

## Original Article

# Design, formulation, and physicochemical evaluation of periodontal propolis mucoadhesive gel

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## ABSTRACT

**Background:** Periodontitis is a disease of tooth supporting tissues, and Gram-negative *Bacteria* are the main cause of this. Propolis has antibacterial, anti-inflammatory, and antioxidant effects due to its high polyphenol and flavonoids content. The aim of this study is the formulation of a mucoadhesive gel containing concentrated extract of propolis for treatment of periodontitis.

**Materials and Methods:** Formulations containing carbopol 940, sodium carboxymethylcellulose (NaCMC), hydroxypropyl methylcellulose K4M, and propolis extract were prepared. Among ten prepared formulations, five formulations had acceptable and proper physical appearance and uniformity; thus, they were selected for physicochemical tests (centrifugal, thermal change, cooling and heating, freeze and thaw, thermal stress, and pH evaluation), quantification of flavonoids, viscosity, mucoadhesion, drug release, and syringeability tests. We investigated the antibacterial activity of F<sub>10</sub> (carbopol 940 1%, NaCMC 3%) against *Porphyromonas gingivalis* using the disk diffusion method.

**Results:** Phenolic content was measured 39.02 ± 3.24 mg/g of concentrated extract as gallic acid and flavonoid content was determined 743.28 ± 12.1 mg/g of concentrated extract as quercetin. Highest viscosity (3700 cps) and mucoadhesion (21 MPa) were seen in F<sub>10</sub>. Study of release profile in F<sub>10</sub> also revealed that propolis could release from this system in more than 7 days. All of the five selected formulations had ease of syringeability in 21-gauge needle for drug delivery into periodontal pocket. In the disk diffusion method, F<sub>10</sub> produced significant growth inhibition zones against *P. gingivalis*.

**Conclusion:** Controlled release of drug into periodontal pocket helps effective treatment and recovery, higher persistence and reduces drug use frequency. Increase of carbopol 940 leads to viscosity and mucoadhesion elevation and accordingly decreases of release rate. F<sub>10</sub> was the best formulation because of highest viscosity and mucoadhesion and lowest release rate. It had efficient function in treatment of periodontitis, so we recommend it for clinical evaluation.

**Key Words:** Antibacterial, compounds, drug delivery systems, effects, flavonoids, oral, periodontitis, propolis

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## INTRODUCTION

Gingivitis and periodontitis are the most common oral diseases; the first stage of the disease is inflammation of the soft tissue besides the teeth which is called gingivitis.<sup>[1]</sup> However, periodontitis is characterized by inflammation of supporting tissues of the teeth, progressive destruction of periodontal ligament, and alveolar bone along with formation of periodontal pocket and gingival recession.<sup>[2,3]</sup>

The immune response from interaction between microorganisms and inflammatory cells in the surrounding tissue of the teeth leads to this disease. Released enzymes and toxins from these microorganisms make periodontium destructed.<sup>[3]</sup> Seven important signs of periodontal disease are red and swelling gums, gingival bleeding, unpleasant breath and bad taste, gaps which gradually form between teeth, loose teeth that eventually falling out if not treated, gingival receding or recession, and itchy or painful gums.<sup>[4]</sup> The main microorganisms involved in disease progression are *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Actinobacillus actinomycetemcomitans* which are very important in etiology of chronic periodontal disease. More than 500 different bacterial species are found in dental plaque. Many of periodontal pathogens are obligatory anaerobic and Gram-negative *Bacteria*.<sup>[5]</sup>

*Bacteria* in periodontal pockets live in biofilms (sticking to the surface) that help them resist against antibiotics. Highly resistant *Bacteria* with pumps that help remove antibacterial drugs from cell have recently been identified. *Bacteria* can connect to each other in biofilm; this action helps transmission of genes that play the role of resistance against antibiotics and growth of the resistant microorganisms species.<sup>[6]</sup>

Timely treatment of the disease and necessary care reduce the disease progression and prevent progressive bone erosion. Without essential care and interventions, bone erosion could cause teeth loss.<sup>[7]</sup> The most important treatment modality is controlling the inflammation by removing dental plaque and calculus. The first stage of treatment is a nonsurgical procedure which includes health education, dental root planing and scaling to reduce pathogen microorganisms.<sup>[8]</sup>

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as flurbiprofen, naproxen, and mefenamic acid have been proven to be effective in the treatment

of periodontal disease.<sup>[9]</sup> Furthermore, due to special role of some microorganisms in disease progression, antibiotics and antiseptics are used too. These antibiotics include tetracyclines, clindamycin, metronidazole, penicillins, and antiseptics such as chlorhexidine.<sup>[10]</sup>

Misuse of systemic antibiotics can lead to Bacterial resistance. Since the causes of disease are a wide range of Bacteria, there are no ideal and effective antibiotics for all the pathogens and concomitant use of different antibiotics may intensify medical side effects.<sup>[11]</sup> Short-term efficacy and inconstancy of antiseptic mouthwashes and side effects of systemic antibiotics led to use of local therapy systems. These new systems have advantages of reduction in prescribed drugs, increasing drug concentration in target tissue, reduction in drug side effects and reduce drug use frequency.<sup>[12]</sup> Local tetracycline fibers at subgingival area, minocycline gel at subgingival area, and chlorhexidine and metronidazole gels at subgingival area are the examples of these novel drug delivery systems.<sup>[13,14]</sup>

Natural products such as propolis can also prevent infection development. They are very safe and cost-effective and can reduce side effects. Local drug delivery of natural products to periodontal pocket is an adjunctive therapy in addition to dental root planing and scaling for the treatment of periodontal disease.<sup>[7]</sup>

Propolis is a brown, viscous, and wax-like resin which is very sticky (bee's glue) and is collected by bees and extracted from hives. Bees use propolis to fill the gaps, smooth inner surfaces of the hive and prevent entering other insects to the hive.<sup>[15]</sup> Extracted propolis is mixed with hypopharyngeal gland secretions, beeswax, and pollen. It is a very complex mixture which its composition differs from one region to another.<sup>[16]</sup> Propolis composition contains 55% resinous compounds, 30% wax, 10% aromatic oils, and 5% honeybee pollen. Propolis is full of Vitamins A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub>, biotin, bioflavonoids (very important content of propolis and the reason for its properties), and polyphenols.<sup>[17]</sup>

Polyphenol and flavonoid compounds have antimicrobial activity and are indirectly involved in response to microorganisms. The mechanisms of producing hydrogen peroxide, protein, and bacterial enzymes inhibition and antiseptic activity are well identified for these compounds.<sup>[16]</sup> Due to reduction

in antioxidant activity of saliva and salivation in these patients, using an antioxidant can be beneficial to prevent disease progression. Polyphenols and flavonoids increase antioxidant activity of saliva and inhibit periodontal diseases. They inhibit prostaglandins production too.<sup>[18]</sup>

Propolis has antibacterial, anti-inflammatory, antioxidant (because of artemillin C and caffeic acid) activity. Nowadays, propolis is very applicable in new formulations of dentistry, dermatology, gastroenterology, and veterinary.<sup>[7,17,19,20]</sup>

Mucoadhesive drug delivery systems have been introduced as new dosage forms because of their ability to remain on the mucous membranes and a slow release of their drug content. One of the new dosage forms is mucoadhesive gel. Some properties of ideal formulation are ease of entrance to periodontal pocket by syringe, controlled release of drug to oral mucosa, the ability to remain in the pocket without mechanical connectivity to teeth surfaces, and being nonallergic and nontoxic.<sup>[7]</sup> It seems that this drug delivery system can let us save more time and money because the patient needs to visit the physician just once for applying drug to the periodontal pocket.

The aim of this study is designing and formulation of propolis mucoadhesive gel from carbomer 940, hydroxypropyl methylcellulose (HPMC), and NaCMC as adjuvant therapy for periodontal disease alongside dental root planing and scaling with antibacterial, anti-inflammatory, and antioxidant effects.

## MATERIALS AND METHODS

### Materials

Propolis was provided and authenticated by the Agricultural Research Center (Isfahan, Iran) on April 2014.

Folin–Ciocalteu reagent, quercetin, gallic acid, sodium carbonate anhydrous, carbopol 940, HPMC K4M, NaCMC, polyethylene glycol 400 (PEG 400), triethanolamine, methanol, and 96% ethanol were purchased from Merck Company (Germany).

Potassium sorbate, aluminum chloride 20%, and glacial acetic acid were obtained from Sigma-Aldrich Chemie GmbH, (Steinheim, Germany).

### Methods

#### *Propolis extraction*

Propolis particles were mechanically cleaned and placed in freezer for fragmentation into fine

particles. To produce propolis extract, 3000 ml of 96% ethanol was mixed well with 1 kg propolis by maceration method of extraction. This mixture was retained at room temperature and dark place. It was mixed 3 times a day. After 10 days, it was filtered through filter paper, grade 589 (Whatman GmbH, Dassel, Germany). For extract concentration, a rotary evaporator (Heidolph VV 2000) was used at 40°C to remove extra solvent. For further concentration, water bath was used.<sup>[7,21]</sup>

#### *Determination of pH extract*

pH of the extract was measured by pH meter (Metrohm 632 Swiss). First measurements were done 24 h after extraction, next pH measurements were prepared 1 week, 2 weeks, 1 month, 3 months, and 6 months after extraction.<sup>[22]</sup> The pH was measured 3 times and the average value was reported.

#### *Determination of polyphenols in ethanolic extract of propolis*

In this study, the Folin–Ciocalteu method for determination of polyphenol contents was used. Folin–Ciocalteu colorimetry method is based on a chemical reduction of the reagent (a mixture of tungsten and molybdenum oxides). Gallic acid solution is used as the standard solution. To prepare stock solution, 500 mg of dry gallic acid was dissolved in 10 ml of 96% ethanol; then, it was diluted to 100 ml by purified water. To draw gallic acid calibration curve, 1, 2, 3, 5 and 10 ml of stock solution were added to distinct 100 ml volumetric flask and were diluted with purified water. Reference and blank solutions were also prepared using Folin–Ciocalteu's reagent and sodium carbonate. After shaking and incubation at 20°C–25°C for 2 h, the absorbance of each sample was measured at  $\lambda_{\max}$  765 nm by the ultraviolet-visible (UV-VIS) spectrophotometer (UV mini-1240, Shimadzu). Then, by means of calibration curve, we measured polyphenol concentrations and their percentages. The polyphenol content was reported as mg gallic acid equivalent (GAE) per 1 g of the extract.<sup>[23]</sup>

To prepare the sample, 500 mg of propolis was dissolved in 10 ml of 96% ethanol and then was diluted to 100 ml with purified water in a volumetric flask. Other steps were repeated as for standard sample. Finally, the absorbance of our sample was measured at 765 nm.<sup>[24]</sup> The test was repeated 3 times and the average of absorbances was applied.

#### *Determination of flavonoids in ethanolic extract of propolis*

Aluminum chloride colorimetric method was used to determine flavonoid content in propolis extract. Quercetin

is considered as the standard solution. Standard curve of quercetin solution was drawn using 12.5, 25, 50, 75, and 100 concentrations as  $\mu\text{g/ml}$  in methanol.<sup>[24]</sup>

Preparation of propolis standard solution was as follows: 50 mg of ethanolic extract of propolis was dissolved in 25 ml methanol, 100  $\mu\text{l}$  of this solution was mixed with 100  $\mu\text{l}$  of 20% aluminum chloride, and 2 drops of glacial acetic acid was added and diluted with methanol to 3 ml. It was mixed well and kept at room temperature for 40 min. Blank solution with only propolis extract and glacial acetic acid was also prepared. Then, absorbances of samples were measured at  $\lambda_{\text{max}}$  415 nm, and flavonoid concentration in extract was determined by the standard curve. The flavonoid content was reported as mg of quercetin (QE) equivalent per 1 g of extract.<sup>[23]</sup> The results were reported in average after triplicate experiments.

#### Formulation preparation of propolis mucoadhesive gel

Some polymers such as NaCMC, carbopol 940, and HPMC were used to obtain propolis mucoadhesive gel. The amount of concentrated extract in gel base is 10% w/w.

#### Carbopol 940 gel

For preparation of carbopol gels, we applied three different amounts of carbopol. Potassium sorbate was dissolved in purified water at 50°C.<sup>[25]</sup> Then, specified amounts of carbopol 940 were dispersed in purified water at 40°C and were mixed by mixer at 1200 rpm for 30 min [Table 1]. Propolis extract was dispersed in PEG 400, and then, it was gradually added to the base. The neutral pH was obtained by required amount of triethanolamine to gain a transparent gel.<sup>[26,27]</sup>

#### HPMC gel

For preparation of this gel, three different amounts of HPMC were used. Potassium sorbate was dissolved in purified water at 50°C.<sup>[25]</sup> Specified amounts of HPMC were dispersed in purified water at 50°C and were mixed by mixer at 1200 rpm for 30 min [Table 1]. Then, the remaining amount of purified water was added coldly and mixed to gain a homogenous gel. The prepared gel is kept in refrigerator for 1 night (hot/cold technique). Propolis extract which was dispersed in PEG 400 was then added to the base and mixed well to obtain a homogenous gel.<sup>[28]</sup>

#### NaCMC gel

For preparation of this gel, two distinct amounts of NaCMC were used. Potassium sorbate was dissolved

**Table 1: Composition of gel formulations with different polymers (Carbopol 940, Sodium carboxymethylcellulose, hydroxypropyl methylcellulose)**

| Ingredients (g)   | Formulations   |                |                |                |                |                |                |                |                |                 |
|-------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|
|                   | F <sub>1</sub> | F <sub>2</sub> | F <sub>3</sub> | F <sub>4</sub> | F <sub>5</sub> | F <sub>6</sub> | F <sub>7</sub> | F <sub>8</sub> | F <sub>9</sub> | F <sub>10</sub> |
| Carbopol 940      | 0.5            | 1              | 1.5            | -              | -              | -              | -              | -              | 0.5            | 1               |
| Sodium CMC        | -              | -              | -              | 3              | 4              | -              | -              | -              | 3              | 3               |
| HPMC              | -              | -              | -              | -              | -              | 2              | 3              | 4              | -              | -               |
| Propolis extract  | 10             | 10             | 10             | 10             | 10             | 10             | 10             | 10             | 10             | 10              |
| PEG 400           | 13             | 13             | 13             | 13             | 13             | 13             | 13             | 13             | 13             | 13              |
| Potassium sorbate | 0.2            | 0.2            | 0.2            | 0.2            | 0.2            | 0.2            | 0.2            | 0.2            | 0.2            | 0.2             |
| Triethanolamine   | qs             | qs             | qs             | -              | -              | -              | -              | -              | -              | -               |
| Purified water    | 100            | 100            | 100            | 100            | 100            | 100            | 100            | 100            | 100            | 100             |

in purified water at 50°C.<sup>[25]</sup> Then, specified amounts of NaCMC were dispersed in purified water at 50°C and were mixed by mixer at 1200 rpm for 30 min [Table 1]. Propolis extract was dispersed in PEG 400, and then, it was gradually added to the base.<sup>[27]</sup>

#### Carbopol 940 and NaCMC gel

Two different amounts of carbopol and NaCMC were used. Potassium sorbate was dissolved in purified water at 50°C.<sup>[25]</sup> The weighed amounts of carbopol and NaCMC were dispersed in purified water at 50°C and mixed well [Table 1]. Propolis extract which was dispersed in PEG 400 was then added to the gel bases.

### Evaluation tests for formulations

#### Macroscopic and microscopic study

This test is carried out 48 h after gels preparation. Macroscopic balance is checked in this evaluation test (color, homogeneity, transparency, lack of particles presence, and consolidation).<sup>[27]</sup> Microscopic reviews are done by 10 X and 40 X microscopes to check the gel texture, uniformity, and presence of bubbles. Evaluation of consolidation is by pressing a small amount of gel between fingers to consider its consolidation.<sup>[22]</sup>

#### pH evaluation of gel formulations

pH meter, which was recently calibrated with standard buffer solution at pH 4 and 7, was used to measure the pH. One gram of each formulation was dissolved in 10 ml purified water.<sup>[29]</sup> The test was repeated 3 times and the average was reported. pH of samples was measured 48 h, 1 week, 2 weeks, 1 month, 3 months, and 6 months after preparation.

### Centrifugal test

This test was carried out to evaluate the resistance of gel against gravity 48 h after preparation. Five grams of each sample was added to test tubes, and then, they were centrifuged at 1200 rpm for 5, 15, 30, and 60 min by centrifugal device (centrifuge 5430). Each formulation was evaluated in terms of precipitation.<sup>[22]</sup> For each formulation, the test was repeated 3 times.

### Thermal changes test

This test is to evaluate gel stability against climatic conditions. Forty-eight hours after gel preparation, three samples from each formulation were prepared and placed at 4°C, 25°C, and 45°C. The samples were evaluated considering appearance, homogeneity, and quality of gel after 24 h, 1 week, 2 weeks, 1 month, 3 months, and 6 months.<sup>[22]</sup>

### Cooling and heating test

Extreme thermal changes lead to investigate formulations stability. Fifteen grams of each formulation was placed at 45°C for 48 h and at 4°C for 48 h for 6 consecutive periods. The apparent changes were then reported.<sup>[22]</sup>

### Freeze and thaw test

This test is to investigate the physical stability of semisolid products in extreme thermal changes. Forty-eight hours after gel preparation, 15 g of each sample was placed in 6 consecutive periods which include 48 h at -8°C and 48 h at 25°C.<sup>[22]</sup>

### Thermal stress test

Stability studies are done at accelerated conditions and also at 30°C ± 2°C and relative humidity of 60% ± 5% for 6 months.<sup>[22]</sup>

## Study of *ex vivo* adhesion strength of the formulations

Mucoadhesion is a principal property to treat periodontal disease. The SANTAM instrument (STM-1, Iran) is used to measure gel mucoadhesion. In this study, mucosal lining of the cow cheek was employed as a model to determine the adhesion strength of the gel. Certain weight of gels (200 mg) was spread over a piece of mucosal lining of the cow (20 mm × 20 mm) after wetting it by some drops of purified water. Then, it attached to constant surface of instrument while another piece of mucosal lining of the cow was attached to the upper surface of the SANTAM instrument. Then, the gel was kept in full contact with the mucosa for 2 min. The force required for detachment of the gel from the mucosal

surface with the rate of 10 mm/min was calculated and reported as the adhesion force of the gel. The detachment force was measured in terms of MPa.<sup>[30]</sup> The test was repeated 3 times for each formulation.

### Determination of flavonoids in gel formulations

Forty-eight hours after preparation of formulations, 1 g of each formulation was dispersed in purified water and diluted to 10 ml in volumetric flask.<sup>[31]</sup> Flavonoid content was quantified as QE as defined before.<sup>[23]</sup>

### *In vitro* drug release

Drug release test of gels is done by Franz diffusion cell using a cellulose acetate membrane. 500 mg of each formulation was placed on cellulose acetate membrane. The device was filled by phosphate buffer, pH 6.2 as dissolution medium at 37°C ± 0.5°C. One milliliter aliquots of medium were removed at times of 6, 24, 48, 72, 96, 120, 144, and 168 h since the beginning of test. The aliquots were replaced by fresh phosphate buffer subsequently. The absorbances of samples were measured at 415 nm by UV-VIS spectrophotometer. The released drug was calculated by the quercetin standard curve. Concentrations obtained are apparent concentrations. To determine the actual amount of released drug, following equation is used:<sup>[22]</sup>

$$C_n = C + (C_{n-1}) V/V_t$$

$C_n$ : Actual concentration in sample  $n$

$C$ : Apparent concentration in sample  $n$

$C_{n-1}$ : Actual concentration in sample  $n - 1$

$V_t$ : Volume of receive phase

$V$ : Sample volume.

### Drug release kinetic studies of gel formulations

To investigate the mechanism of flavonoids release from gel formulations, the release data were analyzed with the following mathematical models: zero-order kinetic (Equation 1), first-order kinetic (Equation 2), and Higuchi kinetic (Equation 3).

$$Q_t = K_0 t \quad (1)$$

$$\ln Q_t = \ln Q_0 - K_1 t \quad (2)$$

$$Q_t = K_h t^{1/2} \quad (3)$$

In these equations,  $Q_t$  is the percent of drug released at time  $t$ ,  $Q_0$  is the percent of drug present in the gel.  $K_0$ ,  $K_1$ , and  $K_h$  are the constants of the equations. In the zero-order kinetic model, diagram of cumulative

percentage of drug release was plotted against time and a linear plot was obtained. In the first-order kinetic model, log of cumulative percentage of drug remaining was plotted against time and a linear plot was obtained. In Higuchi kinetic model, cumulative percentage of drug release was plotted against square root of time and a linear plot was obtained.<sup>[22]</sup>

Furthermore, drug release mechanism was determined according to the Korsmeyer–Peppas equation.

$$\text{Log} (M_t/M_\infty) = \log k + n \log t$$

Where  $M_\infty$  is the amount of drug released after infinite time,  $M_t$  is the cumulative amount of drug released at any specified time ( $t$ ),  $k$  is the release rate constant, and  $n$  is the release exponent.

When  $n$  value is 0.5 or less, the Fickian diffusion phenomenon dominates, and  $n$  value between 0.5 and 1 is non-Fickian diffusion (anomalous transport). The mechanism of drug release follows Case-II transport when the  $n$  value is 1, and for the values of  $n$  higher than 1, the release is characterized by super-Case-II transport.<sup>[30]</sup>

#### Determination of viscosity

Viscosity of samples was measured by Brookfield DV-3 viscometer at 100 rpm, using spindle number 74 at 25°C. The spindles of the device rotate in the sample which is placed in the container. Before determination of viscosity and start of the device, the samples were put at room temperature for 30 min.<sup>[22]</sup> A triplicate experiment was done for each formulation.

#### Syringeability

Injectable systems are preferred for drug delivery to periodontal pocket. Injection of gels is evaluated by 21-gauge needle. Ease of syringeability is the scale of measure.<sup>[7,22]</sup>

#### In vitro evaluation of antibacterial activity of gel formulation (F<sub>10</sub>) against *Porphyromonas gingivalis*

The most common etiological agent of chronic periodontitis is *P. gingivalis*.<sup>[5]</sup> In this study, antibacterial effect of gel formulation (F<sub>10</sub>) against *P. gingivalis* using the disk diffusion method was studied. *P. gingivalis* was prepared by sampling from periodontal pocket of a patient (a 46-year-old man from dental clinic of Islamic Azad University of Isfahan, Isfahan, Iran). Then, they were cultured on supplemented Brucella agar plates (prepared from the Iranian research organization for science and technology) under sterile conditions. Culture media were incubated for 72 h in anaerobic condition

at 37°C ± 2°C to allow bacterial growth.<sup>[32]</sup> After bacterial growth, a microbial suspension was prepared and transferred to the plates containing culture medium by a sterile swap. Then, sterile paper discs with a diameter of 5 mm (Blank disc, Patan Teb, Iran) were smeared with gel formulation and were placed on the surface of the plates containing culture medium in equal distances. Then, the plates were incubated at 37°C ± 2°C for 72 h. After this period, the diameter of the zone of inhibition around the disc was measured in millimeters and the average of diameters noted. The test was conducted in triplicate. Sterile discs free of any material were used as negative control in culture medium. For positive control, tetracycline disc (30 µg/ml) was used.<sup>[22]</sup>

## RESULTS

#### Analyzing the propolis extract

About 500 ml of concentrated extract of propolis was obtained from maceration and removing of alcoholic solvent by rotary evaporator. Its pH was in the range of 6–7. The content of phenolics as GAE was expressed as 39.02 ± 3.24 mg/g of concentrated extract. The content of flavonoids as QE was expressed as 743.28 ± 12.1 mg/g of concentrated extract. The curve linear equation for gallic acid was  $y = 0.122x + 0.002$  ( $R^2 = 0.979$ ) and for quercetin was  $y = 0.0067x + 0.008$  ( $R^2 = 0.997$ ).

#### Propolis gel analysis

10% propolis extract was added to gel base of carbopol 940, HPMC, and NaCMC [Table 1]. After adding propolis extract to formulations containing HPMC (F<sub>6</sub>–F<sub>8</sub>), they developed phase separation and precipitation and propolis ethanolic extract was appeared as some drops on the surface of gel. The reason of phase separation was incompatibility between extract and HPMC so that these formulations were not tested for further experiments. Viscosity of F<sub>3</sub> and F<sub>5</sub> was high, so ethanolic extract was not dispersed uniformly. Their physical appearance and uniformity were also inappropriate, so no more tests were carried out.

F<sub>1</sub>, F<sub>2</sub>, F<sub>4</sub>, F<sub>9</sub>, and F<sub>10</sub> had good physical appearance and uniformity; thus, they were selected for further tests. In centrifugal tests, thermal changes test, cooling and heating test, freeze and thaw test, and thermal stress test, all of the five selected formulations passed all of the tests. pH of selected formulations is mentioned in Table 2. They were in the range of 5–6.

Results of drug content of the formulations, mucoadhesion, and viscosity measurements are shown in Table 2. As considered, F<sub>10</sub> has the highest viscosity and mucoadhesion. Results of drug release are shown as diagrams of cumulative percentage of drug release against time [Figure 1]. Drug release from F<sub>10</sub> was slower than other formulations. Time needed for 50% release of whole drug content from F<sub>10</sub> is approximately 75 h as displayed in Figure 1. This time is 65, 60, 50 and 46 h for F<sub>2</sub>, F<sub>9</sub>, F<sub>1</sub>, and F<sub>4</sub> respectively.

The kinetic of drug release for selected formulations was determined by fitting the data into various kinetic models [Table 3]. In all the formulations, correlation coefficients of the Higuchi's models were higher than correlation coefficients of other kinetics. Thus, for drug release of all formulations, the Higuchi's kinetics was dominant.

To describe the mechanism of drug release from the gels, *in vitro* release data were fitted into Korsmeyer–Peppas equation. Drug diffusion for all formulations was non-Fickian type. Non-Fickian drug release means that the drug is released from the gel via diffusion mechanism and also another process called chain relaxation.<sup>[30]</sup>

Results of drug content determination of selected formulations are shown in Table 2. Results of inhibition zones diameter of F<sub>10</sub> and tetracycline disc

**Table 2: Results of determination of pH, drug content, mucoadhesive strength, and viscosity (at 100 rpm, 25°C) in formulations (mean±SD)**

| Physicochemical characteristics | F <sub>1</sub> | F <sub>2</sub> | F <sub>4</sub> | F <sub>9</sub> | F <sub>10</sub> |
|---------------------------------|----------------|----------------|----------------|----------------|-----------------|
| pH 48 h after preparation       | 6.2±0.2        | 6±0.2          | 6.6±0.1        | 6.3±0.2        | 6.4±0.2         |
| Drug content (mg QE/g)          | 57.3±0.3       | 58.1±0.3       | 57.8±0.3       | 57.4±0.3       | 57.5±0.3        |
| Mucoadhesive strength (MPa)     | 9±0.7          | 17±0.8         | 10±0.7         | 16±0.8         | 21±0.8          |
| Viscosity (cps)                 | 1100±8         | 2000±28        | 1050±16        | 1700±23        | 3700±32         |

**Table 3: Drug release and drug release kinetics of gel formulations in Franz diffusion cell through a cellulose acetate membrane, at 37°C, during 168 h (n=3)**

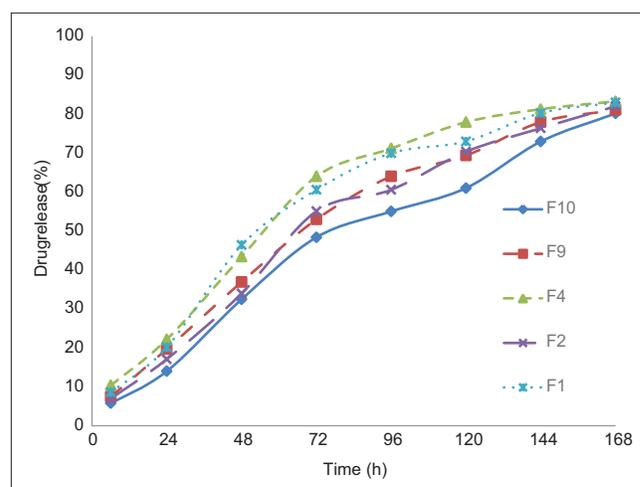
| Formulations    | Cumulative drug release (%) | Kinetic of drug release |                |                   |                |                |                |                   |       |                |
|-----------------|-----------------------------|-------------------------|----------------|-------------------|----------------|----------------|----------------|-------------------|-------|----------------|
|                 |                             | Zero-order model        |                | First-order model |                | Higuchi model  |                | Peppas parameters |       |                |
|                 |                             | K <sub>0</sub>          | R <sup>2</sup> | K <sub>1</sub>    | R <sup>2</sup> | K <sub>n</sub> | R <sup>2</sup> | n                 | K     | R <sup>2</sup> |
| F <sub>1</sub>  | 83.74±2.1                   | 0.531                   | 0.868          | 0.0096            | 0.908          | 7.322          | 0.981          | 0.845             | 1.475 | 0.97           |
| F <sub>2</sub>  | 82.12±1.2                   | 0.462                   | 0.872          | 0.0071            | 0.882          | 6.265          | 0.966          | 0.893             | 1.011 | 0.961          |
| F <sub>4</sub>  | 83.34±3.1                   | 0.572                   | 0.872          | 0.0141            | 0.918          | 7.864          | 0.975          | 0.82              | 1.77  | 0.973          |
| F <sub>9</sub>  | 81.12±2.8                   | 0.467                   | 0.861          | 0.0071            | 0.872          | 6.476          | 0.961          | 0.852             | 1.258 | 0.953          |
| F <sub>10</sub> | 80.23±1.7                   | 0.422                   | 0.887          | 0.0053            | 0.911          | 5.659          | 0.974          | 0.951             | 0.68  | 0.941          |

against *P. gingivalis* are shown in Table 4. All of the F<sub>1</sub>, F<sub>2</sub>, F<sub>4</sub>, F<sub>9</sub>, and F<sub>10</sub> had ease of syringeability in 21-gauge needle.

## DISCUSSION

Periodontitis is the inflammation of gums and supporting tissues of the teeth. It is one of the most common human diseases. Periodontitis is caused by certain *Bacteria* and local inflammation is triggered by those *Bacteria*.<sup>[30]</sup> As described before, treatment methods are dental root planing and scaling, NSAIDs, antibiotics, and antiseptics.<sup>[8,9]</sup> Propolis is a natural and nontoxic beehive product that prevents disease progression. Propolis contains flavonoids and polyphenols that have antimicrobial, anti-inflammatory, and antioxidant activity. It can help with dental root and scaling which is very effective in treatment of periodontal disease.<sup>[16,17]</sup>

In the present study, the content of phenolics and flavonoids was evaluated 39.02 ± 3.24 mg GAE/g



**Figure 1: Percentage of cumulative drug release of formulations F<sub>1</sub>, F<sub>2</sub>, F<sub>4</sub>, F<sub>9</sub>, and F<sub>10</sub> in Franz diffusion cell through a cellulose acetate membrane, in pH 6.2 phosphate buffer at 37°C, during 7 days.**

**Table 4: Inhibition zones diameter of gel formulation F<sub>10</sub> against *Porphyromonas gingivalis***

| Bacterial species               | Formulation F <sub>10</sub> | Positive control |
|---------------------------------|-----------------------------|------------------|
| <i>Porphyromonas gingivalis</i> | 7.3±0.2                     | 10±0.1           |

Numbers are mean diameter of inhibition zones in (mm), tetracycline disk (30 µg/ml) were used as reference antimicrobial compound

of concentrated extract and 743.28 ± 12.1 mg QE/g of concentrated extract, respectively.<sup>[23]</sup> According to Yaghoubi *et al.*, the percentage of phenolic and flavonoid contents of Iranian propolis was calculated 36% and 7.3%.<sup>[33]</sup> In addition, the concentration to phenolic and flavonoid compounds in Brazilian propolis was 100.59 ± 2.27 mg GAE/g of concentrated extract and 69.35 ± 0.38 mg QE/g of concentrated extract, respectively.<sup>[20]</sup> This difference in their chemical compounds could be due to different geographic origins with different climates, vegetation, and the time that the samples collected.<sup>[16]</sup>

Periodontal pocket has the pH of 6.2, so the pH of product should approach to local pH to decrease local irritations.<sup>[12]</sup> The pH of selected formulations was all in the range of 5–6 which is nearly ideal.

Comparison of mucoadhesion results revealed that the highest mucoadhesion first belongs to F<sub>10</sub>. Mucoadhesion strength represents a power which makes mucoadhesive membranes stick to mucous membrane. Proper polymers should have groups forming hydrogen bonds, wetting characteristics, and swelling ability to create mucoadhesion power.<sup>[27]</sup> Mucous surface is coated by mucin. Mucin has negative charge at physiological pH. A prerequisite for mucoadhesion is forming hydrogen bond between hydrophilic functional groups of mucoadhesive polymer and mucosal layer. More link points increase mucoadhesion strength. Higher molecular weights and viscosity elevate mucoadhesion strength.<sup>[34]</sup>

Carbopol is carboxy vinylic derivatives that are widely used in the manufacture of hydrogel dosage forms. It has high molecular weight and swells up to 1000 times of its original volume while neutralizing the system. It permits ionization of carboxyl groups, and as a consequence, a strong gel is formed.<sup>[27]</sup> Carbopol connection to mucin is secondary type while other polymers establish primary connection to mucin.<sup>[22]</sup> Carbopol as a mucoadhesive polymer increases gel's durability which is the most important matter about periodontitis.

As shown in Table 2, F<sub>10</sub> has the highest viscosity. It reveals that carbopol 940 increases gel's viscosity

and consolidation. Release rate in F<sub>10</sub> is slower than F<sub>1</sub>, F<sub>2</sub>, F<sub>4</sub>, and F<sub>9</sub>. When the amount of polymers increases, gel gets more solid and water penetration gets harder and thus drug release rate decreases and gel's durability in periodontal pocket rises, that is, the most significant thing in treatment of periodontitis.<sup>[9]</sup> The most important role in drug release rate belongs to carbopol due to its high molecular weight and high cross-linking, so increase of carbopol leads to increase of viscosity and mucoadhesion and decrease of release rate.<sup>[27,34]</sup>

It is important that the product could be delivered from a syringe to fulfill the requirement of ease of application. All of the formulations (F<sub>1</sub>, F<sub>2</sub>, F<sub>4</sub>, F<sub>9</sub>, and F<sub>10</sub>) had ease of delivery from syringe in 21-gauge needle.

Propolis was reported to have antibacterial activity on some Gram-negative *Bacteria*. Antibacterial activity against periodontal pathogens was exhibited by flavonoid compounds such as quercetin, kaempferol, pinocembrin, and galangin.<sup>[5,35]</sup> The data obtained in the present study revealed antibacterial activity of formulation F<sub>10</sub> against *P. gingivalis*.

Ideal formulation should easily get into grooves of tooth and should have proper mucoadhesion and persistence in periodontal pocket. Controlled release of drug into periodontal pocket helps effective treatment and recovery and higher persistence and reduces drug use frequency.<sup>[7]</sup> According to these results, F<sub>10</sub> was the best formulation in the treatment of periodontitis.

## CONCLUSION

Propolis extract contains polyphenols and flavonoid considerably that can be used in periodontal pocket therapy due to antioxidant, antibacterial, and anti-inflammatory activity. Based on the *in vitro* drug release, viscosity, and mucoadhesion studies, F<sub>10</sub> containing 1% carbopol 940 and 3% NaCMC was selected as the best formulation. F<sub>10</sub> showed satisfying mucoadhesion and viscosity and optimum release profile.

Increase of polymers leads to increase of viscosity and mucoadhesion and as a result, decrease of release rate. Study of release profile in F<sub>10</sub> also revealed that propolis could release from this system in longer time (more than 7 days). F<sub>10</sub> produced significant growth inhibition zone against *P. gingivalis*. This formulation

has efficient function in the treatment of periodontitis besides dental root planing and scaling, so we recommend it for clinical evaluation.

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### Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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