

PERIODONTAL CHITOSAN-GELS DESIGNED FOR IMPROVED LOCAL INTRA-POCKET DRUG DELIVERY

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Abstract

This study proposed a drug delivery system based on a series of formulations with chitosan gels, designed for local, intra-pocket treatment of periodontal disease, containing two drugs (an antibiotic and a chemiotherapeutic-antimicrobial agent: tetracycline hydrochloride (T) and metronidazole benzoate (M), respectively). The formulations varied from the chitosan concentration (3-4%), and the drug loading (1,2 and 3%) point of view. The objectives of the study were to outline the rheological profiles of these formulations and also to evaluate the drug release. All formulations exhibited pseudoplastic and thixotropic behaviour. The drug kinetic profiles and the release mechanisms according to Peppas equation were set. Based on the experimental data, an optimum concentration of chitosan in gel (3%) for useful modulation of drug loading, as a success factor in local therapy of periodontitis, was proposed.

Rezumat

Acest studiu propune un sistem de cedare a substanței medicamentoase, bazat pe o serie de formulări cu geluri de chitosan, proiectate în scopul cedării locale, în sacul periodontal, conținând două substanțe active: un antibiotic – clorhidrat de tetraciclină și un agent chimioterapic antimicrobian – benzoat de metronidazol. Formulările variază din punct de vedere al concentrației de chitosan (3% și 4%) și al încărcării cu substanța medicamentoasă (1, 2 și 3%). Obiectivele studiului au fost stabilirea profilelor reologice ale acestor formulări și evaluarea cedării substanței medicamentoase. Toate formulările prezintă comportare pseudoplastică și tixotropă. Profilele și mecanismele de cedare ale substanței medicamentoase s-au stabilit în conformitate cu modelul Peppas. S-a propus o concentrație optimă de 3% chitosan, care să permită modularea dozei de substanță medicamentoasă, ca factor de succes în cazul terapiei locale a parodontopatiilor.

Keywords: chitosan-gels, tetracycline, metronidazole, local therapy periodontitis.

Introduction

Periodontal diseases describe an ensemble of pathological conditions (degeneration and inflammation) of the gums and bone surrounding and supporting the teeth. Nowadays periodontitis is considered

one of the world's most frequent chronic dental disease [1,2]. This collective term encompasses gingivitis and periodontitis caused by a plaque-associated bacteria infections (*Bacteriodes spp.*, *Actinobacillus*, *Porphyromonas gingivalis*) accumulated in the periodontal pocket [1, 3].

For the management of periodontal disease, an optimal local drug delivery system opposite to systemic administration of antibiotics is needed (fewer adverse effects, less chance of developing bacterial resistance and better compliance) [3,4]. Recent pathways focused on the use of controlled release intra-pocket devices and systems as fibers, strips, films, gels and semisolids with micro- or nanoparticles, vesicular systems, etc. [1,4]. The local administration of drugs in periodontology is considered to be more efficient, since the pathogen-specific drug can be placed directly in the periodontal pocket achieving effective concentrations [2].

The use of intra-pocket injectable gels with biodegradable polymers represents a valuable choice for the local antibiotic therapy in periodontal disease [5-8]. Chitosan is a widely used non-toxic, stable, sterilizable and biocompatible polymer, and it displays an unique and attractive profile with its own antimicrobial, immunostimulatory, haemostatic and wound-healing properties [7,9-16]. Chitosan is a mucoadhesive natural polysaccharide formed through N-deacylation of chitin obtained from shrimp or crab shells [5,11]. Chitosan-based hydrogels have been largely described in literature as drug vehicle [9-16] and its characteristics could be considered as a potentially beneficial approach in the formulation of periodontal systems for local, intra-pocket delivery of antibiotics for periodontitis [1-3].

The aim of this work was to formulate some periodontal chitosan-based gels, outline their rheological profile and evaluate the drug delivery of two antibiotics widely used in the treatment of periodontal diseases: tetracycline and metronidazole.

Materials and methods

Chemicals

Chitosan from shrimp shells (with >75% deacetylated degree), tetracycline hydrochloride and metronidazole benzoate were purchased from Sigma Aldrich Chemie GmbH. All other chemicals used in this study were of analytical grade.

Preparation of chitosan gels

The chitosan gels (3% and 4% w/w) were prepared by continuous manual mixing of chitosan with an adequate amount of citric acid 0.33M. Chitosan (C) is soluble at pH<7. Tetracycline hydrochloride (T) (1% and 3%, respectively), as well as metronidazole benzoate (M) (1% and 2%,

respectively) were dissolved in citric acid 0.33M, and those were manually incorporated to the chitosan-gels. Homogeneous, translucent hydrogels of chitosan were obtained. All formulations were stored at 4°C for 24h prior to all the analyses. Depending on chitosan / antibiotic compositions, the hydrogels prepared were coded as follows: G1-T to G4-T for chitosan gels with tetracycline (T) and G1-M to G4-M for chitosan gels with metronidazole (M) as shown in table I.

Table I

Experimental plan regarding the periodontal chitosan-gels composition

Periodontal chitosan-gel	Chitosan (C % w/w)	Drug loaded (% w/w)
G1-T	3	1
G2-T	3	3
G3-T	4	1
G4-T	4	3
G1-M	3	1
G2-M	3	2
G3-M	4	1
G4-M	4	2

Evaluation of in vitro drug release

The *in vitro* tetracycline hydrochloride (T) and metronidazole benzoate (M) release kinetics from chitosan gels were evaluated using a “sandwich” device fitted to a dissolution apparatus in conjunction with paddle stirrers. The operational steps are described in our previous works [17]. Briefly, the phosphate buffer pH=6.8 (PB) was used as release medium and maintained at 37°±0.5C. At certain time periods aliquots (5mL) were withdrawn from the release medium and replaced by the same volume of fresh pre-heated phosphate buffer solution. The amount of tetracycline hydrochloride (T) and metronidazole benzoate (M) released were spectrophotometrically assayed at 361 nm and 320 nm respectively, using the calibration curves ($A_{1\text{cm}}^{1\%}=337$ for tetracycline hydrochloride, and $A_{1\text{cm}}^{1\%}=506$ for metronidazole benzoate, both evaluated in PB, pH 6.8).

Three replicate measurements were performed for each designed formulation.

Rheoviscosimetry analyses

Rheological profiles for the chitosan-gels formulations were performed with a rotational viscometer Multi Visc–Rheometer Fungilab with standard spindle TR9. The operational conditions were described in our previous studies [18-19]. Samples were allowed to equilibrate for 30

minutes prior to analysis. Rheograms were produced under controlled stress by gradual increasing of the shear rate from 0.3 to 60 rpm, and decreasing it in the same manner.

The gels flow ability were determined at 23°C and 37°C, respectively (the temperatures corresponding to storage and kinetic/local delivery conditions), using a ThermoHaake P5 Ultrathermostat.

In all cases three replicate measurements were performed.

The rheological and kinetic parameters were evaluated using Table Curve 2D v5.01 software.

Results and Discussion

The *in vitro* release of tetracycline hydrochloride expressed as the cumulative percent of drug released as function of time from the designed formulations of periodontal gels, is presented in figure 1.

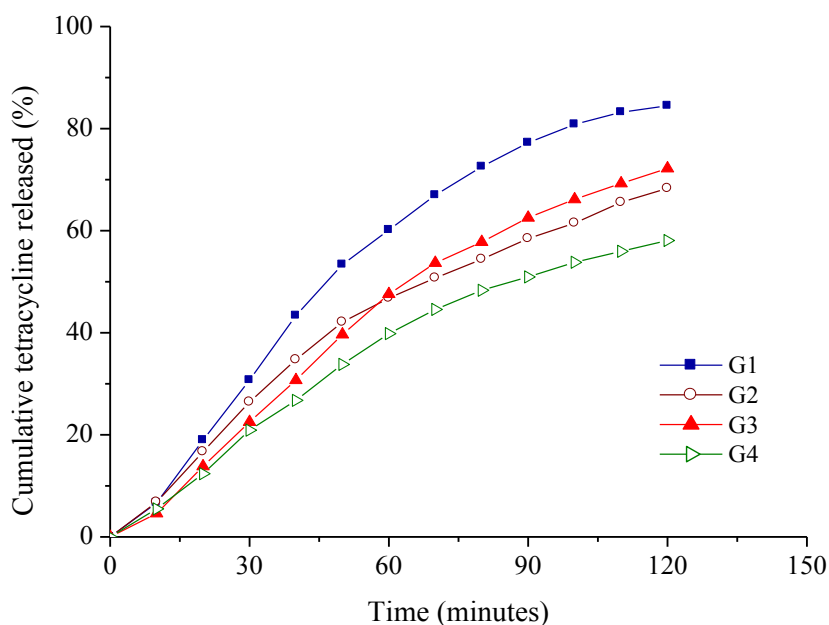
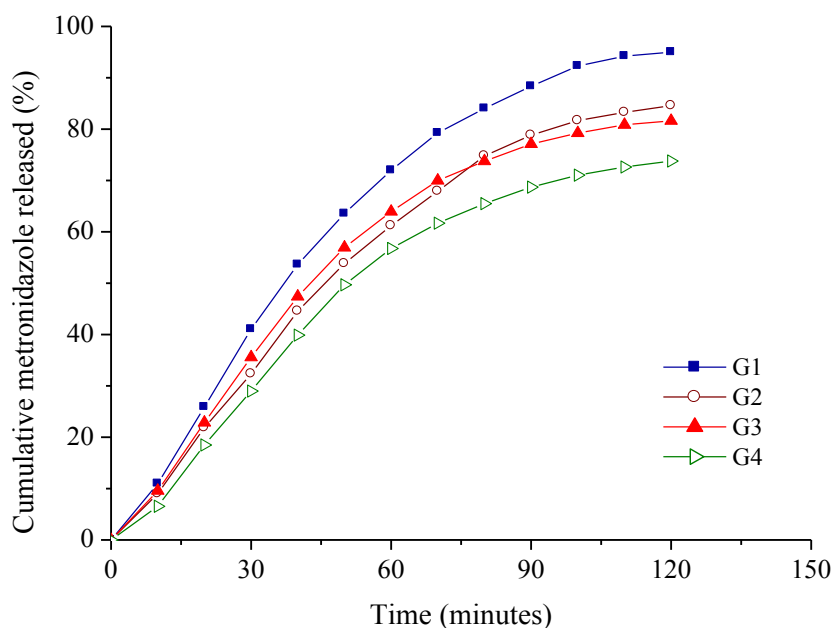


Figure 1

Cumulative release of tetracycline hydrochloride from periodontal chitosan gels

The kinetic profiles plotted as drug cumulative released percent *versus* time, for metronidazole benzoate are illustrated in figure 2.

**Figure 2**

Cumulative release of metronidazole benzoate from periodontal chitosan gels

The cumulative released percentages of tetracycline and metronidazole from chitosan gels are given in table II.

Table II

The percentage of drug released from chitosan gels; the correlation coefficients values and the kinetic parameters specific to Peppas model

Periodontal chitosan gel	Percent released (%)	Kinetic constant ($1/\text{min}^n$)	Release exponent (n)	Determination coefficient (R)
G1-T	84.49	0.029	0.721	0.9869
G2-T	68.30	0.025	0.696	0.9928
G3-T	72.21	0.015	0.813	0.9916
G4-T	58.07	0.017	0.747	0.9913
G1-M	95.01	0.048	0.641	0.9861
G2-M	84.59	0.034	0.687	0.9879
G3-M	81.62	0.045	0.622	0.9823
G4-M	73.79	0.032	0.669	0.9819

As we can see, the increase of chitosan concentration decreased the percent of drug released. Thus, for the same tetracycline concentration (T-1% w/w) the decay is 14.53% and for 3% (w/w) the decay is 14.97%. During the same time period of 120 minutes for metronidazole benzoate the decay is 14.09% (M-1%), and 12.76% (M-3%) respectively.

A larger amount of drug loaded does not necessarily favor a higher percentage of drug released.

The experimental drug release data were fitted according with Peppas equation [20], as follows:

$$\frac{m_i}{m_t} = k \cdot t^n \quad (\text{eq. 1})$$

where, m_i/m_t is the fraction of drug released at time t , k is the release constant, n is the release exponent, as measure of primary mechanism of drug release.

In all cases the correlation coefficient values for Peppas model (R) are larger than 0.9819, being superior to other verified models (such as Higuchi, zero-order, first-order).

In table II the parameters characteristic for Peppas model both for tetracycline and metronidazole chitosan gels are presented.

The release exponent for each chitosan gel formulation ranged between 0.5 and 1.0. We can assume that the mechanism of drug release, for both tetracycline and metronidazole is a non-Fickian diffusion, the polymer relaxation rate being approximately equal to drug diffusion rate though polymer network [21-22].

Further, the flow rheometry analyses for all periodontal gels were applied. For each chitosan gel formulation the rheogram viscosity ($\text{Pa} \cdot \text{s}$) as a function of shear rate (s^{-1}) was built at 23°C and 37°C (Fig. 3-4, a and b).

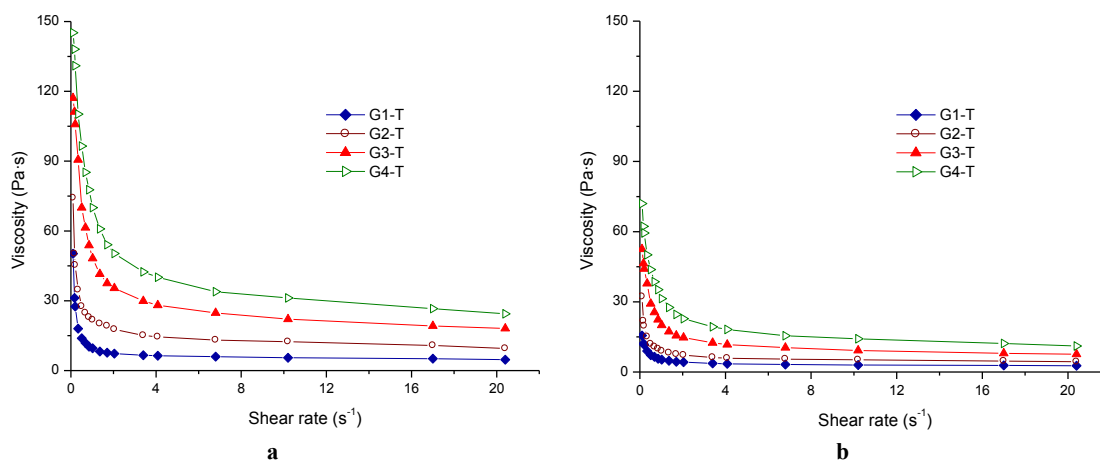


Figure 3

Plots of viscosity as a function of shear rate for the chitosan-tetracycline periodontal gels analyzed at 23°C (a) and 37°C (b).

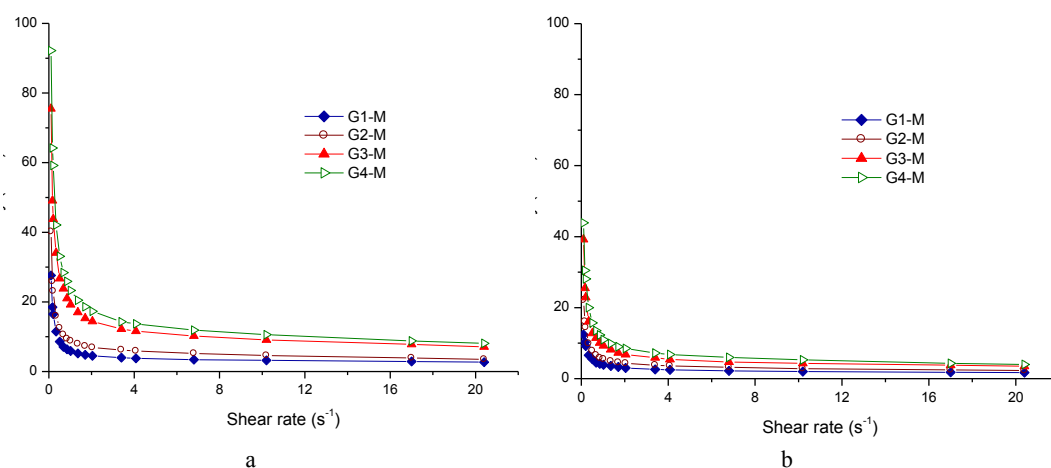


Figure 4

Plots of viscosity as a function of shear rate for the chitosan-metronidazole periodontal gels analyzed at 23°C (a) and 37°C (b).

All the designed periodontal gels present a non-newtonian pseudoplastic behaviour at both temperatures, the viscosity (η) decreasing for shear rate ($\dot{\gamma}$) increase. This variation pattern facilitates the formulations flow and syringeability capacity, important characteristics for local, intra-pocket administration [3].

In order to enable the comparison of the rheological properties for gel formulations, the equation applied was:

$$\eta = m \cdot \dot{\gamma}^{-n} \quad (\text{eq. 2})$$

where, m (viscosity index) and n (flow index) are parameters correlated with the investigated chitosan gels formulation factors and determined through the linearization of eq. (2) by double logarithmic method. It is assumed that m value matches to the viscosity obtained for the shear rate of 1 s⁻¹ [19].

The experimental values for the rheological parameters in the above mentioned flow model and for the correlation coefficient R are given in table III.

Analysing the results presented in table III, we can assume that the higher values of viscosity index were recorded when chitosan concentration increases. At the same concentration of chitosan, a significant increase of this rheological parameters was noticed for the tetracycline periodontal gels (more evident at 3% C, the increase was approximately of 2.32 times). This antimicrobial agent could have a supplementary cross-linking effect, which

is not so obvious to the metronidazole periodontal gels (the increase was of only 1.46 times).

Table III

The values for flow model parameters and the correlation coefficients specific to rheological model applied to periodontal chitosan gels

Periodontal chitosan-gel	Temperature					
	23°C			37°C		
	m	n	R	m	n	R
G1-T	9.981	0.671	0.9810	5.859	0.411	0.9902
G2-T	23.176	0.460	0.9794	9.774	0.477	0.9810
G3-T	52.163	0.393	0.9870	21.719	0.409	0.9923
G4-T	71.007	0.349	0.9909	32.250	0.365	0.9965
G1-M	6.369	0.614	0.9871	4.275	0.461	0.9913
G2-M	9.311	0.603	0.9843	5.984	0.551	0.9891
G3-M	20.047	0.540	0.9882	9.543	0.582	0.9886
G4-M	24.622	0.554	0.9940	11.852	0.547	0.9925

The higher values for the viscosity index at 23°C in comparison with 37°C for the all designed hydrogels are due to the destructure induced by the temperature, noticing an increase of about 1.70-2.40 times for tetracycline-chitosan gels and 1.48-2.10 times for metronidazole-chitosan ones.

We can also observe a thixotropic character for all investigated periodontal gels (exemplified in figures 5 and 6).

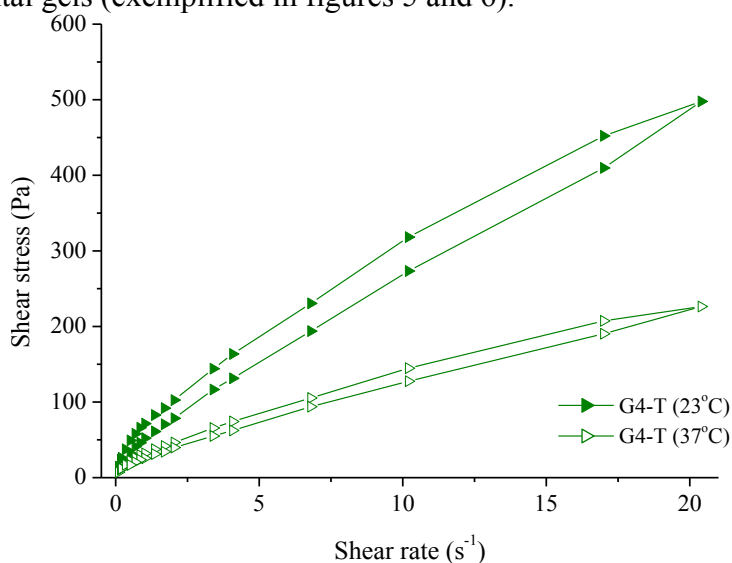
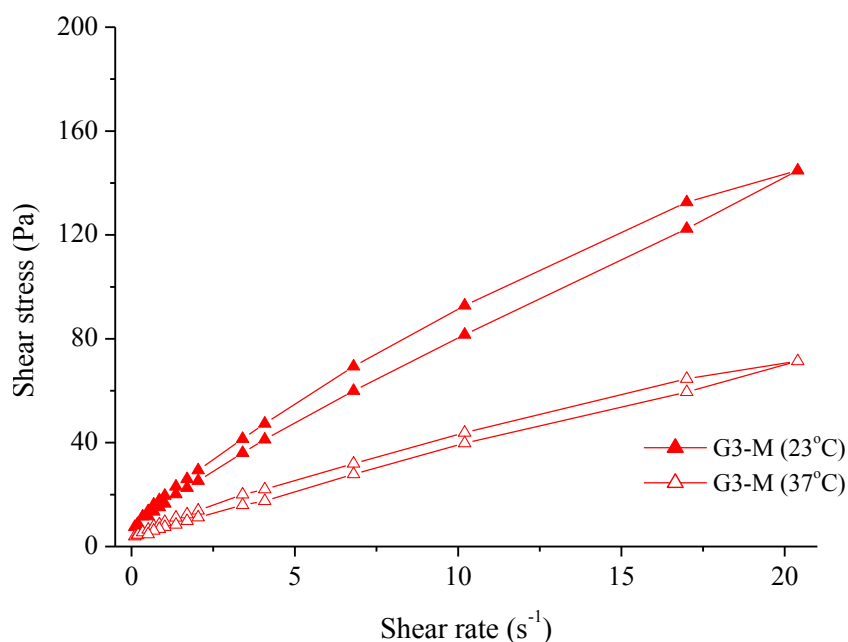


Figure 5

Flow curves (up and down) for hydrogel G4-T tested at 23°C and 37°C

**Figure 6**

Flow curves (up and down) for hydrogel G3-M tested at 23°C and 37°C

Rheological profiles for the same polymer concentration are consistent with kinetic patterns.

Conclusion

The chitosan gels proposed in this study have particular characteristics making them an adequate system for local, intra-pocket drug delivery. After administration with a syringe, the gel formulations are exposed to non-deformable stresses, thus the rheological profile and properties are key aspects in their clinical performance. Following administration, it is desirable to retain the gel within the pocket and, moreover, to present controlled release of antibiotic/antimicrobial agent into the crevicular fluid. The increase of chitosan concentration sequentially decreased the drug release.

Based on the kinetic release data and on the rheological profiles for periodontal gels, we can assume that a chitosan concentration of 3% w/w could offer a base for an optimum modulation in drug dose, and make them efficient in periodontal disease local strategy of treatment.

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Manuscript received: December 17th 2011