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Comparative analysis of tetracycline-containing dental gels: poloxamer- and monoglyceride-based formulations

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Abstract

The aim of the paper was to develop tetracycline-containing formulations for the treatment of periodontitis by direct periodontal intrapocket administration. Two different semi-solid formulations were prepared, based on poly(oxyethylene)poly(oxypropylene) block copolymer (poloxamer) and monoglycerides, respectively. Both formulations possess interesting properties as delivery systems. They are easily administered by syringe equipped with needles appropriate for intrapocket delivery, they are characterized by a sol–gel transition, becoming semi-solid once in the periodontal pocket and, finally, they represent biocompatible formulations eliminated from the body by normal routes. A rheological characterization of both formulations was performed in the presence or in the absence of tetracycline, determining the sol–gel transition temperature (T_c) by 'time cure tests' and the z coefficient by 'frequency sweep test'. In addition, the in vitro tetracycline release from formulations was determined. Comparative in vivo studies were conducted, in order (a) to compare the persistence of the gels on the gum and (b) to evaluate the clinical performances of the gels. These latest studies indicated that both poloxamer and monoglycerides gels, when applied subgingivally, produce a significantly improved outcome in moderate to deep periodontal pockets.

Keywords: Tetracycline; Periodontitis; Drug delivery systems; Monoglycerides; Poloxamer

1. Introduction

Gingivitis and periodontitis are pathological states affecting the gingival, subgingival, periodontal and adjacent tissues (Listgarten, 1986, 1987). Together with conventional therapy, based

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Table 1
Pharmaceutical characteristics of drug delivery systems for the treatment of periodontal diseases

Characteristic	Gels	Solid devices (fibres or microparticles)
Preparation method	Easy	Complex (instruments needed)
Bioadhesivity	Yes	No
Release period	Days, weeks	Weeks, months
Biodegradability	Yes	Yes[a]
Biocompatibility	Yes	Yes (risk of inflammatory or adverse reactions)
Application modality	Easily administrable by appropriate syringe and needles	Special syringe needed (microspheres) or application and removal by specialist (fibres)

[a]Only by using biodegradable polymers.

on scaling and surgery, the use of antibiotics or antimicrobials (e.g. tetracycline, minocycline, clindamycin, metronidazole and chlorhexidine) has been proposed (Van der Ouderaa, 1991). In particular, the tetracycline family of antibiotics was found effective against the microorganisms associated with periodontitis in the gingival crevice (Slots, 1979). The antibiotic therapy of periodontal diseases is mainly based on two different approaches: extensive oral rinses with solutions and systemic administration. On the other hand, both approaches can be unsuccessful and/or produce adverse problems. In fact, the first one could result in a failure of antibiotics to reach the deeper subgingival tissues, while the second one could present disadvantages such as (a) bacterial resistance to the administered antibiotic and (b) unpleasant or toxic side effects as a consequence of the systemic regimen (Okuda et al., 1992). Because of these considerations, a variety of specialized local delivery systems (i.e. intrapocket devices) were designed to maintain the antibiotic in the gingival crevicular fluid at a concentration higher than that achieved by systemic administration (Kornman, 1993). Fibres, films and microparticles made of biodegradable or non-degradable polymers have been recently proposed as effective methods to administer antibacterial agent for periodontal therapy (Medlicott et al., 1994). Together with these solid devices, recently, semi-solid formulations such as Elyzol® 25% dental gel, consisting of metronidazole crystals suspended in a lipid matrix, have been proposed (Stoltze and Stellfeld, 1992).

With respect to solid devices, semi-solid (gel) formulations can indeed have some advantages (see Table 1). In fact, in spite of relatively faster release of the incorporated drug (with respect to fibres or microparticles), gels can be more easily prepared and administered. Moreover, they possess a higher biocompatibility and bioadhesivity, allowing adhesion to the mucosa in the dental pocket and, finally, they can be rapidly eliminated through normal catabolic pathways, decreasing the risk of irritative or allergic host reactions at the application site.

In this paper we propose and compare two different semi-solid formulations designed for administration of tetracycline to the periodontal pocket, namely a poly(oxyethylene) poly(oxypropylene) block copolymer (poloxamer 407)-based gel and a monoglyceride–water system. In particular, this report describes: (a) the preparation of the tetracycline-containing semi-solid formulations, (b) their rheological characterization, (c) the in vitro release kinetics of tetracycline from the gels, (d) the persistence of gels on the human gum and, finally, (e) a comparative in vivo evaluation of their clinical performances for the treatment of periodontal disease.

2. Materials and methods

2.1. Materials

Poly(oxyethylene)poly(oxypropylene) block copolymer (Poloxamer 407), was a kind gift of BASF (Lutrol F 127®) Wyandotte Corporation

(Cheshire, UK). Distilled glyceryl monooleate (Myverol® 18-99) was from Eastman Chemical (Kingsport, TN, USA). Tetracycline hydrochloride was obtained from Sigma (Osterode, Germany).

2.2. Methods

2.2.1. Preparation of poloxamer gel

Poloxamer gel was prepared according to the 'cold technique' (Garcia Sagrado et al., 1995). Briefly, a weighed amount of poloxamer 407 was gradually added to cold water (5–10°C) under magnetic stirring up to a final concentration of 30% (w/w) poloxamer. The container was sealed and left in a refrigerator overnight at 5°C. Tetracycline hydrochloride dissolved in water was then added to the preformed gel before in vitro or in vivo studies, obtaining a final tetracycline content of 20 mg/ml in 25% w/w poloxamer gel. The drug was added just prior to use in order to minimize epimerization of tetracycline, leading to loss in therapeutic activity (Walton et al., 1970). For the rheological studies, gels with a poloxamer concentration of 20, 25 and 30% (w/w) without tetracycline were also prepared.

2.2.2. Preparation of monoglyceride gel

Monoglycerides (4.5 g) (m.p. 35°C) were melted. Subsequently, 5 parts of water for every 90 parts of monoglycerides were added. An opaque and uniform paste was formed by stirring and the container was sealed afterwards to avoid water evaporation. The mixture was then placed in an oven at 42°C for several days. Tetracycline hydrochloride dissolved in water was added to the preformed gel and vigorously stirred immediately before in vitro or in vivo studies. A monoglycerides gel with a final tetracycline content of 20 mg/ml in 90% (w/w) gel was obtained. For the rheological studies, gels with a monoglycerides concentration of 90% and 95% (w/w) without tetracycline were also prepared.

2.2.3. Rheological studies

Rheological measurements have been carried out by using two different instruments, depending on the sample viscosity. Low and high viscosity samples were measured by using Rheometrics RFS2 and Rheometrics RMS800 rheometers (Rheometrics, Possum Town, NY, USA) respectively. Parallel plates (25 mm diameter; 1.5 mm gap) and couette geometries (1 mm gap) were used. Both oscillatory and monodimensional steady shear flow have been considered. Oscillatory measurements were carried out at a low amplitude (within the linear viscoelastic region) with an angular velocity (ω) of between 0.1 and 100 rad/s. Measurements were conducted at four different temperatures, namely 10, 20, 30 and 37°C. According to the Bohlin theory that considers flow as a cooperative phenomenon, the coordination coefficient z was calculated from the slope of the curve obtained by plotting the elastic modulus (G') vs. ω in a log–log plot. The coordination coefficient was calculated for all poloxamer concentrations and temperatures.

The sol–gel transition temperature (T_c) was calculated by 'time cure tests' obtained by plotting elastic (G') and loss (G'') moduli as function of temperature. Determinations were performed at 1 Hz and at a low amplitude, the temperature range was 4–40°C and the temperature ramp was 1°C/min. The viscosity has been measured at a low shear rate (0.1–10 s^{-1}), in order to avoid slipping effects at the wall surface, possibly caused by high shear rates.

2.2.4. Determination of in vitro tetracycline release kinetics

The in vitro release kinetics of tetracycline from the gels were determined by dialysis method. Typically, 3 g of gel were placed into a dialysis tube (MW cut-off 10 000–12 000; Medi Cell International, UK). The dialysis tube was then poured into sealed vials containing 20 ml of receiving solution consisting of isotonic phosphate buffer (pH 7.4). The release experiments were performed at 37°C. At different time intervals from 0.25 to 7 h, 200 ml of receiving buffer were withdrawn and replaced with an equal volume of fresh buffer. Samples were analyzed for tetracycline content by UV spectroscopic analysis.

The tetracycline calibration curve was made by preparing solutions containing increasing amounts of tetracycline (range 1.8–18 mg/ml) and

plotting their respective UV absorption at 365 nm against their respective drug concentrations. The release profiles were determined five times in independent experiments.

2.2.5. Tetracycline release data analysis

The kinetic analysis of the release profiles was carried out according to the general equation (Peppas, 1985):

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

where M_t is the cumulative amount of drug released at time t, M_∞ is the total amount of drug incorporated, k is the proportionality constant (the value of which depends on the structural and geometrical properties of the matrix) and n is the release exponent (its value depends on the mechanism of drug release).

2.2.6. Determination of gel persistence on human gum

Poloxamer- and monoglycerides-based gels containing 6% w/w of an aqueous solution of the dye toluidine blue (Sigma, Milan, Italy) were simultaneously applied on the lower gum of one person by using a plastic applicator containing a defined amount of both gels. After different lengths of time, photographs were taken and the area stained by blue dye was evidenced and quantitated by a computerized scanning analysis of the digitalized images. For this analysis, 'NIH Image', a public domain image processing and analysis program for the Macintosh, was used.

2.2.7. Clinical evaluation

Nine patients, two males and seven females (age range: 27–63 years; mean age: 42.1 years), referred to the Department of Periodontology, University of Ferrara, for treatment of moderate to severe adult periodontitis, were considered. Selection criteria included the presence of at least two non-adjacent sites with probing depth greater than or equal to 5 mm in single-rooted teeth of the same arch. Patients received complete periodontal examination including oral hygiene standards and gingival health recordings, probing measurements, occlusal and radiographic evaluation. A full-mouth supra- and subgingival scaling session to remove soft and calcified plaque accumulation was then performed by means of ultrasonic instruments (Cavitron, Dentsply, York, PA, USA). At the same session, experimental sites received a single subgingival application of tetracycline-containing poloxamer gel, or tetracycline-containing monoglyceride gel, according to a randomization list. Tetracycline gel preparations were slowly delivered to the bottom of the periodontal pocket by using a disposable syringe equipped with a 23-gauge blunt needle. The gels were injected into the pocket until the gel overflowed from the gingival margin. Both the patient and the operator (VC) were blind to the treatment modality. Patients were instructed not to use chlorhexidine mouthwashes or other antiplaque agents during the observation interval. No attempt was made to improve patients' performance in mechanical plaque control.

Clinical recordings, assessed immediately before treatment (baseline) and after 4 weeks, were as follows: (1) probing depth (PD), defined as the distance from the gingival margin to the bottom of the pocket; (2) clinical attachment level (CAL), defined as the distance from the cemento-enamel junction (CEJ) to the bottom of the pocket; (3) recession depth (RD), defined as the distance from the CEJ to the gingival margin; and (4) bleeding on probing (BoP), recorded as the presence of bleeding occurring 10 s after the probe insertion into the pocket.

Probing measurements were recorded to the nearest millimetre with a standard periodontal probe (UNC 15, Hu Friedy, Chicago, IL). The BoP score was expressed as the proportion (%) of bleeding sites out of the total number of experimental sites for each treatment group. All the recordings were assessed by one examiner (A.S.) blinded to the treatment performed. For statistical comparisons between treatments, the subject was considered as the statistical unit. The Wilcoxon matched pairs signed test was used to evaluate mean differences in clinical recordings within and between treatment modalities. The level of significance was set at 5%.

3. Results and discussion

3.1. Tetracycline-containing gels

The aim of the present investigation was to produce and to compare, both in vitro and in vivo, semi-solid formulations for periodontal diseases. The formulations were designed in order to achieve the following requisites.

(a) Formulations should be biocompatible and easily eliminated from the body. The easy elimination appears particular important since degradable delivery systems erode or dissolve in the gingival crevice, avoiding removal after treatment. For instance, monoglycerides are prone to esterase activity and they can be easily cleared from the body.

(b) Formulations should display low viscosity at the moment of in vivo application, in order to be administrable by syringe equipped with needles of appropriate size for intrapocket delivery.

(c) Formulations, once applied, should undergo a sol–gel transition, resulting in semi-solid gels. In this way, bioadhesion to gingival tissue and long-lasting release of the drug could be achieved.

With the aim of obtaining the requested features, two different formulations were designed, one based on a hydrophilic–hydrophobic block copolymer (poloxamer gel) and the other based on a supramolecular association of monoglycerides (monoglycerides gel).

Both these semi-solid formulations possess peculiar characteristics.

Poloxamer 407 is a commercially available poly(oxyethylene)poly(oxypropylene) block copolymer, containing 73% polyoxyethylene units (see specifications in Table 2). Poloxamer 407 has low toxicity, high compatibility with other chemicals and can solubilize drugs with different chemico-physical properties. Moreover, aqueous solutions of poloxamer 407 possess interesting rheological properties. When used at concentrations above 20%, the polymer forms thermoreversible gels characterized by a critical temperature (T_c). At temperatures below T_c, the poloxamer solution is a low-viscosity sol, whilst above T_c, a transparent viscous gel forms. The sol–gel transition is a reversible process and it occurs whenever cooling and heating cycles are conducted, without any appreciable alteration in the gel characteristics and viscosity (Attwood et al., 1983; Tung, 1994).

In this respect, poloxamer solution, liquid at room temperature, can form viscous gels approaching body temperature (e.g. in mouth cavity) (Garcia Sagrado et al., 1995). Myverol 18-99 is a mixture of different monoglycerides (see its composition and specific characteristics in Table 2). These polar lipids are insoluble in water but able to swell, giving rise to different lyotropic liquid crystalline phases described by a complex phase diagram, depending on water content and temperature (Engstrom et al., 1992). When a small amount of water is added to monoglycerides (usually less than 5% w/w), reverse micellar structures are formed with a relatively low viscosity. At this stage a further addition of water leads to the formation of

Table 2
Characteristics of poloxamer and monoglycerides used in gel preparation[a]

Poloxamer 407	
Hydroxyl value	8 – 11
Molecular weight[b]	9840 – 14600
pH (2.5% in water)	5.0 – 7.5
Melting point (°C)	53 – 57
Polyoxyethylene mass fraction (%)	73.2 ± 1.7
Monoglycerides	
Physical form	Semi-plastic
Fatty acid distribution (%)	
Palmitate (C16:0)	4.1
Stearate (C18:0)	1.8
Oleate (C18:1)	60.9
Linoleate (C18:2)	21.0
Linolenate (C18:3)	8.8
Gadoleate (C20:1)	1.0
Melting point (°C)	35
Iodine value (max.)	90–95

[a]As provided by the manufacturers.
[b]Calculated from the hydroxyl value.

Table 3
Rheological characterization of tetracycline-containing gels

Gel	Composition (%)	Guest molecule	T_c [°C]	G' [Pa]				G'' [Pa]				z [Pa·s]				η [$\times 10\ s^{-1}$]
				10°C	20°C	30°C	37°C	10°C	20°C	30°C	37°C	10°C	20°C	30°C	37°C	37°C
Poloxamer	20	No	15.7	0.0004	19 970	26 240	30 060	0.6	1531	1703	1053	—	31.4	73.8	185.4	68.6
	25	No	21.0	0.0007	1841	12 440	15 390	0.5	1430	1149	1443	4.1	7.6	11.2	23.6	46.6
	25	Yes	15.8	0.0063	17 320	24 090	24 120	1.1	1372	1869	1040	0.7	25.2	61.5	106.6	67.5
	30	No	12	992	18 950	24 120	24 770	177	1776	1992	2316	8.8	27.7	40.0	37.0	63.1
Myverol	95	No	—	11 910	4982	475 400	3718	1401	1148	12 270	2135	15.4	9.2	6.5	4.0	10.1
	90	No	—	55 540	2849	0.17	0.026	17 360	531	2.3	1.2	3.97	10.0	1.2	0.8	0.18
	90	Yes	—	7371	3386	808	436	1053	523	162	110	16.1	14.4	12.9	11.0	1.63

*Percentage w/w of poloxamer or myverol used for gel preparation (for further details on gel preparation see Section 2).

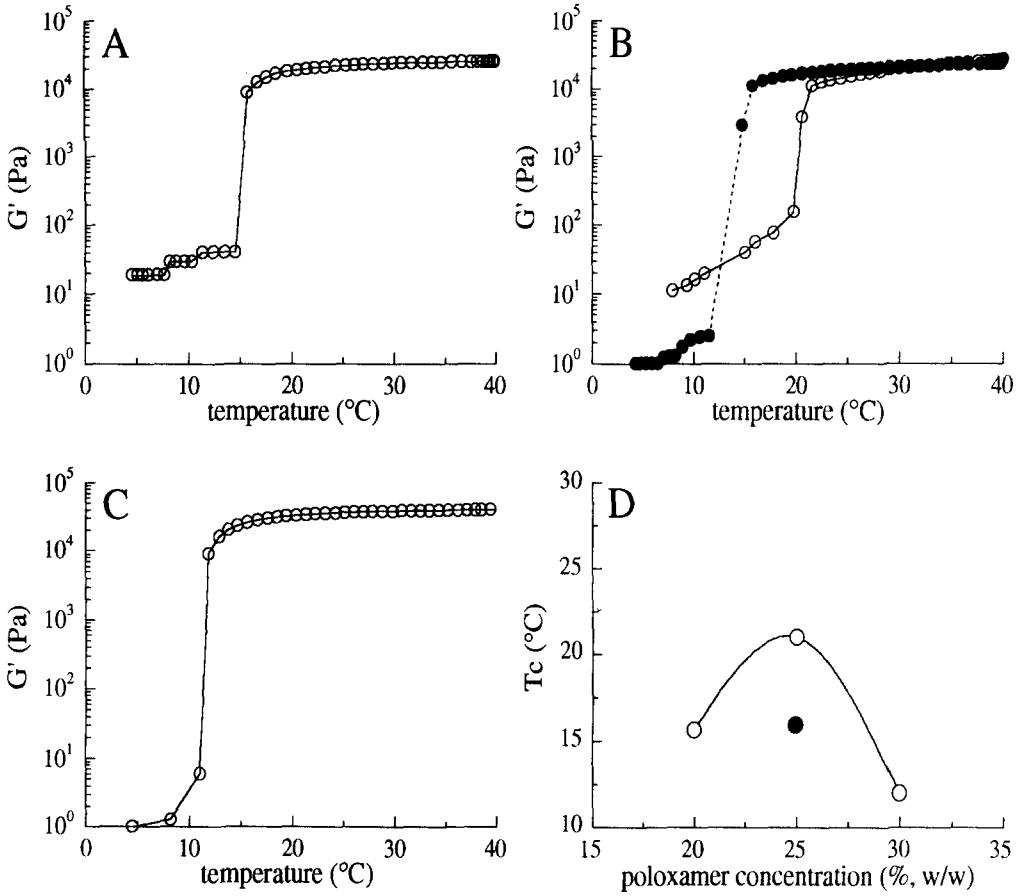

Fig. 1. A–C: Time cure tests, showing the temperature dependence of the elastic modulus G' for (A) 20%, (B) 25% and (C) 30% poloxamer gels. In the case of B, both empty (○) and tetracycline-containing (●) gels were analyzed. D: Influence of poloxamer concentration and tetracycline presence (●) on transition temperature (T_c).

highly viscous lamellar or cubic phases. In particular, we produced a tetracycline gel incorporating 10% w/w of water, since its viscosity allowed expression through the 23-gauge blunt needle used for in vivo administration. After application, the monoglycerides–water system adsorbs the biological fluids, swells and increases its viscosity to a semi-solid form (Engstrom et al., 1995).

In conclusion, both formulations, even with different mechanisms, are administrable by syringe and become semi-solid once in the periodontal pocket, allowing the drug to be released in a sustained manner (Norling et al., 1992).

3.2. Rheological studies

In this study, a series of preliminary results on the rheological characterization of both poloxamer- and monoglycerides-based gels is presented (Table 3). This study was performed in order to define the general rheological behaviour of these relatively novel materials and to provide information on their structure, as a function of temperature and of the presence of solubilized guest molecules (i.e. tetracycline). In particular, we determined the sol–gel transition temperature (T_c) by 'time cure tests', the frequency dependence of the elastic modulus G' by 'frequency sweep tests',

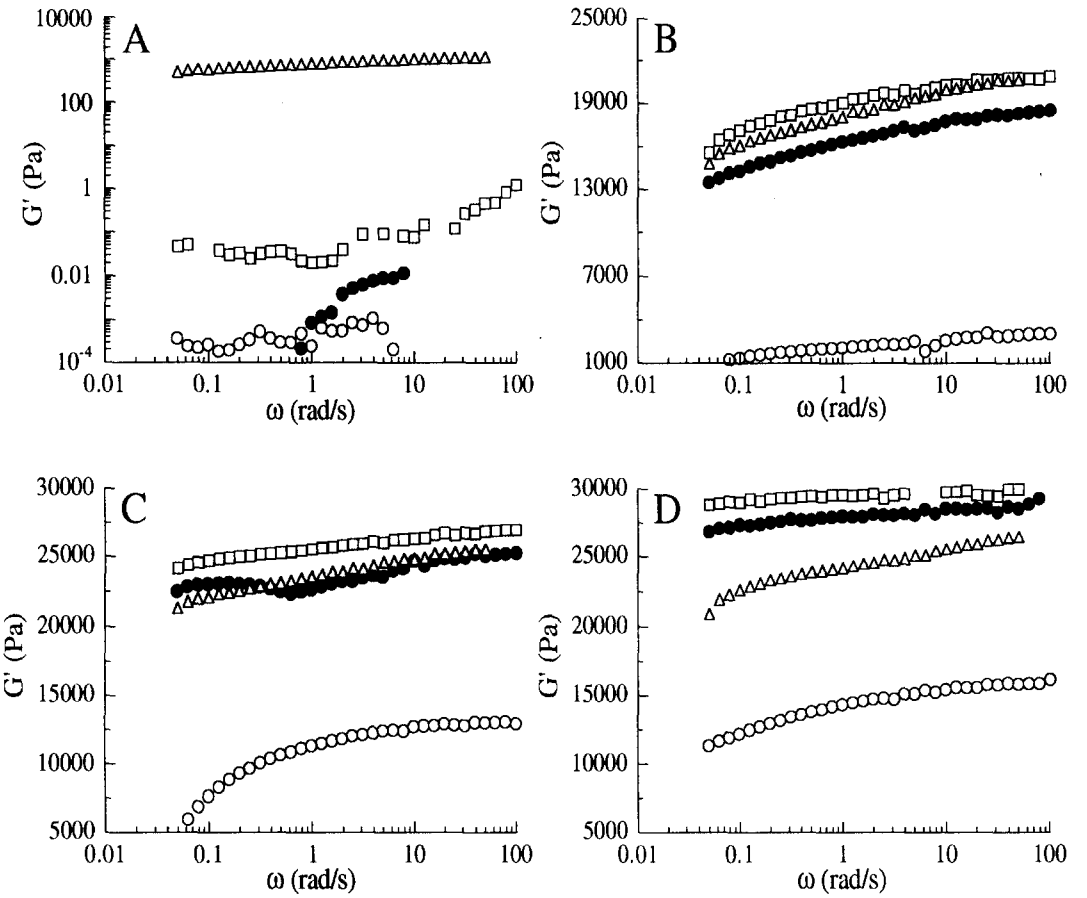

Fig. 2. Frequency sweep tests showing the frequency dependence of the elastic modulus G'. Determinations were performed at (A) 10, (B) 20, (C) 30 and (D) 37°C. (□), 20% poloxamer gel; (○), 25% poloxamer gel; (●), 25% poloxamer gel + tetracycline; (△), 30% poloxamer gel.

and the temperature dependence of G' and the z coefficient. (see Figs. 1–6).

From the analysis of the results obtained by this series of experiments, the following general considerations can be drawn.

3.2.1. Poloxamer-based gels:

(a) Fig. 1 shows that poloxamer gels are characterized by a sharp transition from a liquid (sol) to a structured (gel) behaviour at a well-defined temperature (T_c) determined by the analysis of T vs. G' curves. The highest value of T_c (21°C) was found in the case of 25% poloxamer gel whilst 20% and 30% gels show a lower T_c value (see Fig. 1D).

(b) In all samples, both elastic modulus G' and z coefficient increase as temperature increases (see Figs. 2 and 3). In this regard, it should be noted that G' and z give indications about the structure strength and the structure coordination, respectively.

(c) At temperature above 15°C, samples show a pseudo-plastic behaviour characterized by a typical shear thinning behaviour.

(d) The presence of tetracycline causes a shift of T_c to a lower value ($\Delta T_c = 6.8$°C) (Fig. 1D) and a concomitant increase of both G' and z value (Fig. 3).

Taken together, these results indicate that tetracycline can have a positive effect on both gel

structuration and strength. This feature was tentatively explained by a facilitation of the interactions between the hydrophobic portions of the

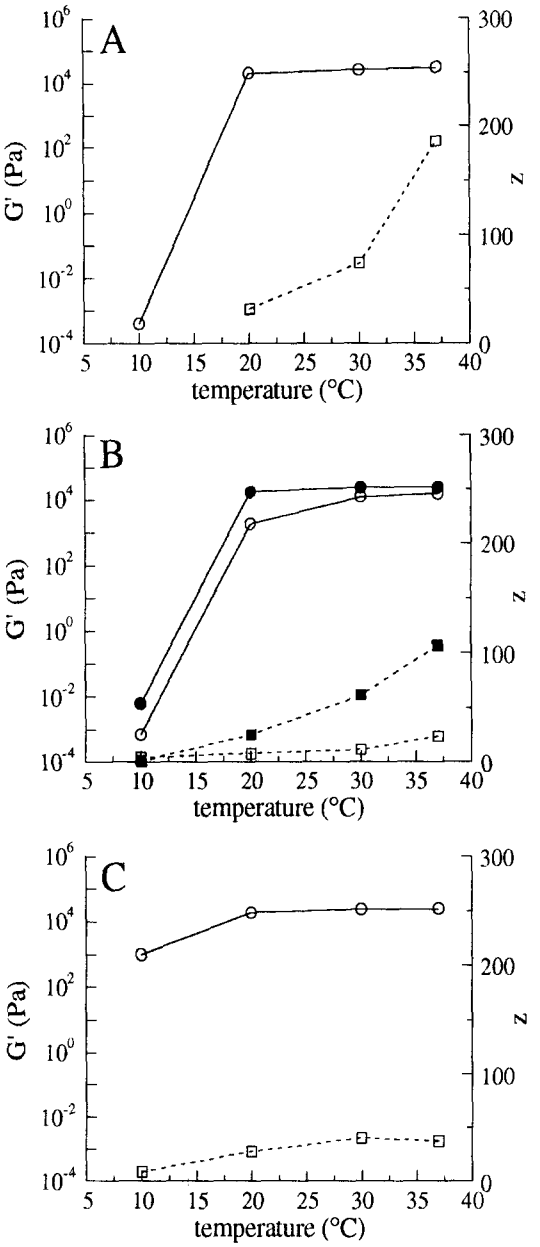

Fig. 3. Temperature dependence of the elastic modulus G' (○——○) and z coefficient (□- - -□) for (A) 20%, (B) 25% and (C) 30% poloxamer gels. In the case of B, both empty (open symbols) and tetracycline-containing (solid symbols) gels were analyzed.

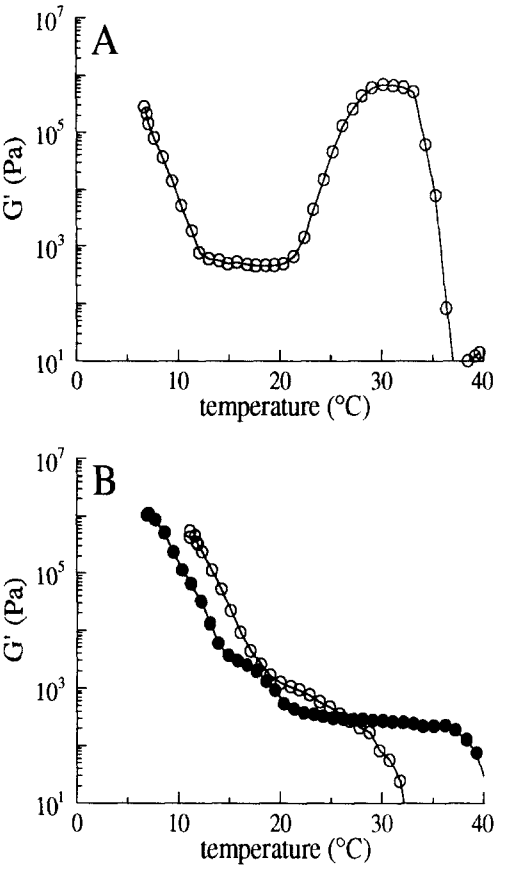

Fig. 4. Time cure tests, showing the temperature dependence of the elastic modulus G' for (A) 95% and (B) 90% monoglyceride gels. In the case of B, both empty (○) and tetracycline-containing (●) gels were analyzed.

polymer molecules responsible for the gelation process. This facilitation could be due to the insertion of the planar tetracycline molecule within the polyoxypropilenic fractions.

3.2.2. Monoglycerides-based gels:

(a) Fig. 4 shows that monoglycerides gels, in contrast to poloxamer ones, do not present a well-defined transition temperature. In this case, elastic and loss moduli are very close in the temperature range 5–40°C. In particular, the rheological behaviour of 95% monoglycerides is quite interesting. Here the G' and G'' curve trends are not monotonic but show a well-defined minimum region between 12 and 18°C.

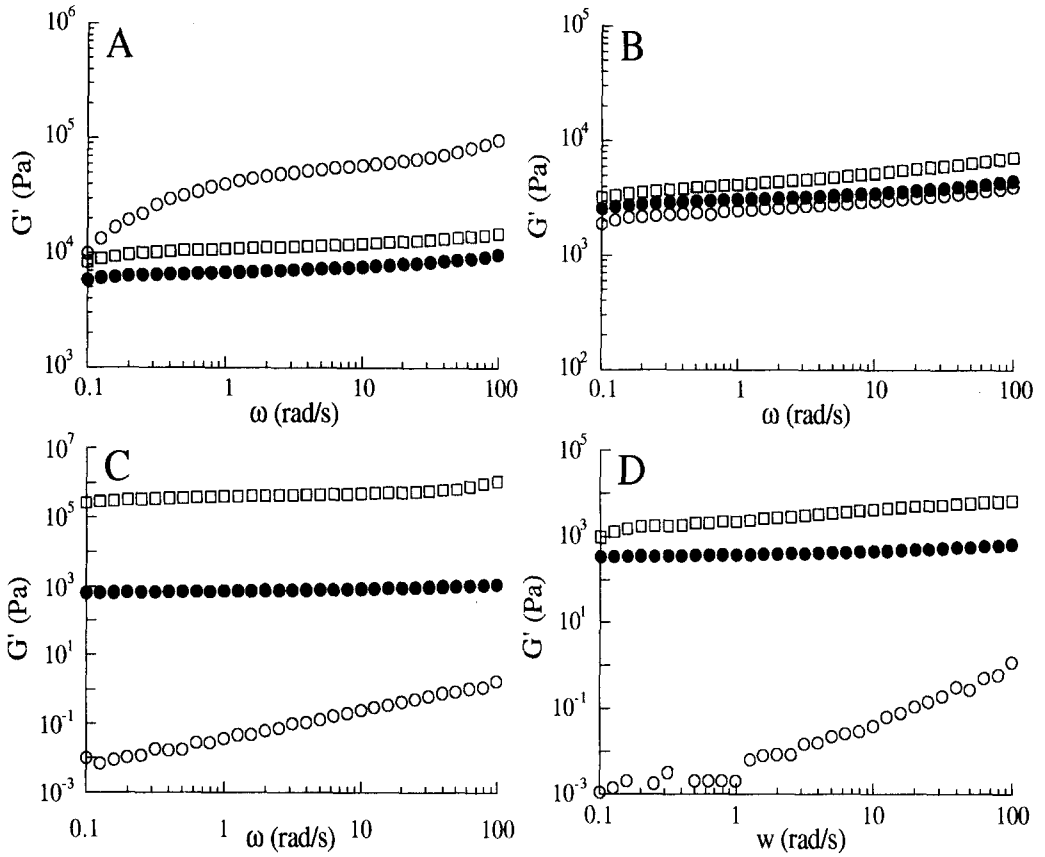

Fig. 5. Frequency sweep tests showing the frequency dependence of the elastic modulus G'. Determinations were performed at (A) 10, (B) 20, (C) 30 and (D) 37°C. (□), 95% Monoglyceride gel; (○), 90% monoglyceride gel; (●), 90% monoglyceride gel + tetracycline.

(b) Both elastic modulus and z coefficient decrease as temperature increases (see Figs. 5 and 6).

(c) The presence of tetracycline causes an increase of the coordination coefficient, indicating that, in the case of monoglycerides gels also, tetracycline leads to the formation of a more 'structured' gel.

3.3. *In vitro tetracycline release kinetics*

in vitro release profiles give important information on the efficiency of a delivery system proposed for controlled release of drugs. The choice of an appropriate in vitro model has to take into account the need to resemble as strictly as possible the 'in vivo' behaviour. In this way, the bioavailability parameters may be reliably predicted from the in vitro studies. Among the different experimental protocols proposed for the determinations of drug release profiles, we utilized the dialysis method (Nastruzzi et al., 1994) for its simple experimental procedure and high degree of reproducibility.

It should be pointed out that the dialysis method was criticized (Washington, 1990) because of its low in vivo predictivity in the case of intravenously or orally administered delivery systems, where biological sink conditions are predominant. Nevertheless, in our opinion, dialysis technique could reproduce the situation of a formulation applied into the periodontal pocket; in this case, the carrier is presumably surrounded by a stagnant layer, causing a slow diffusion of the drug (i.e. non-sink conditions).

Fig. 7 shows the release profiles of tetracycline from poloxamer and monoglycerides gels, determined by using the dialysis method at 37°C. In the case of monoglycerides gel, tetracycline release is slower than from poloxamer gel. After 7 h, the released tetracycline was equal to 18% and 65% of the entrapped drug for monoglycerides and poloxamer gels, respectively. In this view, monoglycerides gel appears to be more suitable for obtaining long-term release kinetic, assuring a constant and prolonged concentration at the application site.

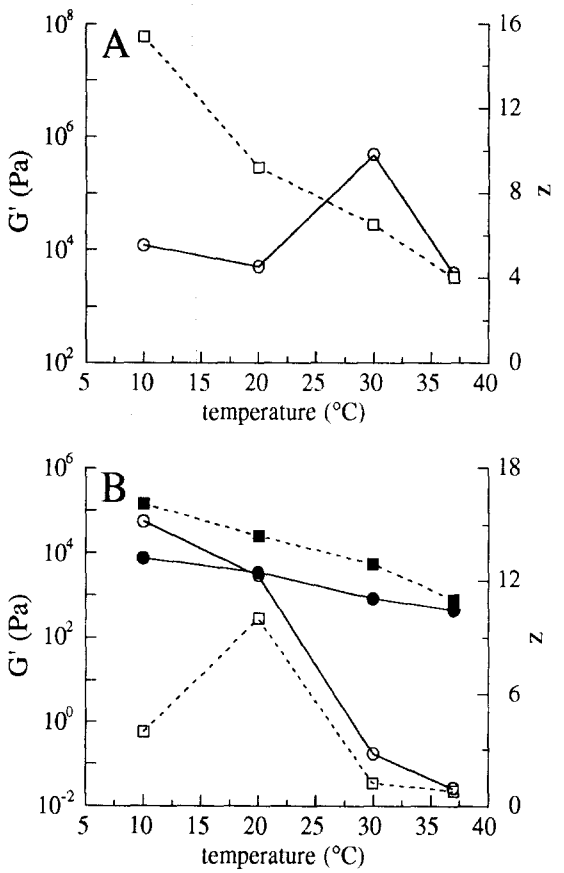

Fig. 6. Temperature dependence of the elastic modulus G' (○——○, ●——●) and z coefficient (□- - -□, ■- - -■) for (A) 95% and (B) 90% monoglyceride gels. In the case of B, both empty (open symbols) and tetracycline-containing (solid symbols) gels were analyzed.

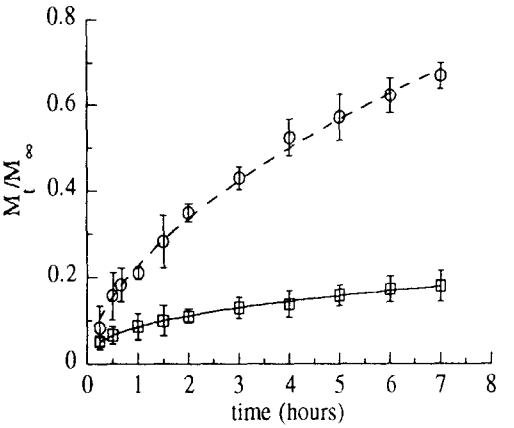

Fig. 7. Tetracycline release profiles from poloxamer (- - -) and monoglyceride (——) based gels. Kinetics were determined by equilibrium dialysis, as reported in Section 2. The reported values represent the average of five independent experiments, bars = S.D..

3.4. Tetracycline release data analysis

In order to investigate the mechanism of drug release from controlled delivery formulations, the values of the kinetic parameters n, K and R from Eq. (1) were calculated (see Table 4). The results of this analysis indicated both for poloxamer and monoglycerides gels an anomalous (non-Fickian) release process (see n-values in Table 4). Nevertheless, in the case of poloxamer, the n-value approaches 0.5, suggesting a tetracycline release that approximates a Fickian diffusional release. On the contrary, in the case of monoglycerides gel, an value of 0.378 indicates a situation where drug release occurs by means of diffusion, partially through a swollen matrix and partially through water-filled spaces (Peppas, 1985).

Table 4
Values of the kinetic release parameters for poloxamer and monoglyceride gels

Gel	k	n	R
Poloxamer	0.228	0.564	0.997
Monoglyceride	0.085	0.378	0.998

Parameters k, n and R were calculated from Eq. (1), see Section 2.

3.5. Gel persistence after in vivo application

In order to evaluate the persistence of tetracycline-containing gels after in vivo application, the following experiment was performed. Poloxamer- and monoglyceride-based gels containing the toluidine blue dye were simultaneously applied on the lower gum by using a specifically designed plastic applicator (applied area 1.5 cm^2). After different lengths of time, photographs were taken and the area stained by blue dye was evidenced and quantitated by a computerized scanning analysis of the digitalized images. The results reported in Fig. 8 clearly indicate that the persistence of monoglyceride gel is more prolonged than that of poloxamer gel. In fact, in the case of monoglyceride gel, after 8 h, the stained region is still 80% of the initial (time 0) stained area, whilst for poloxamer gel, a complete disappearance of the staining occurs after 1 h.

3.6. Clinical evaluation

A total of 24 sites in nine patients were treated: 12 sites received the tetracycline poloxamer gel (group A) and 12 sites received the tetracycline monoglyceride gel (group B). Safety side-effects of the gels were monitored and recorded. At the end of the 4-week observation interval, all the sites healed uneventfully. Neither complications nor allergic reactions which could be related to the experimental treatment modalities were observed. Both gel preparations appeared to be safe and easy in clinical handling. They were fluid enough to allow subgingival placement using a simple syringe, requiring only a few seconds to completely fill the periodontal pocket.

Table 5 shows the mean values for PD, CAL, RD and BoP scores measured at baseline and at week 4 following treatment. PD and CAL were significantly improved after treatment with both gel formulations, while no significant changes in RD were observed. Mean PD reduction was 2.3 and 1.7 mm for poloxamer and monoglycerides gel, respectively. This reduction paralleled a mean CAL gain ranging from 1.2 to 2.3 mm. Subjects treated with tetracycline poloxamer gel showed a trend towards greater PD reduction and CAL gain compared with those treated with the tetracycline monoglyceride gel. However, these differences did not reach statistical significance.

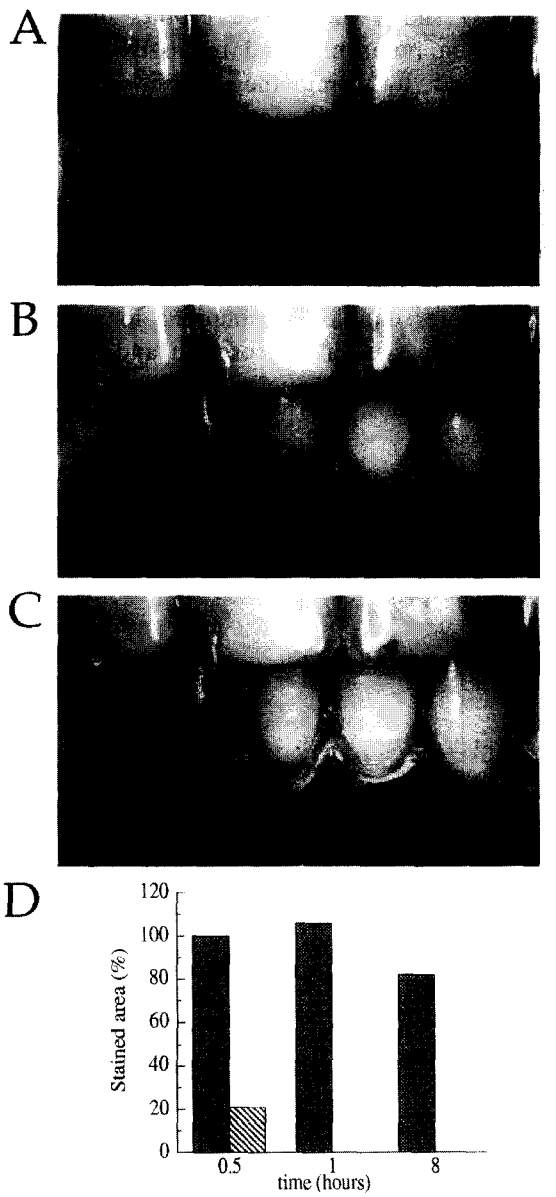

Fig. 8. In vivo evaluation of poloxamer and monoglyceride gels bioadhesivity. A–C: Photographs taken (A) 0.5, (B) 1 and (C) 8 h after the application. Monoglyceride (left) and poloxamer (right) gels were applied on the lower gum. D: Densitometric analysis of the area stained by gels. Results are reported as percentage of the stained area at time 0. (■), Monoglyceride gel; (□), poloxamer gel.

Table 5
Comparative analysis of the clinical performances of poloxamer and monoglyceride gels

Parameter	Baseline mean (S.D.)	Week 4	
		Mean (S.D.)	Change (S.D.)
Probing depth (mm)			
Group A[a]	6.2 (0.9)	3.9 (1.1)	2.3 (1.1)*
Group B[b]	5.7 (0.9)	4.0 (0.8)	1.7 (0.7)*
Clinical attachment level (mm)			
Group A[a]	6.5 (1.3)	4.2 (1.2)	2.3 (1.1)*
Group B[b]	6.3 (1.3)	5.1 (1.6)	1.2 (1.1)*
Recession depth (mm)			
Group A[a]	0.3 (0.8)	0.3 (0.7)	0.0 (0.2)
Group B[b]	0.6 (0.8)	1.0 (1.3)	−0.4 (0.8)
Bleeding on probing (%)			
Group A[a]	75	17*	
Group B[b]	50	0*	

[a]Sites treated with poloxamer-based gel.
[b]Sites treated with monoglyceride-based gel.
*Statistically significant difference from corresponding baseline ($P<0.05$, Wilcoxon signed rank test).

In the pathogenesis of periodontal disease, toxic effects of subgingival bacteria and destructive effects of the host inflammatory response lead to the loss of connective attachment from the root surface and to the apical migration of the junctional epithelium, thus resulting in the formation of a periodontal pocket.

Lack of attachment is demonstrated by insertion of a periodontal probe and changes in the attachment level and pocket depth were monitored to assess disease progression.

Pocket depth reduction and clinical attachment gain, in association with clearance of subgingival infection, are considered a priority in the treatment of periodontal disease, in order to slow down or arrest the progression and to prevent the recurrence of disease.

Bleeding on probing is a clinical parameter generally used to facilitate diagnosis of periodontal disease progression. Previous studies have revealed that the absence of BoP represents a clinical sign of rather high negative predictive value, i.e. non-bleeding periodontal sites may indicate periodontal stability (Lang et al., 1990). In our study, BoP frequencies before treatment were 75% and 50% for group A and group B, respectively. At the end of the 4-week period of observation, BoP was reduced to 17% and 0%. The reductions in BoP were statistically significant within both treatments (see Table 5). However, the difference in change of BoP sites during the observation interval was not significant between treatments.

Recently, a biodegradable gel (Elyzol® Dental Gel, Dumex, Copenhagen, Denmark), based on a mixture of glycerilmono-oleate and triglyceride (sesame oil), has been developed to locally deliver metronidazole 25% (w/w). Previous studies have evaluated this gel formulation in the treatment of adult periodontitis. Pedrazzoli et al. (1992) showed that the topical application of metronidazole 25% gel was effective in reducing PD and BoP in pockets 5 mm or more deep. At the end of the follow-up period, the mean PD reduction from baseline was 1.14 mm and the percentages of sites bleeding on probing were reduced from 35 to 19%. In a multicentre study (Ainamo et al., 1992), subgingival application of metronidazole 25% gel in periodontal pockets with PD of 5 mm or more resulted in significant improvement of clinical parameters. Mean PD reduction was 1.3 mm and the number of bleeding sites was reduced from 88 to 56%.

These results are consistent with those reported in the present study, where a different antimicrobial agent, although vehiculated by a similar gel formulation (monoglycerides-based gel) was used. In the above mentioned studies, however, no mechanical treatment was performed before gel application. It is possible that the additional ultrasonic scaling may have minimized plaque retaining factors which cofluld lead to the bacterial recolonization of the periodontal pocket. Whether and to what extent the observed changes in parameters of healing outcome are due to combined mechanical/antibiotic treatment or to tetracycline treatment alone needs further investigation.

4. Concluding remarks

In conclusion, the rheological and pharmaceutical characterization of both poloxamer and monoglycerides-based gels has demonstrated that they possess appropriate properties as intrapocket tetracycline delivery system for periodontal therapy. Both systems are characterized by a peculiar rheological behaviour, as a function of polymer concentration, temperature and presence of tetracycline.

In addition the results of the short-term split-mouth clinical trial have indicated that the subgingival application of both poloxamer and monoglyceride tetracycline gels, in conjunction with ultrasonic scaling, produced clinically and statistically significant improvement outcome in moderate to deep periodontal pockets. A long-term study is at present in progress and will be the subject of a further paper.

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