Formulation and Release of Doxycycline HCL from an Ion Activated *In Situ* Gelling Delivery System for the Treatment of Periodontal Disease

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Received 27 February 2009; accepted 29 July 2009
DOI 10.1002/app.31204
Published online 10 September 2009 in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: This study reports on the formulation of doxycycline HCL in an ion activated *in situ* gelling delivery system and its implications for the treatment of periodontal disease. The system is based on the use of alginate as the gelling agent and hydroxypropyl methylcellulose (HPMC) as a viscosity enhancing agent. The ion activated gelling mechanism in this system is based on the concept of interaction with the divalent calcium ions present in the gingival crevicular fluid (GCF). The gelling capacity of the prepared formulations was assessed visually and by investigating their rheological behavior upon mixing with human blood serum since it has the same composition as the GCF. The rheological behavior of all formulations was not affected by incorporation of the drug. *In vitro* release studies showed that the alginate/HPMC mixture, upon gelling after mixing with serum, can sustain the release of doxycycline HCL for an extended period of time which was more than 12 days. These results indicated that this system can be used as an *in situ* gelling local delivery system for the treatment of periodontal disease.
 J Appl Polym Sci 115: 811–816, 2010

Key words: drug delivery systems; gelation; viscosity

INTRODUCTION

Gingivitis and periodontitis are two forms of infectious diseases caused by several pathogenic anaerobic microflora of the oral cavity. These diseases are known to affect the teeth supporting structures. Accumulation and invasion of the surrounding tissues by dental plaque at the gingival margin induces inflammatory response leading to the formation of pockets between gingiva and tooth. The retraction of the gingival margin will create an ideal environment for growth of anaerobic bacteria responsible for the disease. Consequently, this will lead to loss of connective tissue attachment and destruction of alveolar bone resulting in periodontal pocket formation and eventually tooth loss.[1–3]

A number of antimicrobial agents administered orally have been used for the treatment of periodontitis. They have been studied both as adjunctive therapies with scaling and root planing and as stand-alone chemotherapies.[4–6] However, they have little long term efficacy in the treatment of periodontitis in addition to side effects and development of bacterial resistance due to systemic administration of these agents.[7,8]

Therefore, local delivery of antimicrobial agents has been investigated as a possible approach for treatment of periodontal disease.[9] Furthermore, patients' compliance can be enhanced and systemic complications of drug administration can be avoided.[10] Treatment of periodontits requires a high concentration of antimicrobials to be available in the periodontal pocket as an adjunctive therapy with scaling and root planing.[5,11] Using a sustained local antimicrobial delivery system will be more beneficial regarding the ease of application and selectively targeting a limited number of diseased sites that were unresponsive to conventional therapy.[9,12]

Many researchers have demonstrated that sustained delivery of antimicrobial agents can be effective in reducing signs of periodontitis. Antimicrobial agents studied were tetracycline,[13,14] doxycycline,[15,16] amoxicillin,[17] metronidazole,[5,7,18] chlorhexidine,[19] and others.[20] Such agents were studied in a variety of specialized systems like fibers, films, microparticles, semisolids, and gels to maintain the antimicrobial agents in the gingival crevicular fluid (GCF) at a bactericidal concentration for the required period of therapy.[21]

Ion activated *in situ* gel-forming delivery systems have been studied mainly for ophthalmic drug

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Contract grant sponsor: Deanship of Scientific Research at Jordan University of Science and Technology; contract grant number: 62/2007.

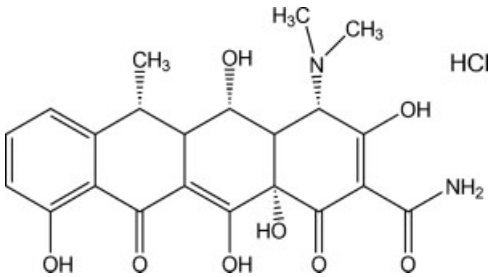

Figure 1 Chemical structure of doxycycline HCL.

delivery where they exhibit reversible phase transition (sol-gel-sol) and pseudoplastic behavior.[22,23] Such systems were aiming at gel formation upon exposure to physiological conditions, and thus increasing the pre-corneal residence time of the delivery system and improving the ocular bioavailability. Recent studies have proposed the use of alginate and hydroxypropyl methylcellulose (HPMC) as a vehicle for ion activated *in situ* ocular delivery systems.[24,25]

Doxycycline HCL has a molecular weight of 480.9 and is known to be freely soluble in water, slightly soluble in alcohol, and practically insoluble in chloroform and ether. It has no significant stability problems but has to be protected from light. Gastrointestinal disturbances are the most common adverse effects of doxycycline after oral administration but are reported to be less frequent than with tetracycline.[26] The chemical structure of doxycycline HCL is shown in Figure 1.

The main objective of our study was to utilize the alginate/HPMC ion activated *in situ* gelling system to formulate doxycycline HCL, a commonly used broad spectrum antibiotic for treatment of periodontitis. In our study, this idea has been extended and applied as a novel approach toward a gelling delivery system for the treatment of periodontal disease. No similar gel for periodontal drug delivery has been investigated by others. This formulation is supposed to undergo gelation upon injection in solution form in the periodontal pocket by taking advantage of the presence of the divalent calcium ions in the GCF and provide sustained release of the drug over an extended period of time. Alginate and HPMC were investigated as a vehicle in this formulation which undergoes sol-gel phase transition upon exposure to human serum which is exactly similar to the GCF in its composition, mainly the presence of the divalent calcium ions. The proposed formulation was evaluated *in vitro* by studying its rheological properties and the release kinetics of doxycycline HCL from the formulation as well as the effect of formulation variables on such parameters.

MATERIALS AND METHODS

Materials

Doxycycline HCL was a generous gift from the Jordanian Pharmaceutical Manufacturing Co. (JPM), Amman-Jordan. Sodium alginate (composed of 65% mannuronic acid (M) and 35% guluronic acid (G)) was purchased from Acros Organics, Belgium. HPMC (Methocel E15 LV and E50 LV) was purchased from Colorcon, UK. Human serum with calcium concentration of 2.3 mmol/L (normal range is 2.0–2.6 mmol/L) was obtained from the blood bank at King Abdullah University Hospital, Irbid-Jordan. All other reagents were of analytical grade and used as received.

Preparation of formulations

Different aqueous solutions containing different concentrations of alginate and HPMC of different grades (Methocel E15 LV and E50 LV) were prepared (formulation codes were F1, F2,..., F9). They were evaluated for the gelling capacity to identify the suitable composition for use as *in situ* gelling system. The components of the formulations are shown in Table I.

TABLE I
Composition of the Formulations and Their Gelling Capacity

Formulation	HPMC grade	HPMC (w/v%)	Alginate (w/v%)	Gelling capacity
F1	–	0	1	+
F2	–	0	2	++
F3	–	0	3	+++
F4	E15 LV	6	1	+
F5	E15 LV	8	1	+
F6	E15 LV	12	1	+
F7	E50 LV	1	1	+
F8	E50 LV	2	1	++
F9	E50 LV	3	1	+++

+, gelling within minutes, dissolving after few hours; ++, immediate gelling, dissolving after two weeks; +++, immediate gelling, dissolving after more than 2 weeks.

The alginate solutions were prepared by dispersing the required amount of alginate in 75 mL of distilled de-ionized water slowly with continuous stirring to prevent clumping and until completely dissolved. The alginate/HPMC solutions were prepared by dissolving the required amount of HPMC in the desired concentration of alginate solution slowly with continuous stirring until completely dissolved. Doxycycline HCL was dissolved in distilled de-ionized water and the pH was raised from 3 to 6.8 using sodium hydroxide solution. This is to avoid precipitation of alginate upon addition of doxycycline HCL solution since it is insoluble in solutions of pH lower than 3. Finally, the drug solution was added to the alginate or alginate/HPMC solution under constant stirring until uniform and clear solution was obtained. The final volume was completed with distilled de-ionized water to have a 100 mL solution. The concentration of doxycyline HCL in the final preparations was 40 mg %.

Visual assessment of gelling

The gelling capacity was determined by placing 2 mL of the prepared formulation in a small beaker containing 2 mL of human serum and equilibrated at 37°C. The gelling was assessed visually by noting the time for gelation and the time taken for the formed gel to dissolve.

Rheological studies

Viscosity determination was performed using a rotational programmable viscometer (Brookfield DV-II+Pro, Brookfield Engineering Laboratories) on 15 mL aliquot of the sample. Viscosity experiments were divided into two parts. The first part was performed to study the viscosity before gelling has taken place in the absence of drug and serum. Four formulations were studied in this part and they were F1, F2, F8, and 2% HPMC E50 LV (formulation H). These four formulations were chosen to study the effect of increasing the alginate concentration on the gelling process (F1 and F2) and to study the effect of the presence of HPMC (F8) and to confirm that HPMC has no effect on the gelling process by studying formulation H.

The second part of the viscosity experiments was performed on the same formulations but with 40 mg of doxycycline HCL and serum to confirm that the sol-gel phase transition does occur for the formulations. Viscosity measurements were conducted at different speeds at certain time intervals. From 5 to 100 rpm at controlled ramp speed for 10 s for each speed. The same run was then conducted from 100 rpm to 5 rpm. The temperature was maintained at 37°C. The average of the two readings from the increasing and decreasing ramp speeds was taken as to be the viscosity reading since two slightly different viscosity readings could be measured at the same speed. Viscosity evaluations were performed in triplicate.

Drug release studies

The *in vitro* release of doxycycline HCL from the formulations (drug concentration in the formulations was 40 mg%) was performed using the dialysis method according to a previously published procedure.[14] Dialysis bags of 12 KD molecular weight cut-off were used to contain 8 mL of the gel and securely tied from both sides and placed in a vessel containing 100 mL of phosphate buffer (pH 7.4). The medium was stirred at a rotation speed of 100 rpm and the temperature was maintained at 37 ± 0.5°C. Aliquots, each 5 mL in volume, were withdrawn through a 0.45 μm membrane syringe filter at specified time intervals and replaced by an equal volume of the dissolution medium. Samples were analyzed for doxycycline HCL using UV spectrophotometry at 270 nm. The duration of the study was maintained until the drug release reached more than 90%.

RESULTS AND DISCUSSION

Visual assessment of gelling

The gelling capacity was assessed visually at 37°C by adding human serum to the alginate system in a ratio of 1 : 1. This ratio was determined based on preliminary trials to give the fastest gel formation. The results of gelling capacity are summarized in Table I along with the composition of each formulation. The mark (+) in Table I refers to gelling within few minutes and dissolving after few hours. The mark (++) refers to the immediate gelling and dissolving after two weeks, and the mark (+++) refers to immediate gelling and dissolving after more than two weeks. As shown in Table I, increasing the alginate concentration had an obvious effect on increasing the gelling capacity. In addition, using Methocel E50 LV is more effective in gel formation than Methocel E15 LV. This can be attributed to the higher molecular weight of Methocel E50 LV which will lead to the formation of a more viscous solution by using smaller amount of this polymer. The results also indicated the transformation of the alginate system to the gel phase upon exposure to the serum which is exactly similar in its composition to the GCF.

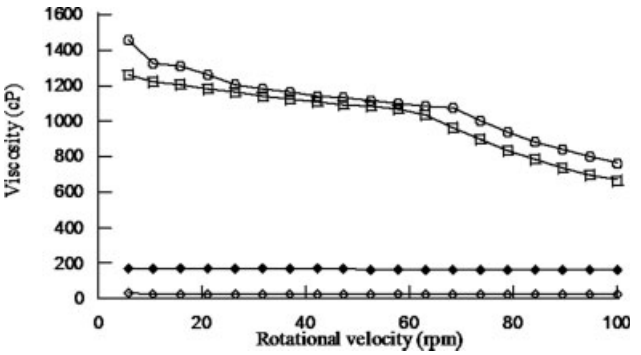

Figure 2 Rheological profiles of formulations F1, F2, F8, and 2% HPMC E50 LV without addition of doxycycline HCL and serum. ◆, formulation F1; □, formulation F2; ○, formulation F8; ◇, 2% HPMC E50 LV.

Rheological studies

Initially, viscosity experiments were performed on F1, F2, F8, and H formulations without the addition of the drug or serum. The rheological profiles of viscosity in cP versus speed in rpm for these formulations are shown in Figure 2. Pseudo-plastic rheology was exhibited by the formulations without the drug. They showed shear thinning and a decrease in viscosity with increased speed. As shearing stress or spindle speed is increased, the normally disarranged molecules begin to align their long axes in the direction of flow since alginate is a linear polymer. This orientation reduces the internal resistance of the system and allows a greater rate of shear at each successive shearing stress. The addition of doxycycline HCL did not affect the rheological properties of all formulations. The order of viscosity of all formulations was F8 > F2 > F1 ≈ H (2% Methocel E50 LV), respectively.

The second part of the viscosity experiments involves the study of the sol-gel phase transition of the alginate system in the presence of the drug and serum. Forty mg of doxycycline HCL were added to formulations F1, F2, F8, and H. Serum was added to each formulation in a volume equivalent to the volume of formulation. Figure 3 shows the resultant rheological profiles of these formulations after the addition of the drug and serum. The viscosity of the formulations was significantly increased in the presence of serum except for formulation H. These results indicated that sol-gel phase transition occurred to these formulations upon exposure to the serum. This is due to the cross-linking of the alginate by the divalent calcium ions present in the serum that replaced the monovalent sodium ions. As shown in Figure 3, the viscosity of formulation H decreased by adding the drug and serum. This might be due to the dilution of the formulation upon addition of the serum. The decrease in viscosity is an evidence that HPMC has no effect on the gelling process. It acts as a viscosity enhancer when added to the alginate system.

The rheological studies showed that formulation F8 has the best properties regarding viscosity. It is better than formulation F2 since it contains half the amount of alginate which requires less amount of calcium ions to cross-link it when it is applied to the periodontal pocket. Therefore, HPMC can enhance the viscosity of the preparation and decrease the amount of alginate required in the preparation.

Drug release studies

The same formulations used in rheological studies were further studied for the release of doxycycline HCL. These were F1, F2, F8, and H formulations. They were all compared to an aqueous solution containing the same amount of doxycycline HCL. These formulations were chosen to study the effect of increasing the alginate concentration (1% in F1 and 2% in F2) and to study the effect of the presence of HPMC where F8 contains 2%HPMC and 1% alginate. To exclude the effect of HPMC as a retardant for the release of the drug, formulation H was studied which contains 2% HPMC only without alginate.

The release profiles from all these formulations compared to aqueous solution of doxycycline HCL are shown in Figure 4. The release of the drug from formulation H and the aqueous solution was rapid and it was almost complete within few hours (about 7 h from the aqueous solution and 9 h from formulation H). As shown in Figure 4, 90% of the drug was released in 12 days from formulation F1 while about 70% was released from formulation F2 during the same time. This agrees with the amount of alginate present in each formulation; 1% in F1 and 2% in F2. Higher viscosity is expected with higher alginate content which will lead to more available sites to be cross-linked with calcium ions and low diffusion of the drug through the network of the gel, and thus,

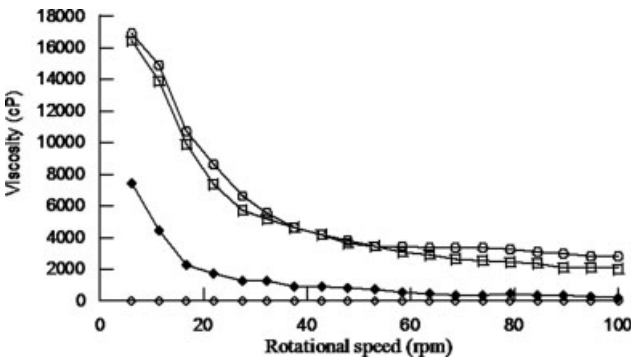

Figure 3 Rheological profiles of formulation F1, F2, F8, and 2% HPMC E50 LV after addition of 40 mg doxycycline HCL and serum. ◆, formulation F1; □, formulation F2; ○, formulation F8; ◇, 2%HPMC E50 LV.

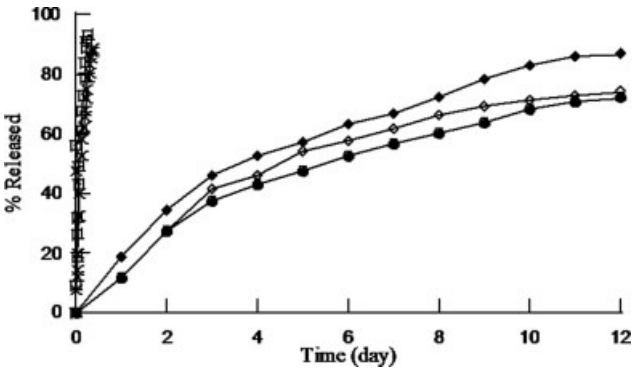

Figure 4 Release profiles of doxycycline HCL from formulations F1, F2, F8, 2% HPMC E50 LV, and control (aqueous solution of doxycycline HCL). ◆, formulation F1; ◇, formulation F2; •, formulation F8; ×, 2% HPMC E50 LV; □, control.

sustained release of the drug over an extended period of time. Formulation F8 also showed the same release profile as formulation F2 where about 70% of the drug was released over 12 days although it contains the same concentration of alginate as in formulation F1. This is due to the presence of 2% HPMC in formulation F8 which increased the viscosity so that it produces a similar release profile to formulation F2. Therefore, formulation F8 could be preferable over formulation F2 where less amount of alginate as well as less amount of calcium ions are required for formation of the gel.[27] In this formulation we were able to reduce the amount of alginate used by one half compared to F2 and that was due to the use of HPMC by 2% (w/v) in the formulation.

Sodium alginate plays a major role in the formulation as the gel forming agent by forming the gel network due to cross-linking by calcium ions. Therefore, it is expected that it will retard the release of the drug and this will be proportional to its concentration in the formulation. HPMC has been used in the formulation as a viscosity enhancing agent where in addition to sodium alginate it will increase the viscosity of the formed gel. Thus, the extent of drug release will be further reduced by adding HPMC to the formulation.

When doxycycline HCL is mixed with the alginate solution before cross-linking, it becomes immobilized within the polymer. Slower release of the drug is expected since its diffusion is limited by the pore size of the alginate gel. Shang et al.[28] showed that the immobilization of the drug by copolymerization is effective in decreasing the drug release rate from membrane based, environmental sensitive drug delivery system. Another study by Liu et al.[29] showed that the diffusion of drug molecules immobilized in cylindrical gels is affected by the density of the gel network. The alginate used in our study is composed of 65% M blocks and 35% of G blocks. A gel with low porosity is expected to be formed with this low G/M ratio, and therefore, more prolonged release of the drug is expected.

The drug release profile provides an insight into the efficiency of the proposed drug delivery system in controlling the release of the drug. To investigate the release mechanism of doxycycline HCL from the alginate gels, the release data were fitted into Korsmeyer-Peppas equation.[30] The results of the release kinetics according to this equation suggested that all the formulations followed an anomalous (non-Fickian) release pattern. This means that diffusion of the drug from the gel is not the only factor controlling its release and other mechanisms could be involved. Other mechanisms could involve gel shrinkage or erosion of the gel itself. The erosion mechanism is very complex because it depends on the degradation, swelling, dissolution or diffusion of the oligomer and monomer residues.[31] In our system, erosion could take place since the gel network starts to dissolve at later times due to slow exchange of the divalent calcium ions (cross-linker) with the monovalent sodium ions present in the gel. Furthermore, gel shrinkage could play a role in the release mechanism where water is expelled from the system with time due to the release of the water soluble drug. Similar findings were reported by McLennan et al.[32] who showed that gel shrinkage may account for the release mechanism of heparin from alginate gels. Since the alginate used in our system is of low G/M ratio, then diffusion is less likely to be the dominant mechanism in drug release due to low porosity of the gel.[33]

CONCLUSIONS

The utility of an ion-activated *in situ* gel forming system has been successfully used in this study to formulate doxycycline HCL which is a broad spectrum antibiotic used in the treatment of periodontitis. The system is based on using alginate as a gel forming agent in combination with HPMC as a viscosity enhancing agent. The formulation underwent sol-gel phase transition upon mixing with serum which has similar composition to the GCF. An extended *in vitro* release of doxycycline HCL has been maintained over a 12 day period from the gel formed. Therefore, this system is promising in sustaining the release of doxycycline HCL over an extended period of time to treat periodontitis.

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