Infarma CIÊNCIAS FARMACÊUTICAS
e-ISSN: 2318-9312
Vol.: 38, ano 2026
e202613
Original

Thermoresponsive Hydrogels for Buccal Eugenol Delivery

Hidrogéis termorresponsivos para liberação bucal de eugenol

doi: 10.14450/infarma.e202613
Recebido em: 17/4/2026
Aceito em: 5/8/2026

Elaine Ferreira[1]; Patricia Mazureki Campos[2]; Paulo Vitor Farago[2]
[1]University Center UGV; [2]Universidade Estadual de Ponta Grossa
e-mail: patriciamazurekicampos@yahoo.com.br

ABSTRACT

The action of drugs on the oral mucosa can be improved through delivery systems that provide longer local residence time for the treatment of diseases or procedures in the oral cavity. The aim of this study was to develop poloxamer-chitosan hydrogel formulations containing eugenol, based on poloxamer 407 20% (w/w) and chitosan 1% (w/w), namely: poloxamer-hydrogel (HP), poloxamer-chitosan hydrogel (HPQ), poloxamer-chitosan hydrogel with eugenol 1% (w/w) (HPQEU1); poloxamer-chitosan hydrogel with eugenol 2% (w/w) (HPQEU2); and poloxamer-chitosan hydrogel with eugenol 4% (w/w) (HPQEU4). The characterization were made by sol/gel transition (Tsol/gel), pH, spreadability, rheology and texture, turbidimetry, drug quantification by UV-Vis spectrophotometry and stability test. The Tsol/gel were 29.33 ± 0.57, 29.66 ± 0.7, 25.33 ± 0.57, 19.33 ± 0.57 °C for HP, HPQ, HPQEU1, HPQEU2, respectively. Hydrogels with chitosan exhibited pH of 5.3. HPQEU1 and HPQEU2 showed elastic behavior, spreadability and texture profile suitable for oral application. The stability study evidenced storage at 5 ± 2 °C as favorable. HPQEU1, HPQEU2 and HPQEU4 were able to adhere to the mucosa evidenced by *ex vivo* pig cheek model. Thus, thermoresponsive hydrogels may constitute effective alternatives for localized delivery of eugenol to the oral mucosa, a remarkable feature in dental clinical practices.

Keywords: Drug delivery system. Oral mucosa. Biopolymers. Local administration. Dentistry.

RESUMO

A ação de fármacos na mucosa oral pode ser aprimorada por meio de sistemas de liberação que proporcionem maior tempo de permanência local para o tratamento de doenças ou procedimentos na cavidade oral. O objetivo deste estudo foi desenvolver formulações de hidrogel de poloxamer-quitosana contendo eugenol, baseadas em poloxamer 407 20% (p/p) e quitosana 1% (p/p), a saber: hidrogel de poloxâmer (HP), hidrogel de poloxamer-quitosana (HPQ), hidrogel de poloxamer-quitosana com eugenol 1% (p/p) (HPQEU1); hidrogel de poloxamer-quitosana com eugenol 2% (p/p) (HPQEU2); e hidrogel de poloxamer-quitosana com eugenol 4% (p/p) (HPQEU4). A caracterização foi realizada por meio da transição sol/gel (Tsol/gel), pH, espalhabilidade, reologia e textura, turbidimetria, quantificação do fármaco por espectrofotometria UV-Vis e teste de estabilidade. Os valores de Tsol/gel foram de 29,33 ± 0,57, 29,66 ± 0,7, 25,33 ± 0,57 e 19,33 ± 0,57 °C para HP, HPQ, HPQEU1 e HPQEU2, respectivamente. Os hidrogéis com quitosana apresentaram pH de 5,3. HPQEU1 e HPQEU2 demonstraram comportamento elástico, espalhabilidade e perfil de textura adequados para aplicação oral. O estudo de estabilidade evidenciou que o armazenamento a 5 ± 2 °C é

favorável. HPQEU1, HPQEU2 e HPQEU4 foram capazes de aderir à mucosa, conforme demonstrado pelo modelo *ex vivo* em bochecha de porco. Assim, os hidrogéis termossensíveis podem constituir alternativas eficazes para a administração localizada de eugenol na mucosa oral, uma característica notável nas práticas clínicas odontológicas.

Palavras-chave: Sistema de liberação de fármaco; Mucosa oral; Biopolímeros; Administração local; Odontologia.

1. INTRODUCTION

In the search for alternatives to oral drug administration, topical delivery via the buccal mucosa presents a promising route for the sustained release of therapeutic agents[1]. The buccal mucosa offers several advantages, including a highly vascularized, non-keratinized stratified epithelium that enables enhanced local permeability and direct access to systemic circulation, thus bypassing hepatic first-pass metabolism and gastrointestinal degradation. Additionally, the buccal route is characterized by ease of access and reduced enzymatic activity. However, limitations such as short residence time and inadequate absorption hinder clinical utility. Therefore, the development of suitable mucoadhesive formulations is essential to enhance drug retention and ensure sustained therapeutic effect at the site of application[2,3].

Mucoadhesive drug delivery systems represent a non-invasive approach capable of prolonging residence time and improving bioavailability for both local and systemic effects[4]. These systems may establish intimate contact with the mucosal surface, interacting with the mucus layer and mucin molecules to enhance drug absorption and therapeutic efficacy[5]. Effective mucoadhesive formulations must demonstrate increased contact with the mucosal surface, followed by a consolidation phase characterized by adequate spreadability and swelling, promoting firm adhesion and sustained release[6]. Additionally, such systems must exhibit suitable mechanical properties, resistance to salivary clearance, and protection of the drug against enzymatic degradation in the buccal environment[3].

Hydrogels, composed of hydrophilic homo- or copolymer networks, can swell in the presence of aqueous or physiological fluids and allow for controlled swelling kinetics. Their three-dimensional structure, formed via physical or chemical crosslinking, enables drug entrapment and release[7]. *In situ* gelation is induced by environmental triggers such as pH, temperature, or ionic strength[8]. Poloxamer 407, a non-ionic triblock copolymer of poly (ethylene oxide) and poly (propylene oxide), exhibits thermoreversible gelation and forms micellar structures at physiological temperatures[9]. Although considered safe and widely used in various pharmaceutical formulations (oral, ophthalmic, intravenous, topical), poloxamer 407 alone lacks adequate mucoadhesive strength and requires additional agents to improve retention at the application site[10]. Studies have shown that its combination with active molecules such as levodopa can modify the gelation temperature and enhance bioadhesive properties, with sol–gel transitions ranging from 22 to 31 °C depending on concentration, in comparison with non-modified poloxamer formulations at 15 to 35 °C sol-gel transition[9,11].

Chitosan, a biodegradable and biocompatible biopolymer, demonstrates cationic behavior in acidic environments, forming electrostatic complexes with negatively charged biological surfaces. Its broad bioactivity, including antibacterial, antifungal, antitumoral, and antioxidant effects, combined with its non-decreasing mucoadhesive performance over time without inactivation in the presence of mucin, makes it an attractive polymer for transmucosal drug delivery[12,13].

Previous studies on buccal films containing miconazole nitrate have shown that chitosan–hydroxypropylmethylcellulose-based films provided superior mucoadhesion and enhanced drug dissolution compared to the free drug[14]. Eugenol (4-allyl-2-methoxyphenol), widely used for its local anesthetic and anti-inflammatory properties, has emerged as a candidate for the treatment of oral diseases such as gingivitis and periodontitis. These conditions require a formulation capable of delivering effective concentrations of therapeutic agents with prolonged residence time and minimal side effects—criteria that synthetic compounds alone fail

to meet. Due to its lipophilic nature, eugenol requires formulation strategies that improve its retention, spreadability and absorption at the application site[15,16,17,18].

In this context, mucoadhesive *in situ* gelling hydrogels represent an effective strategy for buccal drug delivery. The present study aimed to develop and evaluate a poloxamer–chitosan-based hydrogel incorporating varying concentrations of eugenol. The formulation was designed for buccal applications, with the goal of achieving suitable mechanical strength, mucoadhesion, and extended retention time for the local treatment of oral diseases.

2. MATERIAL AND METHODS

2.1 MATERIAL

Eugenol (≥ 99% purity, Sigma-Aldrich, MO, USA), Poloxamer 407- Lutrol® F127 [Poly (ethylene glycol) – co – poly (propylene glycol) – co – poly (ethylene glycol)], Sigma- Aldrich (Steinheim, Germany), Chitosan – Chitosan [β-(1,4) -2-acetamido – D-glucose and β-(1,4) -2-amino – D-glucose] (Fagron, São Paulo, Brazil), Glacial acetic acid P.A. (Dinâmica Química, São Paulo, Brazil); Deionized water by reverse osmosis (Gehaka, model OS10LX, São Paulo, Brazil), Ethanol 96ºGL (All Chemistry, São Paulo, Brazil); Potassium sorbate (Embrafarma, São Paulo, Brazil).

2.2 PREPARATION OF HYDROGELS

Hydrogel base formulations were prepared, in triplicate, composed of the copolymer poloxamer 407 - Lutrol® F127 20% (w/w) and potassium sorbate 0.2% (w/w) in ice-cold purified water, according to a previously proposed methodology[8].

To prepare the poloxamer-chitosan (HPQ) hydrogel, chitosan (1%, w/w) was initially dissolved in a 1% (v/v) glacial acetic acid solution with constant homogenization (500 rpm) until gel formation. Afterwards, poloxamer 407® was added to the chitosan solution and homogenized. The formulations were kept in closed containers, protected from light and in the refrigerator (4 °C), for 24 h, until a clear solution was formed. Subsequently, eugenol was incorporated into the formulations HPQEU1, HPQEU2 and HPQEU4, as shown in Table 1.

Table 1 – Chemical composition of hydrogels in percentage (%).

	HP	HPQ	HPQEU1	HPQEU2	HPQEU4
Components			**(%, w/w)**		
Poloxamer 407®	20	20	20	20	20
Potassium sorbate	0.2	0.2	0.2	0.2	0.2
Chitosan	------	1	1	1	1
Acetic acid	------	1	1	1	1
Purified water (*qsp*)	100 g	100 g	100 g	100 g	100 g
Eugenol	------	------	1	2	4

2.3 CHARACTERIZATION OF THE HYDROGELS

The organoleptic characterization was evaluated through the parameters of color, odor and appearance. The transition of the liquid phase (Sol) to the gel phase (Tsol/gel) was verified with 10 g of samples in test tubes with an internal diameter of 10 mm, being initially subjected to 4 °C and followed by heating in a water bath. Every 2 °C, the samples were analyzed visually. The Tsol/gel was determined when the liquid sample in the tube presented itself as a motionless solid phase at the bottom of the tube[19].

The pH of the hydrogels was determined with a calibrated potentiometric meter, at room temperature, 24 hours after preparation. For the turbidimetric assay, the hydrogels, without dilution, were analyzed in a UV-VIS spectrophotometer at a wavelength of 650 nm to determine %T. The sample readings were determined by adjusting the %T of water to 100% (blank)[19].

2.4 STABILITY STUDY

The study of hydrogels stability was carried out in accordance with international guidelines[20] through physicochemical analyses. Initially, the hydrogels (10 g) were centrifuged at room temperature at 3000 rpm for 30 minutes. The stability test was carried out at different storage temperatures, for 30 days, in sealed packaging (plastic tubes). The characteristics evaluated were those mentioned above at times 1, 7, 15, and 30 days. The percentage of eugenol content and the percentage of loss were also determined.

2.5 SPREADABILITY EVALUATION

The spreadability of the hydrogels was assessed at 30 °C, according to the thermoresponsive behavior and by methodology described, using graph paper and a glass slide[21]. 50 mg of the sample was applied to the center of the slide, and subsequently glass slides of determined mass were added to the sample, with their respective diameters measured. The procedure was followed, always at 1-minute intervals, adding weight as shown in Table 2. The spreadability determination was designated by the equation:

$$\mathbf{Ei = (d^2 \times \pi) / 4}, \text{ where}$$

Ei = spreadability of the sample for weight *i* in mm^2
d = average diameter (mm)

Table 2 – Spreadability measurement and weight of glass slides.

Measures	Weight of glass slides
1ª	Glass slide *1* (4.780 g)
2ª	Glass slide *1* (4.780 g) + glass slide *2* 4.745 g
3ª	Glass slide *1* (4.780 g) + glass slide *2* 4.745 g + glass slide *3* 5.011 g
4ª	Glass slide *1* (4.780 g) + glass slide *2* 4.745 g + glass slide *3* 5.011 g + glass slide *4* 4,968 g

2.6 RHEOLOGICAL EVALUATION

Rheological characterization was performed using an oscillatory rheometer, and the procedures were calibrated through the TRIOS® software at a temperature range of 15–60 °C with a heating rate of 5 °C/min. The samples were positioned in the center of the lower plate. The values of the elastic or storage mode (G' in

Pa), viscous or loss mode (G'' in Pa) were obtained. The analyses of hardness, compressibility, adhesiveness, cohesiveness and elasticity of the *in situ* hydrogel formulations were determined using a texture analyzer[8].

2.7 EUGENOL QUANTIFICATION

To quantify the drug, spectrophotometric scanning (UV-Vis) was performed at wavelengths from 200 to 400 nm to identify the wavelength of maximum absorption of eugenol (λ max). For this purpose, a eugenol solution (100 mg/mL) was prepared in ethanol/water, 60/40 (v/v). The analytical curve of eugenol was determined by linearity (n=3), through eugenol concentrations at 5, 10, 15, 20, 25, 30 and 35 µg/mL.

To determine the eugenol content in the formulations, solutions of samples HPQEU1, HPQEU2 and HPQEU4 were prepared at the following concentrations: HPQEU1 1:200, v/v (5 µg/mL); HPQEU2 1:100, v/v (10 µg/mL); HPQEU4 1:50, v/v (20 µg/mL), using ethanol/water solution (60/40, v/v). The readings in UV-Vis spectrophotometer were made at appropriate wavelength after scanning. The eugenol contained in the formulations was determined through the equation extracted from the analytical curve. The analyses were carried out in triplicate.

2.8 *EX VIVO* MUCOAHESION STUDY

The *ex vivo* mucoadhesion test of the *in situ* hydrogels was performed using a texture analyzer with 5 kg load cell, 32 mm aluminum cylindrical probe, and a mucoadhesive support[8]. The buccal mucosa (1.5 cm^2 samples) was obtained from the cheek of a freshly slaughtered pig, after removal of all muscle and other tissues. The buccal mucosa was frozen at -20 °C and packaged in plastic film and aluminum foil (This type of biological sample is exempt from review by an animal research ethics committee). At the time of the experiment, the buccal mucosa was thawed at room temperature in saline solution.

The mucosa was fixed with cyanoacrylate glue in a glass container (Paladon jar) and positioned on the plate heated to 37 °C, at the lower end of the probe. The mucosa was moistened with artificial saliva over its entire surface. Afterwards, the hydrogels (HP, HPQ, HPQEU1, HPQEU2 and HPQEU4) were applied to mucosa. A constant contact force of 0.5 N was maintained for 120 s on the mucosa/mucoadhesive system. After this time, the equipment raised the probe at a constant speed until the system was separated to obtain measurements of interest. Using a force-distance graph (mN/mm), the separation force was measured, indicating the mucoadhesive performance of the samples.

2.9 STATISTICAL ANALYSIS

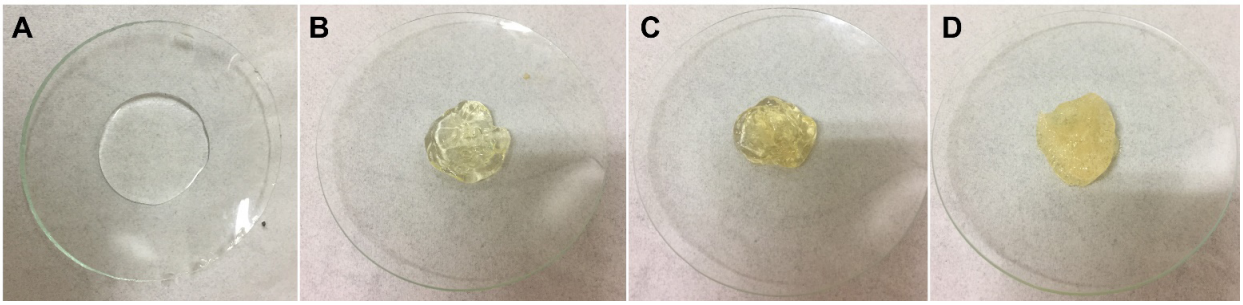
For statistical analysis, GraphPad Prisma software version 5 was used. Data were expressed as percentage or mean ± standard deviation (SD). Data for physicochemical characterization were analyzed by one-way analysis of variance (ANOVA) followed by Newman Keuls post-hoc test. Data for stability was analyzed by a two-way analysis of variance (ANOVA) followed by Bonferroni post-hoc test. The significance level was $p < 0.05$.

3. RESULTS

3.1 CHARACTERIZATION OF HYDROGELS

The HP and HPQ hydrogels presented a homogeneous semi-solid, odorless and translucent appearance. The HPQEU1, HPQEU2 and HPQEU4 hydrogels presented a homogeneous semi-solid appearance, slightly yellowish color and characteristic odor (eugenol). Opacity was observed for HPQEU2 and HPQEU4 (Figure 1).

Figure 1 – Organoleptic characteristics of hydrogels.



Legend: poloxamer 407® hydrogel (HP), poloxamer 407® hydrogel with chitosan (HPQ), poloxamer 407® hydrogel with chitosan + eugenol 1% (w/w) (HPQEU1), poloxamer 407® hydrogel with chitosan + eugenol 2% (w/w) (HPQEU2), poloxamer 407® hydrogel with chitosan + eugenol 4% (w/w) (HPQEU4).

3.2 SOL → GEL TRANSITION (T SOL/GEL)

For the HP and HPQ formulations, the T sol/gel was 29.33 ± 0.57 and 29.66 ± 0.7 °C, respectively. For the formulations containing eugenol, HPQEU1 and HPQEU2, the results changed significantly ($p<0.05$) to 25.33 ± 0.57 and 19.33 ± 0.57 °C, respectively. HPQEU4 did not change its physical state in the temperature range evaluated (4 to 50 °C).

3.3 pH

The pH values of the hydrogel formulations HP and HPQ were 6.49 ± 0.04 and 5.33 ± 0.02, respectively. The values of the formulations containing eugenol such as HPQEU1, HPQEU2 and HPQEU4 were 5.35 ± 0.02; 5.37 ± 0.02 and 5.40 ± 0.01, respectively.

3.4 TURBIDIMETRIC EVALUATION

HPQEU1, HPQEU2 and HPQEU4 showed transmittance values of 34.56 ± 0,6%, 30.66 ± 0.61% and 16,53 ± 0.46%, respectively, disclosing increased turbidity in relation to the base formulations (72.83 ± 0.25 % for HP and 48.13 ± 0.40 for HPQ) with their turbidimetry values being proportional to the increase in eugenol concentration in the formulations ($p<0.05$).

3.5 STABILITY STUDY

After centrifugation, no phase separation and/or changes in the physical characteristics of the formulations were observed, with the initial appearance maintained, thus, suitable for stability testing. The evaluation of the organoleptic characteristics of the hydrogels at different temperatures for 30 days is presented in Table 3.

Table 3 – Macroscopic evaluation of the hydrogels stored in different temperatures for 30 days.

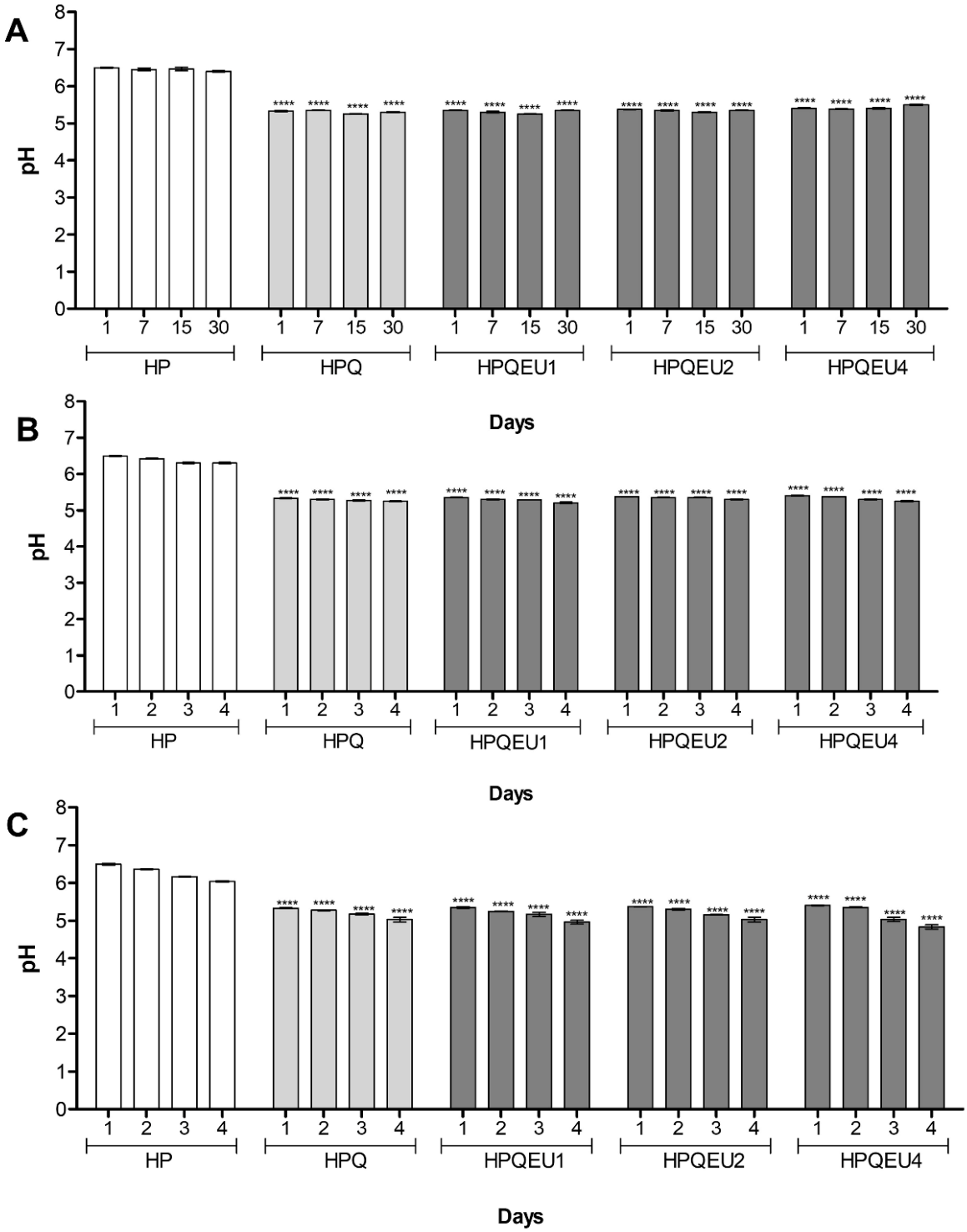
	5 ± 2 °C				25 ± 2 °C				45 ± 2 °C			
	Days				Days				Days			
Hydrogels	1	7	15	30	**1**	**7**	**15**	**30**	**1**	**7**	**15**	**30**
HP	N	N	N	N	N	N	N	N	N	N	LA	LA
HPQ	N	N	N	N	N	N	N	LA	N	LA	LA	LA
HPQEU1	N	N	N	LA	N	N	LA	LA	N	LA	LA	LA
HPQEU2	N	N	N	LA	N	N	LA	LA	N	LA	LA	LA
HPQEU4	N	N	N	LA	N	N	LA	LA	N	LA	LA	LA

Legend: poloxamer 407® hydrogel (HP), poloxamer 407® hydrogel with chitosan (HPQ), poloxamer 407® hydrogel with chitosan + eugenol 1% (w/w) (HPQEU1), poloxamer 407® hydrogel with chitosan + eugenol 2% (w/w) (HPQEU2), poloxamer 407® hydrogel with chitosan + eugenol 4% (w/w) (HPQEU4). N = normal, no changes; LA = slightly changed; IM = intensely changed.

At 5 °C, the HP and HPQ hydrogels were stable for 30 days. The formulations containing eugenol maintained the same initial characteristics until the 15th day of storage. However, on the 30th day, they presented slight changes in color and appearance. At room temperature, the hydrogels HPQ, HPQEU1, HPQEU2 and HPQEU4 presented changes in their organoleptic characteristics from the 15th day of storage. For the samples containing eugenol, the yellow color became more intense with increasing turbidity. At 45 °C, the hydrogels HPQ, HPQEU1, HPQEU2 and HPQEU4 presented changes in their organoleptic characteristics from the 7th day of storage, with only the hydrogel HP presenting changes from the 15th day of storage.

The graphs presented in Figure 2 display a comparison of the pH variation during the evaluated period.

Figure 2 – Values of pH for the hydrogels stored at 5 °C (A), 25 °C (B) e 45 °C (C) for 30 days.



Legend: poloxamer 407® hydrogel (HP), poloxamer 407® hydrogel with chitosan (HPQ), poloxamer 407® hydrogel with chitosan + eugenol 1% (w/w) (HPQEU1), poloxamer 407® hydrogel with chitosan + eugenol 2% (w/w) (HPQEU2), poloxamer 407® hydrogel with chitosan + eugenol 4% (w/w) (HPQEU4).

$p < 0.001$ = *** (compared to HP).

Related to the pH values of the formulations when stored at 5 °C (Figure 2A), it was noted that the pH remained close to 5 during the evaluated period, demonstrating stability. The same was true for the formulations stored at room temperature (Figure 2B). Despite slight acidification in the formulations stored in oven, the pH remained constant (Figure 2C).

The turbidimetric evaluation of the hydrogels subjected to different storage conditions (Table 4) shows the oscillation of transmittance as a function of time and storage condition. The formulations showed increased turbidity due to the increase in the concentration of lipophilic substances and the tendency for micelles due to the polymeric surfactant of the hydrogel. Meanwhile, there was no significant difference throughout the storage period, evaluating each formulation individually for storage in the refrigerator and at room temperature. Fluctuations in transmittance were observed in the formulations stored in oven.

Table 4 – Turbidimetric evaluation of the hydrogels stored in different temperatures for 30 days.

		Formulations				
	Days	HP	HPQ	HPQEU1	HPQEU2	HPQEU4
5 ± 2 °C	1	75,71 ± 0,38	49,57 ± 0,71	36,56 ± 0,47	31,57 ± 0,10	16,44 ± 0,14
%T ± SD	7	74,19 ± 0,29	48,58 ± 0,35	36,24 ± 0,66	30,94 ± 0,57	16,11 ± 0,65
	15	72,71 ± 0,35	47,85 ± 0,35	35,87 ± 0,62	30,32 ± 0,59	15,79 ± 0,50
	30	70,53 ± 0,13	46,89 ± 0,21	34,79 ± 0,62	29,72 ± 0,42	15,48 ± 0,36
25 ± 2 °C	1	72,80 ± 0,59	48,13 ± 0,30	35,56 ± 0,44	30,66 ± 0,37	16,53 ± 0,11
%T ± SD	7	69,16 ± 0,31	46,20 ± 0,36	34,13 ± 0,60	29,74 ± 0,26	15,86 ± 0,43
	15	66,39 ± 0,43	44,81 ± 0,49	33,61 ± 0,09	28,84 ± 0,16	15,55 ± 0,14
	30	64,40 ± 0,47	43,47 ± 0,43	32,12 ± 0,64	28,28 ± 0,62	15,08 ± 0,49
45 ± 2 °C	1	71,34 ± 0,35	46,85 ± 0,60	34,49 ± 0,43	30,05 ± 0,56	16,06 ± 0,35
%T ± SD	7	69,20 ± 0,14	45,91 ± 0,51	33,80 ± 0,36	29,29 ± 0,59	15,55 ± 0,35
	15	67,12 ± 0,25	44,99 ± 0,62	33,12 ± 0,44	28,71 ± 0,60	15,24 ± 0,09
	30	65,11 ± 0,27	44,09 ± 0,33	32,46 ± 0,42	27,85 ± 0,28	14,70 ± 0,65

Legend: poloxamer 407® hydrogel (HP), poloxamer 407® hydrogel with chitosan (HPQ), poloxamer 407® hydrogel with chitosan + eugenol 1% (w/w) (HPQEU1), poloxamer 407® hydrogel with chitosan + eugenol 2% (w/w) (HPQEU2), poloxamer 407® hydrogel with chitosan + eugenol 4% (w/w) (HPQEU4).

The eugenol content during the storage in the formulations HPQEU1, HPQEU2 and HPQEU4 kept with a loss of 5% of the initial value[20] for HPQEU1 after 30 (5 °C), 15 (25 °C) and 7 (45 °C) days. For HPQEU2, a significant loss was observed from the 15th day of storage in the refrigerator and at room temperature. For storage in oven, the significant variation in loss occurred from the 7th day. HPQEU4 was the formulation that presented suited values within the recommended range, with losses during storage in the refrigerator and at room temperature, around 5%. This variation is due to the higher eugenol concentration in the formulation. However, oven storage resulted in over 5% eugenol loss by the 15th day.

3.6 SPREADABILITY EVALUATION

The spreadability values of the hydrogels are presented in Table 5. The analysis was performed at a temperature above the T sol/gel for all formulations (30 ºC), mimicking the temperature of the formulation at the application site.

Table 5 – Spreadability evaluation of the hydrogels

	HP	HPQ	HPQEU1	HPQEU2	HPQEU4
Weight (g)	**Average area (mm²) ± SD**				
4,780	78,50 ± 9,51	78,50 ± 9,51	50,24 ± 6,8	50,24 ± 0	50,24 ± 0
9,525	78,50 ± 9,51	78,50 ± 0	50,24 ± 0	50,24 ± 6,8	50,24 ± 6,8
14,536	113,04 ±10,4	113,04 ± 0	78,50 ± 9,51	78,50 ± 0	78,50 ± 9,51
19,504	153,86±12,23	113,04±10,42	113,04 ± 0	78,50 ± 9,51	78,50 ± 9,51

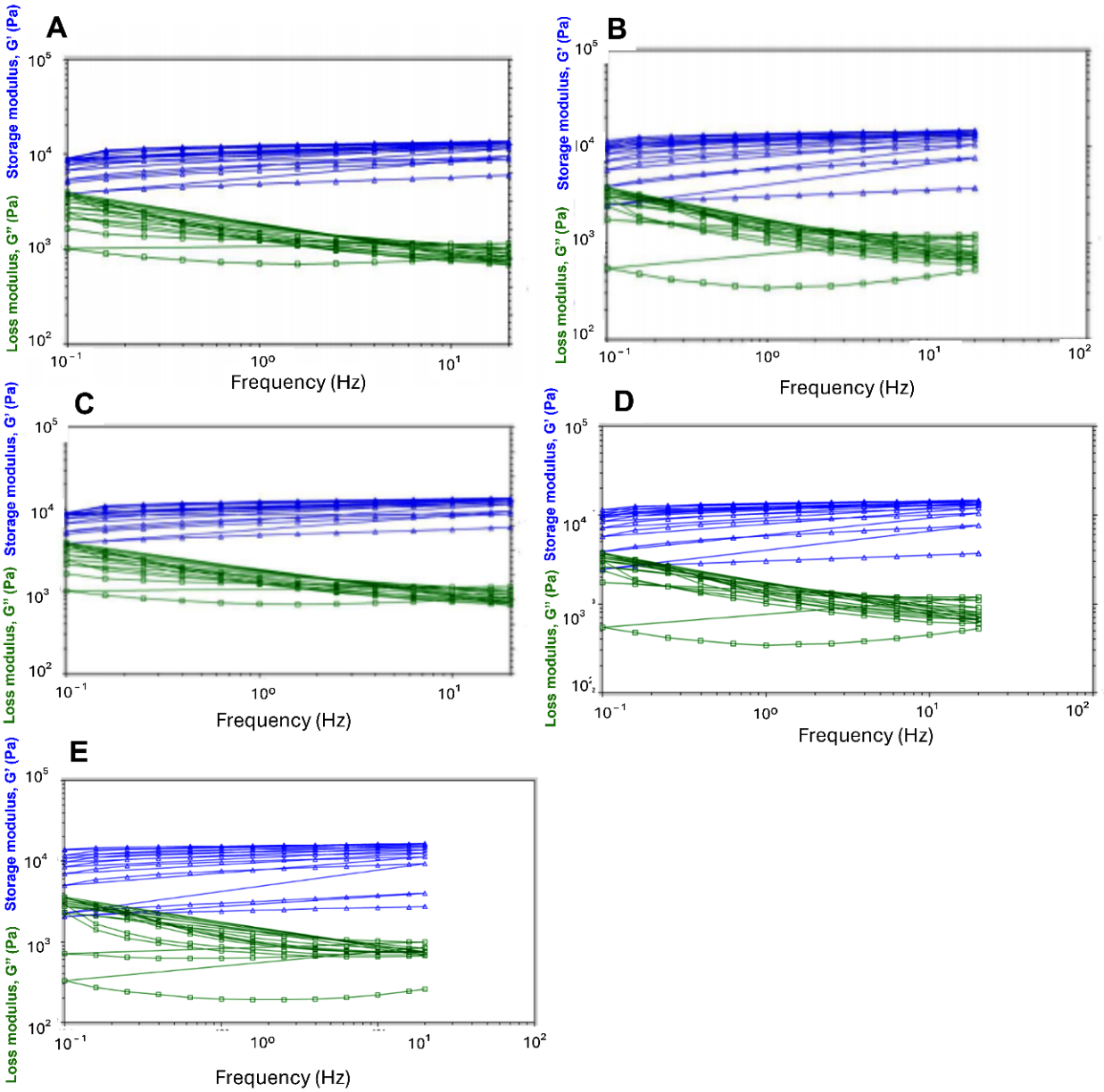
Legend: poloxamer 407® hydrogel (HP), poloxamer 407® hydrogel with chitosan (HPQ), poloxamer 407® hydrogel with chitosan + eugenol 1% (w/w) (HPQEU1), poloxamer 407® hydrogel with chitosan + eugenol 2% (w/w) (HPQEU2), poloxamer 407® hydrogel with chitosan + eugenol 4% (w/w) (HPQEU4).

When comparing the values between the base formulations – HP and HPQ and the formulations containing eugenol – HPQEU 1, 2 and 4, a decrease in the spreadability of the latter was observed. Hydrogels HPQEU2 (T sol/gel 19.33 ± 0.57 ºC) and HPQEU4 (no transition value) presented lower spreadability values. The differences between T sol/gel values, as well as spreadability values between the base formulations HP and HPQ, may be related to the addition of positively charged chitosan and due to the change in the surface charge of the polymeric micelles. Therefore, considering the spreadability characteristics of all formulations, linked to the results of the sol/gel transition analysis, the HPQEU1 formulation (T sol/gel 25.33 ± 0.57 ºC) proved to be the most favorable formulation, including the attribution of retention capacity at the application site without loss of material.

3.7 RHEOLOGICAL STUDY

The rheological behavior of the hydrogels (Figure 3) was studied through oscillatory measurements to determine their viscoelastic properties. The elastic modulus was greater than the loss modulus at all applied frequencies, indicating the presence of a stable gel suitable for application to the oral mucosa.

Figure 3 – Rheological analysis of the hydrogels: HP (A); HPQ (B); HPQEU1 (C); HPQEU2 (D) e o HPQEU4 (E).



Legend: G'(*storage modulus*): elastic or storage modulus in blue; G" (*loss modulus*): viscous or loss modulus in green.

3.8 TEXTURE PROFILE

The analysis of the texture profile of hydrogels provides the mechanical properties of the oral semi-solid formulations, which should have appropriate mechanical properties such as lower hardness and compressibility and higher adhesiveness, cohesion and elasticity. The results for texture analysis are shown in Table 6.

Table 6 – Mechanical properties of the hydrogels

Formulations	Hardness (N) ± SP	Compressibility (N. s^{-1}) ± DP	Adhesiveness (N.s^{-1}) ± DP ± DP	Cohesiveness	Elasticity ± DP
HP	3,87 ±0,005	26,05 ±0,064	29,56 ±0,352	1,044 ±5,85	0,981 ±0,001
HPQ	4,30 ±0,015	30,62 ±0,041	30,79 ±0,994	0,983 ±0,003	0,977 ±0,002
HPQEU1	4,46 ±0,01	34,93 ±0,036	31,53 ±0,11	0,955 ±0,001	0,973 ±0,001
HPQEU2	4,97 ±0,026	35,33 ±0,023	31,90 ±0,1	0,930 ±0,004	0,969 ±0,001
HPQEU4	5,75 ±0,01	40,87 ±0,015	38,55 ±0,55	0,919 ±0,003	0,960 ±0,001

Legend: poloxamer 407® hydrogel (HP), poloxamer 407® hydrogel with chitosan (HPQ), poloxamer 407® hydrogel with chitosan + eugenol 1% (w/w) (HPQEU1), poloxamer 407® hydrogel with chitosan + eugenol 2% (w/w) (HPQEU2), poloxamer 407® hydrogel with chitosan + eugenol 4% (w/w) (HPQEU4).

Among the formulations containing eugenol, HPQEU1 presented the lowest hardness value and HPQEU4 presented the highest value. Compressibility was lower for HPQEU1, which, in parallel, presented greater spreadability compared to the hydrogels containing eugenol. The addition of chitosan and the eugenol concentration increase caused higher values of adhesiveness for the gels, with greater adhesion for HPQEU4. The greatest cohesiveness was observed for HPQEU1 compared to HPQEU2 and HPQEU4. When comparing the formulations regarding elasticity, the formulations were presented in the order HP > HPQ > HPQEU1 > HPQEU2 > HPQEU4.

3.9 DRUG CONTENT

Drug quantification was obtained after scanning, what resulted the maximum absorption wavelength of 261 nm for eugenol. The construction of the analytical curve showed linearity in the range of 0.005 to 0.035 mg/mL with an adequate correlation coefficient of r = 0.9976 (y = 6.5714x + 0.0069), demonstrating suitability for eugenol quantification[20,27]. The eugenol content quantified in the formulations was determined through the calculation obtained by the straight-line equation. The theoretical concentrations of 1, 2 and 4% for HPQEU1, HPQEU2 and HPQEU4 corresponded to the experimental concentrations of 0.8 ± 0.001%, 2 ± 0.001% and 3.6 ± 0.001%, respectively.

3.10 *EX VIVO* MUCOADHESION STUDY

The mucoadhesion test, assessed by means of a tensile test, used the maximum force and the work of adhesion to separate the mucosa from the bioadhesive formulation. The maximum force and adhesion parameters are directly proportional (Table 7).

Table 7 – Mucoadhesive properties of the hydrogels

	Adhesion force (N.s)	**F** *max* (N)
HP	0,554 ± 0,62	0,231 ± 0,69
HPQ	0,672 ± 0,52	0,249 ± 0,62
HPQEU1	1,55 ± 0,56	0,308 ± 0,50
HPQEU2	1,795 ± 0,25	0,366 ± 0,58
HPQEU4	1,895 ± 0,38	0,381 ± 0,62

Legend: poloxamer 407® hydrogel (HP), poloxamer 407® hydrogel with chitosan (HPQ), poloxamer 407® hydrogel with chitosan + eugenol 1% (w/w) (HPQEU1), poloxamer 407® hydrogel with chitosan + eugenol 2% (w/w) (HPQEU2), poloxamer 407® hydrogel with chitosan + eugenol 4% (w/w) (HPQEU4).

The HPQEU4 hydrogel presented higher adhesion values than the other formulations, intermediate values were observed for the other hydrogels tested and HP presented the lowest adhesion value. When evaluated the maximum force, the HPQEU4 hydrogel presented a higher value than the others, and HP presented the lowest values. Higher values of adhesion and maximum force ensure good contact between the hydrogel and the mucosa.

4. DISCUSSION

Poloxamer 407® and chitosan hydrogels were produced with organoleptic characteristics varying according to the chemical composition as well as the opaque appearance due to the addition of eugenol, a lipophilic compound[17,18].

The adequate Tsol/gel should be in the range of 25–37 °C to avoid degradation during preparation and for administration to the oral mucosa, in which the influence of the body temperature on the formulation gelled state[22]. There was a decrease in the Tsol/gel of the HPQEU1 and HPQEU2 formulations, what may be related to the interference of the added components, such as eugenol, on the micellization of Poloxamer 407®. This was evident for HPQEU4 with a higher concentration of eugenol and no transition in Tsol/gel, regardless of the temperature change[9,10,11,18]. It has already been reported that decreasing the concentration of Poloxamer 407® in the formulations can increase the Tsol/gel[9].

Regarding pH, formulations with eugenol were considered suitable for administration to the oral mucosa, since the pH of the oral cavity with the presence of saliva is between 5.5 and 7.0, and formulations with pH values outside this range can cause injury or irritation to the mucosa[23]. Another important factor is the absorption rate of the drug, which is influenced by the permeabilities of the oral and sublingual mucosas, as well as its physical and chemical properties[23]. Furthermore, such pH values of the formulations maintain the low ionization of eugenol with pKa = 10.19, what may contribute to its absorption at the site of application[18,24].

For turbidimetry, the values increased due to the tendency of chitosan incorporated in hydrogel to form crystalline domains and the addition of lipophilic substance in a hydrophilic medium, being both able to reduce light transmittance[25,26].

According to guidelines from regulatory agencies[20,27], the organoleptic characteristics of formulations subjected to storage under different conditions must remain intact under all conditions, except at high temperatures, where small changes are acceptable. Regarding color and odor, the formulations must remain stable for at least 15 days, and small changes are acceptable at high temperatures, since under these conditions the rates of degradation reactions are increased[21].

In general, when evaluating the behavior of the formulations stored under the three different conditions, the best storage temperature was 5 ± 2 ºC, followed by 25 ± 2 ºC. Furthermore, packaging that

offers less interference with the environment, such as aluminum tubes, and strict storage away from light, can ensure the stability of the organoleptic properties of the formulations.

Variations in pH above 10% in relation to time zero are considered inappropriate, and this is the limit for excluding formulations[20]. Thus, all oscillations in pH values found in the formulations stored at room temperature and refrigerator are within as recommended, except for the formulation HPQEU4 that was above 10%.

Apart from that, some amendments would be favorable to minimize losses and variations in eugenol concentration in the hydrogels, such as changing the packaging and establishing storage in the refrigerator, which proved to be more appropriate. Further, the addition of adjuvants to maintain the physicochemical characteristics of eugenol, related to oxygen and unsaturation in chemical structure[18,24].

The decreased spreadability of hydrogels containing eugenol can be explained by the lower mobility of the polymeric micelles that encapsulate the lipophilic substance. This property is essential for the permanence of the formulation at the application site, such as the oral mucosa, with less movement and favoring the absorption of eugenol[21,28].

The oscillatory frequency test reveals the elastic or storage modulus G' (elastic response) or loss G" (viscous response)[17]. The rheological study demonstrated an increase in elastic behavior, important for retention at the application site and prolonged release of the active ingredient. After administration of the thermosensitive gels, the system gels (G'>G") and resists intraoral shear forces[29].

For the texture profile of the hydrogels, the lower hardness expresses the facilitated applicability on the oral mucosa[30]. Low compressibility indicates the removal of the gel from the packaging and its spreading during application[29]. High adhesiveness was influenced by chitosan, demonstrating the appropriate composition for retention in the mucosa through electrostatic interaction[8]. The product's performance is determined by the high cohesion value, which indicates complete structural recovery after application of the gel. Elasticity indicates the deformation and returns of the gel structure, with lower values indicative of this property[30].

Ideally, poloxamer-chitosan hydrogels, as a formulation for application to the oral mucosa, should have high adhesiveness and appropriate elasticity[31]. The hydrogel formulation HPQEU1 followed by the formulation HPQEU2 were the formulations, with thermoresponsive ability and release system, that presented the best mechanical properties for transporting eugenol to the oral mucosa.

The mucoadhesive characteristic of formulation is important for prolonging retention at the application site, such as the oral mucosa, therefore, with less loss of the formulation[32]. Hydrogels formulated by binary systems consisting of different polymers, which are bioadhesive and thermosensitive, promote a combination of advantages. Thus, there is an improvement in the adhesion and administration performance of these systems *in vitro* and *in vivo*[31], which was achieved with the developed formulations, based on Poloxamer 407® and chitosan, containing eugenol.

5. CONCLUSION

The hydrogels containing different concentrations of eugenol presented adequate organoleptic characteristics, pH values, turbidity and texture profile favoring the application in oral mucosa. The sol/gel transition was shown to be more suitable for HPQEU1. The stability study established refrigeration to preserve the physicochemical properties of the hydrogels. The rheological profile demonstrated elastic behavior, ideal for remaining retained in place as well as ease of application. The mucoadhesion test demonstrated that the formulations HPQEU1, HPQEU2 and HPQEU4 presented adequate values regarding the ability to adhere to the mucosa, with the hydrogel HPQEU4 presenting superior mucoadhesion. Among all the parameters evaluated, the formulation with the most suitable profile was HPQEU1 followed by HPQEU2. Therefore, they constitute alternatives for delivery of eugenol in oral mucosa with thermos responsiveness, being a promising release system for dental application.

REFERENCES

1. Batheja P, Thakur R, Michiniak B. Basic biopharmaceutics of buccal and sublingual absorption. In: Touitou E, Barry BW. Enhancement in Drug Delivery. Florida: Crc Press, 2006, p. 175-202. Doi: 10.1201/9780849332036.ch9

2. Dwivedi P, Kalra P, Zhou H, Alam K, Tsakalozou E, Al-Ghabeish M, Kelchen M, Pauletti GM. In vitro oral cavity permeability assessment to enable simulation of drug absorption. Pharmaceutics. 2025; 17(7):924. Doi: 10.3390/pharmaceutics17070924

3. Tran PHI, Duan W, Tran TTD. Recent developments of nanoparticle-delivered dosage forms for buccal delivery. Int J of Pharmaceutics. 2019; 571: 118697. Doi: 10.1016/j.ijpharm.2019.118697

4. Carvalho FC, Bruschi ML, Evangelista RC, Gremião MPD. Mucoadhesive drug delivery systems. Brazilian J Pharm Sci. 2010; 46 (1) 1-17. Doi: 10.1590/S1984-82502010000100002.

5. Kulkarni R, Fanse S, Burgess DJ. Mucoadhesive drug delivery systems: a promising non-invasive approach to bioavailability enhancement. Part I: biophysical considerations. Expert Opin Drug Deliv. 2023; 20(3):395-412. Doi: 10.1080/17425247.2023.2181331.

6. Hagerstrom H, Edsman K, Stromme M. Low-frequency dielectric spectroscopy as a tool for studying the compatibility between pharmaceutical gels and mucous issue. J Pharm Sci. 2003; 92(9): 1869-1881. Doi: 10.1002/jps.10451

7. El-Husseiny HM, Mady EA, Hamabe L, Abugomaa A, Shimada K, Yoshida T, Tanaka T, Yokoi A, Elbadawy M, Tanaka R. Smart/stimuli-responsive hydrogels: Cutting-edge platforms for tissue engineering and other biomedical applications, Materials Today Bio. 2022; 13: 100186. Doi: 10.1016/j.mtbio.2021.100186.

8. Tugcu-Demiroz F. Development of in situ poloxamer--chitosan hydrogels for vaginal drug delivery of benzydamine hydrochloride: Textural, mucoadhesive and in vitro release properties. Marmara Pharma J. 2017; 21(4): 762-770. Doi: 10.12991/mpj.2017.3

9. Giuliano E, Paolino D, Fresta M, Cosco D. Mucosal applications of poloxamer 407-based hydrogels: an overview. Pharmaceutics. 2018; 10(3):159. Doi: 10.3390/pharmaceutics10030159.

10. Fakhari A, Corcoran M, Schwarz A. Thermogelling properties of purified poloxamer 407. Heliyon. 2017; 3(8): 390. Doi: 10.1016/j.heliyon.2017.e00390.

11. Ci L, Huang Z, Liu Y, Liu Z, Wei G, Lu W. Amino-functionalized poloxamer 407 with both mucoadhesive and thermosensitive properties: preparation, characterization and application in a vaginal drug delivery system. Acta Pharm Sin B. 2017; 7(5):593-602. Doi: 10.1016/j.apsb.2017.03.002.

12. Mishra A, Omoyeni T, Singh PK, Anandakumar S, Tiwari A. Trends in sustainable chitosan-based hydrogel technology for circular biomedical engineering: A review. Int J Biol Macromol. 2024; 276:133823. Doi: 10.1016/j.ijbiomac.2024.133823.

13. Andersen T, Bleher S, Eide Flaten G, Tho I, Mattsson S, Škalko-Basnet N. Chitosan in mucoadhesive drug delivery: focus on local vaginal therapy. Mar Drugs. 2015; 13(1):222-36. Doi: 10.3390/md13010222.

14. Tejada G, Barrera MG, Piccirilli GN, Sortino M, Frattini A, Salomón CJ, Lamas MC, Leonardi D. Development and evaluation of buccal films based on chitosan for the potential treatment of oral candidiasis. AAPS PharmSciTech. 2017;18(4):936-946. Doi: 10.1208/s12249-017-0720-6.

15. Park CK, Kim K, Jung SJ, Kim MJ, Ahn DK, Hong SD, Kim JS, Oh SB. Molecular mechanism for local anesthetic action of eugenol in the rat trigeminal system. Pain. 2009;144(1-2):84-94. Doi: 10.1016/j.pain.2009.03.016.

16. Jadhav BK, Khandelwal KR, Ketkar AR, Pisal SS. Formulation and evaluation of mucoadhesive tablets containing eugenol for the treatment of periodontal diseases. Drug Dev Ind Pharm. 2004;30(2):195-203. Doi: 10.1081/ddc-120028715.

17. Pokharkar VB, Shekhawat P, Dhapte VV, Mandpe LP. Development and optimization of eugenol loaded nanostructured lipid carriers for periodontal delivery. Int J Pharm Pharmaceutical Sci. 2011; 3(4): 138-143.

18. Chung G, Oh SB. Eugenol as Local Anesthetic. In: Ramawat KG, Merillon J M. Natural products: phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes. London: Springer, 2013. p. 4001-4015. Doi: 10.1007/978-3-642-22144-6_171

19. Liu Y, Zhao ZQ, Liang L, Jing LY, Wang J, Dai Y, Chen BZ, Guo XD. Toward a solid microneedle patch for rapid and enhanced local analgesic action. Drug Deliv Transl Res. 2024;14(7):1810-1819. Doi: 10.1007/s13346-023-01486-6.

20. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): QiA (R2). (2003). Stability testing of new drug substances and products. London:

European Medicines Agency. Disponível em: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-1-r2-stability-testing-new-drug-substances-products-step-5_en.pdf.

21. Giarola LR. Explorando a biodiversidade brasileira e os conhecimentos populares: desenvolvimento de formulação para uso tópico com extrato de *Pterodon pubescens benth.* (sucupira). 2015. 138 f. Dissertação (Mestrado) - Curso de Ciências, Universidade Estadual de Campinas, Campinas, 2015.

22. Shapiro RL, Bockley KM, Hsueh HT, Appell MB, Carter DM, Ortiz J, Brayton C, Ensign LM. Hypotonic, gel-forming delivery system for vaginal drug administration. J Control Release. 2024; 371:101-110. Doi: 10.1016/j.jconrel.2024.05.037.

23. Shinkar DM, Dhake AS, Setty CM. Drug Delivery from the Oral Cavity: A Focus on Mucoadhesive Buccal Drug Delivery Systems. Pda J Pharm Sci Technol. 2012; 66(5): 466-500. Doi: 10.5731/pdajpst.2012.00877

24. PUBCHEM. Eugenol. National Library of Medicine - Center for Biotechnology Information. PubChem Compound Summary for CID 3314. Disponível em: https://pubchem.ncbi.nlm.nih.gov/compound/Eugenol.

25. Amiri MA, Amiri D, Hamedani S. Thermosensitive hydrogels for periodontal regeneration: a systematic review of the evidence. Clin Exp Dent Res. 2024;10(6):e70029. Doi: 10.1002/cre2.70029.

26. Meher JG, Yadav NP, Sahu JJ, Sinha P. Determination of required hydrophilic–lipophilic balance of citronella oil and development of stable cream formulation. Drug Develop Ind Pharm. 2012; 39(10): 1540-1546. Doi: 10.3109/03639045.2012.719902

27. BRASIL. ANVISA. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Resolução da Diretoria Colegiada - RDC Nº 166, de 24 de julho de 2017. Guia para validação de métodos analíticos.

28. Patel VF, Liu F, Brown MB. Advances in oral transmucosal drug delivery. J Control Release. 2011; 153(2): 106-116. Doi: 10.1016/j.jconrel.2011.01.027

29. Baloglu E, Karavana SY, Senyigit ZA, Guneri T. Rheological and mechanical properties of poloxamer mixtures as a mucoadhesive gel base. Pharm Dev Technol. 2011;16(6):627-36. Doi: 10.3109/10837450.2010.508074.

30. Zhao H. Progress of research on oral mucosal adhesive agents. Hua Xi Kou Qiang Yi Xue Za Zhi. 2023;41(1):1-10. Doi: 10.7518/hxkq.2023.01.001.

31. Jones DS, Bruschi ML, de Freitas O, Gremião MP, Lara EH, Andrews GP. Rheological, mechanical and mucoadhesive properties of thermoresponsive, bioadhesive binary mixtures composed of poloxamer 407 and carbopol 974P designed as platforms for implantable drug delivery systems for use in the oral cavity. Int J Pharm. 2009;372(1-2):49-58. doi: 10.1016/j.ijpharm.2009.01.006.

32. Bonacucina G, Cespi M, Misici-Falzi M, Palmieri GF. Rheological, adhesive and release characterisation of semisolid Carbopol/tetraglycol systems. Int J Pharm. 2006; 307(2):129-40. Doi: 10.1016/j.ijpharm.2005.09.034.