, D., Bernkop-Schnürch, A., & Pongjanyakul, T. (2016). Chitosan–gum arabic polyelectrolyte complex films: Physicochemical, mechanical and mucoadhesive properties. *Pharmaceutical Development and Technology, 21*(5), 590-599. https://doi.org/10.3109/10837450.2015.1035727

Sakwanichol, J., Sungthongjeen, S., & Puttipipatkhachorn, S. (2019). Preparation and characterization of chitosan aqueous dispersion as a pharmaceutical film forming material. *Journal of Drug Delivery Science and Technology, 54*, 101230. https://doi.org/10.1016/j.jddst.2019.101230

Shariatinia, Z. (2019). Pharmaceutical applications of chitosan. *Advances in Colloid and Interface Science, 263*, 131-194. https://doi.org/10.1016/j.cis.2018.11.008

Silva, F. C., Marto, J. M., Salgado, A., Machado, P., Silva, A. N., & Almeida, A. J. (2017). Nystatin and lidocaine pastilles for the local treatment of oral mucositis. *Pharmaceutical Development and Technology, 22*(2), 266-274. https://doi.org/10.1080/10837450.2016.1221424

Sun, W., Chen, G., Wang, F., Qin, Y., Wang, Z., Nie, J., & Ma, G. (2018). Polyelectrolyte-complex multilayer membrane with gradient porous structure based on natural polymers for wound care. *Carbohydrate Polymers, 181*, 183-190. https://doi.org/10.1016/j.carbpol.2017.10.068

Şenel, S., İkinci, G., Kaş, S., Yousefi-Rad, A., Sargon, M., & Hıncal, A. (2000). Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery. *International Journal of Pharmaceutics, 193*(2), 197-203. https://doi.org/10.1016/S0378-5173(99)00334-8

Tolaimate, A., Desbrieres, J., Rhazi, M., Alagui, A., Vincendon, M., & Vottero, P. (2000). On the influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin. *Polymer, 41*(7), 2463-2469. https://doi.org/10.1016/S0032-3861(99)00400-0

Wang, J., & Kinsella, J. (1976). Functional properties of novel proteins: Alfalfa leaf protein. *Journal of Food Science, 41*(2), 286-292. https://doi.org/10.1111/j.1365-2621.1976.tb00602.x

Wang, Q. Z., Chen, X. G., Liu, N., Wang, S. X., Liu, C. S., Meng, X. H., & Liu, C. G. (2006). Protonation constants of chitosan with different molecular weight and degree of deacetylation. *Carbohydrate Polymers*, 65(2), 194-201. https://doi.org/10.1016/j.carbpol.2006.01.001

Yaşayan, G., Karaca, G., Akgüner, Z. P., & Bal Öztürk, A. (2021). Chitosan/collagen composite films as wound dressings encapsulating allantoin and lidocaine hydrochloride. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 70(9), 623-635. https://doi.org/10.1080/00914037.2020.1740993

Yeddes, W., Djebali, K., Wannes, W. A., Horchani-Naifer, K., Hammami, M., Younes, I., & Tounsi, M. S. (2020). Gelatin-chitosan-pectin films incorporated with rosemary essential oil: Optimized formulation using mixture design and response surface methodology. *International Journal of Biological Macromolecules, 154*, 92-103. https://doi.org/10.1016/j.ijbiomac.2020.03.092

Younes, I., & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine Drugs, 13*(3), 1133-1174. https://doi.org/10.3390/md13031133

How cite this article

Demirtürk, E., Çevikelli, T., Güven, U.M., Boran, T., & Özhan, G. (2024). Preparation of lidocaine hydrochloride containing chitosan-based buccal films for mucositis: In-Vitro evaluation and cytotoxicity assay. *İstanbul Journal of Pharmacy, 54*(1), 20–31. DOI: 10.26650/IstanbulJPharm.2024.1372602