Latin American Journal of Pharmacy
(formerly *Acta Farmacéutica Bonaerense)*
Lat. Am. J. Pharm. **27** (3): 360-3 (2008)

Original Article

Received: March 6, 2008
Accepted: April 5, 2008

Tetracycline Release from Chitosan Films

Alexandre BELLA CRUZ*, Marla SURDI, Makerli M.P. CENCI & Clóvis A. RODRIGUES

Programa de Mestrado Acadêmico em Ciências Farmacêuticas
& Núcleo de Investigações Químico-Farmacêuticas (NIQFAR),
Universidade do Vale do Itajaí (UNIVALI), 88302-202, Itajaí, SC, Brazil.

SUMMARY. A drug release system of a tetracycline loaded chitosan film with sustained antibiotic effect is described. Chitosan films (CF) containing tetracycline were prepared by casting method using acetic acid. The films were exposed to heat treatment at 90 ºC, 120 ºC or 150 ºC (HCF). The effect of heat treatment on the water resistance to the chitosan films and tetracycline release was investigated. Heat treatment increase to a greater extent the water resistance of chitosan films. Heat treatment decreases the releases of tetracycline of the HCF when compared with CF. The antibiotic activity of tetracycline in chitosan film and heat-treated chitosan film at 150 ºC was prolonged for 23 days approximately. The results indicated that the chitosan film was useful in tetracycline release such as for site-specific tetracycline controlled release in treatment of periodontal disease.

RESUMEN. "Liberación deTetraciclina de Películas de Quitosano". El trabajo describe un sistema de dispositivo de liberacíon lenta de droga de una película de quitosano cargada de tetraciclina con efecto antibiótico sostenido. Las películas de quitosano (CF) que contenían tetraciclina fueron preparadas empleando ácido acético. Las películas fueron expuestas al tratamiento de calor a 90 ºC, 120 ºC o 150 ºC (HCF). El efecto del tratamiento térmico en la resistencia al agua de las películas de quitosano y sobre la liberacíon de tetraciclina fue investigado. El tratamiento de calor aumentó a un mayor grado la resistencia de agua de las películas de quitosano. El tratamiento de calor disminuyó la liberación de la tetraciclina del HCF en comparación con CF. La actividad antibiótica de la tetraciclina de la película de quitosano y la película de quitosano sometida al tratamiento térmico a 150 ºC fue prolongada por 23 días aproximadamente. Los resultados indicaron que la película de quitosano puede ser de utilidad en la liberacíon controlada de tetraciclina en el tratamiento sitio-específico de la enfermedad periodontal.

INTRODUCTION

Chitosan, a natural polysaccharide, is being widely used as a pharmaceutical excipient. It is obtained by the partial deacetylation of chitin [1]. It is obtained from the alkaline deacetylation of chitin which is a glucose-based unbranched polysaccharide widely distributed in nature as the principal component of exoskeletons of crustaceans and insects as well as of cell walls of some bacteria and fungi. Chitosan exhibits a variety of physicochemical and biological properties resulting in numerous applications. In addition to its absence of toxicity and allergenicity, and its biocompatibility, biodegradability and bioactivity, make it a very attractive substance for diverse applications as a biomaterial in pharmaceutical and medical fields, where it has been used for systemic and local release of drugs [2].

In dentistry and oral medicine, various applications of chitosan have been proposed due to its favorable properties such as biocompatibility and biodegradability [3].

Periodontal disease (periodontitis) refers to the inflammatory processes that occur in the tissues surrounding the teeth in response to bacterial accumulations (dental plaque) on the teeth [4,5]. It is well established that periapical disease is the result of bacteria, their product, and the host response to them. Periradicular disease will occur after microorganisms and their metabolic products affect the periradicular tissue [6]. The introduction of local release antibiotics specifically for the treatment of periodontitis offers an alternative treatment of localized disease [7].

Fibers, films, chips, strips and microparticles made of biodegradable or non-biodegradable

KEY WORDS: Chitosan, Drug delivery, Release, Tetracycline.
PALABRAS CLAVE: Liberación lenta de drogas, Quitosano, Tetraciclina.

* Author to whom correspondence should be addressed: *E-mail:* bellacruz@univali.br

ISSN 0326-2383

polymers have been reported as effective methods to administer antibacterial agents for periodontal therapy [4,6].

Aim of using antibiotics as part of a treatment regimen is to achieve, within the periodontal environment, a concentration of the drug that is sufficient either to kill (bactericidal) or arrest the growth (bacteriostatic) of pathogenic microorganisms [6].

In the current study, a tetracycline loaded chitosan films were developed, and the release test was conducted *in vitro* to determine the antibiotic diffusion from the film and antimicrobial activity.

MATERIAL AND METHODS

Materials

Chitosan was obtained through basic hydrolysis of chitin according to the method described by Rinaudo *et al.* [8]. After process, the deacetylation degree was 85% (determined by potentiometric titration) and molecular weight 1.10 x 10^6. Tetracycline.HCl (TC) was purchased from Aldrich. All other reagents are analytical grade and used without further purification.

Simulated saliva was prepared by mixing (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8.00 g NaCl per liter of distilled water adjusted with phosphoric acid to pH 6.75) [9].

Preparation of Chitosan Films

Chitosan films (CF) were produced by a casting/solvent evaporation technique 10. Chitosan solution (1% w/v) containing tetracycline (0.2% w/v) was prepared by dissolving chitosan and tetracycline in (1% w/v) acetic acid. Solution is then poured into mould and allowed to evaporate at 50 °C to form a film. The dried film was cut in disc form with diameter of 6.2 mm and area of 0.30 cm2. The thickness of dried film were determined to be 470 ± 17 µm (means ± sd) measured with a micrometer (Mitutoyo 103-259) and was taken as the mean of 20 films.

Chitosan heat-treated films (HCF) were obtained by heat treatment of films in an oven at 90 °C (HCF-90), 120 °C (HCF-120) and 150 °C (HCF-150) for 4 h [11]. Chitosan film without tetracycline was prepared and it was used as control test.

To ensure uniform distribution of TC in mold, a content uniformity test was performed. Samples representing different regions within mold were cut and weighed, dissolved in HCl 0.01 M (25 mL). The resultant TC solution was determined spectrophotometrically at 276 nm with an UV/Vis spectrophotometer Shimadzu model 1601 and the actual value were calculed based on a calibration curve.

Assay of Tetracycline Chitosan Film

Film (50 mg) was dissolved in HCl 0.01 M (25 mL) and resultant TC solution was determined spectrophotometrically (at 276 nm) with an UV/Vis spectrophotometer Shimadzu model 1601 and the actual value were calculed based on a calibration curve [12].

Equilibrium Water Content and Swelling Ratio of the Chitosan Film

Three films (CF, or HCF), were tested by placing in distilled water (30 mL) and incubated at 37 °C. At an appropriate time interval, the films were taken out, and excess water was removed carefully with filter paper from the film, and weighed immediately. The swelling was expressed as a swelling ration (SR) where:

$SR = (W_t - W_0) / W_0$, were W_0 is weight of dry sample (g) and Wt is weight of sample (g) at time t after incubation (30 min) [10].

"In vitro" Release Study

In three test tubes, two films CF or HCF, were covered with 30 mL saliva simulated solution and stored a 37.5 ± 0.5 °C. At certain time intervals, sample (1 mL) were removed from the release medium and tetracycline content was measured as mentioned above and solution were returned. The cumulative amount of TC was obtained from the calibration curves in salive simulated medium. Results of triplicate test were used to calculate the released tetracycline [12].

Test of Antibiotic Activity

The bacteriostatic effect of the films containing tetracycline was determined by agar diffusion assay. Cotton swab charged with *Staphylococcus aureus* (ATCC 25923) suspension (10^6 CFU/mL) was inoculated on plates and bacteria was spread evenly over the surface of the Mueller-Hinton agar media. Twenty films were divided into five groups (Control, CF, HCF-90, HCF-120 and HCF-150) and placed on agar plates inoculated. These plates were incubated at 37 °C for 24 h, when the zones of inhibition were measured and recorded. The films were removed individually from the plates, dabbed on a sterile swab to remove surface moisture, and immediately transferred to another fresh plate inoculated with the same bacteria. This

procedure was repeated until no zone of inhibition was seen (> 10 mm). The bacteriostatic effect of the 4 films without tetracycline was studied by the same method as the control group.

RESULTS AND DISCUSSION

Tetracycline Dose in Chitosan Film

Chitosan could be used, as film former, since it is soluble in dilute organic acid medium, attempt to prepare in insoluble form is to expand its use in controlled release systems. Films obtained from evaporation the acetic acid solution were transparent and flexible and had a yellow color due presence of TC. The final amount of TC that resided in the film was 3.2 mg cm^{-2}.

Expose to heat treatment for 1 h led to the development of a uniform color in HCF-90 (yellow intense), HCF-120 (yellowish brown) and HCF-150 (brown) samples. The coloration change observed in the chitosan films is similar the those related for the formation of crosslinking of chitosan chain by glutaraldehyde and the Maillard reaction. Like theses two reactions, heat-induced reactions in chitosan may involve the NH2 groups and the formation of crosslinks [13].

Swelling Ratio of the Chitosan Films

Table 1 shows the influence of heat treatment on the swelling chitosan film. An equilibrium was reached within 30 min and no weight increase was observed after up to 24 h. Was observed change in diameter of the CF, greater expansion and swelling were observed, this behavior is associated with the solubility of chitosan-acetate in water. On the other hand, heat treatment reduces significantly the water absorption of HCF in distilled water when compared with CF, Table 1. The water absorption was reduced at 90 °C, but further raises in the temperature of treatment (120 and 150 °C) no produce an increase effect. Similar result, were related by Lim and Wan [11], for chitosan films.

Dry heat treatment to chitosan acetate film was reported to increase its water resistance and additionally decreased their percentage of water sorption and dissolution, this was possible due to the intramolecular and intermolecular condensations of carboxylic acid in chitosan salt film [10].

The increases in water resistance of an aqueous soluble polymer (heat-induced) can be caused by increase in film crystallinety and/ or the formation of crosslink between the polymer molecules [11,13,14]. Toffey *et al.* [15] suggested, that chitosan acetate films could be thermally converted to chitin by heating at 120 °C and Jarry *et al.* [16] showed that sterilization (autoclaved at 120 °C) of chitosan in the dry state, decrease its solubility in water.

Film	diameter (mm)*	Swelling ratio (g/g)
CF	8.59 ± 0.66	1.11 ± 0.08
HCF-90	6.95 ± 0.19	0.35 ± 0.09
HCF-120	7.45 ± 0.52	0.75 ± 0.10
HCF-150	7.47 ± 0.18	0.81 ± 0.07

Table 1. Swelling ratio of films in distilled water with time of contact 30 min.

In vitro tetracycline release

Figure 1 shows the release percentage of TC from chitosan films against the square root of time. The releases rates of TC were dependent on the heat treatment that films were submitted. A total of 45% of TC was released from the CF in 1 h, whereas with heat treatment chitosan films, 25%, 32% and 35% of TC were release respectively of HCF-120, HCF-150 and HCF-90 after the same time. The release of TC from CF was higher than that HCF. This difference can be due to solubility of CF in the saliva-simulated medium and may be attributed to the solubilization of chitosan acetate films.

The heat treatment of the films led to the decrease of swelling and consequently the release of TC from films to be decreasing. However, the heat treatment greatly prolonged TC release, the time period for 75% TC released was extended from 2 to 5 and 8 h after heat treatment of films at 120 and 150 °C, respectively.

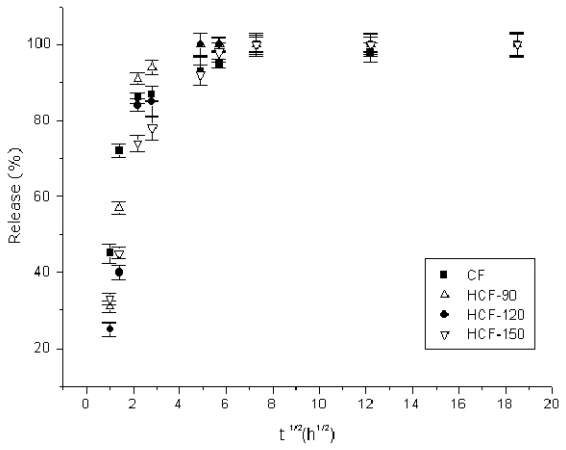

Figure 1. Amount of TC released from chitosan film versus square root of time (means ± sd). (*) = means ± sd for 3 films.

Antibiotic Activity

The results showed that tetracycline in the chitosan film had good bacteriostatic activity to bacteria *Staphylococcus aureus*. TC release was measured by antimicrobial activity during the period of the assay and it was determined by agar diffusion. The control film (Chitosan film without tetracycline) did not demonstrate antibacterial activity. Chitosan film and HCF-150 had demonstrated ability in antibiotic release for long time (until 27 and 29 days, respectively), while that film prepared at temperature at 90 °C (HCF-90) and 120 °C (HCF-120), had presented comparatively low antibiotic release index (until 9 and 17 days, respectively), see Figure 2.

The differences of antibacterial activities during their time courses should be due behavior of respective chitosan film in media. The softening of film CF and the hardening with fragmentation of film prepared at 150 °C (HCF-150), collaborate to continuous antibiotic release until 27 and 29 days of assay, approximately. However the chitosan film HCF-90 and HCF-120 presented behavior without significative shape alteration and short time antibacterial activity that should be relationed with only superficial antibiotic release and apparent low antibiotic release of inside from those film.

The gradual release of TC in the films of CF can be attributed to the slow swelling of the film, dissolution of TC and posterior liberation for the agar media. On the other hand, the rapid release of TC in the films submitted to the heat treatment can be attributed the liberation of TC present in surface of film, once they are resistant to the swelling and solubilization. In addition, after the heat treatment the film is brittle and this way TC is released for the agar media.

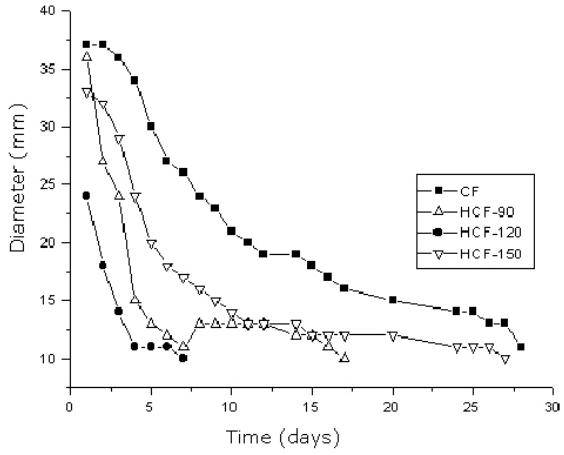

Figure 2. Time (day) *versus* antibacterial activity (diameter) from chitosan film prepared at different temperatures against *Staphylococcus aureus*.

Heat treatment change the solubility and appearance of chitosan films. Heat treatment increase the water resistance of chitosan films by inducing the formation of crosslinks between chitosan molecules, therefore the heat treatment decrease the rate of release of tetracycline from films, specially in HCF-150. The incorporation of TC into film no eliminates its antibacterial activity. The results demonstrated what TC chitosan film could be a potential matrix to use as the adjunctive treatment of periodontitis and moreover chitosan it is a biodegradable polymer, lack of toxicity and allergenicity make it a very attractive.

Acknowledgements. We are grateful to FAPESC (Fundação de Apoio à Pesquisa Científica e Tecnológica do Estado de Santa Catarina) for financial support.

REFERENCES

1. Singla, A.K. & M. Chawla (2001) *J. Pharm. Pharmacol.* **53**: 1047-67.
2. Senel, S. & S.J. McClure (2004) *Adv. Drug Deliv. Rev.* **56**: 1467-80.
3. Ikinci, G., S. Senel, H. Akincibay, S. Kas, S. Ercis, C.G. Wilson & A.A. Hincal (2002) *Int. J. Pharm.* **235**: 121-7.
4. Loesche, W.J. & N.S. Grossman (2001) *Clin. Microbiol. Review* **14**: 727-52.
5. Etienne, D. (2003) *Oral Dis.* **9** Suppl 1: 45-50.
6. Lacevic, A., E. Vranic & I. Zulic (2004) *Bosn. J. Basic Med. Sci.* **4**: 73-8.
7. Walker, C. & K. Karpinia (2002) *J. Periodontol.* **73**: 1188-96.
8. Rinaudo, M., M. Milas & P. Le Dung (1993) *Int. J. Biol. Macromol.* **15**: 281-5.
9. Peh, K.K. & C.F. Wong (1999) *J. Pharm. Pharmaceut. Sci.* **2**: 53-61.
10. Ritthidej, G.C., T. Phaechamud & T. Koizumi (2002) *Inter. J. Pharm.* **232**: 11-22.
11. Lim, L. Y. & L.S.C. Wan (1995) *Drug. Dev. Ind. Pharm.* **21**: 839-846.
12. Lin, D.M., S. Kalachandra, J. Valiyaparambil & S. Offenbacher (2003) *Dental Materials* **19**: 589-90.
13. Lim, L. Y., E. Knor & C.E. Ling (1999) *J. Biom. Mater. Res. (Appl. Biochem.)* **48**: 111-6.
14. Ogawa, K. (1991) *Agric. Bio. Chem.* **55**: 2375-9.
15. Toffey, A., G. Samaranayake, C.E. Frazier & W.G. Glasser (1996) *J. Appl. Polym. Sci.* **60**: 75-85.
16. Jarry, C., C. Chaput, A. Chenite, M.A. Renuad, M. Buschmann & J.C. Leroux (2001) *J. Biomed. Mater. Res. (Appl. Biomater.)* **58**: 127-35.