İSTANBUL UNIVERSITY PRESS

RESEARCH ARTICLE / ARAŞTIRMA MAKALESİ
Journal of Advanced Research in Health Sciences / Sağlık Bilimlerinde İleri Araştırmalar Dergisi 2025
DOI: 10.26650/JARHS2025-1517952

DEVELOPMENT AND EVALUATION OF BIOADHESIVE MUCOSAL DOSAGE FORMS OF PILOCARPINE HCL FOR XEROSTOMIA THERAPY

KSEROSTOMİ TEDAVİSİ İÇİN PİLOKARPİN HCL'NİN BİYOADEZİF MUKOZAL DOZAJ FORMLARININ GELİŞTİRİLMESİ VE DEĞERLENDİRİLMESİ

Özlem AKBAL DAĞISTAN[1*], Nur Sena BAŞARIR[1], Sinem Yaprak KARAVANA[2], Ayca YILDIZ PEKÖZ[1]

[1]İstanbul University, Faculty of Pharmacy, Department of Pharmaceutical Technology, İstanbul, Türkiye
[2]Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology, İzmir, Türkiye
*Özlem AKBAL DAĞISTAN has passed away

ORCID ID: Ö.A.D. 0000-0002-6524-3535; N.S.B. 0000-0002-0695-5292; S.Y.K. 0000-0001-6010-5902; A.Y.P. 0000-0003-2243-6684

Citation/Atıf: Akbal Dağıstan Ö, Başarır NS, Karavana SY, Yıldız Peköz A. Development and evaluation of bioadhesive mucosal dosage forms of pilocarpine HCL for xerostomia therapy. Journal of Advanced Research in Health Sciences 2025;8(1):34-41. https://doi.org/10.26650/JARHS2025-1517952

ABSTRACT

Objective: Saliva maintains vital mouth functions and acts as a barrier to dental health. Xerostomia, which is characterised as a feeling of dry mouth, adversely affects the patient's quality of life. Palliative therapies, such as sialagogues, form the based on treatment of xerostomia. The FDA-approved sialagogue pilocarpine is currently recommended as the first-line medication for patients. Owing to its wide range of action, oral pilocarpine users may experience several negative side effects. The purpose of this study was to increase the duration of action and prevent systemic side effects of pilocarpine hydrochloride by designing and evaluating prolonged-release formulations of the drug using either xanthan gum, hydroxyethyl cellulose, or a combination of these two natural polymers buccal bioadhesive films.

Material and Methods: The films were analysed for their physicochemical, mechanical, bioadhesive, swelling, in vitro release, and in vitro cytotoxicity.

Results: Physicochemical and mechanical feature examinations revealed that the Xanthan-HEC combinations showed better results compared to the single polymer-used formulations. The *in vitro* dissolving profiles of all the optimal formulations showed a sustained release pattern with a steady-state plateau after an initial fast release. Using various release kinetic models to assess drug release kinetics revealed that the Higuchi and Korsmeyer-Peppas correlations are primarily followed by drug release from buccal films.

Conclusion: The findings show that the mucoadhesive buccal formulation is a viable method for pilocarpine localised distribution that is both safe and effective in treating xerostomia. Further in vivo studies are planned to assess the pharmacokinetic and histopathological effects of the formulation.

Keywords: Buccal delivery, buccal film, pilocarpine hydrochloride, sialogogues, xerostomia

ÖZ

Amaç: Tükürük salgısı hem ağız içi fonksiyonları hem de diş sağlığını korumaktadır. Bu nedenle ağız kuruluğu hissi ile karakterize olan kserostomi durumu hastanın yaşam kalitesini olumsuz yönde etkilemektedir. Sialagoglar gibi palyatif tedaviler, kserostominin tedavisinin temelini oluşturmaktadır. Mevcut hastalar için birinci basamak ilaç tedavisi olarak, Amerikan ilaç ve Gıda Dairesi tarafından onaylı pilokarpin HCl önerilmektedir. Oral yoldan pilokarpin kullanımı, geniş etki yelpazesi nedeniyle çeşitli olumsuz yan etkinin ortaya çıkmasına neden olabilmektedir. Çalışmanın amacı, pilokarpin hidroklorürün etki süresini arttırmak ve sistemik yan etkilerini önlemek için; ksantan sakızı, hidroksietil selüloz veya bu iki doğal polimerin kombinasyonunun kullanıldığı bukkal biyoadhezif filmlerinin hazırlanması ve ilacın uzun süreli salınımını sağlayan formülasyonlarının tasarlanıp değerlendirilmesidir.

Gereç ve Yöntemler: Bukkal filmler; fizikokimyasal, mekanik, biyoadheziflik, şişme, *in vitro* salım ve *in vitro* sitotoksisite açısından analiz edimiştir.

Bulgular: Fizikokimyasal ve mekanik özellikler Ksantan-HEC kombinasyonlarının tek polimerin kullanıldığı formülasyonlara göre daha iyi sonuçlar verdiğini göstermiştir. Tüm optimal formülasyonların *in vitro* çözünürlük profilleri, başlangıçtaki hızlı salımdan sonra kararlı durum platosuyla birlikte sürekli salım modeli göstermiştir. İlaç salım kinetiğini değerlendirmek için kullanılan çeşitli salım kinetik modelleri öncelikli Higuchi ve Korsmeyer-Peppas korelasyonlarının gerçekleştiğini bukkal filmlerden ilaç salımının takip ettiğini göstermiştir.

Sonuç: Bulgular mukoadezif bukkal formülasyonun lokalize dağılımı için pilokarpinin kserostomi tedavisinde hem güvenli hem de etkili olarak uygulanabilir bir yöntem olduğunu göstermektedir. İleri çalışmalar olarak formülasyonun farmakokinetik ve histopatolojik etkilerini değerlendirmek için in vivo deneyler planlanmaktadır.

Anahtar kelimeler: Bukkal uygulama, bukkal film, pilokarpin hidroklorür, sialagog, kserostomi

Corresponding Author/Sorumlu Yazar: Ayca YILDIZ PEKÖZ **E-mail:** aycay@istanbul.edu.tr

Submitted/Başvuru: 17.07.2024 • **Revision Requested/Revizyon Talebi:** 26.09.2024 • **Last Revision Received/Son Revizyon:** 03.10.2024 • **Accepted/Kabul:** 28.10.2024 • **Published Online/Online Yayın:** 24.01.2025

INTRODUCTION

Saliva is a protective factor for oral health and provides support for crucial oral functions as a digestion lubricant and buffering agent that modulates the pH levels in the mouth (1). Therefore, any kind of salivary dysfunction such as "xerostomia", generally used to describe the sense of dryness felt in the mouth (2). Currently, pilocarpine is indicated as the first-line drug for patients with radiation-induced xerostomia and Sjögren's syndrome (1). Due to its broad range of activity, various adverse effects , i.e. flushing, sweating, and nausea can be seen in oral pilocarpine users. Furthermore, the oral pilocarpine's stimulation effect is relatively short with a 5 to 10 mg dosage and a 3/4 times daily administration, which has a negative impact on the patient adherence to the therapy regime (3). Localised buccal drug delivery system increases the patient's compliance with ease of administration, reduced dose frequency, high drug accumulation, and prolonged residence time at the target site (3).

In the frame of this knowledge, the study aimed to design and *in vitro* characterise prolonged-release formulation of pilocarpine hydrochloride buccal bioadhesive films using either xanthan gum, hydroxyethyl cellulose, or a combination of these two natural polymers to increase the duration of action and extend the release duration, which may prevent systemic side effects of pilocarpine.

MATERIALS AND METHODS

Compounds and reagents

Pilocarpine Hydrochloride was kindly provided by the Bilim Pharmaceutical Company, Türkiye. Hydroxyethyl Cellulose (HEC; 250000 Mw, 145 mPas, 1% in H_2O/ 20°C) was purchased from Sigma-Aldrich (Darmstadt, Germany) and the Xanthan gum (1000-1400 mPas, 1% in H_2O/ 20°C) was purchased from the Doga Drug Company (Istanbul, Turkey). All other reagents were of analytical grade.

Preparation of the bioadhesive mucosal buccal films

Buccal films of the pilocarpine hydrochloride were prepared using the solvent casting technique. Various amounts of each matrix polymer were added to the required amount of water and left overnight under magnetic stirring to allow the polymers to obtain a bubble-free polymer dispersion. Pilocarpine hydrochloride (0.04% w/w) was dissolved in distilled water. As a penetration enhancer and a plasticising agent, propylene glycol (10% w/w) was added to the pilocarpine solution. Then, this mixture was added to the polymer dispersion and stirred on a magnetic stirrer until a homogeneous mixture was obtained. Afterwards, the homogenised gel was cast onto a glass petri dish (*r*=9 cm) and left to dry at 40 °C in a hot air oven (Nüve EN 40, Turkey) until a formed dry film was obtained. After drying, the films were stored in a desiccator at room temperature with 40% relative humidity for further experiments (4, 5).

Characterisation of Pilocarpine Hydrochloride Films

Physical appearance

The films' physical characteristics, including colour (visual inspection), transparency, softness, peelability (removal of the film from the Petri dish after drying), and homogeneity were visually examined (6).

Initial polymer solution viscosity measurement

The RV6 spindle Brookfield viscometer (Brookfield DV2, USA) was used to take measurement of the matrix polymers' viscosity were used to assess the formulations' pourability and spreadability (5).

Weigh uniformity and film thickness

The films were cut with a diameter of 1 cm from six different regions. The weight of the individual cut films was recorded, and the average and standard deviation of the film weight were calculated. A manual digital micrometre (QLR digit, IP4, PRC) was used to gauge the thickness of the produced films, and the samples for the thickness measurement were chosen similarly to the weight variation analysis (7).

Folding endurance

For each formulation, a little 4-4 cm strip of film was taken and folded in the same spot repeatedly creating an angle of 180° until it broke. The folding endurance of a film was measured by the number of times it could be folded in the same spot without tear (n=3) (8).

Surface pH

To measure the film's surface pH, it was allowed to swell in a glass Petri dish for 1 h while in contact with 10 mL of simulated saliva fluid (SSF) (KH_2PO_4 12 mM, NaCl 40 mM, $CaCl_2$ 1,5 mM adjusted with H_3PO_4 to pH 6.75). Using a pH meter, the surface pH was measured by placing a glass electrode close to the film's surface for one minute (n=3) (9).

Drug content and content uniformity

The buccal films were cut with a diameter of 0,6 cm and dissolved in 15 mL SSF in an orbital shaker (IKA, HS501, Germany) with the rotation speed adjusted to 200 rpm for 4 h. Then, the collected samples were filtered through membrane filters. The drug content of the films was quantified by the developed HPLC method (LC-20 AT model, Shimadzu, Japan). The chromatographic separation was accomplished on a C18 column (150x4,6mm, 5µm: Shim-pack VP-ODS, Shimadzu, Japan). HPLC was performed using an isocratic gradient; a mobile phase of 25:75 [MeOH: Buffer (10 µM sodium hexane sulphonate, 0.2% v/v Trimethylamine pH adjusted to 2.8 with o-phosphoric acid) at a flow rate of 1 mL/min. The pilocarpine hydrochloride peak was detected by a photodiode array detector at 214 nm (n=3, α=0.05) (10).

Measurement of Mechanical Properties

Mechanical properties

The mechanical properties of the pilocarpine films were measured using a texture profile analyser (TA.XT Plus, Stable Micro Systems, Surrey, UK) equipped with a 5-kg load cell (11). The tensile strength and elongation at break will be calculated as shown in the Equations;

$$\text{Eq1: Tensile Strength } (mm^2) = \frac{\text{Breaking Force (N)}}{\text{Initial Cross} - \text{Sectional Area of Sample } (mm^2)}$$

$$\text{Eq 2: Elongation at Break } (\%) = \left(\frac{\text{Increase in lenght at breaking point(mm)}}{\text{initial lenght}}\right) x100$$

***In vitro* bioadhesion studies**

The measurement was carried out using a texture analyser that was outfitted with a bioadhesion test rig and a 500 g load cell. The bioadhesive properties of the formulations were determined using bovine buccal mucosal tissue obtained from a local slaughterhouse. Mucosal membrane sections were attached to the holder of the texture analyser at 37±0.5°C. Formulations were placed at the lower end of the probe and the probe was lowered onto the bladder mucosa surface at a constant speed (1 mm/s). The contact force (0.05 N) was applied for 2 min. The area under the curve (mucoadhesion) was determined from the resultant force–distance graph (n=6) (12).

Swelling studies

A portion of each mucosal dosage film was divided into portions of 4 cm^2 (2x2 cm) and cut, placed in a stainless steel wire mesh, and the total weight (W1) was measured. Then, the mixture was submerged into a beaker containing 20 mL SSF at pH 6.75 (13). The samples were measured at predetermined periods (5, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 300 and 360 min). The samples were carefully withdrawn from the medium and the excess surface water was wiped off with filter paper and weighed (W2). After the experiment, the swollen films were dried at 60 °C for 24 h and kept in desiccators for over 48 h, and after they fully dried, the weighing was repeated (W3). These experiments were performed in triplicate (n=3, α =0,05) (5). The percentage of hydration and matrix erosion were calculated using the following equations (Eq.3-4):

$$\text{Eq 3: } \%\ of\ Hydration = \frac{W2-W1}{W2} X100$$

$$\text{Eq 4: } \%\ of\ Matrix\ Erosion = \frac{W1-W3}{W1} X100$$

***In Vitro* Drug-Release Studies**

***In vitro* drug-dissolution studies**

The PL release studies were assessed with a design closely similar to the USP 23 dissolution test apparatus 5 (paddle over disk) method using a dissolution tester (SOTAX, AT 7 Smart V230, Switzerland). The dissolution medium comprised 900 ml of SSF at 37±0.5 °C and paddle rotation speed was adjusted to 50 rpm. The buccal films were cut with a diameter of 2 cm and were fixed in a glass slide with a self-fabricated basket (50 mm diameter and 6 mm height) made from stainless steel with a sieve opening of approximately 850 µm (size No. 20, USP 23). The basket containing the sample was submerged in the dissolution medium. The manually collected samples at intervals of 0, 5, 1, 2, 3, 4, 5 and 6 h. were filtered through a 0.45 µM Millex syringe-driven filter (Millipore Cooperation, Bedford MA, USA) and quantified by HPLC (n=3). The samples were replaced with an equal volume of SSF maintained at the same temperature (14).

Drug Release Mechanisms

Based on the *in vitro* release data of the film formulations, four kinetic models with their corresponding relationships were constructed, as shown in Table 1 (15-17). The kinetic models' associated mathematical equations are shown in Eqs. (5)–(8) below;

Table 1: Various plots with corresponding kinetic mechanisms used to evaluate the in vitro dissolution data

Plot parameters	Kinetic Model	Release Dependency
Cumulative percentage drug release vs. time	Zero Order	The rate of drug release is independent of its concentration.
Log cumulative of percentage drug remaining vs Time	First Order	The rate of drug release is dependent on its concentration.
Cumulative percentage drug release vs. square root of time	Higuchi	Drug release through a matrix via diffusion based on Fick's law, which is square root time-dependent
Log cumulative percent drug release vs log of time	Korsmeyer-Peppas	The release regime depends on the "n" exponent value

Zero order; $Q_t = Q^0 + K_0 t$

Where Q_0 is the initial amount of the drug, Q_t is the cumulative amount of the drug released time *(t),* K_0 is the zero-order rate constant, and t is time in minutes.

$$Log\ Q_t = LogQ_0 + {K_1 t}/{2.303}$$

Where Q_0 is the initial amount of the drug, Q_t is the cumulative amount of the drug released time (*t*), K_1 is the first-order rate constant, and *t* is time in minutes.

Higuchi; $Q = K_H t^{1/2}$

where Q is the cumulative amount of drug released in time *(t),* K_H is the Higuchi release rate constant, and *t* is time in minutes.

Korsmeyer–Peppas;

$$F = (M_t/M) = K_p t^n$$

where F is the fraction of the drug released in time *(t), Mt* is the amount of the drug released at time *(t), M* is the total amount of the drug in the dosage form, K_p is the release rate constant, *n* is the diffusion or release exponent, *t* is the time in minutes, *'n'* is estimated from the linear regression of log *(Mt/M)* versus log *t.*

***In vitro* cell viability assay**

The tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on Caco-2 was used

to evaluate the cytotoxicity of pure Pilocarpine/Xanthan/HEC and selected buccal film formulations. Caco-2 cells (ATCC® HTB-37TM) are adherent cells derived from the human colorectal adenocarcinoma. Cells were incubated in Eagle's Minimum Essential Medium supplemented with 5% foetal bovine serum, 100 IU/mL penicillin, 100 µg/mL streptomycin. The cells were grown in an incubator used for cell culture (Thermo Scientific, Hessen, Germany) at 37°C, 5% CO2. After attaining 80%–85% confluence, the cells were harvested. The cells (Caco-2, $4x10^4$ cells/mL) were seeded in a sterile, flat-bottomed 96-well tissue culture plate. After 24 h, the cells (except those in the control wells) were exposed to either pure Pilocarpine/Xanthan/HEC or buccal film formulations in the cell culture medium and incubated for 24 h. MTT stock solution (5 mg/mL) was prepared and 30 µL of the solution was added to each well and the plate was incubated for a further 4 h at 37 °C, 5% CO_2. At the end of the incubation time, formazan crystals formed by the mitochondrial dehydrogenase reduction activity were dissolved by adding 100 µL DMSO to each well. The optical density was measured on a Multiscan EX Micro-plate reader (Thermo Scientific, Essex, UK) at 570 nm. The obtained results were expressed as percentage inhibition relative to the control cells in which cell survival was taken as 100 %. The experiments were performed in triplicate (18).

Statistical analysis

In-vitro data obtained from each experiment will be subjected to statistical analysis using a computer programme, GraphPad-Prism 9.0 software, for a one-way analysis of variance (ANOVA) followed by the Newman–Keuls multiple comparisons test. $P<0.05$ will be considered to be indicative of significance.

RESULTS

Preparation of the bioadhesive mucosal buccal films

Table 2 displays the preliminary formulation details of the pilocarpine hydrochloride buccal films.

Characterisation of pilocarpine hydrochloride films

The weight of the drug-loaded buccal film formulations was determined using electronic balance, and the average weight ±SD of films was given in Table 3.

The polymer type and viscosity are responsible for the variance in the thickness (Table 3). Each film's pH was measured to be between 6.0 and 6.5, which is within the normal range for salivary pH (Table 3) (19). The folding endurance was increased with the increased amount of XG. All the films showed a good value of folding endurance, which is above 300 times/per film.

An HPLC method for the quantification of PL in the buccal film formulation was developed and validated. The determination correlation coefficient (r^2) was found to be 0.9997 with the linear regression equation. The limits of detection and quantification were determined as 0.162 and 0.256 µg/mL, respecti-

Table 2: Formulation components of the buccal film formulations

Formulation	PL(%)	HEC(%)	XG(%)	PG(%)	DW
F1	0.04	2.5	-	10	q.s.
F2	0.04	-	2.8	10	q.s.
F3	0.04	1.25	1.4	10	q.s.
F4	0.04	1.25	2.8	10	q.s.
F5	0.04	1.25	4.2	10	q.s.
F6	0.04	1.25	5.6	10	q.s.
F7	0.04	2.5	1.4	10	q.s.
F8	0.04	3.75	1.4	10	q.s.
F9	0.04	5.0	1.4	10	q.s.

(% w/w), PL: Pilocarpine, HEC: Hydroxyethyl Cellulose, XG: Xanthan Gum, PG: Propylene Glycol, DW: Distilled water

Table 3: Physicochemical characteristics of the prepared buccal films of PL

Formulations	Weigh (mg)	Thickness (mm)	Folding endurance (time/film)	Surface pH	Drug content (%)
F1	40.89±0.67	0.040±0.008	332±12	6.17±0.05	96.45±1.01
F2	56.64±0.54	0.056±0.002	315±11	6.23±0.08	97.75±1.02
F3	41.56±0.78	0.037±0.005	324±06	6.35±0.06	97.04±1.54
F6	58.21±1.12	0.045±0.006	321±04	6.45±0.05	98.77±1.03
F9	42.38±0.84	0.039±0.002	328±08	6.51±0.09	98.54±1.26

All values are expressed as mean ±S.D; n=6.
[a] percentage of the drug concerning the film weight.

Table 4: Mechanical properties of the prepared Bioadhesive buccal films of PL

Formulations	Tensile Strength (N/cm^2)	Elongation at the break (%)	Work of adhesion (mJ/ cm^2)
F1	1.659±0.154	4.260±0.089	4.016±0.026
F2	3.956±0.148	1.524±0.015	1.523±0.036
F3	2.827±0.295	3.260±0.024	3.625±0.049
F6	4.154±0.389	2.019±0.056	1.756±0.056
F9	1.896±0.256	5.126±0.045	5.260±0.025

All values are expressed as mean ±S.D; n=3.

vely. In pharmaceutical formulation content uniformity, one of the most important characteristics to guarantee the presence and consistency of the drug is the film's formulation (20). The percent drug content in the films was found to be between 96.45±1.01 and 98.77±1.03%. The data shows uniform distribution (Table 3). It can be concluded that the content homogeneity was not affected by the polymer or the polymer ratio.

Measurement of the mechanical properties

The Texture Profile Analyser was used to assess the mechanical characteristics of the buccal films based on Xanthan gum and HEC. Table 4 displays the findings of our measurements for the tensile strength, elongation at break, *and in vitro* bioadhesion work.

The mechanical strength of the films prepared with HEC varied between 1.659±0.154 and 1.896±0.256 N/cm^2, whereas xanthan gum contributed a greater mechanical strength to the films (3.956±0.148-4.154±0.389 N/cm^2). The results of the *in vitro* mucoadhesion study using PL buccal film formulations are given in Table 4.

Swelling studies

A correlation has been demonstrated between the swelling index and the mucoadhesive strength (Figures 1, 2) (21)-sufficient swelling guarantees that the polymer chains unfold and establish a connection with the buccal mucosa. The percentage hydration of the drug-loaded films (F1–F9) was measured for 6 h. The findings are shown in Figure 1. Notably, a steep slope during this phase indicates that the hydration rate was rapid in the first hour (Figure 1).

Comparisons between the films' initial and final weights upon immersion in simulated saliva were used to calculate the matrix erosion (%) profiles shown in Figure 2. Comparing the HEC-based formulation to the xanthan-based films revealed a substantial ($p \leq 0.05$) increase in matrix erosion (per cent), and the ratio of matrix erosion increased in direct proportion to the amount of HEC present (Figure 2).

***In vitro* drug-release studies**

In terms of the release profile (Figure 3), PL release ranged between 59.15±2.69% and 72.09±0.09% in the first 30 min in the HEC-based films (Figure 3). At 2 h, the release profile reached its maximum level (87.67±0.85 to 79.99±6.56%).

Within the first 30 minutes, 39.92±4.98% of PL was released

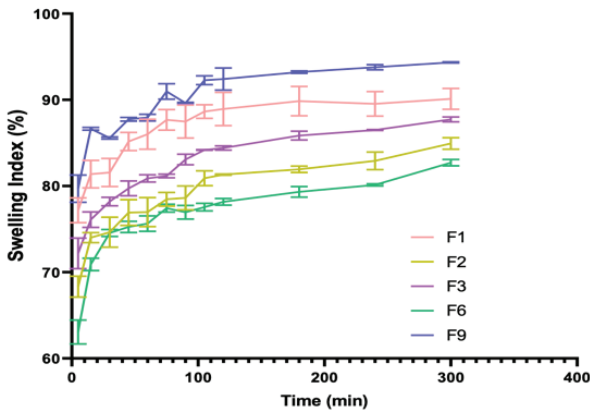

Figure 1: Swelling index of the mucoadhesive films
All values are mean ±SD, n=6

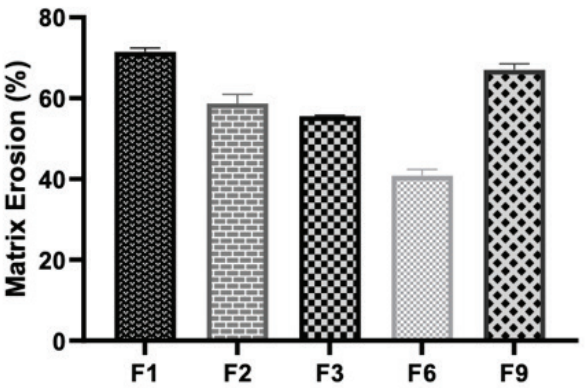

Figure 2: Matrix erosion of the mucoadhesive films
All values are mean ±SD, n=6

from the buccal film F3. The release profile reached its maximum level after 4 h (89.49±9.10%), and the release level was maintained until 6 h. Table 5 summarises the release parameters that were acquired through the process of fitting the experimental dissolution release data to the several kinetic equations that were assessed (22).

In our study, the xanthan-based films F2 and F6 fulfilled both the Higuchi and Korsmeyer–Peppas correlations (Table 5) (15). In the Higuchi model, the xanthan-based film exhibited the

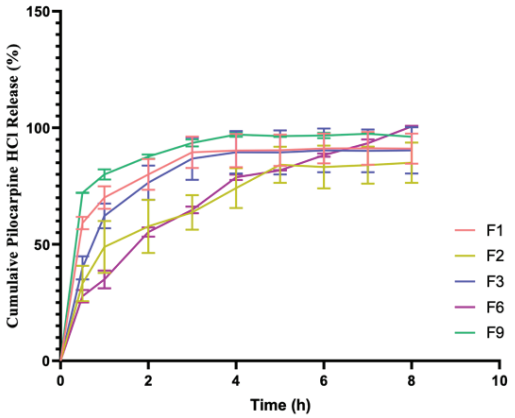

Figure 3: Pilocarpine hydrochloride release from the bioadhesive films over 6 h. Each film formulation contained the same amount of PL (10 mg) as the active agent.
All values are mean ±SD, n=6

Table 5: Estimated values of pilocarpine-release determination coefficient (r^2) and the Korsmeyer-Peppas kinetic model release exponent (n) for the buccal film formulations

Formulations	Zero-order Kinetics (r^2)	First-order Kinetics (r^2)	Higuchi Kinetics (r^2)	Korsmeyer-Peppas Kinetics (r^2.n)
F1	0.6871	0.6860	0.7819	0.8055 0.3281
F2	0.6372	0.8781	0.9909	0.9909 0.5768
F3	0.7259	0.7317	0.8475	0.9723 0.5129
F6	0.6871	0.8998	0.9905	0.9974 0.6178
F9	0.6998	0.7014	0.8114	0.8375 0.3425

best linear correlation, with an r^2 value of 0.9909 and 0.9905, respectively. In the Korsmeyer–Peppas model, the n-value of F2 was 0.5768 with a linear correlation of 2r ¼ 0.9909 while F6 was with an r^2 value of 0.6178 with a linear correlation of 2r ¼ 0.9974 whereas the combination of the polymers (F3) had an r^2 value of 0.5129 with a linear correlation of 2r ¼ 0.9723, indicating non-Fickian behaviour 40,52.

***In vitro* cell viability assay**

The effective and safe use of polymers as drug carriers in humans depends on several aspects, with toxicity being a key consideration (22). MTT was used in this investigation to assess the short-term cytotoxic effects of the chosen buccal film formulations. Based on the metabolically active cells reducing MTT to a coloured formazan product, the assay can be measured using spectrophotometry (23). The study formulations exhibited no cytotoxic impact and PL presented no physical risks to the endothelial cells. Additional research is required to shed light on the entire toxicological profile.

DISCUSSION

Because of its superior rheological characteristics, XG was employed as a thickening agent and mucoadhesive controlled-release excipient for the buccal formulations (24) along with HEC. Depending on the type, content, and temperature of the polymer, HEC is dissolved under different conditions to produce transparent solutions with different viscosities. With increasing temperature, the viscosity of the solution reduces (25). In addition to these mucoadhesive polymers/combinations, propylene glycol (PG) was also chosen as a hydrophilic permeation enhancer to improve drug partition into the mucosa to solubilise the drug and act as a plasticiser.

The thickness of the films was directly related to the dose accuracy, and the optimal thickness was necessary for adequate bioadhesion, whereby increased thickness reduces the mucoadhesion capability of the films (8). Each film's pH was measured to be between 6.0 and 6.5, which is within the normal range for salivary pH (Table 3) (19). The folding endurance was increased with the increased amount of XG. All the films showed a good value of folding endurance, which was above 300 times/per film. Mechanical studies were performed, where the tensile strength is the stretching force given to the sample at the time of breakage, while the elongation at break is the maximum change in the length of the polymeric film before breaking (6). These findings showed that films prepared with xanthan gum were more resilient to tension stress and underwent a plastic deformation, which is why the elongation at break happened

faster and earlier. Because the xanthan-based formulation has a smaller area under the force-distance curve than the HEC-based formulation, its work of adhesion is also lower. The HEC-based formulation, in contrast, exhibited an elastic deformation and more lasting elongation at break. It has a far wider region beneath the force-distance curve. As a result, the HEC-based formulation requires more force to adhere to it (26).

For the release studies, as the HEC concentration rose from 2.5% to 5%, the adhesiveness of the HEC films increased. The literature has demonstrated that when the concentration of polymer in the formulation increases, so does the work of adhesion value, which is compatible with our findings (27). For the film swelling, water entering the matrix allows drug molecules to diffuse out of the film and become available for mucosal penetration in the interim. As it may facilitate rapid film mucoadhesion with the buccal mucosa, rapid hydration is important during the first phase. It is assumed that HEC causes this quick hydration. However, after that, in the first phase, the hydration remained largely unchanged during the analysis. Moreover, there was no distortion or erosion, and the films maintained sufficient physical integrity until the completion of the experiment (9). The findings imply that greater matrix erosion may occur from the HEC-based films' with comparatively high swelling index. Lower matrix erosion was shown by the xanthan-based films, and this was correlated with a lower swelling index. PL release and HEC concentration were inversely correlated. The observed change in the viscosity of the polymer due to rapid hydration and the gelation process that affects the rate of drug dissolution could be the cause of this observation (5). In contrast, when xanthan and HEC were used together, the PL release was maintained more sustainably. The development of a thick gel layer that raised the viscosity of the polymeric film may have contributed to the decreased *in vitro* release during the first 4 h. This observation aligns with the findings of Akash et al., who suggested that thick gel formation and a dry interior core could cause a delay in drug release from xanthan films (28). Additionally, the drug's in-vitro release behaviour is impacted by the high viscosity of xanthan gum (29). This result also implies that PL partially diffuses with increasing diffusion path length across the gradually expanding hydrated matrix and the inflated polymer matrix. The phenomenon known as anomalous or non-Fickian diffusion is noted when the rates of liquid diffusion and polymer relaxation are equally large (30). However, according to various *in vitro* release models, the HEC-based films F1 and F9 were deemed inadequate and did not follow the Higuchi kinetics.

CONCLUSION

Hyposalivation can be caused by either long- or short-term cases that may be triggered by auto-immune diseases such as Sjögren syndrome, psychologic complications stimulated by stress, or radiotherapy of the neck and head region, which in turn may cause a syndrome named xerostomia. The objective of this study was to formulate a long-acting buccal dosage form to minimise the side effects and develop an alternative route of administration. Based on the results, it can be concluded that the mucoadhesive buccal formulation is a potential approach for effective as well as safe localised delivery of pilocarpine to treat xerostomia.

Data Availability: Data may be made available upon reasonable request to the corresponding author of the study. However, it must comply with the applicable legal restrictions.

Ethics Committee Approval: This manuscript does not contain any experiment that requires ethics committee approval.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- A.Y.P.; Data Acquisition- Ö.A.D., S.Y.K., N.S.B.; Data Analysis/Interpretation- A.Y.P., Ö.A.D.; Drafting Manuscript- Ö.A.D., N.S.B.; Critical Revision of Manuscript- A.Y.P.; Final Approval and Accountability- A.Y.P.; Material and Technical Support- Ö.A.D., S.Y.K., N.S.B.; Supervision- A.Y.P.

Conflict of Interest: The authors declare that there is no conflict of interest.

Financial Disclosure: This work was supported by Scientific Research Projects Coordination Unit of Istanbul University (Project number 54173).

REFERENCES

1. Salum FG, Medella-Junior FAC, Figueiredo MAZ, Cherubini K. Salivary hypofunction: An update on therapeutic strategies. Gerodontology 2018;35(4):305-16.
2. Nakagawa Y. Management of dry mouth in Sjögren's syndrome. Japanese Dental Science Review 2011;47(2):115-23.
3. Malallah OS, Garcia CMA, Proctor GB, Forbes B, Royall PG. Buccal drug delivery technologies for patient-centred treatment of radiation-induced xerostomia (dry mouth). Int J Pharm 2018;541(1-2):157-66.
4. Shipp L, Liu F, Kerai-Varsani L, Okwuosa TC. Buccal films: A review of therapeutic opportunities, formulations & relevant evaluation approaches. J Controlled Release 2022;352:1071-92.
5. Yildiz Pekoz A, Sedef Erdal M, Okyar A, Ocak M, Tekeli F, Kaptan E, et al. Preparation and in-vivo evaluation of dimenhydrinate buccal mucoadhesive films with enhanced bioavailability. Drug Dev Ind Pharm 2016;42(6):916-25.
6. Dinte E, Muntean DM, Andrei V, Boşca BA, Dudescu CM, Barbu-Tudoran L, et al. In Vitro and In Vivo Characterisation of a Mucoadhesive Buccal Film Loaded with Doxycycline Hyclate for Topical Application in Periodontitis. Pharmaceutics 2023;15(2):580.
7. Rençber S, Karavana SY, Yilmaz FF, Eraç B, Nenni M, Gurer-Orhan H, et al. Formulation and evaluation of fluconazole loaded oral strips for local treatment of oral candidiasis. J Drug Delivery Sci Tech 2019;49:615-21.
8. Nafee NA, Ismail FA, Boraie NA, Mortada LM. Mucoadhesive buccal patches of miconazole nitrate: in vitro/in vivo performance and effect of ageing. Int J Pharmaceutics 2003;264(1):1-14.

9. Nair AB, Al-Dhubiab BE, Shah J, Vimal P, Attimarad M, Harsha S. Development and evaluation of palonosetron loaded mucoadhesive buccal films. J Drug Delivery Sci Tech 2018;47:351-8.
10. Desai SD, Blanchard J. A Simplified and Rapid High-Performance Liquid Chromatographic Assay for Pilocarpine Hydrochloride*. J Chromatographic Sci 1992;30(4):149-52.
11. Kalapathy U, Proctor A, Shultz J. Production and properties of flexible sodium silicate films from rice hull ash silica. Bioresource Tech 2000;72(2):99-106.
12. Coşkunmeriç N, Üstündağ Okur N, Okur ME, Ayla Ş, Yoltaş A, Karavana SY. Promising nanogels loaded with usnic acid for oral ulcer treatment: development, characterization, and in vivo evaluation in rabbits. Pharmaceutical Development Tech 2021;26(4):431-43.
13. Peh KK, Wong CF. Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. J Pharm Pharm Sci 1999;2(2):53-61.
14. Arslan D, Akbal Dağıstan Ö, Sagirli O, Mulazimoglu L, Cevher E, Yildiz-Pekoz A. Development and Evaluation of Combined Effect Buccal Films for Treatment of Oral Candidiasis. AAPS PharmSciTech 2022;24(1):23.
15. Higuchi T. Rate of Release of Medicaments from Ointment Bases Containing Drugs in Suspension. J Pharmaceutical Sci 1961;50(10):874-5.
16. Korsmeyer RW, Gurny R, Doelker E, Buri P, Peppas NA. Mechanisms of potassium chloride release from compressed, hydrophilic, polymeric matrices: effect of entrapped air. J Pharm Sci 1983;72(10):1189-91.
17. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers. Pharm Acta Helv 1985;60(4):110-1.
18. Nazlı H, Mesut B, Akbal-Dağıstan Ö, Özsoy Y. A Novel Semi-Solid Self-Emulsifying Formulation of Aprepitant for Oral Delivery: An In Vitro Evaluation. Pharmaceutics 2023;15(5).
19. Abu-Huwaij R, Assaf S, Salem M, Sallam A. Mucoadhesive Dosage form of Lidocaine Hydrochloride: I. Mucoadhesive and Physicochemical Characterization. Drug Development and Industrial Pharmacy 2007;33(8):855-64.
20. Nair AB, Kumria R, Harsha S, Attimarad M, Al-Dhubiab BE, Alhaider IA. In vitro techniques to evaluate buccal films. J Control Release 2013;166(1):10-21.
21. Nair AB, Al-ghannam AA, Al-dhubiab BE, Hasan AA. Mucoadhesive Film Embedded with Acyclovir Loaded Biopolymeric Nanoparticles: In vitro Studies. J Young Pharmacists 2017;9:100-5.
22. Khan S, Trivedi V, Boateng J. Functional physico-chemical, ex vivo permeation and cell viability characterization of omeprazole loaded buccal films for paediatric drug delivery. Int J Pharmaceutics 2016;500(1):217-26.
23. Weyermann J, Lochmann D, Zimmer A. A practical note on the use of cytotoxicity assays. Int J Pharm 2005;288(2):369-76.
24. Singh M, Tiwary AK, Kaur G. Investigations on interpolymer complexes of cationic guar gum and xanthan gum for formulation of bioadhesive films. Res Pharm Sci 2010;5(2):79-87.
25. Kapoor D, Maheshwari R, Verma K, Sharma S, Ghode P, Tekade RK. Chapter 14 - Coating technologies in pharmaceutical product development. In: Tekade RK, editor. Drug Delivery Systems: Academic Press; 2020. p. 665-719.
26. Pamlényi K, Kristó K, Sovány T, Regdon G, Jr. Development and evaluation of bioadhesive buccal films based on sodium alginate for allergy therapy. Heliyon 2022;8(8):10364.
27. Ghosh S, Seelbinder B, Henderson JT, Watts RD, Scott AK, Veress AI, et al. Deformation Microscopy for Dynamic Intracellular and Intranuclear Mapping of Mechanics with High Spatiotemporal Resolution. Cell Rep 2019;27(5):1607-20.4.
28. Akash MSH, Khan IU, Shah SNH, Asghar S, Massud A, Qadir M, et al. Sustained release hydrophilic matrices based on xanthan gum and hydroxypropyl methylcellulose: development, optimization, in vitro and in vivo evaluation. J Applied Pharm 2010;2:89-103.
29. Jadav M, Pooja D, Adams DJ, Kulhari H. Advances in Xanthan Gum-Based Systems for the Delivery of Therapeutic Agents. Pharmaceutics 2023;15(2).
30. Perioli L, Ambrogi V, Angelici F, Ricci M, Giovagnoli S, Capuccella M, et al. Development of mucoadhesive patches for buccal administration of ibuprofen. J Control Release 2004;99(1):73-82.