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# Precursor System of Liquid Crystalline Phase Containing Propolis Microparticles for the Treatment of Periodontal Disease: Development and Characterization

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**Precursor systems of liquid crystalline phase were prepared using the surfactant PPG-5-Ceteth-20, isopropyl myristate, and water; gelatin microparticles containing propolis were then added into these systems. Homogeneity of dispersion, the in-system microparticle morphology, and sedimentation behavior of each formulation were evaluated. The rheological and mechanical properties (hardness, compressibility, and adhesiveness), the work of syringing, and the propolis release profile were also evaluated. All the formulations exhibited pseudoplastic flow and thixotropy, and they displayed storage modulus, loss modulus, dynamic viscosity, and loss tangent that depended on temperature, frequency, and composition. Mechanical properties varied significantly among the formulations being affected by changes in the composition and temperature. Raising the concentration of surfactant and adding propolis microparticles significantly decreased the work of syringing. The drug release was non-Fickian (anomalous) and there was no significant difference between the tested systems in the times required for 10%, 30%, and 50% release of the initial drug loading.**

**Keywords** periodontal disease; precursor system of liquid crystalline phase; propolis microparticles; intra-periodontal pocket drug delivery systems; propolis release

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## INTRODUCTION

Periodontal disease is defined as a group of conditions, including gingivitis and periodontitis, that affect the supporting structures of the teeth, gingiva, periodontal ligament, and alveolar bone (Listgarten, 1987). Gingivitis is the inflammation of the gingiva, whereas extension of inflammation into deeper tissues is termed periodontitis (Addy & Langeroudi, 1984; Lindhe, Haffajee, & Socransky, 1983). The inflammation is in response to plaque bacteria residing on both the tooth surface and beneath the gingiva (Jones, Irwin, Woolfson, Djokic, & Adams, 1999). Due to the direct actions of both plaque and the induced inflammatory response within the deeper tissues, a space (pocket) develops between the roots of the affected teeth and the soft tissues. In this protected environment, bacteria accumulate and flourish. If the disease is not treated, increased tooth mobility, and ultimately tooth loss, can occur (Medlicott, Rathbone, Tucker, & Holborow, 1994; Slots, 1979).

Treatment of the disease is mainly through the mechanical cleaning of the tooth surface to remove bacterial plaque and calculus (Lindhe & Nyman, 1984). However, as specific bacteria (Ciancio, 1996; Löe, Theilade, & Jensen, 1965; Wolff, Dahlen, & Aeppli, 1994) and the host response are thought to play a major role in the development of periodontal disease, antimicrobial (e.g., tetracycline, minocycline, clindamycin, metronidazole, and chlorhexidine) and anti-inflammatory (e.g., flurbiprofen) drugs have also been used as adjuncts to mechanical treatment, particularly in treating early-onset and refractory cases (Jones et al., 1999; Van Der Ouderaa, 1991). In

particular, propolis (bee-glue), a strongly adhesive resinous beehive product collected by honeybees from trees, has been used in dentistry for its pharmaceutical properties, including antimicrobial (Koo et al., 2000; Kujumgiev et al., 1999; Marcucci et al., 2001; Moreno, Isla, Cudmani, Vattuone, & Sampietro, 1999; Santos et al., 2002a, 2002b; Sforcin, Fernandes, Lopes, Bankova, & Funari, 2000), anti-inflammatory (Burdock, 1998; Song et al., 2002a, 2002b), and antioxidant (Isla, Moreno, Sampietro, & Vattuone, 2001; Marquele et al., 2005; Moreno et al., 1999; Nagai, Sakai, Inoue, Inoue, & Suzuki, 2001) activities. Alone, or incorporated in another dosage form, ethanol extract of propolis is commonly used in the treatment of periodontal disease, owing to its security and efficacy (Bruschi, Panzeri, & Lara, 2005; Burdock, 1998). However, this extract has some disadvantage, such as strong and unpleasant taste, aromatic odor, and the high concentration of ethanol, resulting in difficulties for its incorporation into another dosage form and for patient acceptance of the treatment as well (Bruschi, Cardoso, Lucchesi, & Gremião, 2003). Thus, gelatin microparticles containing propolis ethanol extract have been prepared (Bruschi, Cardoso, et al., 2003), exhibiting modified release of propolis into an aqueous medium (Bruschi, Lopes, Franco, & Gremião, 2004) and microbiological activity against oral pathogens (Bruschi et al., 2006), these being useful features for the development of propolis delivery systems for the treatment of periodontal disease.

The therapy of periodontal disease is mainly based on extensive oral rinses with solutions and systemic administration of drugs (Esposito, Cortesi, & Nastruzzi, 1996). However, to obtain an effective concentration of therapeutic agents in the pocket after systemic administration, repeated high doses over a prolonged period of time is required. Hence, this type of treatment contains the risk of systemic side effects and the emergence of resistant strains and superimposed infections (Norling et al., 1992; Van Winkelhoff, Cortesi, & Nastruzzi, 2000). These problems have evoked an interest in the development of localised drug delivery systems to offer prolonged delivery of therapeutic agents into the periodontal pocket (Jones, Woolfson, Djokic, & Coulter, 1996b).

For these reasons, a variety of intra-pocket devices have been designed (Medlicott et al., 1994; Hao & Heng, 2003). More recently, the use of insertable biodegradable drug delivery systems (films and semisolids) has been suggested and several types have been clinically evaluated (Bruschi & de Freitas, 2005; Jones et al., 1996b; Medlicott et al., 1994; Repka, Prodduturi, & Stodhill, 2003). The practical problems associated with and attributes of such drug delivery systems have been described by several authors (Fiorellini & Paquette, 1992; Hao & Heng, 2003; Jones, Brown, & Woolfson, 2001; Jones et al., 1996b, 1999; Medlicott et al., 1994; Okonogi, Khongkhunthain, Bunyaratavej, Thusaphorn, & Umpriwan, 2004). Moreover, buccal semisolid formulations have the advantage of being deliverable with a syringe, with a consequent easy placement in periodontal pockets and easy dispersion throughout the mucosa of oral cavity (Bruschi & de Freitas, 2005). On the other hand, these systems create considerable technical problems (Hao & Heng, 2003). One particular problem common to many drug delivery systems designed for use in the periodontal pocket is poor retention at the site of application (Hao & Heng, 2003; Jones et al., 1999). These problems may be resolved by the use of bioadhesive compounds or substances that increase their viscosity with increasing temperature or when in contact with saliva and crevicular fluid, improving the retention and, hence, clinical performance of the formulation (Hao & Heng, 2003; Jones et al., 1999, 2000; Okonogi et al, 2004; Stoltze & Stellfed, 1992).

Semisolid formulations consisting of glycerylmonooleate as main principle have been reported to deliver metronidazole or tetracycline into the periodontal pocket (Esposito et al., 1996; Norling et al., 1992; Okonogi et al., 2004). By contact with the saliva and gingival crevicular fluid, these systems are transformed into an adhesive gel of high viscosity, a so-called liquid crystal or mesomorphic phase, improving their retention within the pocket (Bruschi & de Freitas, 2005; Esposito et al., 1996; Lee, Young, & Kellaway, 2001). However, various additives (glycerylmonostearate, methylcellulose, Tween 80, Span 80, sesame oil) have been used in these systems to obtain suitable rheologic and mechanical properties in relation to the buccal and periodontal administration (Esposito et al., 1996; Lee et al., 2001; Norling et al., 1992; Okonogi et al., 2004).

New studies have shown that the mixture of the surfactant PPG-5-Ceteth-20 (ethoxylated and propoxylated cetyl alcohol) and isopropyl myristate swells when in contact with water, increasing the viscosity and giving rise to different kinds of liquid crystalline phases, which may coexist with excess water (Urban, Bruschi, Chiavacci, & Gremião 2004). These results have shown that the mixture of the surfactant and isopropyl myristate can be used for intra-pocket drug delivery. The present study describes the development and physicochemical characterization of a novel precursor system for a liquid crystalline phase containing propolis microparticles that may be applied easily to the periodontal pocket using a periodontal syringe.

## MATERIALS AND METHODS

### Materials

Propolis was collected at the experimental farm of State University of Maringá, Paraná State, Brazil. Type A gelatin, Royal (Brazil), was used without further purification. PPG-5-Ceteth-20 (Procetyl AWS$^{TM}$), isopropyl myristate, hydroxyethylcellulose 4400, and sodium chloride were purchased from Croda Chemicals Ltd. (Campinas, Brazil), Henrifarma (Sao Paulo, Brazil), Union Carbide Corporation (Sao Paulo, Brazil), and Carlo Erba (Milano, Italy), respectively. All other chemicals were purchased from Merck (Darmstadt, Germany) and were of AnalaR or equivalent quality.

### Preparation of Systems

Gelatin microparticles containing propolis (PM) were obtained by spray-drying, as described by Bruschi, Cardoso, and colleagues. (2003). Propolis extract (PE) was prepared with a propolis/ethanol ratio of 30/70 (w/w) by turbo extraction, filtered through filter paper, and made up to the initial weight with the ethanol. PE was dispersed in a gelatin solution containing 20% (w/w) of mannitol, using dripping technique, at 25ºC and with magnetic agitation by 30 minutes. The amount of gelatin utilized was a function of the dryness residue of PE (6/1). The final dispersion was spray-dried in a BÜCHI Mini Spray Dryer model B-191 (Büchi, Switzerland) the following spray-drying conditions: inlet temperature (160ºC), feed rate (6%), aspiration (80%), and pressure (3%). PPG-5-Ceteth-20 (PPG; 80%, 85%, 90% w/w) was warmed (50–60°C) with slow stirring. Isopropyl myristate (IM; 5%, 10%, 15% w/w) and water (0%, 5%, 10% w/w) were added to the PPG to give the desired compositions (Table 1) and the mixture was stirred until homogenization. Finally, PM (particle size 2.48 ± 0.81 μm) were mixed thoroughly into these preparations to form systems containing 6.41% (w/w) microparticles, equivalent to 4% (w/w) of propolis extract, the concentration normally utilized (Burdock, 1998; Bruschi, Cardoso, et al., 2003, 2005). All formulations were analyzed visually and by optical microscopy and were stored in amber glass ointment jars until required.

### Evaluation of Sedimentation Characteristics

To study the sedimentation of microparticles in the formulations, the sedimentation volume was determined as a function of time. The sedimentation volume $F$ is defined as the ratio of the final, equilibrium volume of the sediment, $Vu$, to the total volume $V_0$ before settling, as expressed in the following equation (Martin, Bustamente, & Chun, 1993):

$$F = [V_u/V_0] \quad (1)$$

TABLE 1
Composition of Precursor Systems of Liquid Crystalline Phase

| | Concentration (% w/w) | | |
|---|---|---|---|
| Systems | PPG-5-Ceteth-20 (PPG) | Isopropyl Myristate (IM) | Water |
| S1 | 90 | 10 | 0 |
| S2 | 90 | 5 | 5 |
| S3 | 85 | 15 | 0 |
| S4 | 85 | 10 | 5 |
| S5 | 85 | 5 | 10 |
| S6 | 80 | 15 | 5 |
| S7 | 80 | 10 | 10 |

The sedimentation volume was determined as a function of time. Five (5.0) ml of each formulation was decanted into a 7.5-ml cylinder with a diameter of 1.0 cm. After 1, 2, 4, 8, 12, 24, and 168 hours, the sedimentation volume $F$ was determined. Moreover, the capacity of formulations to redisperse was assessed by stirring the cylinder using 180° movements, after 168 hours. Formulations were evaluated according to the number of movements necessary to convert the sediment to a homogeneous dispersion (Patel, Kennon, & Levinson, 2001).

### Morphological Analysis

The systems were evaluated for homogeneity of dispersion and in-system microparticle morphology using image capture with a Leica DMRXA optical microscope (Wetzlan, Germany).

### Continuous Shear Analysis

Continuous shear analysis of formulations was performed at 25 and 37 ± 0.1°C using an AR 2000 controlled stress/controlled rate rheometer (T.A. Instruments, Surrey, England), in flow mode, and in conjunction with parallel steel plate geometry (40 mm, separated by a fixed distance of 1.0 mm) or with standard-size double concentric cylinder geometry (rotor outer radius of 21.96 mm and inner radius of 20.38 mm, stator inner radius of 20.00 mm, cylinder height of 59.50 mm, and gap of 0.5 mm), according to the consistency of each formulation. Samples were carefully applied to the lower plate or to the inside stator of the rheometer, taking care to cause minimal shearing, and allowed to equilibrate for at least 5 minutes prior to analysis. Upward and downward flow curves were measured over shear rates ranging from 10 up to 2000 $s^{-1}$. In each case, the shearing rate was increased over a period of 150 seconds, held at the upper limit for 10 seconds, and then decreased over a period of 150 seconds. At least five replicates were analyzed for each formulation. Upward flow curves were modelled using the Oswald-de-Waele equation (Power Law) (Martin et al., 1993; Malkin, 1994):

$$\sigma = k\gamma^n \quad (2)$$

where $\sigma$ is the shear stress, $k$ is the consistency index, $\gamma$ is the rate of shear, and n is the flow behavior index.

### Oscillatory Rheological Analysis

Oscillatory analysis of formulations was performed using an AR 2000 controlled stress/controlled rate rheometer (T.A. Instruments, Surrey, England) in oscillation mode at 25 and 37 ± 0.1°C. The geometry used was a 40-mm parallel steel plate (separated by a fixed distance of 1.0 mm) or was standard-size double concentric cylinders (rotor outer radius of 21.96 mm and inner radius of 20.38 mm, stator inner radius of 20.00 mm, height of cylinder 59.50 and gap 0.5 mm),

according to the consistency of each formulation (Jones et al. 2001). Samples of each formulation were carefully applied to the lower plate or to the inside stator of the rheometer, ensuring minimal formulation shearing, and allowed to equilibrate for at least 5 minutes prior to analysis. After determination of the linear viscoelastic region of each formulation, where stress was directly proportional to strain and the storage modulus ($G'$) remained constant, a frequency sweep analysis was performed over the frequency range of 0.1 to 10.0 Hz, following the application of a constant stress. The storage modulus ($G'$), loss modulus ($G''$), dynamic viscosity ($\eta'$) and loss tangent (tan $\delta$) were then determined, using Rheology Advantage software provided by T. A. Instruments. In each case, the dynamic rheological properties of at least five replicates were determined.

### Texture Profile Analysis

Texture profile analysis (TPA) of all formulations was performed using a TA-XTplus Texture Analyser (Stable Micro Systems, Surrey, United Kingdom) in TPA mode, as previously described (Andrews, Gorman, & Jones, 2005; Jones, Woolfson, & Brown, 1997b). Formulations (16 g) were packed into McCartney bottles, avoiding the introduction of air bubbles. In TPA, an analytical probe (10-mm diameter) was twice compressed into each sample at a defined rate (2 $mms^{-1}$) and to a defined depth (15 mm), allowing a delay period (15 seconds) between the end of the first and the beginning of the second compressions. At least five replicate analyses of each sample were performed at temperatures of 25 and 37°C. From the resultant force-distance and force-time plots, the hardness (the force required to attain a given deformation), compressibility (the work required to deform the product during the first compression of the probe), and adhesiveness (the work necessary to overcome the attractive forces between the surface of the sample and the surface of the probe) were derived (Jones, Lawlor, & Woolfson, 2002; Jones et al., 2000).

### Determination of Syringeability

The work of syringing of each system was performed using a TA-XTplus Texture Analyser (Stable Micro Systems, Surrey, United Kingdom) in compression mode, as previously described (Jones et al., 1996b, 1999, 2000). Each formulation was carefully packed into identical 1-ml plastic syringes to a height of 30 mm, avoiding the introduction of air bubbles. The syringe was then vertically clamped and the probe lowered until initial contact with the plunger of the syringe was observed. The probe was then lowered at a constant speed (2.0 $mms^{-1}$) through a distance of 30 mm and the resistance to expression of the syringe contents (work done) was determined from the area under the force-distance plot recorded during compression of the plunger. All measurements were performed at 25°C, with at least five replicates.

### In Vitro Release of Propolis from the Systems

In vitro release of propolis from the formulations was determined (at least in triplicate) using a periodontal pocket simulator apparatus (Figure 1), through a flow system. The release medium was artificial saliva composed of potassium phosphate dibasic (0.082%), potassium phosphate monobasic (0.036%), sorbitol solution 70% (4.27%), potassium chloride (0.063%), sodium chloride (0.087%), magnesium chloride hexahydrate (0.013%), calcium chloride dihydrate (0.007%), metilparaben (0.180%), hidroxyethylcellulose 4400 (0.025%), and water (95.24%) (Nakamoto, 1979). Moreover, it was used at the temperature of 37 ± 0.5°C and at a constant flow of 0.9 ± 0.05 µl/min. Formulations (0.5 g) were placed in the flow cells (total volume 1.0 ml) and, after 10 minutes, to reach the thermal equilibrium, the artificial saliva flow was turned on. At predetermined time intervals (24, 48, 72, 96, 120, 144, and 168 hours), samples of the release fluid were collected and the propolis concentration (total flavonoids drift) was analyzed by a spectrophotometric technique ($\lambda$ = 425 nm), as previously described by Bruschi, Franco, and Gremião (2003). The presence of formulation and artificial saliva components was observed not to interfere with the analysis. The data from these experiments were fitted to the general release equation (Eq. 3), using logarithmic transformations and least squares regression analysis (Jones et al., 2000; Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983):

$$\frac{M_t}{M_\infty} = kt^n \tag{3}$$

where $M_t/M\infty$ is the percentage of propolis released at time $t$, $k$ is a constant incorporating structural and geometric characteristics of the delivery system, and $n$ is the release exponent, a measure of the primary mechanism of drug release.

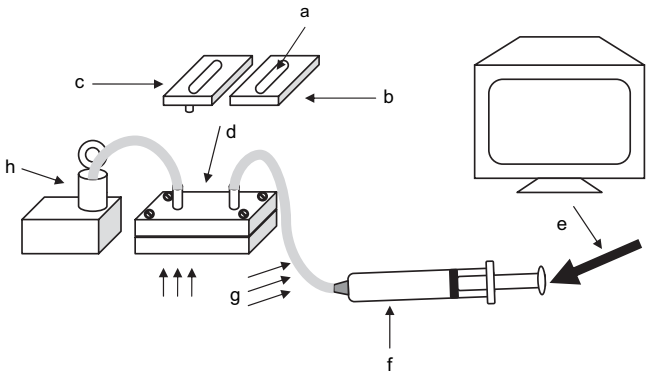


FIGURE 1. Periodontal pocket simulator apparatus for in vitro drug release studies from periodontal pocket drug delivery systems: (a) sample place, (b) lower part of flow cell, (c) upper part of flow cell, (d) closed flow cell, (e) flow control, (f) release medium, (g) temperature control, and (h) sample collector.

### Statistical Analysis

Effects of the PPG/IM ratio in the formulation, of propolis microparticles presence and temperature on the consistency index, the flow index and the viscoelastic properties ($G'$, $G''$, tan $\delta$, $\eta'$) at five representative frequencies (0.60, 2.55, 5.04, 7.53, 10.0 Hz) were statistically compared using a three-way Analysis of Variance (ANOVA). Similarly, the effects of PPG/IM proportions, presence of propolis microparticles, and temperature on textural/mechanical properties (hardness, compressibility, and adhesiveness) were also statistically evaluated using three-way ANOVA. Furthermore, the effects of PPG/IM and propolis microparticles presence on the work of syringing were statistically evaluated using two-way ANOVA. Finally, the effects of PPG/IM proportion on the time required for the release of defined percentages (10%, 30%, and 50%) of the original mass of propolis from each system were evaluated statistically using a one-way ANOVA. In all cases of ANOVA analysis, post hoc comparisons of the means of individual groups were performed using Tukey's Honestly Significant Difference test. In all cases, a significance level of $p < .05$ was accepted to denote significance (60) utilizing Statview software (Abacus Concepts, California).

## RESULTS AND DISCUSSION

### Preparation and Macroscopic Behavior of the Samples

The use of anti-inflammatory or antimicrobial agents is very important in the treatment of periodontal disease (Jones et al., 1996b) and propolis ethanolic extract has been much used for this application (Burdock, 1998). In response to the problems associated with this preparation, the microencapsulation technique can be used to develop propolis delivery systems to treat periodontal disease (Bruschi et al., 2004). Formulations designed for administration into the periodontal pocket should eliminate the inflammation and/or prevent the recolonization of the periodontal pocket with pathogenic microorganisms for a period of time sufficient to ensure reattachment of the gingiva to the alveolar bone (Medlicott et al., 1994; Slots, 1979). Thus, the ideal candidate formulation for the controlled delivery of an agent to the periodontal pocket should exhibit a variety of characteristics. These include ease of application into and retention within the periodontal pocket, controlled (prolonged) drug release, ease of manufacture, and eventual clearance from the periodontal pocket by either product biodegradation and/or dissolution (Jones et al., 1996b). While there have been several reports of controlled drug delivery systems for the treatment of periodontal disease (Bruschi & de Freitas, 2005; Medlicott et al., 1994), few of these have an ideal product profile and none use propolis. Moreover, precursor systems of liquid crystalline phase facilitate the insertion and improve the intimacy contact and the retention time of the formulation in the periodontal pocket (Esposito et al., 1996). Therefore, this study presents the formulation of semisolid devices based on the use of a surfactant (PPG-5-Ceteth-20), isopropyl myristate (IM), and gelatin microparticles containing propolis. Crucially, the components PPG and IM were chosen for their capacity, when mixed, to form different kinds of liquid crystalline phase in contact with water (Urban et al., 2004), safety, lack of irritancy, and biodegradation/dissolution (Croda, 2002).

The preparations described in this study were simple to manufacture. The utilized concentrations of PPG and IM on the structure of such products can provide a series of formulations with a wide range of physical and physicochemical properties, as described by Urban and colleagues (2004). During the manufacturing process, the visual and optical microscopy analysis showed that only the systems containing 90/5/5 (S2) and 85/10/5 (S4) of PPG/IM/water yielded preparations containing dispersed and complete PM. These two systems displayed integrity of the microparticles (Figure 2) and similar and suitable sedimentation characteristics (Table 2), with easy and fast redispersion. In contrast, the other formulations showed clusters, destruction of microparticles, and phase separation. It was described that amounts of water in this system can modify the phases and swell the gelatin of PM (Bruschi et al., 2004; Urban et al., 2004). Thus, only the systems S2 and S4 were tested.

To obtain an acceptable dispersion, the sedimentation volume $F$ should remain at or above 0.9 for 1 hour, but a longer period is preferred for our purpose (Gabriëls & Plaizier-Vercammen, 2004). For the two formulations, first signs of sedimentation were noticed after 8 hours. At this point, the value $F$ was 0.98, which means that the dispersions are stable.

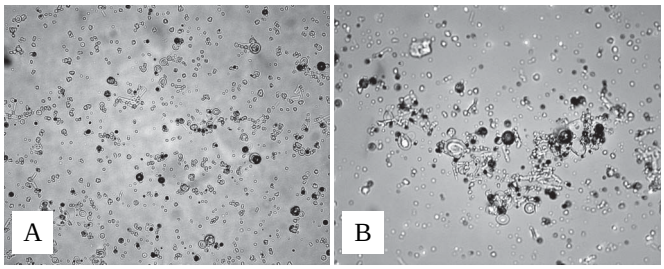


FIGURE 2. Optical microscopy photomicrographs of the systems containing propolis microparticles: (A) S2 and (B) S4. Original magnification 400x.

TABLE 2
Sedimentation Behavior of Precursor Systems of Liquid Crystalline Phase Containing Propolis Microparticles (PM)

| | Sedimentation Volume ($F$)[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Sample | 1h | 2h | 4h | 8h | 12h | 24h | 48h | 96h | 168h |
| S2 + PM | 1 | 1 | 1 | 0.98 | 0.97 | 0.96 | 0.93 | 0.90 | 0.89 |
| S4 + PM | 1 | 1 | 1 | 0.98 | 0.97 | 0.97 | 0.94 | 0.91 | 0.90 |

[a]Standard deviation has been omitted for clarity (in all cases the coefficient of variation [CV] < 6%).

Both of the systems showed easy and fast redispersion, with a maximum of four normalized 180° movements.

## Rheological Study

Pharmaceutical semisolids can display a wide range of rheological behaviors. Dispersions (suspensions and emulsions) can display flow and viscoelastic characteristics depending on the volume fraction, droplet/particle size distribution, and viscosity of the dispersed phase (Woolfson, Malcon, Campbell, Jones, & Russell 2000). It is very important to develop an understanding of the rheological behavior of the developed systems since this will affect a range of performance parameters, including drug release and administration characteristics, spreadability and retention in the periodontal pocket, and manufacturing operations.

Flow properties of the periodontal formulations determine the ease of product administration into the periodontal pocket and the (time-dependent) recovery of the products following administration (Jones et al., 2001). In continuous shear rheometry, both the systems (with and without propolis microparticles) exhibited shear-thinning behavior (pseudoplastic flow), with low degrees of thixotropy at the two temperatures. Thixotropy was more marked at 25°C and in the system S2 containing microparticles, this behavior being characteristic of suspensions (Falkiewicz, 1996; Jones et al., 2001). To enable statistical comparisons of the effects of each component on the flow properties of the formulations, the upcurve of each rheogram was mathematically defined using the Power Law model (Martin et al., 1993; Malkin, 1994), from which the consistency index and flow behavior index of each formulation were derived (Table 3).

As the PPG concentration was increased, the consistency index of the systems increased significantly. On the other hand, there was no significant difference in the flow index. The steady shear behavior of the systems was also influenced by the temperature and the presence of propolis microparticles. There was a significant decrease in the consistency index as the temperature was increased, while the flow behavior index increased significantly. The presence of propolis microparticles led to a significant increase in the consistency index and a significant decrease in the flow behavior index. The flow properties of the systems, at temperatures of 25 and 37°C, are displayed graphically in Figures 3 and 4.

The nonlinear responses to shear stresses exhibited by the formulations under study at two temperatures were probably a result of structural changes caused by shearing. The formulations consisted primarily of high-molecular-weight components (PPG and IM), organized in micelles with the water (Urban et al., 2004), in which the propolis microparticles were dispersed. Following exposure to a shear stress, the dispersion could flow, the chains of the PPG and IM could align along the direction of shear, releasing water or water and propolis microparticles (to the formulations containing microparticles). As a result, subsequent shearing occurred more readily and the apparent viscosity was decreased. Shear thinning is a desirable property in formulations intended for administration to the periodontal pocket (Jones et al., 2001). At high rates of shear, such as those experienced during expulsion from a syringe, the material flows readily, facilitating successful clinical administration. However, under conditions of low shear, such as those experienced in the periodontal pocket, the material adopts a higher consistency, recovering the original rheological properties that it possessed before administration. Furthermore, the low degree of thixotropy observed for the systems containing microparticles, especially at 37°C, indicates that the restoration of the original configuration would require only a short time after removal of the shear stress (after the administration). This attribute is an advantage in formulations that are designed for administration to the periodontal pocket, because it will enhance retention therein.

Viscoelastic properties provide information concerning the structural nature of the formulation and its rheological response to sub-destructive stresses, features that have been reported to influence directly the performance of semisolid systems (Jones et al., 2001). In this study, the viscoelastic properties of the systems were characterized by oscillatory rheometry. The effects of the PPG/IM ratio, presence of propolis microparticles, and temperature on the oscillatory properties, namely, storage modulus ($G'$), loss modulus ($G''$), dynamic

TABLE 3
Power Law Parameters of Formulations with and without Propolis Microparticles (PM)

| | $k$ (Pa.s)[a] | | $n$ (Dimensionless)[a] | |
|---|---|---|---|---|
| Sample | 25°C | 37°C | 25°C | 37°C |
| S2 | 0.2524 ± 0.0164 | 0.1300 ± 0.0065 | 0.9712 ± 0.0092 | 0.9823 ± 0.0038 |
| S2 + PM | 1.1354 ± 0.1315 | 0.4757 ± 0.0462 | 0.8590 ± 0.0009 | 0.9066 ± 0.0131 |
| S4 | 0.2030 ± 0.0058 | 0.1104 ± 0.0028 | 0.9814 ± 0.0023 | 0.9815 ± 0.0008 |
| S4 + PM | 0.8745 ± 0.0699 | 0.4536 ± 0.0075 | 0.8676 ± 0.0012 | 0.8923 ± 0.0003 |

[a]Each value represents the mean (± standard deviation) of five replicates.

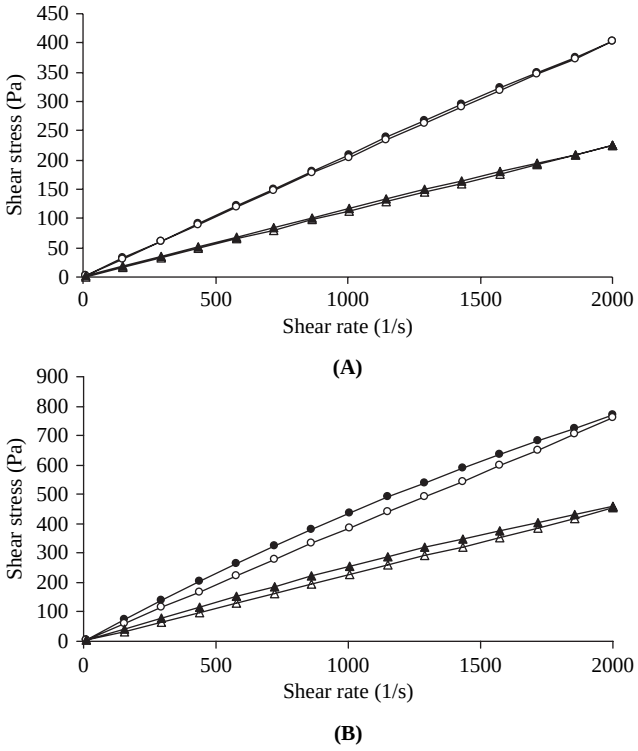


FIGURE 3. Flow rheograms of system S2 (A) without and (B) with propolis microparticles: (❍) 25°C and (Δ) 37°C. Closed symbol represents upcurve and open symbol represents downcurve. Standard deviations have been omitted for clarity; however, in all cases the coefficient of variation of replicate analyses was less than 3%.

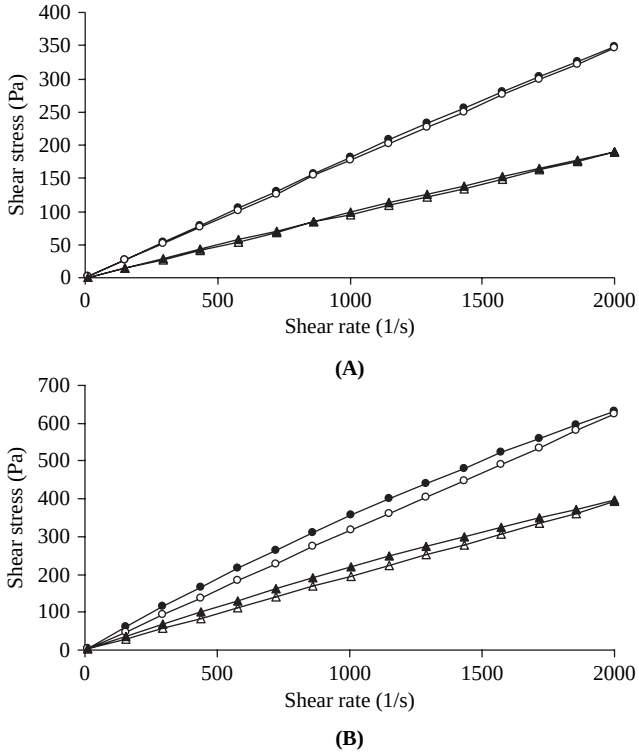


FIGURE 4. Flow rheograms of system S4 (A) without and (B) with propolis microparticles: (❍) 25°C and (Δ) 37°C. Closed symbol represents upcurve and open symbol represents downcurve. Standard deviations have been omitted for clarity; however, in all cases the coefficient of variation of replicate analyses was less than 8%.

viscosity ($\eta'$), and loss tangent (tan $\delta$), of four systems in the oscillatory frequency range are displayed in Figures 5 through 8, respectively.

The four formulations displayed $G'$ and $G''$ that were temperature, PPG/IM ratio, microparticle presence, and frequency dependent. Typically, increasing the temperature significantly decreased the $G'$ and $G''$, except in system S2 with microparticles, where the $G''$ was increased significantly. Moreover, $\eta'$ was significantly decreased as the temperature increased, mainly to the preparations without microparticles. The increasing temperature also decreased the tan $\delta$ of formulations without microparticles, but significantly increased the tan $\delta$ of systems containing microparticles. Storage and loss modules of all preparations increased as the oscillatory frequency increased, whereas the tan $\delta$ significantly decreased. For example, increasing the oscillatory frequency from 0.6 to 10.0 Hz increased the $G'$ of S2-containing microparticles from 111.42 to 139.40 Pa (25°C) and from 4.18 to 22.06 Pa (37°C), and of S4-containing microparticles from 119.06 to 160.52 Pa (25°C) and from 13.11 to 35.21 Pa (37°C). Moreover, $\eta'$ was significantly decreased as the frequency increased.

The rheological behavior of the preparations also changed as a function of PPG/IM proportions. Increasing the PPG concentration significantly increased the $G'$, $G''$, and $\eta'$ of microparticle-free preparations over the entire frequency range, at two temperatures, and may be accredited most likely to the increasing of interactions between the PPG molecules and the increased number of PPG molecules on the micelles, resulting in the increased resistance to deformation of the system. On the other hand, raising the PPG concentration of formulations containing microparticles, at two temperatures, significantly decreased $G'$, $G''$, and $\eta'$ over the entire frequency range, probably due the presence of microparticles that decreased the organization of the PPG/IM/water system.

The presence of microparticles increased $G'$, $G''$, and $\eta'$, but decreased tan $\delta$. Interestingly, at temperature of 25°C, in systems containing microparticles, $G'$ exceeded $G''$ over the entire frequency range and accordingly these systems may be described as viscoelastic (semisolid or gel) (Barnes, Hutton, & Walters, 1989; Jones et al., 2001). To the other cases, tan $\delta$ was greater than 1 across the entire frequency range and therefore these systems are more appropriately described as elastoviscous (Barnes et al., 1989; Jones et al., 2001). Considering the proposed usage of these formulations, it is held that their elasticity is important to ensure resistance to deformation and, hence, the good retention of the system within the periodontal pocket (Jones et al., 2001). In this context, the use of the formulations containing microparticles would be appropriate.

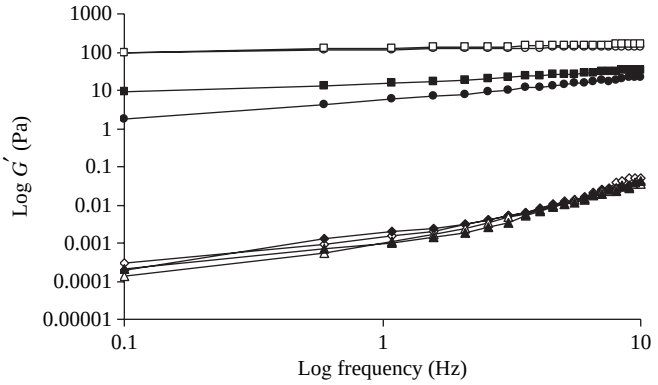


FIGURE 5. The effect of PPG/IM proportion, presence of propolis microparticles, and temperature on the storage modulus ($G'$) of formulations: (-♦-) S2; (-▲-) S4; (- ● -) S2 + PM; (-■-) S4 + PM. Open symbol represents the results at 25ºC and closed symbol represents the results at 37ºC. Standard deviations have been omitted for clarity, however, in all cases the coefficient of variation of replicate analyses was less than 10%.

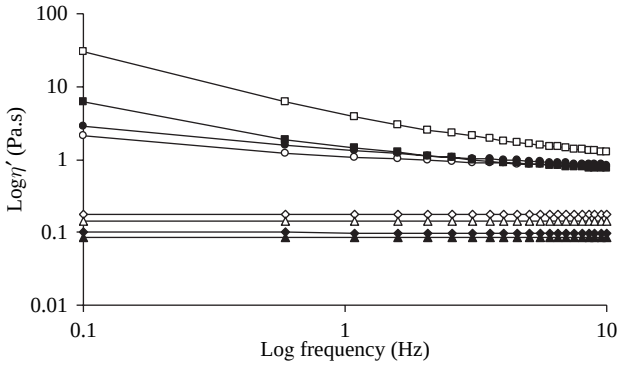


FIGURE 7. The effect of PPG/IM proportion, presence of propolis microparticles, and temperature on the dynamic viscosity ($\eta'$) of formulations: (-♦-) S2; (-▲-) S4; (- ●-) S2 + PM; (-■-) S4 + PM. Open symbol represents the results at 25ºC and closed symbol represents the results at 37ºC. Standard deviations have been omitted for clarity, however, in all cases the coefficient of variation of replicate analyses was less than 10%.

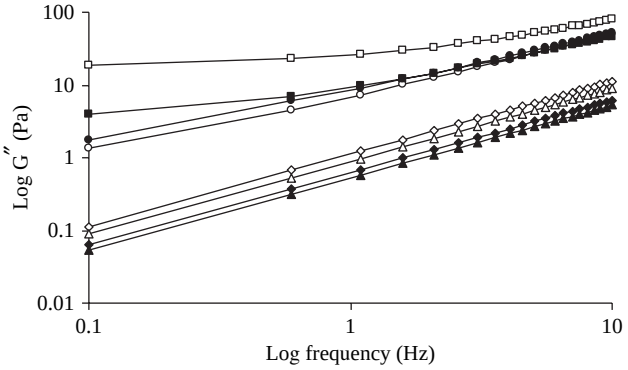


FIGURE 6. The effect of PPG/IM proportion, presence of propolis microparticles, and temperature on the loss modulus ($G''$) of formulations: (-♦-) S2; (-▲-) S4; (-●-) S2 + PM; (-■-) S4 + PM. Open symbol represents the results at 25ºC and closed symbol represents the results at 37ºC. Standard deviations have been omitted for clarity, however, in all cases the coefficient of variation of replicate analyses was less than 10%.

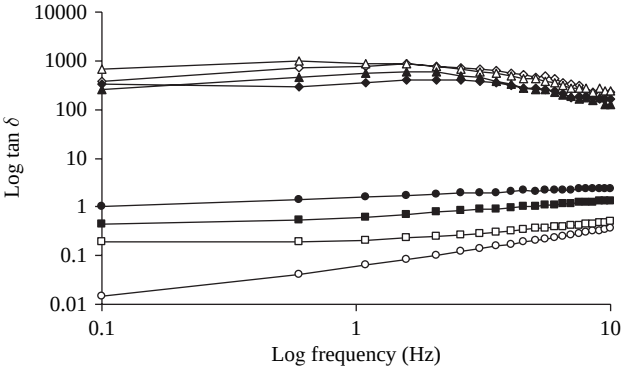


FIGURE 8. The effect of PPG/IM proportion, presence of propolis microparticles, and temperature on the loss tangent (tan $\delta$) of formulations: (-♦-) S2; (-▲-) S4; (-●-) S2 + PM; (-■-) S4 + PM. Open symbol represents the results at 25ºC and closed symbol represents the results at 37ºC. Standard deviations have been omitted for clarity, however, in all cases the coefficient of variation of replicate analyses was less than 10%.

Moreover, the dynamic viscosity is commonly employed to characterize the viscous nature of viscoelastic systems (Jones et al., 2001; Martin et al., 1993). The formulations containing microparticles displayed a dynamic viscosity that depended on the oscillatory frequency, in accordance with the Maxwell model for the response of viscoelastic materials to oscillatory stresses (Martin et al., 1993). As the oscillatory frequency was increased, the magnitude of $\eta'$ decreased because of the relatively short time available during each oscillation cycle for (time-dependent) viscous deformations. Thus, at high frequencies, the viscous properties of each system dominated, whereas at lower frequencies the elastic contribution to the viscoelastic response was considerable.

## Texture Profile Analysis

Mechanical properties (hardness, compressibility, and adhesiveness) of topical formulations directly influence their clinical performance. Therefore, it is very important to characterize these properties fully. Much has been published on the application of TPA for mechanical characterization of a range of semisolid systems designed to be administered into the periodontal pocket (Jones, Woolfson, & Brown, 1997a, 1997b; Jones, Woolfson, & Djocic, 1996a; Jones et al., 1996b, 1997c, 1999, 2000). The mechanical properties of the four formulations tested in this study are presented in Table 4. Typically, these formulations differed appreciably in their mechanical properties, which were significantly affected by changes in PPG/IM, presence of propolis microparticles, and temperature. Increasing the concentration of PPG in the systems significantly increased

TABLE 4
Mechanical Properties of Formulations with and without Propolis Microparticles Determined by Texture Profile Analysis

| | Hardness (N) | | Compressibility (N.mm) | | Adhesiveness (N.mm) | |
|---|---|---|---|---|---|---|
| Sample | 25°C | 37°C | 25°C | 37°C | 25°C | 37°C |
| S2 | 0.0378 ± 0.0016 | 0.0358 ± 0.0019 | 0.2916 ± 0.0247 | 0.2760 ± 0.0216 | 0.2520 ± 0.0188 | 0.2472 ± 0.0196 |
| S2 + PM | 0.0373 ± 0.0024 | 0.0385 ± 0.0035 | 0.3060 ± 0.0262 | 0.3235 ± 0.0214 | 0.1955 ± 0.0132 | 0.1560 ± 0.0134 |
| S4 | 0.0250 ± 0.0023 | 0.0368 ± 0.0011 | 0.1652 ± 0.0108 | 0.2880 ± 0.0179 | 0.1256 ± 0.0056 | 0.2636 ± 0.0180 |
| S4 + PM | 0.0375 ± 0.0017 | 0.0425 ± 0.0041 | 0.2850 ± 0.0219 | 0.4795 ± 0.0167 | 0.0610 ± 0.0060 | 0.2125 ± 0.0150 |

their adhesiveness at 25°C, decreased at 37°C, but there was no significant difference in hardness and compressibility. In contrast, the addition of propolis microparticles to the formulations significantly increased their hardness and compressibility, as well as reducing their adhesiveness at two temperatures. Finally, increasing the temperature significantly increased the compressibility and adhesiveness of systems containing microparticles, while reducing these values in systems without microparticles.

The lowest hardness and compressibility were exhibited by formulation without microparticles S4, at 25°C. This formulation also showed the highest value of adhesiveness, at 37°C. In contrast, the lowest adhesiveness value was shown by formulation S4 containing microparticles, at 25°C. Moreover, maximum hardness and compressibility values were displayed by formulation S4 containing microparticles.

Thus, the systems containing microparticles displayed a rise of compressibility with temperature, possibly indicating a greater organization of the systems, as observed in the viscoelastic properties. Moreover, the hardness of the systems was also influenced by the presence of microparticles, increasing at two temperatures. The systems containing microparticles displayed increased hardness as the temperature increased, corroborating the data on compressibility. Product hardness and compressibility are rheological parameters that quantify product deformation under both compression and shear. Therefore, the effects of temperature, propolis microparticles, and PPG/IM ratio on these parameters may be explained by their effects on product viscosity (Jones et al., 1997b). Adhesiveness in TPA is commonly defined as the work required to overcome the attractive forces between the surface of the sample and the surface of the probe (Jones et al., 1996a). However, this measure may in some cases involve fracture of cohesive bonds within the sample, and therefore is partially dependent on sample tack (Jones et al., 1997b). Therefore, the effect of proportions of components of the formulation on its adhesiveness is due to increased interactions between the molecules and the analytical probe and also due to increased product tack. The addition of microparticles decreased the adhesiveness, probably by decreasing the product tack and the interactions with the analytical probe, according to the other mechanical results. In contrast, the increasing of tack of the formulations and/or interactions between the molecules and the analytical probe may be inferred when the PPG content is increased, at 25°C, and when the temperature is increased in the systems containing microparticles.

### Determination of Syringeability

Syringeability of the formulations was measured using a texture analyzer, as, in many cases, it is appropriate to apply formulations to remote supragingival sites/periodontal pockets using syringe systems (Jones et al., 1999). Thus, the work of syringing the systems was measured at temperature of 25°C, using a texture analyzer in compression mode. The work required to expel each formulation from a syringe is presented in Table 5.

Significantly, decreases in the work of syringing were observed as the PPG concentration was increased. These results are probably due to the concomitant decrease in product viscosity, at 25°C, as previously reported. Moreover, the addition of propolis microparticles also significantly decreased the work of syringing of the formulations. It should be noted that the work values observed for all systems were similar to or lower than those reported for other semisolids designed to be administered into the periodontal

TABLE 5
Effects of PPG-5-Ceteth-20 (PPG)/Isopropyl Myristate (IM) Proportion and Propolis Microparticles (PM) on the Work of Syringing the Formulations

| Sample | Concentration of PPG (% w/w) | Concentration of IM (% w/w) | Work of Syringing (N.mm) |
|---|---|---|---|
| S2 | 90 | 5 | 10.86 ± 0.85 |
| S2 + PM | 90 | 5 | 10.73 ± 0.85 |
| S4 | 85 | 10 | 19.87 ± 1.78 |
| S4 + PM | 85 | 10 | 16.54 ± 1.42 |

pocket (Jones et al., 1996a, 1999, 2000; Kelly, Deasy, Ziaka, & Claffey, 2004).

### In Vitro Release of Propolis from the Systems

The search for the ideal controlled drug delivery system for improved treatment of periodontal disease has showed limitations, mainly in relationship to the control of the drug release (Bruschi & de Freitas, 2005; Jones et al., 2000; Medlicott et al., 1994). Thus, evaluation of the in vitro drug release profile is a fundamental step in the development of an intra-periodontal pocket drug delivery system (Soskolone & Freidman, 1996) and the test method is successful when the experimental conditions (temperature, dissolution medium, and stirring or flow rate) are appropriate, simulating as far as possible the in vivo conditions. Many authors have described the use of equipment and conditions to simulate the periodontal pocket (Esposito et al., 1996; Jones et al., 2000; Norling et al., 1992; Perioli et al., 2004). However, these conditions are very specific, especially in relation to the volume and composition of the dissolving medium (Medlicott et al., 1994). A periodontal pocket simulator apparatus, based on a flow system, with artificial saliva, was used to investigate the release of propolis from the formulations. The results are presented in Figure 9.

In order to investigate the mechanism of drug release from formulations, the values of kinetic parameters *n*, *k*, and *R* in Equation 3 were calculated (Table 6). Moreover, the times required for the release of propolis (10%, 30%, and 50% of the original drug loading) from each formulation are shown in Table 7.

The times required for 10%, 30%, and 50% release of propolis from formulation S2 were not significantly different than those for S4. Therefore, under sink conditions, the in vitro release studies showed that each of the formulations provided a controlled release of propolis.

Application of the general equation (Jones et al., 2000; Korsmeyer et al., 1983) enabled calculation of the release exponent (*n*), shedding light on the mechanism of propolis release from these systems. The systems S2 and S4 examined in this study possessed release exponents of 0.8447 ± 0.0044 and 0.8186 ± 0.0202, respectively, indicating that the release of propolis from these formulations was non-Fickian (anomalous). Propolis release was therefore the result of the exit of total flavonoids from the gelatin microparticles and diffusion through the liquid crystalline phase, combined with relaxing of the PPG structure, leading to entry of water and phase change (Fickian diffusion and structure relaxing) (Ritger & Peppas, 1987). In addition, the times required for the release of 10%, 30%, and 50% of the original propolis content from formulations S2 and S4 were similar.

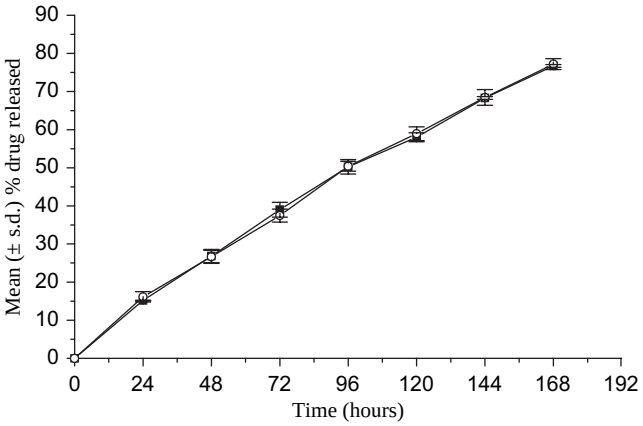


FIGURE 9. The effects of PPG-5-Ceteth-20 and isopropyl myristate on the release of propolis from formulations S2 (closed squares) and S4 (open circles).

TABLE 6
Values of the Kinetic Release Parameters for Formulations

| | Kinetic Release Parameters | | |
|---|---|---|---|
| Sample | *n* | *k* ($min^{-n}$) | *R* |
| S2 + PM | 0.8447 ± 0.0044 | 1.0374 ± 1.0150 | 0.9999 |
| S4 + PM | 0.8186 ± 0.0202 | 1.1703 ± 1.1024 | 0.9985 |

TABLE 7
Effects of PPG-5-Ceteth-20 (PPG)/Isopropyl Myristate (IM) Proportion on the Time Required for the Release of Propolis (10%, 30%, and 50% of Original Drug Loading) From Formulations

| Sample | Concentration of PPG (% w/w) | Concentration of IM (% w/w) | Time (h) Required for Release of Propolis[a] (*M* ± *SD*) | | |
|---|---|---|---|---|---|
| | | | 10% | 30% | 50% |
| S2 + PM | 90 | 5 | 14.64 ± 0.59 | 53.62 ± 2.02 | 98.04 ± 3.58 |
| S4 + PM | 85 | 10 | 13.89 ± 2.81 | 52.97 ± 5.05 | 98.76 ± 5.85 |

[a]Time required for release of a given percentage of the original mass of propolis from the systems.

## CONCLUSIONS

This study has described the design and characterization of a set of syringeable precursor systems of liquid crystalline phase containing propolis microparticles for application to the periodontal pocket. The sedimentation characteristics of these systems were appropriate for the required application and showed good stability. The rheological properties of systems were determined by destructive (flow rheometry), non-destructive (oscillatory rheometry), and mechanical techniques, revealing characteristics that favored easy insertion into the periodontal pocket and subsequent stable retention therein. Furthermore, the release profile studies showed that the propolis can be released from the systems for a prolonged period of time (more than 7 days). These properties of the candidate formulations indicate a possible advantageous role in the treatment of periodontal disease and suggest they are worthy of clinical evaluation.

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