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Formulation and Evaluation of Secnidazole or Doxycycline Dento-Oral Gels

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Local delivery of antibiotics has been shown to be effective in reducing periodontopathic microorganisms. The purpose of this study is to formulate gels containing secnidazole or doxycycline hydrochloride that could be used in the treatment of periodontitis by direct periodontal intrapocket administration. Different mucoadhesive polymers were used as cellulose derivatives, carbopol and eudragit. The prepared gels were evaluated for their in vitro drug release, rheological behavior, and mucoadhesive force. Increasing the concentration of each polymer increased the viscosity, mucoadhesion, and the time required for 30 and 50% release of the original mass of each drug. Gels with appropriate balance of the above-examined parameters were selected for microbiological evaluation. Microbiological studies on selected gels showed faster release of the two drugs (expressed as inhibition zones) than the commercial products of chlorhexidine gel (Elugel®) and miconazole nitrate emulgel (Miconaz®).

Keywords doxycycline; secnidazole; mucoadhesive polymers; rheological properties; release study; periodontitis

INTRODUCTION

Periodontal diseases are a group of inflammatory conditions affecting the supportive structures of the teeth (Kinane & Lindhe, 1997). They are characterized by a destruction of the periodontal ligament, a resorbtion of the alveolar bone and the migration of the junctional epithelium along the tooth surface with the formation of a space (pocket) between the gum and the teeth and can eventually cause teeth loss. This pocket provides an ideal environment for the growth and proliferation of microbes including anaerobic pathogenic bacteria (Haffajee & Socransky, 1994).

Current periodontal therapy includes the removal of the bacterial deposits from the tooth surface and shifting the pathogenic microorganism to healthy one compatible with periodontal health. Conventional root planning and scaling relies on the mechanical debridement of tooth surfaces as the primary antimicrobial measure (Schwach-Abdellaoui, Vivien-Castioni, & Gurny, 2000). However, as specific bacteria are thought to play a major role in disease process (Wolff, Dahlen, & Aeppli, 1994), antimicrobial agents have been used as adjuncts to mechanical treatment (Goodson, 1994). The potential side effects in administering systemic antibiotics and the inability of antiseptic mouthwashes to penetrate the periodontal pocket have raised interest in the localized delivery of therapeutic agents within the pocket, thus ensuring a high effective concentration of antimicrobial agent at the site of infection.

Localized drug-delivery systems based on bioadhesive polymers are effective in delivering antimicrobial agents to the periodontal pocket (Bruschi & De Freitas, 2005; Jones, Woolfson, Brown, & O'Neill, 1997; Jones et al., 1996). Tetracyclines and metronidazole were reported to be effective in the treatment of periodontal diseases (Friesen, Williams, Krause, & Killoy, 2002; Mombelli, Lehmann, Tonetti, & Lang, 1997; Noyan et al., 1997; Pähkla, Koppel, Saag, & Pähkla, 2005; Williams et al., 2001). Among tetracyclines, doxycycline hydrochloride (DH) was chosen because of its activity against putative periodontal pathogens, and it has been shown to be effective in the management of periodontitis in human, also it is the most potent tetracycline for collagenase inhibition (Golub, Sorsa, & Lee, 1995; McCulloch et al., 1990; Sorsa et al., 1995; Walker et al., 2000). Secnidazole (SC) is structurally related to the commonly used 5-nitroimidazoles metronidazole; it has a spectrum of activity against anaerobic microorganisms. However, SC has a longer terminal elimination half-life than commonly used drugs in this class (Gillis & Wiseman, 1996). Therefore, the treatment with SC is shorter and significantly more effective than the treatment using other imidazole drugs. Both DH and SC were selected for this study, and the aim of this work is to formulate DH or SC gels that could be used in the treatment of periodontitis by direct periodontal intrapocket administration.

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MATERIALS AND METHODS

Materials

Secnidazole (SC), methylcellulose (MC) (2% solution has a viscosity of 300 cP), and hydroxypropylmethylcellulose (HPMC) (Methocel E4, high viscosity) were kindly supplied by E.P.I.C.O. pharmaceutical company, Egypt. Hydroxyethylcellulose (HEC) (2% solution has a viscosity of 640.9 cP) was kindly supplied by Memphis Co., Egypt. DH was kindly supplied by El-Nile pharmaceutical Company, Egypt. Eudragit L100 (ED) was purchased from Rhom Pharma, GMBH, Germany. Carbopol of two grades: Carbopol 934P (CP 934) and Carbopol 971P NF (CP 971) were purchased from Goodrich Co., USA. Crude porcine gastric mucin was purchased from Sigma chemical Co., USA. Propylene glycol (analytical grade) was purchased from ADWIC, El-Nasr Chemical Co., Egypt. Elugel® gel (0.2% chlorhexidine) was purchased from Global Napi Pharmaceuticals, Egypt. Miconaz-H® emulgel (2% miconazole nitrate and 1% hydrocortisone) was purchased from Medical Union Pharmaceuticals, Egypt.

Preparation of Gels Containing Drug

All gels were prepared with 5% wt/wt of each drug (SC or DH) separately.

Preparation of Gels Containing Drug Using Different Cellulose Derivative

MC and HEC at concentration 3 and 5% (wt/wt) of each, and HPMC at concentration 1 and 3% (wt/wt) gels were prepared by gradually adding the calculated amounts of the polymers, while stirring to one third of the required amount of freshly prepared distilled water (80°C) (Mitchell et al., 1993a, 1993b). The final volume is made by adding a mixture of water and propylene glycol containing SC or DH. The prepared gels were stored overnight in a refrigerator.

Preparation of Un-Neutralized Carbopol Gels Containing Drug

CP 934 and CP 971 gels were prepared without neutralization at concentrations 1 and 3% (wt/wt) by gradual sprinkling of the polymer into the vortex of distilled water at room temperature, together with SC or DH dissolved in propylene glycol and stirred until no lumps were observed. Gels were left for one day to hydrate completely (Liu, Sheu, Chen, Yang, & Ho, 2007; Tamburic and Craig, 1995).

Preparation of Organogel-Containing Drug

SC or DH was first dissolved in propylene glycol at room temperature and then poured into calculated amounts of ED previously weighed in a mortar and immediately mixed by the pestle to produce the gels. The concentrations of ED were 15, 20, and 25% (wt/wt) of the total base weight (Goto, Kawata, Suzuki, Kim, & Ito, 1991).

Rheological Measurements and Data Analysis

Steady shear measurement was conducted where the rheograms of all prepared gels was performed at 25 ± 0.1°C using cone and plate (the cone with dimensions equal to 2.4 cm external diameter, 1.7 cm internal diameter and 1.3 cm height, and the plate with dimensions equal to 6.3 cm external diameter, 5.4 cm internal diameter and 1.7 cm height) programmable viscometer (Brookfield Engineering Laboratories Inc., Model HADV-II, USA) connected to a digital thermostatically controlled circulating water bath (Polyscience, Model 9101, USA) with spindle 52. The shear rate was ranging from 2 to 400 s^{-1} corresponding to 1–200 rpm with 10 s between each two successive speeds and then in a decreasing order. Equilibration of the sample for 5 min was made following loading of the viscometer. Ramp time for each viscosity stage was reading after 20 s. All studies were performed in triplicates and the average was taken.

Rheological data were fitted to different models (Bingham, Power law, Casson) to examine the pattern of flow and the presence of yield value:

- Bingham: $\tau = \tau_o + \eta\gamma$
- Power law: $\tau = \eta\gamma^n$
- Casson: $\tau^{1/2} = \tau_o^{1/2} + \eta^{1/2}\gamma^{1/2}$

Where τ is the shear stress, τ_o the yield value, η a constant called the apparent viscosity or the consistency index, γ the shear rate, and n the flow index. In case of Newtonian behavior $n = 1$ and $\tau_o = 0$, whereas in case of pseudoplastic (shear thinning) behavior $0 < n < 1$ and $\tau_o = 0$; for plastic behavior, it is the same as pseudoplastic but with $\tau_o > 0$, whereas in case of dilatant flow (shear thickening) $n > 1$ (Steffe, 1996). The software employed was Graph Pad Prism® version 4, and the level of significance was set at 5%.

In-Vitro Release Study

SC or DH release from gels was monitored by USP paddle method with some modifications, (Figure 1). Gels were retained within plastic cups (an inner diameter 3.7 cm, outer diameter 4.2 cm and internal depth 1.2 cm), which were fixed

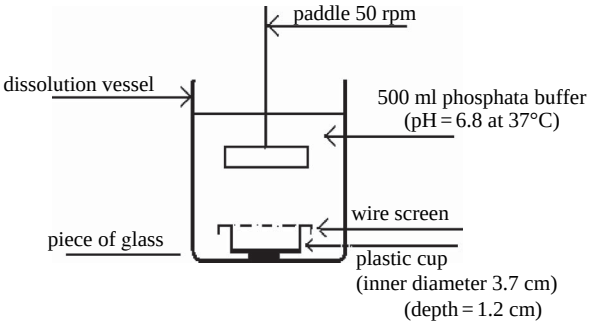

FIGURE 1. Diagrammatic representation of the USP paddle dissolution apparatus.

to a flat squared piece of glass (2 × 2 × 0.5 cm) so as to be anchored to the bottom of the dissolution vessel.. The surfaces of these cups were covered with equally sized wire screen (100 mm mesh size), so that drug release will occur through this mesh. A 0.5-g sample of the gel was weighed in the cup and was gently lowered through 500 mL of the dissolution medium (phosphate buffer; pH 6.8). The paddle of the modified USP Dissolution

Apparatus (Pharma test, type PTW, Hainburg, Germany) was centrally positioned 2.5 cm above the rim of the cup (Abdel-Hamid, Abdel-Hady, El-Shamy, & El-Dessouky, 2006). The release study was carried out at 37 ± 0.5°C, and the stirring paddles were rotated at a speed of 50 rpm. Aliquots of 5 mL were withdrawn at different time intervals. All samples were replaced by the same volumes of phosphate buffer of pH 6.8. Samples were suitably diluted and measured spectrophotometrically at λ_{max} 320 nm for SC and 274 nm for DH using UV visible double beam spectrophotometer (Shimadzu, model UV-1601 PC, Kyoto, Japan). The experiments were conducted in triplicates, the results were averaged, and blank experiments were carried out at the same time using plain bases.

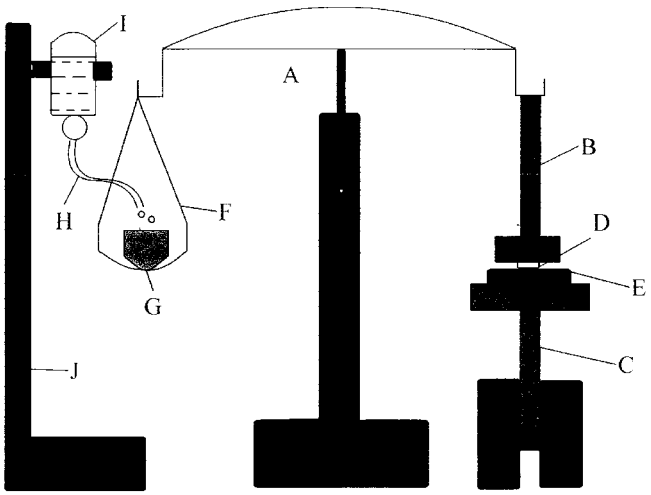

FIGURE 2. Diagrammatic representation of the mucoadhesive force-measuring device. (A) modified balance ; (B) upper stage; (C) lower stage moving on screw; (D) mucin disc; (E) gel preparation; (F) balance pan; (G) plastic jar; (H) IV infusion set; (I) glass bottle containing water; (J) stand-holder.

Statistical Analysis of Drug-Release Data

Drug-release data generated from the dissolution experiments were fitted to the following general release equation (Peppas, 1985) using logarithmic transformations and least squares regression analysis:

$$M_t / M_\infty = K\, t^n \quad (1)$$

$$\text{Log}\,(M_t/M_\infty) = \text{Log}\,K + n\text{Log}\,t \quad (2)$$

Where M_t/M_∞ is the fraction of released drug at time t; k is the release constant incorporating structural and geometrical characteristics of the delivery system; and n is the release exponent, a measure of the primary mechanism of drug release. Statistical analyses were performed on the times required for the release of 30 and 50% ($t_{30\%}$ and $t_{50\%}$) of the original loading of SC or DH from each gel formula using one-way analysis of variance (ANOVA) (Jones, Woolfson, & Brown, 1997b). Post hoc statistical analyses were performed using Tukey–Kramer test for multiple comparisons. The software employed was Graph Pad Instat® V2.04, and the level of significance was set at 5%.

In-Vitro Evaluation of the Mucoadhesion for the Gels

The mucoadhesive strength of the formulations was determined by measuring the force required to detach the formulations from a mucin disc using the mucoadhesive force-measuring device shown in Figure 2. Initially, mucin discs were prepared by compression of crude porcine mucin (50 mg) by a single punch tablet press (10 mm diameter die) (Karishma Pharma machines 400007, Mumbai, India). These discs were horizontally attached to the upper stage of the modified balance by a cynoacrylate adhesive. Before mucoadhesion testing, the mucin disc was hydrated by submersion in a freshly prepared 5% (wt/wt) dispersion of mucin for 60 s. Samples of each gel formulation (0.5 g) were placed on the lower vertically movable stage. The lower stage was then elevated till the surface of the sample became contacted to the mucin disc adhered to the upper stage. A constant weight (5 g) was then applied for 30 s and removed. The sample was left in contact for 4 min with the mucin disc to ensure intimate contact. To the pan on the other side of the used device, water was then added from a glass bottle through an infusion set into a plastic jar at a constant rate of 13–15 drops/min. The addition of water was stopped when mucin disc was detached from the sample. The minimal weight of water required to detach the sample from the mucin disc was noted as the mucoadhesive force. This method is adapted to the methods by Mikos and Peppas (1990); Yong et al. (2001); Desai and Kumar (2004), where the addition of weights were replaced by the addition of water followed by weighing the amount of water. These experiments were carried in four replicates. All detachment tests were carried out at about 35 ± 1.5°C. Mucoadhesive force or the detachment stress (N/m^2) was determined using the following equation stated by Ch'Ng, Park, Kelly, & Robinson, 1985:

$$\text{Detachment stress}\,(N/m^2) = m.g/A$$

Where m is the weight of water (g); g the acceleration due to gravity taken as 9.81 m/s^2; A is the area of the mucin disc (area of contact) and is equal to πr^2 (r is the radius of the mucin disc).

Statistical Analysis of Mucoadhesion Data

The results were statistically evaluated using one-way ANOVA. Post hoc statistical analyses were performed using Tukey–Kramer test for multiple comparisons. The software employed was Graph Pad Instat® V2.04, and the level of significance was set at 5%.

Microbiological Evaluation of the Selected Formulations

Determination of Minimum Inhibitory Concentration of SC and DH

A serial dilution of SC or DH was prepared and mixed with molten Mueller Hinton agar (National Committee for Clinical Laboratory Standards [NCCLS], 1993, 1997). The inoculum of the microorganisms was prepared in a broth solution to be equivalent to 10^5 cfu/mL. One aliquot of 0.2 mL of this broth was inoculated on the surface of Mueller Hinton agar plates containing SC or DH and incubated aerobically or anaerobically according to the tested microorganisms. Also, the broth was inoculated on nutrient agar as a control. The plates were incubated for 24 h at 37°C for visible growth. Growth of one or two colonies was disregarded (Andrews, 2001).

Patients Specimen

This study included 20 patients suffering from periodontal diseases were randomly chosen from the flow of the outpatient Diagnosis Clinic, Department of Oral Medicine, Diagnosis and Periodontology, Faculty of Dentistry, Ain Shams University. After removing of supragingival plaque, two paper points were inserted into a periodontal pocket for 10 s. The first paper point was inoculated on peptone water as transport medium for aerobes, and the second paper point was inoculated on Robertson cooked meat broth, as transport medium for anaerobes (Eick, Pfister, & Straube, 1999).

Isolation and Identification of the Microorganisms

The samples were cultured aerobically from peptone water on Colombia blood agar plate and cultured anaerobically on Colombia blood agar plate with added kanamycin at concentration of 75 μg/mL. (kanamycin is selective for obligate anaerobes). The first plate was incubated aerobically at 37°C for 24 h whereas the second plate was incubated anaerobically in anaerobic jar at 37°C for 2–4 days. Identification of isolated colonies was done using Gram stain, morphology, UV lamp, and biochemical reactions (Kinci et al., 2002).

In Vitro Antimicrobial Activity of the Selected Gels of SC and DH on the Isolated Microorganisms

An inoculum of 1.5 mL of a suspension containing each identified isolated organism was transferred to sterile Petri dish (20 cm in diameter), then 30 mL of Mueller Hinton agar was added to the inoculum, and mixed well and left to solidify (National Committee for Clinical Laboratory Standards [NCCLS], 2002). Wells were done by punching stainless steel cylinders onto the plate and removing the agar to form a well. Each well was aseptically filled with accurate weight of the selected gel. For aerobic organisms; the Mueller Hinton plates were incubated aerobically at 37°C for 24 h. For anaerobic organisms, the plates were incubated anaerobically in anaerobic jar at 37°C for 24 h. After incubation, the inhibition zone diameter was measured using a ruler. The extent of release (zone of inhibition) was measured by taking the average of three readings. Results were analyzed using one-way ANOVA followed by post hoc statistical analysis performed by Tukey–Kramer test for multiple comparisons. The software employed was Graph Pad Instat® V2.04, and the level of significance was set at 5%.

RESULTS AND DISCUSSION

Rheological Measurements and Data Analysis

Formulation designed for administration into the periodontal pocket must be easily administrated using a blunt syringe in order to fulfill the requirements for ease of application (Jones et al., 2000); therefore, the used concentrations of all polymer-forming gels were chosen according to the viscosity required to fulfill this goal.

The rheological parameters of the prepared gels, namely the flow index (n), the consistency index (η), and the apparent viscosity measured at shear rate 50 s^{-1} at 25°C are summarized in Table 1 and illustrated in Figures 3–5 (only rheograms of gels selected for evaluation of their antimicrobial activity are included). The regression coefficient (R^2) values were >0.95 (significant when $p = 0.05$), indicating that the rheological data of the gels are best fitted to Power law model. On the basis of the values of (n), calculated after Power law equation and which are <1, it could be confirmed that all the gels exhibit shear-thinning behavior.

The causes of pseudoplastic flow revealed by the tested gels may be due to progressive rapture of the internal structure of the formulations and its later reconstruction by means of Brownian movement (Dolz, Herraez, Gozalez, & Diez, 1998; Pena, Lee, & Sternes, 1994). Increasing the concentration of each polymer was associated with a decrease in the flow index values. This result is in accordance with what reported with Fresno, Ramirez, & Jimenez (2002); this decrease in the flow index was explained by the formation of full structured three dimensional polymer lattices because of increased polymer concentration. Increasing the concentration of MC, HEC, HPMC, and CP 934 shows a significant decrease in (n) value. Moreover, CP 971 and ED gels show a slight decrease in the (n) value upon increasing the concentration, which indicates that the full structured polymer lattice is already formed at the

TABLE 1
The Rheological Parameters of the Prepared Gels

Formulations	Flow Index (n)	Consistency Index (η)	Viscosity (P)[a]
MC/SC/3	0.6519	37.8	6.41 ± 0.1879
MC/DH/3	0.799	9.275	5.18 ± 0.1065
MC/SC/5	0.4851	353.7	83.33 ± 5.311
MC/DH/5	0.4492	238.2	74.13 ± 4.03
HEC/SC/3	0.5336	149.54	38.86 ± 2.96
HEC/DH/3	0.5595	174.1	28.54 ± 1.05
HEC/SC/5	0.4364	511.6	101.00 ± 9.20
HEC/DH/5	0.44	1003	96.80 ± 8.52
HPMC/SC/1	0.5503	42.35	3.91 ± 0.1658
HPMC/DH/1	0.5416	49.93	44.0 ± 0.2036
HPMC/SC/3	0.3669	755	124.44 ± 10.47
HPMC/DH/3	0.3645	982.9	144.05 ± 9.85
CP 934/SC/1	0.5209	38.67	4.18 ± 0.201
CP 934/DH/1	0.5277	36.75	4.03 ± 0.154
CP 934/SC/3	0.3131	847.5	35.40 ± 2.30
CP 934/DH/3	0.4207	474.9	32.68 ± 1.87
CP 971/SC/1	0.4651	151.1	12.20 ± 4.97
CP 971/DH/1	0.5009	114	11.20 ± 2.05
CP 971/SC/3	0.4437	205.3	17.62 ± 3.99
CP 971/DH/3	0.4932	164	16.09 ± 5.06
ED/SC/15	0.9346	43.924	20.05 ± 2.57
ED/DH/15	0.9464	47.187	20.33 ± 3.77
ED/SC/20	0.9001	110.8	80.57 ± 1.32
ED/DH/20	0.9443	86.73	74.14 ± 2.31
ED/SC/25	0.8919	215.73	161.15 ± 6.43
ED/DH/25	0.921	174.56	148.29 ± 5.94

MC: methylcellulose; HEC: hydroxyethylcellulose; HPMC: hydroxypropyl-methylcellulose; CP: Carbopol; ED: Eudragit; SC: secnidazole; DH: doxycycline hydrochloride.

[a]Average of three determination ± *SD*, measured at shear rate 50 s^{-1} at 25°C.

lower concentration and further increase in its concentration does not affect greatly its structure.

Fitting of the rheological data to Power law revealed that gels have no apparent yield value, indicating the limited resistance to flow at low stress values characteristic of pseudoplastic flow. This could be a useful property that indicates the displacement of the gels from tissue surfaces (Needleman, Martin, & Smales, 1998). Also, non-thixotropic behavior is revealed as shown in Figures 3–5, which indicates a time-independent flow, a property that helps the retention of gels on the buccal mucosa. Thixotropic materials may not have sufficient time to reform again after application (Eouani, Piccerellea, Prinderrea, Bourret, & Joachim, 2001).

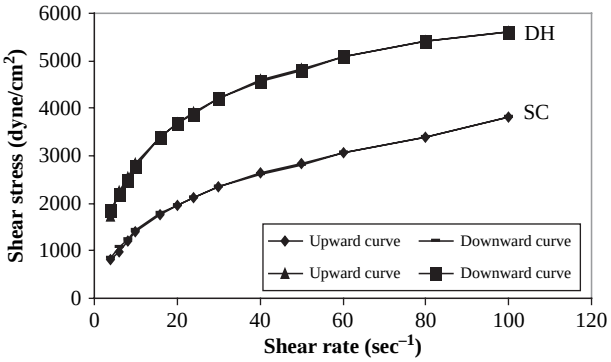

FIGURE 3. Flow curves of 5% HEC gels containing secnidazole (SC) or doxycycline hydrochloride (DH) at 25°C.

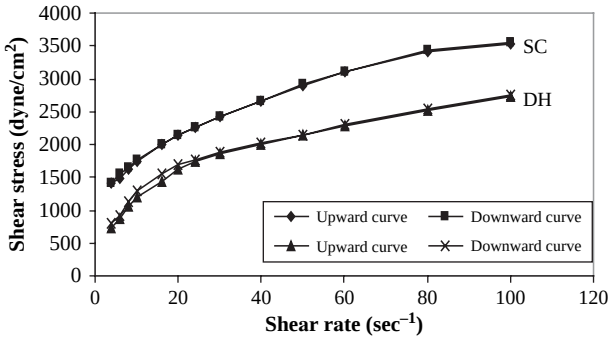

FIGURE 4. Flow curves of 3% CP 934 gels containing secnidazole (SC) or doxycycline hydrochloride (DH) at 25°C.

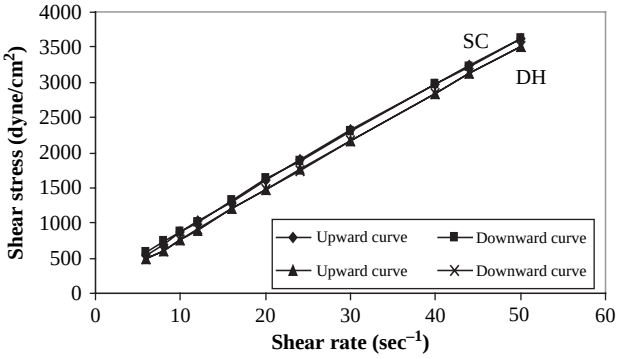

FIGURE 5. Flow curves of 20% ED gels containing secnidazole (SC) or doxycycline hydrochloride (DH) at 25°C.

Table 1 summarizes that increasing the concentration of each polymer significantly increases the apparent viscosity. This is in accordance with Jones, Woolfson, and Brown (1997a), Jones et al. (1997), and Talukder, Vinckier, Moldenaers, and Kinget (1996). This can be explained by macromolecular entanglement phenomena. Because higher

concentrations increase the entanglement density, the viscoelastic properties increase correspondingly.

In-Vitro Release of SC or DH from Gels

The time required for 30 ($t_{30\%}$) and 50% ($t_{50\%}$) release of each drug was calculated and statistically compared for each gel to determine the effect of polymer concentration and type, as summarized in Table 2.

Generally for SC or DH gels, increasing the concentration of each polymer shows a significant increase in $t_{30\%}$ and $t_{50\%}$. This is in accordance with what reported by Jones et al. (1997, 2000). The physical reason for the slower release rate from the more viscous gels is most probably because of the formation of highly viscous diffusion layer of hydrated polymer chains that entrap the access of water and thus reduce the migration of drug molecules (Paavola et al., 1995).

As shown in Figures 6 and 7 for SC and DH, respectively, 3% (wt/wt) MC and 1% (wt/wt) HPMC gels show the fastest release rate with no significant differences observed among their $t_{30\%}$ and $t_{50\%}$ upon statistical analyses at $p < 0.05$. 5% (wt/wt) MC, 3% (wt/wt) HPMC, 3% (wt/wt) HEC, and 5% (wt/wt) HEC gels show a relatively similar rate of release; however, upon comparing each pair, statistical analyses showed no significant differences among $t_{30\%}$and $t_{50\%}$ of 3% (wt/wt) HPMC and 3% wt/wt HEC, whereas 5% (wt/wt) MC and 5% (wt/wt) HEC gels show a significant difference.

Concerning cellulose gels containing SC, the maximum $t_{30\%}$ and $t_{50\%}$ are 20.04 ± 4.84 and 37.63 ± 4.95 min, respectively, and are associated with the formulation containing 5% (wt/wt)

TABLE 2
The Time Required for the Release of 30 and 50% of the Original Drug Loading and the Mucoadhesive Strength of Different Gels

Formulations	Time (min) Required for the Release of the Drug ($M \pm SD$)		Detachment Force (dyne/cm$^2 \times 10^2$) (Mean ± SE)
	$t_{30\%}$	$t_{50\%}$	
MC/SC/3	7.34 ± 1.07	15.09 ± 0.70	112.85 ± 4.95
MC/DH/3	5.99 ± 0.81	14.84 ± 1.44	102.76 ± 2.09
MC/SC/5	15.05 ± 1.61	26.57 ± 2.93	146.57 ± 4.53
MC/DH/5	14.09 ± 0.36	25.16 ± 2.12	149.57 ± 3.65
HEC/SC/3	14.85 ± 0.99	32.19 ± 1.97	88.1 ± 3.94
HEC/DH/3	11.47 ± 1.96	31.29 ± 3.36	76.1 ± 4.21
HEC/SC/5	22.52 ± 3.11	40.37 ± 1.90	96.26 ± 2.29
HEC/DH/5	23.6 ± 1.59	43.56 ± 0.84	89.99 ± 1.54
HPMC/SC/1	7.76 ± 0.88	14.7 ± 0.27	132.94 ± 5.45
HPMC/DH/1	7.63 ± 0.67	14.00 ± 0.36	129.45 ± 4.44
HPMC/SC/3	17.54 ± 3.00	35.11 ± 2.00	153.25 ± 5.39
HPMC/DH/3	14.06 ± 0.22	31.19 ± 2.06	142.25 ± 4.93
CP 934/SC/1	3.20 ± 0.05	8.45 ± 0.33	80.83 ± 1.5
CP 934/DH/1	3.00 ± 0.20	7.98 ± 0.35	79.65 ± 1.17
CP 934/SC/3	48.71 ± 4.36	138.87 ± 0.10	108.76 ± 2.47
CP 934/DH/3	49.77 ± 2.98	151.27 ± 4.64	115.65 ± 3.24
CP 971/SC/1	19.97 ± 2.00	46.20 ± 3.20	89.84 ± 1.17
CP 971/DH/1	19.24 ± 0.08	47.57 ± 0.98	88.48 ± 2.71
CP 971/SC/3	32.5 ± 2.72	79.35 ± 0.27	95.8 ± 2.42
CP 971/DH/3	43.58 ± 5.10	90.63 ± 5.20	92.3 ± 3.59
ED/SC/15	8.11 ± 0.67	16.95 ± 0.35	110.94 ± 3.35
ED/DH/15	24.88 ± 0.64	48.19 ± 2.82	103.59 ± 2.54
ED/SC/20	21.87 ± 0.23	46.14 ± 1.13	120.13 ± 2.77
ED/DH/20	45.18 ± 1.55	84.00 ± 4.55	123.13 ± 3.98
ED/SC/25	56.67 ± 0.70	123.28 ± 0.15	140.66 ± 1.93
ED/DH/25	87.56 ± 5.22	155.55 ± 2.23	135.65 ± 2.19

MC: methylcellulose; HEC: hydroxyethylcellulose; HPMC: hydroxypropyl-methylcellulose; CP: Carbopol; ED: Eudragit; SC: secnidazole; DH: doxycycline hydrochloride.

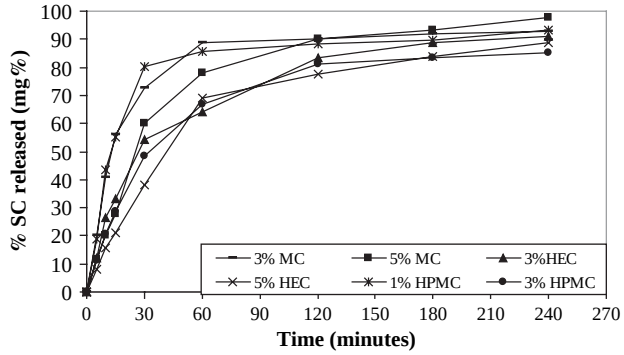

FIGURE 6. In-vitro release of secnidazole (SC) in phosphate buffer (pH°6.8) from cellulose gels.

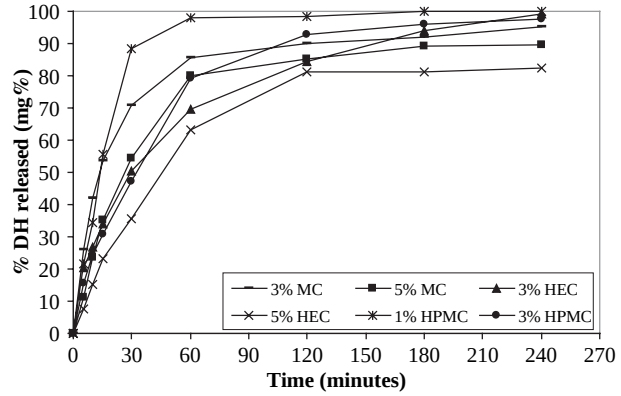

FIGURE 7. In-vitro release of doxycycline hydrochloride (DH) in phosphate buffer (pH°6.8) from cellulose gel.

HEC. Conversely, minimum $t_{30\%}$ and $t_{50\%}$ are associated with the formulations containing 1% (wt/wt) HPMC and 3% (wt/wt) MC and are 7.76 ± 0.88 and 14.7 ± 0.27 min for 1% wt/wt HPMC and 7.68 ± 0.88 and 14.72 ± 1.1 min for 3% (wt/wt) MC, respectively, whereas 5% (wt/wt) MC, 3% (wt/wt) HEC, and 3% (wt/wt) HPMC formulations show intermediate $t_{30\%}$ and $t_{50\%}$.

Concerning cellulose gels containing DH, the maximum $t_{30\%}$ and $t_{50\%}$ are 23.6 ± 1.59, 43.56 ± 0.84 min, respectively, and are associated with the formulation containing 5% (wt/wt) HEC. Conversely, minimum $t_{30\%}$ and $t_{50\%}$ are associated with the formulations containing 3% (wt/wt) MC and 1% (wt/wt) HPMC and are 6.85 ± 0.44 and 14.52 ± 2.18 min for 3% (wt/wt) MC and 7.63 ± 0.67 and 14 ± 0.36 min for 1% (wt/wt) HPMC, respectively, whereas 5% (wt/wt) MC, 3% (wt/wt) HEC, and 3% (wt/wt) HPMC formulations show intermediate $t_{30\%}$ and $t_{50\%}$.

As summarized in Table 2, increasing the concentration of CP significantly increases $t_{30\%}$ and $t_{50\%}$; however, statistical analyses of $t_{30\%}$ and $t_{50\%}$ show significant differences among all formulations. For SC formulations, the maximum $t_{30\%}$ and $t_{50\%}$ are 48.71 ± 4.36 and 138.87 ± 10 min, respectively, and are associated with the formulation containing 3% (wt/wt) CP 934. Conversely, minimum $t_{30\%}$ and $t_{50\%}$ are associated with the formulation containing 1% (wt/wt) CP 934 and are 3.1 ± 0.04 and 7.83 ± 0.35 min, respectively. For DH formulations, the maximum $t_{30\%}$ and $t_{50\%}$ are 49.77 ± 2.98, 151.27 ± 4.64 min, respectively, and are associated with the formulation containing 3% (wt/wt) CP 934. Conversely, minimum $t_{30\%}$ and $t_{50\%}$ are associated with the formulation containing 1% (wt/wt) CP 934 and are 2.8 ± 0.95 and 7.62 ± 1.68 min, respectively.

The rate of release of SC or DH from 3% (wt/wt) CP 934 gels was slower than their rate of release from 3% (wt/wt) CP 971 gels (Figures 8 and 9). This result appears in agreement with the polymers structure and their rheological properties described above. CP 934 is heavily cross-linked whereas CP 971 is lightly cross-linked, and this difference in polymer structure is reflected in their rheological behavior (Bonacucina, Cespi, Misici-Falzi, & Palmieri, 2006; Bonacucina, Martelli, & Palmieri, 2004). Conversely, by comparing the rate of release of SC or DH from 1% (wt/wt) CP 934 and CP 971, it is obvious that the rate of release of SC or DH is faster from 1% (wt/wt) CP 934 than from 1% (wt/wt) CP 971, and this result appears in agreement with their rheological behavior but not

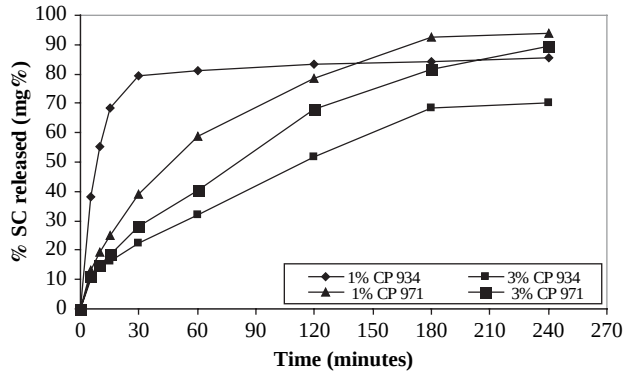

FIGURE 8. In-vitro release of secnidazole (SC) in phosphate buffer (pH 6.8) from CP gels.

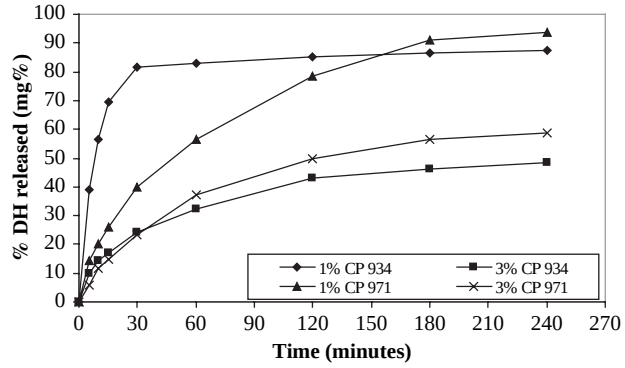

FIGURE 9. In-vitro release of doxycycline hydrochloride (DH) in phosphate buffer (pH 6.8) from CP gels.

with their polymer structure. Therefore, we concluded that the viscosity of the CP gels does not depend only on the degree of cross linking; this appears in agreement with Muramatsu et al. (2000), who compared the viscosities of different grades of CP and revealed that the highest viscosity was observed with CP 971P, which had a medium degree of cross linking.

The release of SC and DH from ED gels is shown in Figures 10 and 11. Statistical analyses of ED gels show significant differences among all formulations. For SC formulations, the maximum $t_{30\%}$ and $t_{50\%}$ are 56.67 ± 0.7 and 123.28 ± 15.16 min and are associated with the formulation containing 25% (wt/wt) ED, respectively. Conversely, minimum $t_{30\%}$ and $t_{50\%}$ are associated with the formulation containing 15% (wt/wt) ED and are 8.11 ± 0.67 and 16.95 ± 0.35 min, respectively. Whereas 20% ED shows intermediate release with $t_{30\%}$ and $t_{50\%}$ equal 21.87 ± 0.23 and 46.14 ± 1.13 min, respectively. For DH formulations, the maximum $t_{30\%}$ and $t_{50\%}$ are 87.56 ± 5.22 and 155.55 ± 2.23 min, respectively, and are associated with the formulation containing 25% (wt/wt) ED. Conversely, minimum $t_{30\%}$ and $t_{50\%}$ are associated with the formulation containing 15% (wt/wt) ED and are 24.88 ± 0.64 and 48.19 ± 2.82 min, respectively. Although 20% (wt/wt) ED shows intermediate release with $t_{30\%}$ and $t_{50\%}$ equal 45.18 ± 1.55 and 84 ± 4.55 min, respectively.

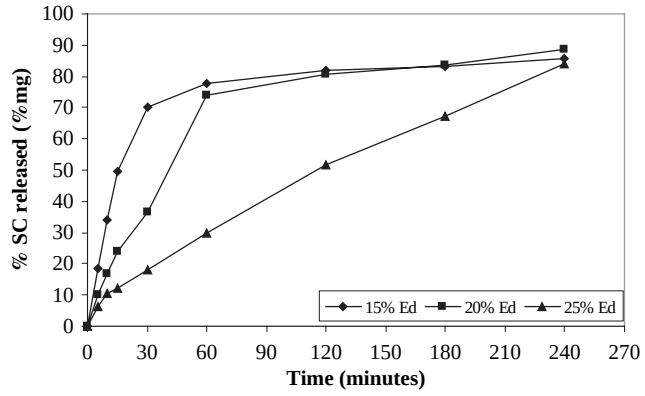

FIGURE 10. In-vitro release of secnidazole (SC) in phosphate buffer (pH 6.8) from ED gels.

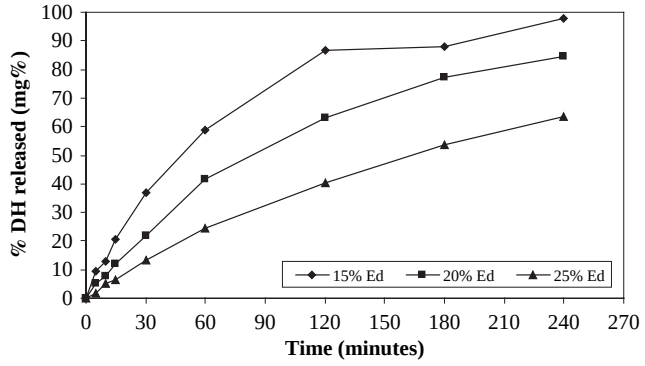

FIGURE 11. In-vitro release of doxycycline hydrochloride (DH) in phosphate buffer (pH 6.8) from ED gels.

The similarity observed among $t_{30\%}$ and $t_{50\%}$ required for SC and DH release from cellulose and CP gels may be attributed to the solubility of both drugs in the release media resulting in similarity in the rate of release. It is revealed that $t_{30\%}$ and $t_{50\%}$ required for the release of SC from ED gels are significantly less than $t_{30\%}$ and $t_{50\%}$ required for the release of DH. ED Ll00 is an anionic co-polymer of methacrylic acid and methyl methacrylate; so, the decrease in the release of DH may be attributed to the interaction that occurred between the COOH group of the polymer and the amino group of the drug. Similar results were observed by Khare and Peppas (1993). They also presented data on modulated drug release from anionic, hydrophilic copolymers as a function of the degree of ionization of the polymer, the pH, the ionic strength, and the nature of the dissolution medium. The data indicated that, contrary to the expected behavior, a water-soluble drug such as oxprenolol hydrochloride exhibited a lower diffusion coefficient into the swollen hydrogel than the less water-soluble theophylline. This observation suggests again a possible interaction between charged groups of the polymer and the drug.

For SC and DH formulations, release kinetics data showed that the release exponents (n) ranged from 0.494 to 0.8991 for gels containing SC, and from 0.4724 to 0.8891 for gels containing DH indicating that the mechanism of release from all gels is anomalous diffusion with correlation coefficient ranging from 0.943 to 0.999.

Evaluation of Mucoadhesive Properties for Gels

The effect of polymer type and concentration on the mucoadhesive strength of different gels are summarized in Table 2. In general, increasing the concentration of each polymer in the gels significantly increases the mucoadhesive strength. This is in accordance with Jones et al. (1997, 2000). This was a result of the greater concentration of polymer chains at the formulation surface and, subsequently, the greater number of entanglements/interactions with mucus glycoprotein.

Results reveal that 5% (wt/wt) MC and 3% (wt/wt) HPMC gels show the highest mucoadhesive strength followed by 1% (wt/wt) HPMC and 3% (wt/wt) MC, whereas 5% HEC and 3% (wt/wt) HEC gels show the least values for mucoadhesive strength. All formulations show a statistically significant difference in their mucoadhesive strength except 5% (wt/wt) MC and 3% (wt/wt) HPMC gels that show no significant differences.

Also, 3% CP 934 gels show the highest mucoadhesive strength followed by 3% (wt/wt) CP 971 and 1% (wt/wt) CP 971 whereas 1% (wt/wt) CP 934 gels show the lowest value. This correlates well with their rheological properties. This is in accordance with previous works (Bonacucina et al., 2004, 2006) who reported that mucoadhesion is greater in systems

with higher elastic components. All CP gels show significant differences among their mucoadhesive strength. Lee et al. (1996) reported the increase in mucoadhesive strength of CP 934 gels as a function of increasing the polymer percent, indicating that the high content of CP 934 polymer leads to strong polymer–mucin interaction.

By comparing the same concentration of two grades of CP, it can be observed that 3% (wt/wt) CP 934 gels show higher mucoadhesive strength than 3% (wt/wt) CP 971, whereas 1% (wt/wt) CP 971 gels show higher mucoadhesive strength than 1% (wt/wt) CP 934, which indicates that different degree of cross-linking does not necessarily lead to differences in bioadhesion (Jones et al., 1997a; Needleman et al., 1998).

Microbiological Evaluation of the Selected Formulations

From the results of the previous measured parameters, it is revealed that among cellulose gels, formulations containing 5% (wt/wt) HEC show the largest $t_{30\%}$ and $t_{50\%}$ with acceptable viscosity and good mucoadhesion properties. Among CP gels, formulations containing 3% (wt/wt) CP 934 show the largest $t_{30\%}$ and $t_{50\%}$ with acceptable viscosity and highest mucoadhesion strength, so both formulations were selected for microbiological evaluation. Formulations containing 20% (wt/wt) ED were selected for further investigation as they show reasonable viscosity and mucoadhesion properties; although they do not show the largest $t_{30\%}$ and $t_{50\%}$ among ED gels.

Determination of Minimum Inhibitory Concentration of SC and DH

The MIC obtained for SC and DH for the tested microorganisms are 0.125–4 μg/mL and 0.5–2 μg/mL, respectively, which indicates their efficiency as antimicrobial agents.

Identification of the Microorganisms

Table 3 summarizes the number and the percent of isolated microorganisms from the mouth of 20 patients suffering from periodontal diseases. It is revealed that anaerobic microorganisms represent 78.57% of the total isolated organisms in which *Bacteroid* species represent 50% of these anaerobes. This is in agreement with Tomazinho and Avila-Campos (2007); they reported that *Bacteroid* species as anaerobic bacteria are predominant components of the bacterial florae of mucous membranes, and therefore, they are a common cause of endogenous infections.

From Table 3, it is revealed that black-pigmented anaerobic rods such as *Prevotella* and *Porphyromonas* are involved in the etiology of endodontic infections. Of a total of 14 isolates from periodontal infections, 28.57% have *Porphyromonas*, 14.28% have *Prevotella*, 7.14% have *Fusobacteria*, 28.57% have *Peptostreptococci*, and 21.43% have *Streptococci*. Van Winkelhoff, Herrera, Oteo, and Sanz, (2005) found that of 261 isolates from patients with periodontal infections, 35% of cases had *Prevotella*, 37.5% had *Fusobacteria*, and 65% had *Streptococci*. This difference in the percentage of isolates may be due to small number of the studied cases. This also appeared in agreement with Eick et al. (1999), who revealed that the majority of 164 species found in patients with periodontitis were black-pigmented *Prevotella* species and *Porphyromonas gingivalis*.

In-Vitro Antimicrobial Activity of the Selected Gels of SC or DH on the Isolated Microorganisms

The selected formulations are tested for their antimicrobial activity against the isolated microbes using cup diffusion method. As summarized in Table 4, results reveal that anaerobic gram-negative bacteria are highly sensitive to SC in which 95–100% of these bacteria are inhibited by SC, whereas gram-positive cocci (*Peptostreptococci*) show only 70% sensitivity to SC. These results agree with Behra-Miellet, Calvet, and Dubreuil (2003), they tested the antimicrobial activity of linezolid and compared it with reference antibiotic; they found that all strains of gram-negative anaerobes were inhibited by metronidazole whereas strains of *Peptostreptococci* acquired resistance to metronidazole. In our study, as expected SC show no activity against aerobic bacteria.

As summarized in Table 4, studying the susceptibility of different organisms to DH reveals that aerobic gram-positive

TABLE 3
The Microorganisms Isolated from 20 Patients Suffering from Periodontal Diseases

Category	Number of Each Category	Percentage of Each Category	Organism	Number of Each Organism	Percentage of Each Organism
Anaerobic Gram negative bacteria (Bacteriods)	7	50	*Porphyromonas*	4	28.57
			Prevotella	2	14.28
			Fusobacteria	1	7.14
Anaerobic Gram positive bacteria	4	28.57	*Peptostreptococci*	4	28.57
Total anaerobes	11	78.57	–	–	–
Aerobic bacteria	3	21.43	*Streptococci*	3	21.43
Total bacteria	14	100	–	14	100

TABLE 4
The Antimicrobial Susceptibility of Different Microorganisms to Secnidazole (SC) and Doxycycline Hydrochloride (DH)

		Antimicrobial Susceptibility Percentage	
Category	Organism	SC	DH
Anaerobic gram-negative bacteria (Bacteriods)	*Porphyromonas*	95	70
	Prevotella	100	75
	Fusobacteria	100	0
Anaerobic gram-positive bacteria	*Peptostreptococci*	70	25
Aerobic gram-positive cocci	*Streptococci*	Zero	100

cocci are highly sensitive to DH (100%), whereas anaerobic bacteria show some resistance, in which 25, 70, and 75% of *Peptostreptococci*, *Porphyromonas*, and *Prevotella* show sensitivity to DH, whereas *Fusobacteria* are resistant to it.

Won-Jun and Bayoumi (1986) reported that the release of drugs from the topical bases into the agar medium has been affected by the solubility of the drug in the ointment base, its solubility in the gel base (agar), and the intermolecular forces of attraction between the drug and the diffusion medium. The effect of different formulations on the in vitro antibacterial activity (expressed as zones of inhibition) of SC and DH against the isolated microorganism (aerobes and anaerobes) using agar diffusion method is investigated and compared with the commercially available products, as shown in Figures 12 and 13. Statistical analyses of the results show significant differences among all the tested bases and the commercial products. It is shown that all formulations show a greater zone of inhibition than commercial products. However, the greater zones of inhibition obtained may be attributed to the greater solubility of the two drugs in gels and hence greater partitioning of the drugs at the boundary between the diffusion medium and the preparation.

The sequence of arrangement of the obtained zones of inhibition appeared in agreement with that obtained from the in vitro release except for 20% (wt/wt) ED gels. It is revealed that 20% (wt/wt) ED gels show the greatest zone of inhibition, although they show an intermediate rate of release when compared with 5% HEC and 3% (wt/wt) CP 934, which may be attributed to the high solubility of drug in the base.

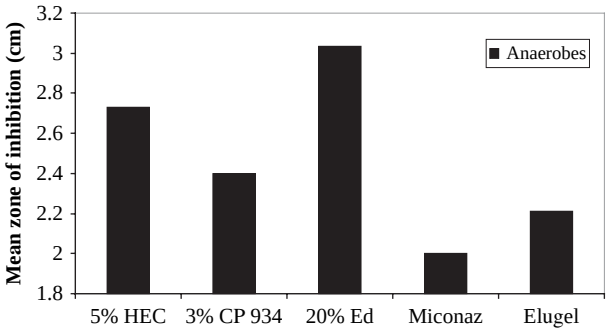

FIGURE 12. Antibacterial activity of selected secnidazole (SC) gels in comparison with Miconaz® and Elugel® using anaerobes as test organisms.

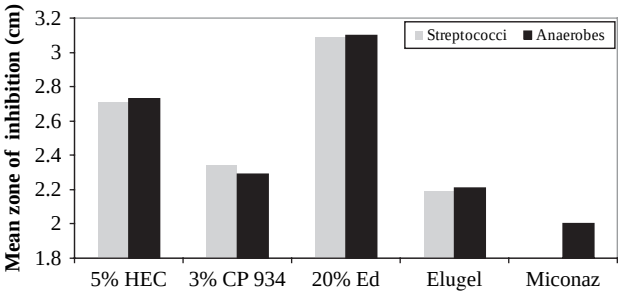

FIGURE 13. Antibacterial activity of selected doxycycline hydrochloride (DH) gels in comparison with Miconaz® and Elugel® using *Streptococci* and anaerobes as test organisms.

CONCLUSION

According to the obtained results, it could be concluded that SC and DH would be successfully formulated as mucoadhesive gels for the treatment of periodontitis. Eudragit (20%, wt/wt) gels showed optimum viscosity to pass through blunt syringe to be applied easily, optimum mucoadhesion properties to be retained locally, with prolonged drug release, and good antibacterial activity compared with the commercial products. So, eudragit-based gels containing each drug could be used for the treatment of periodontitis; however, further clinical investigations are needed.

REFERENCES

Abdel-Hamid, S. M., Abdel-Hady, S. E., El-Shamy, A. A., & El-Dessouky, H. F. (2006). Formulation of an antispasmodic drug as a topical local anesthetic. *Int. J. Pharm., 326,* 107–118.

Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother., 48,* 5–16.

Behra-Miellet, J., Calvet, L., & Dubreuil, L. (2003). Activity of linezolid against anaerobic bacteria. *Int. J. Antimicrob. Agents, 22*, 28–34.

Bonacucina, G., Martelli, S., & Palmieri, G. F. (2004). Rheological, mucoadhesive and release properties of Carbopol gels in hydrophilic cosolvents. *Int. J. Pharm., 282*, 115–130.

Bonacucina, G., Cespi, M., Misici-Falzi. M., & Palmieri G. F. (2006). Rheological, adhesive and release characterization of semisolid Carbopol/tetraglycol systems. *Int. J. Pharm., 307*, 129–140.

Bruschi, M. L., & De Freitas, O. (2005). Oral bioadhesive drug delivery systems. *Drug Dev. Ind. Pharm., 31*, 293–310.

Ch'Ng, H. S., Park, H., Kelly, P., & Robinson, J. R. (1985). Bioadhesive polymers as platforms for oral controlled drug delivery: II. Synthesis and evaluation of some swelling, water-insoluble bioadhesive polymers. *J. Pharm. Sci., 74*, 399–405.

Desai, K. G. H., & Kumar, T. M. P. (2004). Preparation and evaluation of a novel buccal adhesive system. *AAPS Pharm. Sci. Tech., 5*(3), article 35. http://www.aapspharmscitech.org.

Dolz, M., Herraez, M., Gozalez, F., & Diez, O. (1998). Flow behavior of Carbopol 940 hydrogels, the influence of concentration and agitation time. *Pharmazie, 53*(2), 126–130.

Eick, S., Pfister, W., & Straube, E. (1999). Antimicrobial susceptibility of anaerobic and capnophilic bacteria isolated from odontogenic abscesses and rapidly progressive periodontitis. *Int. J. Antimicrob. Agents, 12*, 41–46.

Eouani, C., Piccerellea, P., Prinderrea, P., Bourret, E., & Joachim, J. (2001). In-vitro comparative study of buccal mucoadhesive performance of different polymeric films. *Eur. J. Pharm. Biopharm., 52*, 45–55.

Fresno, M. J. C., Ramirez, A. D., & Jimenez, M. M. (2002). Systematic study of the flow behavior and mechanical properties of Carbopol Ultrez 10 hydroalcoholic gels. *Eur. J. Pharm. Biopharm., 54*, 329–335.

Friesen, L. R., Williams, K. B., Krause, L. S., & Killoy, W. J. (2002). Controlled local delivery of tetracyclines with polymer strips in the treatment of periodontitis. *J. Periodontol., 73*, 13–19.

Gillis, J. C., & Wiseman, L. R. (1996). Secnidazole: A review of its antimicrobial activity, pharmacokinetic properties and therapeutic use in the management of protozoal infections and bacterial vaginosis. *Drugs, 51*, 621–638.

Golub, L. M., Sorsa, T., & Lee, H. M. Ciancio, S. Sorbi, D., Ramamurthy, N. S., Gruber, B., Salo, T. & Konttinen, Y. T. (1995). Doxycycline inhibites neutrophil (PMN)-type matrix metalloproteinases in human adult periodontitis gingival. *J. Clin. Periodontol., 22*, 100–109.

Goodson, J. M. (1994). Antimicrobial strategies for treatment of the periodontal diseases. *Periodontol. 2000, 5*, 142–168.

Goto, S., Kawata, M., Suzuki, T., Kim, N., & Ito, C. (1991). Preparation and evaluation of Eudragit gels. I: Eudragit Organogels containing drugs as rectal sustained-release preparations. *J. Pharm. Sci., 80*, 958–961.

Haffajee, A. D., & Socransky, S. S. (1994). Microbial etiological agents of destructive periodontal disease. *Periodontol. 2000*, 5, 78–111.

Jones, D. S., Woolfson, A. D., & Brown, A. F. (1997a). Textural, viscoelastic and mucoadhesive properties of pharmaceutical gels composed of cellulose polymers. *Int. J. Pharm., 151*, 223–233.

Jones, D. S., Woolfson, A. D., & Brown, A. F. (1997b). Textural analysis and flow rheometry of novel, bioadhesive antimicrobial oral gels. *Pharm. Res., 14*, 450–457.

Jones, D. S., Woolfson, A. D., Brown, A. F., & O'Neill, M. J. (1997). Mucoadhesive, syringeable drug delivery systems for controlled application of metronidazole to the periodontal pocket: in vitro release kinetics, syringeability, mechanical and mucoadhesive properties *J. Control. Rel., 49*, 71–79.

Jones, D. S., Woolfson, A. D., Brown, A. F., Coulter, W. A., McClelland, C., & Irwin, C. R. (2000). Design, characterization and preliminary clinical evaluation of a novel mucoadhesive topical formulation containing tetracycline for the treatment of periodontal disease. *J. Control. Release, 67*, 357–368.

Jones, D. S., Woolfson, A. D., Djokic, J., & Coulter, W. A. (1996). Development and mechanical characterization of bioadhesive semi-solid, polymeric systems containing tetracycline for the treatment of periodontal diseases. *Pharm. Res., 13*, 1734–1738.

Kinane, D. F., & Lindhe, J. (1997). Pathogenesis of periodontitis. In J. Lindhe, T. Karring, & N. P. Lang (Eds.). *Clinical periodontology and implant dentistry*, (pp. 189–225). Copenhagen: Munksgaard Intl Pub.

Kinci, I., Senel, S., Akıncıbay, H., Kas, S., Ercis, S., Wilson, C. G., & Hıncal, A. A. (2002). Effect of chitosan on a periodontal pathogen *Porphyromonas gingivalis G*. *Int. J. Pharm., 235*, 121–127.

Khare, A. R., & Peppas, N. A. (1993). Release behavior of bioactive agents from pH-sensitive hydrogels. *J. Biomater. Sci. Polym. Ed., 4*, 275–289.

Lee, C. H., & Chien, Y. W. (1996). Development and evaluation of a mucoadhesive drug delivery system for dual-controlled delivery of Nonoxynol-9. *J. Control. Release, 39*, 93–103.

Liu, D. Z., Sheu, M. T., Chen, C. H., Yang, Y. R., & Ho, H. O. (2007). Release characteristics of lidocaine from local implant of polyanionic and polycationic hydrogels. *J. Control. Release, 118*(3), 333–339.

McCulloch, C. A. G., Birek, P., Overall, C. *et al.* (1990). Randomized controlled trial of doxycycline in prevention of recurrent periodontitis in high-risk patients: Antimicrobial activity and collagenase inhibition. *J. Clin. Periodontol., 17*, 616–622.

Mikos, A. G., & Peppas, N. A. (1990). Bioadhesive analysis of controlled release systems: IV. An experimental method for testing the adhesion of microparticles with mucosa. *J. Control. Release, 12*, 31–37.

Mitchell, K., Ford, J. L., Armstrong, D. J., Elliott, P. N. C., Rostron, C., & Hogan, J. E. (1993a). The influence of concentration on the release of drugs from gels and matrices containing Methocel®. *Int. J. Pharm., 100*, 155–163.

Mitchell, K., Ford, J. L., Armstrong, D. J., Elliott, P. N. C., Hogan, J. E., & Rostron, C. (1993b). The influence of drugs on the properties of gels and swelling characteristics of matrices containing methylcellulose or Hydroxypropylmethylcellulose. *Int. J. Pharm., 100*, 165–173.

Mombelli, A., Lehmann, B., Tonetti, M., & Lang, N. P. (1997). Clinical response to local delivery of tetracycline in relation to overall and local periodontal conditions. *J. Clin. Periodontol., 24*, 470–477.

Muramatsu, M., Kanada, K., Nishida A., Ouchi, K., Saito, N., Yoshida, M., Shimoaka, A., Ozeki, T., Noriyasu, Yuasa, H., & Kanaya, Y. (2000). Application of Carbopol® to controlled release preparations I. Carbopol® as a novel coating material. *Int. J. Pharm., 199*, 77–83.

National Committee for Clinical Laboratory Standards. (1993). *Methods for antimicrobial susceptibility testing of anaerobic bacteria, 3rd Ed. Approved standard (M11-A3)*. Villanova, PA: National Committee for Clinical Laboratory Standards; NCCLS.

National Committee for Clinical Laboratory Standards. (1997). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard (M7-A4)*. Wayne, PA: NCCLS.

National Committee for Clinical Laboratory Standards. (2002). *Performance standards for antimicrobial disk susceptibility tests. Approved Standard, 7th ed. (M02-A7)*. Villanova, PA: NCCLS.

Needleman, I. G., Martin, G. P., & Smales, F. C. (1998). Characterisation of bioadhesives for periodontal and oral mucosal drug delivery, *J. Clin. Periodontal., 25*, 74–82.

Noyan, U., Yilmaz, S., Kuru, B., Kadir, T., Acar, O., & Buget, E. (1997). A clinical and microbiological evaluation of systemic and local metronidazole delivery in adult periodontitis patients. *J. Clin. Periodontol.*, 24, 158–165.

Paavola, A., Yliruusi, J., Kajimoto, Y., Kalso, E., Wahlström, T., & Rosenberg, P. (1995). Controlled release of lidocaine from injectable gels and efficacy in rat sciatic nerve block. *Pharm. Res., 12* (12), 1997–2002.

Pähkla, E. R., Koppel, T., Saag, M., & Pähkla, R. (2005). Metronidazole concentrations in plasma, saliva and periodontal pockets in patients with periodontitis. *J. Clin. Peridontol., 32*, 163–166.

Pena, L. E., Lee, B. I., & Sternes, J. F. (1994). Structural rheology of model ointment. *Pharm. Res., 11*, 875–881.

Peppas, N. A. (1985). Analysis of Fickian and non-Fickian drug release from polymers. *Pharm. Acta Helv., 60*, 110–111.

Schwach-Abdellaoui, K., Vivien-Castioni, N., & Gurny. R. (2000). Local delivery of antimicrobial agents for the treatment of periodontal diseases. *Eur. J. Pharm. Biopharm., 50*, 83–99.

Sorsa, T., Ding, Y. L., Ongmam, T. et al. (1995). Cellular source, activation and inhibition of dental plaque collagenase. *J. Clin. Periodontol., 22*, 709–711.

Steffe, J. F. (1996). Intoduction to Rheology. In J. F. Steffe (Ed.), *Rheological methods in food process engineering* (pp. 1–91). East Lansing, MI: Freeman Press.

Talukder, M. M., Vinckier, I., Moldenaers, P., & Kinget, R. (1996). Rheological characterization of xanthan gum and hydroxypropylmethyl cellulose with respect to controlled-release drug delivery. *J. Pharm. Sci., 85*(5), 537–540.

Tamburic, S., & Craig, D., Q. M. (1995). An investigation into the rheological, dielectric and mucoadhesive properties of poly (acrylic acid) gel systems. *J. Control. Release, 37*, 59–68.

Tomazinho, L. F., & Avila-Campos, M. J. (2007). Detection of Porphyromonas gingivalis, Porphyromonas endodontalis, Prevotella intermedia, and Prevotella nigrescens in chronic endodontic infection. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod., 103*(2), 285–288.

Van Winkelhoff, A. J., Herrera, D., Oteo, A., & Sanz, M. (2005). Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in The Netherlands and Spain. *J. Clin. Periodontol., 32*(8), 891–892.

Walker, C. B., Godowski, K. C., Borden, L., Lennon, J., Nango, S., Stone, C., & Garrett, S. (2000). The effects of sustained release doxycycline on the anaerobic flora and antibiotic–resistant patterns in subgingival plaque and saliva. *J. Periodontol., 71*, 768–774.

Williams, R. C., Paquette, D. W., Offenbacher, S. et al. (2001). Treatment of periodontitis by local administration of minocycline microspheres: a controlled trial. *J. Periodontol., 72*, 1535–1544.

Wolff, L, Dahlen, G., & Aeppli, D. (1994). Bacteria as risk marker for periodontitis. *J. Periodontol., 64*, 498–510.

Won-Jun, H., & Bayoumi, S. M. (1986). Diffusion model for studying the drug release from semisolid dosage forms. Part 1. Methodology using agar gel as diffusion medium. *Drug. Dev. Ind .Pharm., 12*(6), 899–914.

Yong., C. H., Choi, J. S., Quan, Q. Z., Rhee, J. D., Kim, C. K., Lim, S. J., Kim, K. M., Oh, P. S., & Choi, H. G. (2001). Effect of sodium chloride on the gelation temperature, gel strength and bioadhesive force of poloxamer gels containing diclofenac sodium. *Int. J. Pharm., 226*, 195–205.