

Design of Thymol Loaded Dental In Situ Gels; In Vitro Evaluation Timol Yüklü Diş İn Situ Jellerin Tasarımı; In Vitro Değerlendirilmesi

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ABSTRACT

Objective: The aim of the study was to develop thymol thermosensitive in situ gels based on poloxamers in order to prolong dental contact time, control drug release, and enhance dental bioavailability.

Materials and Methods: Poloxamer 188 (P188) and poloxamer 407 (P407) were used in varying amounts to designed the in situ gels. Mucoadhesive excipient, like hydroxypropyl methylcellulose (HPMC), was transferred to the in situ gels to improve the formulation's ability to adhere to biological surfaces. For the created formulations, in vitro drug release, pH, clarity, and sol-gel transition temperature were all evaluated.

Results: The all of in situ gels gelation temperatures of the developed formulations range from 33 to 37°C, pH values are around 7, and syringeability is defined as the amount of force necessary to discharge each formulation from a syringe fitted with a 20-gauge needle. The quantities of P407 and HPMC with the preparations, decreasing in vitro burst release while also increasing the viscosity but every in situ gel formulation releases for six hours.

Conclusion:The results show that in situ gels containing P407 and P188 show promise for thymol dental application.

Keywords: Thymol, Poloxamer, In Situ Gel, Dental, Drug Delivery System, Hydroxypropyl methylcellulose

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Ö Z E T

Amaç: Bu çalışmanın amacı, dental temas süresini uzatmak, ilaç salımını kontrol etmek ve dental biyoyararlanımı arttırmak için poloksamerlere dayalı timol yüklü in situ jel formülasyonları geliştirmektir.

Gereç ve Yöntem: İn situ jellerin tasarımında Poloksamer 188 (P188) ve Poloksamer 407 (P407) farklı oranlarda kullanılmıştır. Jelin biyolojik yüzeylere yapışma yeteneğini geliştirmek için formülasyonlara hidroksipropil metilselüloz (HPMC) gibi muko-yapışkan bir yardımcı madde eklenmiştir. Oluşturulan formülasyonlar için in vitro ilaç salımı, pH, berraklık ve sol-jel geçiş sıcaklığı değerlendirilmiştir.

Bulgular: Geliştirilen formülasyonların jelleşme sıcaklıkları 33 ila 37°C arasında olduğu, pH değerlerinin yaklaşık 7 olduğu ve 20 numaralı iğne ile şırıngalanabilir olduğu görülmüştür. Preparat içerisindeki, P407 ve HPMC miktarları yükseldikçe, in vitro patlama salım azalırken aynı zamanda viskozitenin arttığı tespit edilmiştir, bütün in situ jel formülasyonlarının altı saat süre ile salım yaptığı belirlenmiştir.

Sonuç: Sonuçlar, timol içeren P407 ve P188 in situ jellerin timol dış uygulaması için umut verici olduğunu göstermektedir.

Anahtar Kelimeler: Timol, Poloksamer, İn Situ Jel, Dental İlaç Taşıyıcı Sistemler, Hidroksipropil metilselüloz



1. Introduction

Up to 90% of people worldwide are affected by periodontal diseases, which are quite frequent. The least serious type of periodontal disease, gingivitis, is a nondestructive periodontal condition brought on by bacterial biofilm that grows on teeth close to the gingiva. The most typical indications of gingivitis are bleeding, sore, or swollen gums as well as bad breath.

Although gingivitis may be treatable, if it isn't treated, it can progress into a serious form of periodontal disease that can obliterate the connective tissue and bone that support the teeth, resulting in tooth loss in adults [1]. Periodontitis has been linked to infectious endocarditis, cardiovascular illness, diabetes mellitus, respiratory problems, and poor pregnancy outcomes.

Various approaches are often used to treat periodontal disorders, namely the use of systemic antibacterial and anti-inflammatory medications [2]. However, prolonged use of systemic medications has possible risks, such as superimposed infections and resistant strains [3]. The interest in finding novel anti-infective natural compounds originating from plant origin increased as many prevalent infections developed growing resistance to treatment medicines already in use. In light of this, thymol has intriguing properties for oral treatment. Thymus, Origanum, and Satureja are plants that contain thymol and carvacrol, have long been utilized, particularly in the treatment of a variety of medical ailments [4]. In diverse locations of the eastern Mediterranean, *Thymus pectinatus* (Lamiaceae) grows wild [5]. Thymol is the most common monoterpene phenol found in *Thymus* sp. volatile oils [6-8]. It possesses appropriate organoleptic qualities for oral administration as well as antibacterial [9, 10] and antioxidant [11] capabilities. It is volatile and only very little soluble in water. Through the avoidance of considerable gut and first pass metabolism and the extension of drug residency at the site of action, local thymol delivery to the mouth cavity has the potential to maximize its local impact. Thus, a highly promising method for treating periodontitis can be achieved by locally injecting thymol into a system while maintaining its chemical stability and improving its solubility.

The use of in-situ gel formulations, which first administer medications in a liquid dosage form before forming strong gels at the delivery site to prolong the time that the active ingredient remains in the body, is currently a novel method for doing so [3]. Thermosensitive systems, such as pluronic, have been investigated as an appropriate dosage form for injection into dental pockets among in-situ gelling polymers. In addition, semisolid formulations containing mucoadhesive polymers as carbopol, polycarbophil, and hydroxypropyl methylcellulose (HPMC) have been suggested to enhance contact intimacy and lengthen the dose form's stay in the periodontal pocket [12].

In this study, thymol in-situ gels were created and evaluated as a local medication delivery system for treating periodontitis in pockets. These formulations included a thermosensitive polymer, " poloxamer 407 (P407) and poloxamer 188 (P188)," as well as a mucoadhesive one, " (HPMC)." The developed formulations were designed to stabilize thymol in order to ensure its effectiveness over the whole application period. In order to improve clinical efficacy and patient compliance, such formulations combine the benefits of simple administration, decreased frequency of administration, and prolonged drug release.

2. Material and Method

A phosphate buffered saline (PBS) tablet, hydroxypropyl methylcellulose (HPMC) (4K), pluronic (poloxamer) 407 (P407) and pluronic poloxamer 188 (P188) were all received from Sigma in Steinheim, Germany.

Plant material

Thymus pectinatus specimens were gathered in Erzincan in 2021 and verified by Prof. Dr. Ali Kandemir of the biology department of Erzincan Binali Yildrm University's Faculty of Science and Art. The dried aerial parts of *T. pectinatus* were chopped into little pieces and powdered in a mill after drying in the shade.

Isolation procedure

The dried aerial parts of *T. pectinatus* (650 g) were hydro-distilled for 4 hours in 2 L of water using a Clevenger-type equipment with a water-cooled oil receiver to stop the formation of artifacts as a result of hydrodistillation overheating. The volatile oils were collected, dried over anhydrous sodium sulfate (Merck), and kept at refrigerator 4-6 °C. The volatile oils (5 mL) which was dissolved hexane:DMSO (9:1) were subjected to column chromatography (CC) over silica gel (200-300 mesh) and eluted with *n*-hexane:ethylacetate (100:0 → 50:50 v/v). This elution gave the fractions Fr. A (520 mg) and Fr. B (1240 mg). Fr. A (520 mg) was subjected to Sephadex LH-20 using MeOH and gave thymol (480 mg). Its structure was compared with the literature information [13] and was identified by means of spectral method [1D-NMR (Varian Mercury Plus 400 MHz, USA) (Figure 1-2).

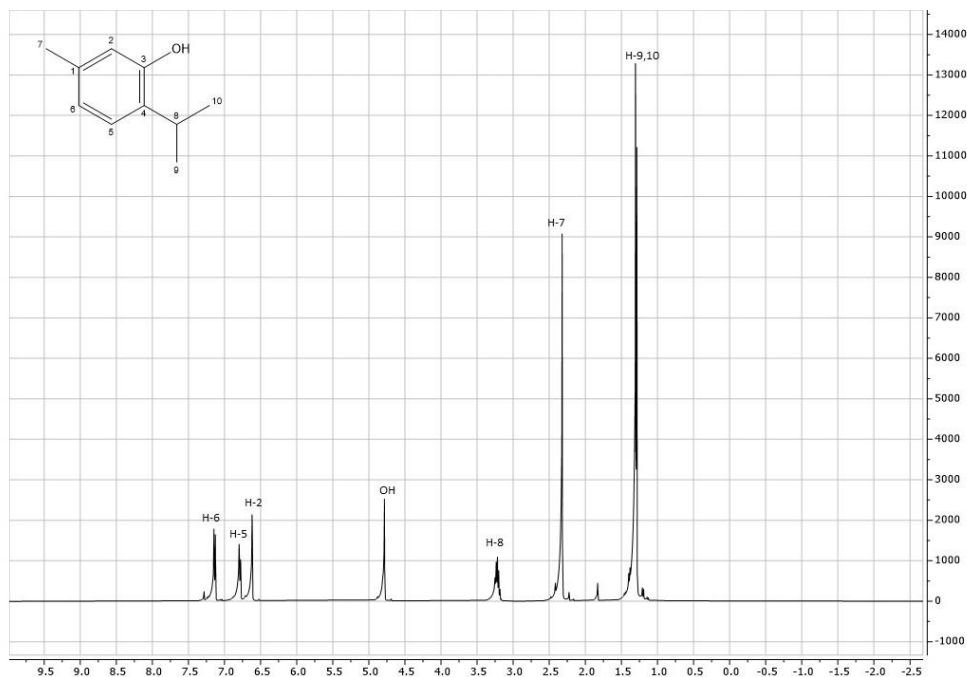


Figure 1: $^1\text{H-NMR}$ Spectra of Thymol

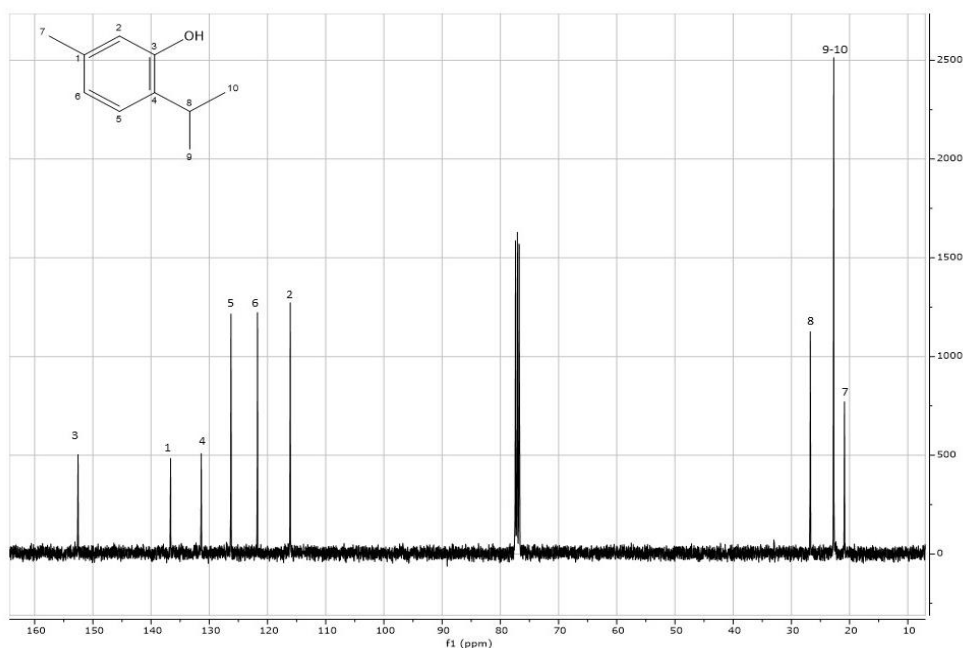


Figure 2: $^{13}\text{C-NMR}$ Spectra of Thymol

The process of making the in-situ gels

Various amount of P188 and P407 with mucoadhesive like HPMC were used to create thymol in situ forming gels. To create medicated in situ forming gels depending on weight, a modified cold method was applied [14]. The ingredients in the produced formulations are listed in Table 1.

In this method, P188 and P407 were dispersed in distilled water and then mixed for 1 hour. The partly dissolved poloxamer solutions were maintained in the refrigerator and sometimes mixed to get clear, kept in the refrigerator at 4°C overnight. During manufacture, additional amounts of the mucoadhesive polymers HPMC were combined with the total poloxamer content, each at concentrations of 0.5-1 % (w/w) (Table 1). Finally, 0.5% thymol was added.

Table 1: Ingredients in in situ gels

Code	Content of ingredients in each formulation (% w/w)				
	Thymol	Poloxamer 407	Poloxamer 188	HPMC (4K)	Water (qs)
FB-1	0.5	15	5	0	100
FB-2	0.5	15	5	0.5	100
FB-3	0.5	15	5	1	100
FB-4	0.5	16	5	0	100
FB-5	0.5	16	5	0.5	100
FB-6	0.5	16	5	1	100
FB-7	0.5	17	5	0	100
FB-8	0.5	17	5	0.5	100
FB-9	0.5	17	5	1	100

pH

The pH was measured with a pH meter (Germany's HANNA). Three measurements (n=3) were taken in total.

Clarity

After gelation, the clarity of the in situ gels was evaluated on a black background. [15].

Syringeability Study

Using the same technique as Maheshwari et al., the produced formulations' capacity to flow readily through a syringe with a 20 gauge needle was evaluated. A 20 gauge needle syringe was loaded with one ml of the cold gel before testing its flowability at standard handling pressure. It has been determined whether the formulation flows from the tip of the syringe number 20 used in dental applications [16]

Gelation Temperature

Each polymer solution (10 ml) was stirred with a magnetic stirrer in a water bath. The heated polymer solutions were swirled at 100 rpm at 1 °C/min (Thermomac-TM19). The temperature at which the magnetic bar stopped moving was marked as the gelling temperature. Each was subjected to three measurements.

Viscosity

The Brookfield, DV2T-RV Viscometer (Essex, UK) was used to gauge the viscosity of in situ gels using a CP 52 spindle. Viscosity at 10 rpm was also shown for comparison (Table 2). Each was subjected to three measurements [17].

Drug Content

To determine the amount of MHL in in situ gel, 1 mL of in situ gel was diluted in 1 mL of ethanol water mixture (Ethanol:Water) 50:50. Thymol concentrations were determined using a UV spectrophotometer (UVmini-1240 Shimadzu) at 278 nm [18, 19]. (Calibration of the method was carried out by measuring a series of standards of diluted stock solutions of Thymol. Absorbance values were plotted against Thymol concentrations over a range of 0,5-15 ppm).

$$\text{Drug Loading (\%)} = \left[\frac{\text{Amount to encapsulated thymol}}{\text{Total Weighted}} \right] \times 100$$

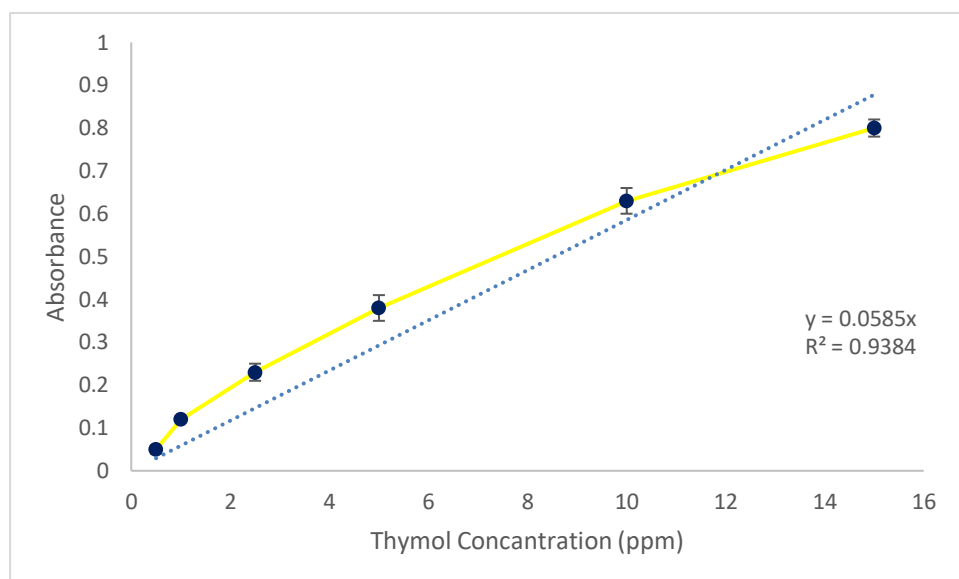


Figure 3:Thymol calibration curve with the calibration equation and correlation coefficient The linear regression equation Thymol was found as $y=0.0585x$ where y is the average and X is concentration (ppm), with a correlation 0.9384.

In Vitro Release Studies

The dialysis bag method was used to examine the in vitro release of thymol [20]. Closing the dialysis bags, adding 100 μ L of thymol, and depositing them at 37 °C in 25 mL of an isotonic phosphate buffer with a pH of 7.4 were the next steps. The sink condition is provided in this way. At several time point (15 minute, 30 minute, 60 minute, 120 minute, 180 minute, 240 minute, and 360 minutes), equal amounts of the medium were taken out and replaced with equal portions of the new buffer media. UV-vis spectrophotometer measurements of thymol concentrations were made. The amount of medication released from in situ gel over time was utilized to develop a thymol release profile. The experiment was repeated three times.

3.Results

Thymol Isolation from *T. pectinatus*

T. pectinatus was used to isolate thymol (Figure 1). Nuclear Magnetic Resonance (NMR) spectroscopy was used to determine the chemical structure. The isolated substance's spectrum data are as follows:¹H NMR (400 Hz), δ : 1.29 (6H, d, $J=6.8$ Hz, H-9, 10), 2.33 (3H, s, H-7), 3.22 (1H, m, $J=6.8$ Hz, H-8), 4.79 (1H, s, -OH), 6.62 (1H, s, H-2), 6.79 (1H, d, $J=7.6$ Hz, H-5), 7.14 (1H, d, $J=7.6$ Hz, H-4);¹³C NMR (400 Hz), δ : 20.9 (C-7), 22.72 (C-9,10), 26.75 (C-8), 116.39 (C-2), 121.95 (C-6), 126.47 (C-5), 131.52 (C-4), 137.08 (C-1), 152.44 (C-3). These data are similar to those previously reported The information is comparable to what has already been published [13].

Characterization of in situ gel formulations

All of the thymol in situ gel were observed to gel between 33 and 37 °C, making them suitable for dental administration. The data in Table 2 demonstrated that the pH of all formulations ranges between 7.01 to 7.11. It has been found to be syringable from a 20 gauge needle.

As a consequence, viscosity coefficients were obtained for all formulations at 10 rpm at both 25 °C and 37 °C. The results indicated that the viscosity values changed depending to the polymer concentrations (Table 2)

Table 2: Results of in situ gels' in vitro characterization analysis

Formulation	pH (\pm SD)	Gelation temperature ($^{\circ}$ C \pm SD)	Viscosity (centipoise) 25 $^{\circ}$ C	Viscosity (centipoise) 37 $^{\circ}$ C	Clarity
FB-1	7.02 \pm 0.01	37 \pm 0.7	224 \pm 19	6842 \pm 124	Clear
FB-2	7.05 \pm 0.02	37 \pm 0.3	263 \pm 25	7338 \pm 163	Clear
FB-3	7.01 \pm 0.03	36 \pm 0.1	287 \pm 32	7545 \pm 212	Clear
FB-4	7.05 \pm 0.02	35 \pm 0.2	268 \pm 19	7152 \pm 128	Clear
FB-5	7.1 \pm 0.08	35 \pm 0.4	296 \pm 38	7948 \pm 132	Clear
FB-6	7.01 \pm 0.02	35 \pm 0.6	312 \pm 29	8442 \pm 222	Clear
FB-7	7.03 \pm 0.07	34 \pm 0.4	310 \pm 31	9041 \pm 432	Clear
FB-8	7.02 \pm 0.05	34 \pm 0.8	324 \pm 35	10874 \pm 467	Clear
FB-9	7.11 \pm 0.03	33 \pm 0.2	344 \pm 43	11231 \pm 424	Clear

Drug Loading

All formulations have loading capabilities of more than 97 percent, according to tests (Figure 4). The 3% loss is thought to occur during the addition of Thymol. This reveals that polymer concentration has no effect on drug loading.

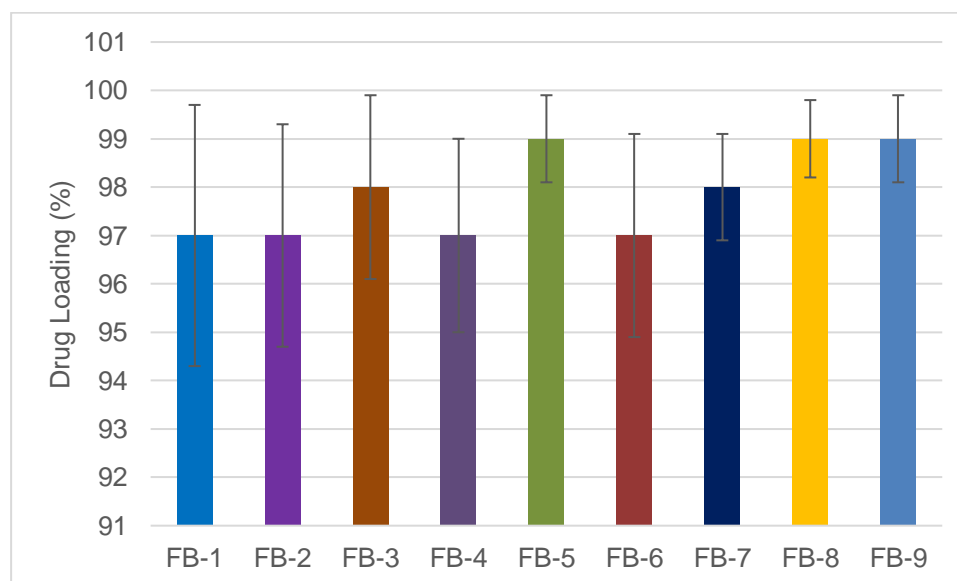


Figure 4: Drug loading given in percentages for Thymol (n=3)

Drug Release

In situ gels were submitted to in vitro drug release experiments at 37 °C and pH 7.4 isotonic phosphate buffer containing thymol (% 0.5). Figure 5 illustrates the in vitro release profiles of thymol. When the two-hour formulation releases are investigated, it is observed that as the HPMC concentration increases, the burst release drops to 56%. The burst impact is reported to approach 70% in formulations that do not include any HPMCs, such as FB-1. However, at the end of the sixth hour, 95 percent of the thymol had been released for all formulations. As a consequence, the sixth hour was considered as a final time the drug was administered.

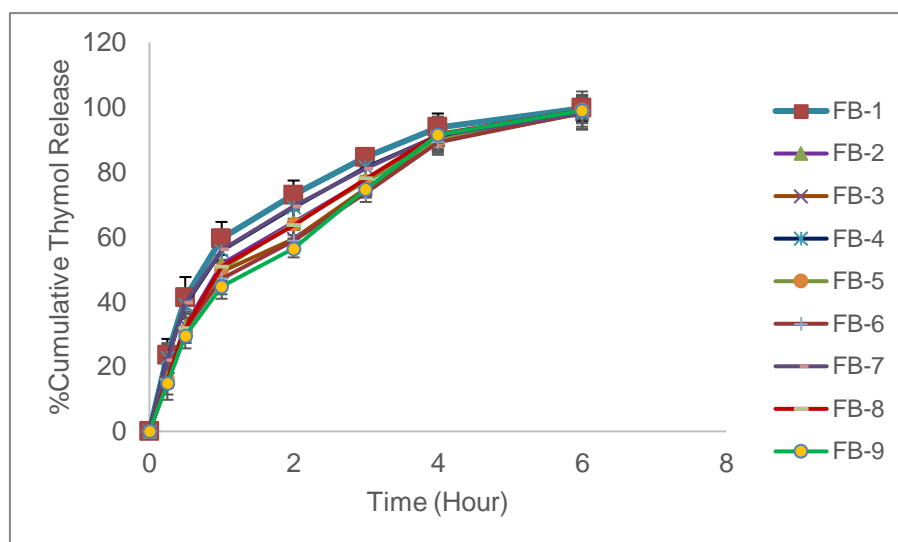


Figure 5: Cumulative release of thymol from in situ gel

4. Discussion and Conclusion

Two different temperatures—room temperature of 25°C and the administration dental pocket area temperature of 37°C—were used in the sol-gel transition studies to determine if the formulations were suitable for in-situ application as well as storage conditions [21]. Depending on the grade, thermoreversible gelation of poloxamer solutions has been seen, concentration, and other formulation factors utilized. When the two poloxamer grades are combined, the gelation temperature may be regulated to fall within an appropriate range (25-37°C) [22].

Two combinations of polymer grades were studied and used to produce in situ forming gels in order to choose formulations with an adequate sol-gel transition temperature and the lowest overall pluronic concentrations. The results of thymol in situ forming gel formulations containing P407/P188 (15/5, 16/5, and 17/5 percent, w/w) were remarkable. All of the thymol in situ forming gel formulations were observed to gel between 33 and 37 °C, making them suitable for dental administration. The data was demonstrated in Table 2.

The inclusion of mucoadhesive polymers, which allow formulations to adhere to the dental surface, would dramatically reduce dental formulation drainage from the dental surface. According to Table 3, the transition temperature of the in situ forming gels gradually reduced as the concentration of the mucoadhesive polymers raised from 0% to 1%. This was caused by the mucoadhesive polymer HPMC, which reduced the gelation temperature of the gels.

The ability of such bioadhesive polymers to attach to polyoxyethylene chains present in pluronic molecules may explain their ability to reduce gelation temperature. This promotes dehydration, which increases intermolecular hydrogen bonding and entanglement of adjacent molecules, resulting in dramatically greater gelation at lower temperatures [22].

Table 2 shows the in vitro characterization findings of in situ gels. The look of all formulations was clear. The pH of all formulations ranges between 7.01 to 7.11. Hypodermic syringes with gauges 19–27 are used for oral injection. An extremely viscous solution requires the use of a needle with a

smaller gauge [23]. Syringeability is defined as the amount of force necessary to discharge each formulation from a syringe fitted with a 20-gauge needle. The syringeability requirements are met by all formulations. The formulation, on the other hand, should have an optimal viscosity to readily infuse in the periodontal pocket.

As a consequence, viscosity coefficients were obtained for all formulations at 10 rpm at both 25 °C and 37 °C. The results showed that the viscosity values changed depending to the polymer concentrations (Table 2). This example demonstrates how polymer concentration has a substantial influence on viscosity. The results are compatible when the findings were examined in the literature [24].

When all in situ gel formulations are examined, it is seen that there is a drug loading over 97%. This situation is similar to the literature.

The release results from in situ gels show that when the concentration of P407 enhanced from 15% to 17%, the amount of medicine discharged decreased. These data reveal that as the quantity of P407 increased, the structure of the gel became a more formidable barrier to drug release. A decrease in the quantity and size of water channels and an enhance in the quantity and size of micelles inside the gel structure could be the mechanisms producing this improved resistance [25]. Higher viscosity and slower drug release are caused by more cross-links between surrounding micelles as a result of the smaller intermicellar distance [17,26].

In conclusion, multiple polymer solutions with varying concentrations of P407, P188, the mucoadhesive polymer HPMC, and thymol were prepared as part of the experiment. All of these formulations were tested in vitro (pH, clarity, gelation temperatures and syringeability). When the gelation temperatures were compared to the poloxamer concentrations, the gelation temperature decreased. The pH of all formulations, however, was found to be near to 7. It has been determined that the formulations would not irritate the dental. Furthermore, both formulations drug loading capabilities surpassed 97 %. Regardless, all formulations were made available for six hours. All formulations can be applied dentally. The dental surface is thought to increase the interaction of a drug with its bioavailability. Thymol is one example of such drug. In vitro characterization tests suggest that all formulations may be used to treat periodontitis effectively

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

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