Journal of Applied Polymer Science

Tetracycline Release from Chitosan/Fish-Scale-Based Membranes

Gracy Karla da R. Cortes,[1] Eunice F. S. Vieira,[2] Antonio R. Cestari,[2] Renata A. Chagas[2]
[1]Núcleo de Ciência e Engenharia de Materiais, Universidade Federal de Sergipe, São Cristóvão 49100-000 SE, Brazil
[2]Laboratory of Materials and Calorimetry, Departamento de Química/Centro de Ciências e Estudos de Tecnologia, Universidade Federal de Sergipe, São Cristóvão 49100-000 SE, Brazil
Correspondence to: E. F. S. Vieira (E-mail: eunice@ufs.br)

ABSTRACT: In this study, we aimed to develop new biocompatible membranes on the basis of chitosan (CHIT) and fish scale powder (ESC) from the species *Leporinus elongatus*. The possibility of using the uncrosslinked membrane (ESC/CHIT) and membrane crosslinked with sodium tetraborate (ESC/CHIT-B) for tetracycline release was investigated. The drug-release kinetics were studied at 30 and 37°C in phosphate buffered saline (pH 7.4). For ESC/CHIT, the drug release was faster, about 6 days, whereas the release time of tetracycline impregnated in ESC/CHIT-B was about 7 days. The *in vitro* release behavior of tetracycline from both membranes followed the Peppas and Higuchi kinetic models. The kinetics of drug release from ESC/CHIT were regarded as a coupled diffusion/polymer relaxation mechanism, whereas drug release from ESC/CHIT-B seemed to be controlled by polymer relaxation. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39943.

KEYWORDS: biopolymers and renewable polymers; biosynthesis of polymers; drug-delivery systems; kinetics; membranes

Received 1 April 2013; accepted 24 August 2013
DOI: 10.1002/app.39943

INTRODUCTION

The study of nonsurgical approaches to periodontal therapy have become intense in modern pharmaceutical design. Periodontal disease is an infection caused by specific bacteria that adhere to the teeth, with subsequent inflammation of the soft tissues surrounding the teeth.[1] The goal of nonsurgical periodontal therapy is to return the tissues to a state of health that can be easily maintained by the client through periodontal debridement procedures.[2] The introduction of locally delivered antibiotics specifically for the treatment of periodontitis offers a novel concept for the treatment of localized disease. It may prove useful in the treatment of recurrent disease activity where only a few individual sites are involved.[3] The major purpose of local drug-delivery systems is to achieve a controlled and low release rate of the drug and thus ensure a constant *in vivo* drug concentration and facilitate prolonged drug delivery.[4] In the absence of drug delivery and controlled release, bioactive factors may undergo rapid diffusion, denature shortly after *in vivo* delivery, and often fail to induce the intended effects on the target cells and tissues.[5]

Abha et al.[6] provided a comprehensive overview of a transbuccal mucoadhesive drug-delivery system. According to them, a buccal film is preferred over various mucoadhesive dosage forms because it protects the wound surface and thus reduces pain and can also treat oral diseases more effectively. Chitosan (CHIT) films for the treatment of periodontitis have been developed, and it was shown that drug-loaded CHIT films were flexible, possessed good tensile strength, and demonstrated satisfactory physicochemical characteristics.[7] Because of their excellent biocompatibility characteristics, natural polymers such as CHIT and other polysaccharides have a long history as drug-delivery systems in many biomedical fields. CHIT, an *N*-deacetylated derivative of chitin, is a linear copolymer polysaccharide consisting of β-(1–4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and 2-acetamido-2-deoxy-D-glucose (*N*-acetyl-D-glucosamine).[8–14]

Hydroxyapatite [HA; $Ca_{10}(PO_4)_6(OH)_2$], as one of the most important inorganic biomaterials, has received long-term attention because of its biocompatibility and osteoconductivity because it is essentially the hard, inorganic component of human bones.[15] Furthermore, the physical and chemical properties of HA, such as its chemical composition, structure, porosity, particle size, and surface area, and the ionic composition of the equilibrating solution are important determinants in drug binding and release. In this study, we report on the preparation of films based on CHIT and fish scales from the *Leporinus elongatus*. These scales were composed of two different phases, an inorganic phase and another, organic one. The inorganic phase is formed of HA, and the organic phase is formed mainly of collagen (~32 mass %).[16] By combining the biocompatibility and controlled release capability of CHIT and the organic and

inorganic components of the fish scales, we designed novel films for the controlled release of tetracycline, a drug already extensively used in the treatment of periodontitis.[1–3,17–19]

EXPERIMENTAL

Materials

Scales of Piau fish, *L. elongatus,* with an average length of about 20 cm (1.5–2.0 kg), were obtained from a free market in Itabaiana, Brazil, in the summer of 2011. CHIT, with a deacetylation degree of 85% and a viscosity-average molecular weight of 3.22×10^5 g/mol, was kindly donated by Primex Ingredients A.S. (Avaldsnes, Norway). Sodium tetraborate decahydrate (borax) and acetic acid were purchased from Synth (Diadema, Brazil). Tetracycline (purity = 88%) and phosphate buffered saline (PBS; pH 7.4) were purchased from Sigma-Aldrich (St. Louis, MO). Sodium hydroxide used to treat the fish scales was from Proquímicos (Bauru, Brazil). Double-distilled water was used throughout.

Treatment and Processing of the Fish Scales

According a procedure previously described,[16] the fish scales were washed thoroughly with running water and immersed in an NaOH solution (pH 9.0) for 4 h at room temperature. After this, the fish scales were washed (for 60 min under ultrasound and for 10 min by stirring in double-distilled water) and were then dried at 60°C for 6 h. The resulting material was triturated in an industrial blender (Skymsen model TA-02); this resulted in a mixture of powder and fibers. The powder was sieved with a 100-mesh sieve, and this resulted in a material hereafter called ESC for simplicity.

Membrane Preparation

We prepared the CHIT membranes by dissolving 3 g of CHIT in 100 mL of a 1% aqueous acetic acid solution. The gel was mixed with 6 g of ESC (ESC/CHIT ratio = 2:1) and kept under stirring for 30 min. The resulting suspension was poured into a Teflon dish, and a homemade spacer was used to prepare membranes with similar thicknesses and dry them at room temperature for 72 h.[20] The resulting membrane (ESC/CHIT) was immersed in a 3.5% w/v sodium tetraborate aqueous solution at room temperature for 24 h; this was followed by washing with distilled water. The crosslinked membrane (ESC/CHIT-B) was then dried at room temperature for 24 h. The dry membranes were cut into squares (1.0×1.0 cm^2), and the thickness of each membrane was measured with a Mitutoyo 103–259 micrometer.

Characterization

The materials were characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM) and thermogravimetric analysis (TGA) and differential thermogravimetry (DTG). The X-ray diffractograms of the materials were obtained in a Shimadzu diffractometer. The equipment was operated in continuous scanning mode with Cu Kα radiation (1.5418 Å) generated at 40 kV with a 30-mA current. The scanning speed used was 0.02°/s at 2θ (10–70°). All of the diffractograms were obtained with powdered samples at room temperature. The surfaces of the materials were morphologically observed by SEM (JCM 5700) at a voltage of 10 kV. The samples were previously coated with gold *in vacuo* by sputtering with a Denton vacuum apparatus. TGA was done with masses of about 10 mg under a nitrogen atmosphere from 25 to 600°C in a TGA–DTA SDT 2960 thermogravimetric analyzer from TA Instruments.

Tetracycline Entrapment in ESC/CHIT and ESC/CHIT-B and Drug Analysis

For impregnation of the drug, several membranes of each material were placed in 50 mL of a 1.0×10^{-4} mol/L tetracycline solution prepared in PBS and then left overnight in a refrigerator. After this time, the tetracycline concentration in PBS was determined by UV spectrophotometry (Femto, 700 Plus) at 272 ± 2 nm with a 1.0-cm quartz cell to obtain the drug entrapment efficiency.[21] A standard linear calibration curve was applied, and the linear relationship in standard solutions with a concentration range of $1.00–8.00 \times 10^{-5}$ mol/L was attained. We did not detect any loading of tetracycline during freezing in the refrigerator.

Tetracycline Release

All drug-release experiments were carried out in isothermal mode with a given membrane impregnated with tetracycline and immersed in various tubes containing 10 mL of PBS at temperatures of 30 and 37°C. At predetermined time intervals, aliquots of 3 mL were withdrawn to determine by UV spectrophotometry the concentration of drug released. The amount of drug released from ESC/CHIT and ESC/CHIT-B in a 9-day period of time was determined. The cumulative degree of tetracycline released (α) was determined with eq. (1):[22]

$$\alpha = C/C_{max} \quad (1)$$

where C is the tetracycline concentration in the solution at time n (mol/L) and C_{max} is the maximal (equilibrium) concentration of tetracycline in the solution (mol/L). The experiments were repeated three times, and mean values were obtained.

RESULTS AND DISCUSSION

The average thicknesses of the membranes were 0.20 ± 0.01 and 0.60 ± 0.02 mm for ESC/CHIT and ESC/CHIT-B, respectively. Mineral–polysaccharide membranes are of widespread importance for the controlled release of drugs.[23] Although HA has received much attention in the field of medical applications, the use of pure HA is very limited because of its slow degradation and brittleness.[24] Because natural bone is a composite mainly consisting of nanosized, needlelike HA crystals and collagen fibers, many efforts have been made to modify HA by polymers, such as CHIT.[24,25] The ESC/CHIT and ESC/CHIT-B inorganic–organic hybrid materials described in this article should offer much promise in the design of biocompatible membranes. The formation of interpenetrating polymer network membranes is due to hydrogen bonds and coordination interactions.[26] Because of the ability of borax to complex with the hydroxyl groups of CHIT, the ESC/CHIT-B membrane was obtained. Borax is considered to be a nontoxic agent and has a long history of medical use.[27]

XRD

XRD patterns of the pure CHIT, ESC, ESC/CHIT, and ESC/CHIT-B are shown in Figure 1. These studies were useful for

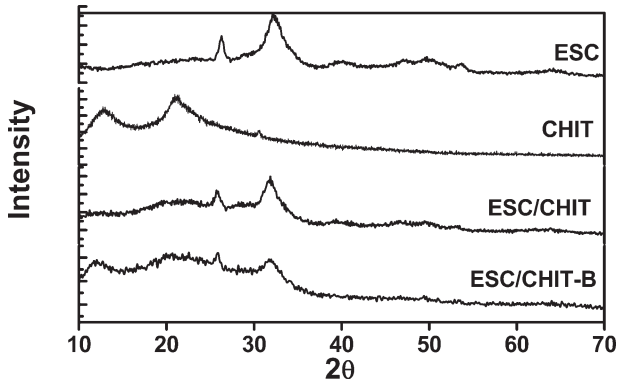

Figure 1. XRD patterns obtained at room temperature with powdered samples of ESC, CHIT, ESC/CHIT, and ESC/CHIT-B.

investigating the crystallinity of the samples. The XRD pattern of pure CHIT gave two major crystalline peak reflections located at $2\theta = 12.72$ and 20.94°, which represented two crystal forms, I and II.[28,29] HA was identified as the only inorganic phase in the ESC, with two broad characteristic peaks at $2\theta = 25.70$ and 31.96°.[30] For ESC/CHIT and ESC/CHIT-B membranes, the typical crystalline peaks of HA were still observed. For the ESC/CHIT-B membrane, the characteristic peak of CHIT at $2\theta = 12.72°$ had the intensity minimized; this left the peak at 2θ between 10.80 and 13.62°. However, for the ESC/CHIT membrane, no peaks from CHIT were detected. It is possible that interaction between CHIT and HA led to difficulty in measuring at the detection limit of the CHIT crystal size in this case. Also, in the diffractograms of the pure CHIT film, it was possible that the crystalline CHIT peaks could not be found because CHIT did not form its own crystalline region but maintained an amorphous state during film formation.[31] For ESC/CHIT-B, the peaks became broader compared to those of ESC/CHIT; this indicated that the crosslinking reaction with sodium tetraborate exercised influence on the structure and crystallinity.

SEM Analysis

SEM was used to examine the morphology of the ESC/CHIT and ESC/CHIT-B membranes. Two representative SEM micrographs obtained with 500× magnification are represented in Figure 2. The SEM micrograph of the pure CHIT membrane is not shown, but generally, the SEM micrographs of the pure CHIT films showed a relatively smooth and homogeneous surface with very sparsely distributed small particles without any phase separation.[32] From the SEM analysis, the distinctions in the structures of the two membranes were obvious. The SEM image of ESC/CHIT [Figure 2(a)] showed a relatively smooth surface with some distributed irregularities. It was possible that the mineralized polysaccharide produced precipitates of HA or mixtures of other types of calcium phosphate. On the other hand, the SEM micrograph of ESC/CHIT-B [Figure 2(b)] showed a different kind of surface morphology, in which the surface was more rough and irregular.

TGA

The TGA/DTG curves for pure CHIT, ESC, ESC/CHIT, and ESC/CHIT-B are shown in Figure 3. For CHIT, we observed two stages of mass loss. The first one started at 39.5°C and reached a maximum at 61.5°C with a weight loss of 8%. The second stage started at 230°C and reached a maximum at 303°C with a weight loss of 48%. These results were previously presented and described.[12] The first event was related to the loss of water, and the second one corresponded to the thermal and oxidative decomposition of CHIT, vaporization, and elimination of volatile products. With regard to ESC, peaks with maxima at 58 and at 332°C were observed. The first event started at 41°C, with a weight loss of 11%. The major weight loss (of 32%) occurred at 200°C. The first event was related to the superficial water release and the denaturation of fish-scale collagen. The second stage of mass loss corresponded to the thermal degradation of the polymeric chains of collagen, the possible dehydroxylation of HA, and carbon material elimination.[33] The TGA and DTG curves of ESC/CHIT and ESC/CHIT-B were equivalent in the first stage, with weight losses of 11 and 12%, respectively. The second stage of degradation showed peaks with maxima at 284°C for ESC/CHIT and 290°C for ESC/CHIT-B and might have been caused by the decomposition of the materials. These differences in the peak area and position might have clearly indicated the formation of distinct materials. The examination

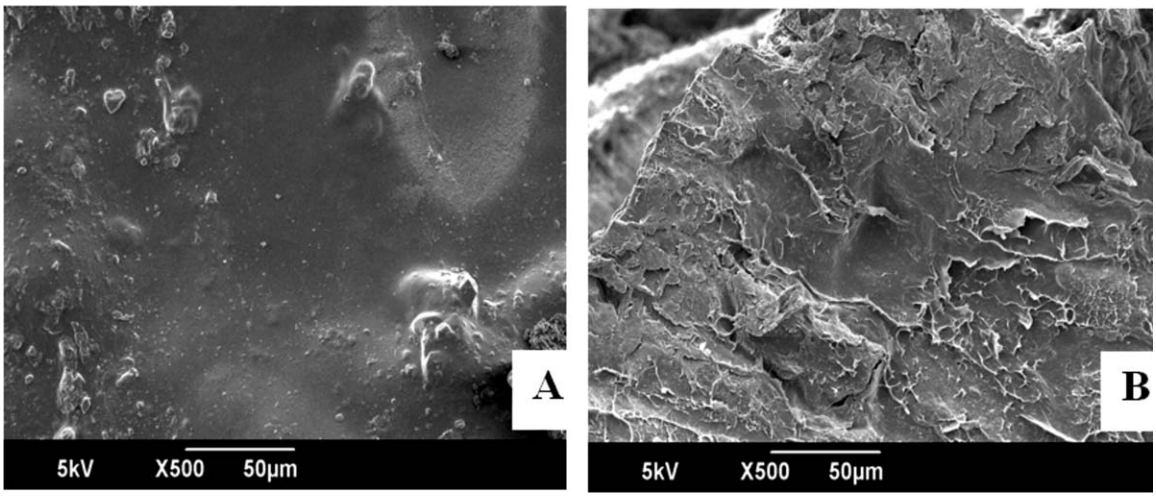

Figure 2. SEM micrographs of (A) ESC/CHIT and (B) ESC/CHIT-B (500× magnification for both).

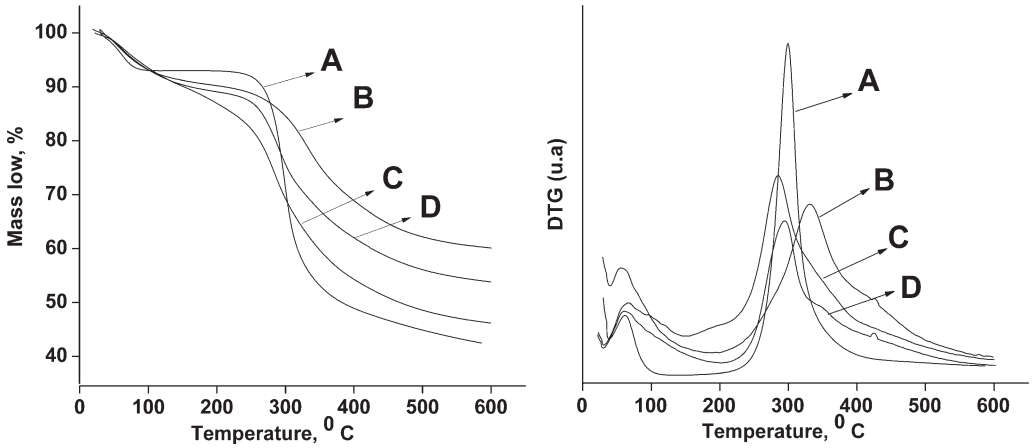

Figure 3. TGA and DTG curves for (A) CHIT, (B) fish scale, (C) ESC/CHIT, and (D) ESC/CHIT-B.

of the TGA curves showed that for ESC/CHIT, the second stage started at 156°C, whereas ESC/CHIT-B was thermally more stable, with the second stage of mass loss at 206°C. This indicated that its thermal properties became different after sodium tetraborate treatment. Furthermore, TGA data revealed that 40 and 46% residue were obtained from ESC/CHIT and ESC/CHIT-B, respectively.

In Vitro Kinetic Release Profiles of Tetracycline

For both materials, ESC/CHIT and ESC/CHIT-B, the entrapment efficiency was 100% because no amount of drug was present in the supernatant PBS solutions after the tetracycline entrapment procedure.

All of the release experiments were carried out in PBS (pH 7.4) at 30 and 37°C. The release profiles are shown in Figure 4. The plots of α versus release time obtained at 30 and 37°C are shown in Figure 5. We observed that for both materials, the amount of drug released increased slightly with increasing temperature. For ESC/CHIT, the drug was released within 6 days approximately at 30 and 37°C, whereas the release was more prolonged for the tetracycline impregnated in ESC/CHT-B (ca. 7 days at both temperatures).

Many important mathematical models have been developed to describe drug release from polymeric systems. However, in many cases, theoretical fundamentals of release experiments with tablets, capsules, and membranes do not exist, and empirical models are used.[34] In addition, the comparison and analysis of the results may be difficult, mainly because of poor mathematical fittings to the experimental data. A very commonly used and easy to apply semi-empirical equation is the so-called power law, as introduced by Peppas and coworkers.[35–38] Its linearized form is shown in the following equation:[22]

$$\ln \alpha = \ln k + n \ln t \quad (2)$$

where t is the drug release time, n is the release exponent, which is indicative of the mechanism of drug release, and k is the apparent release rate, which incorporates structural and geometric characteristics of the release system. A plot of ln α versus ln t should result in a straight line whose slope and intercept give the n and k values, respectively.

We observed that both kinetic models were well fitted the experimental values because the correlation coefficients (r^2's) were greater than 0.99 in almost all cases. From the plots of ln α versus ln n (Figure 6), a straight line was not obtained throughout the full range times of the release processes; thus, the n and k values from the Peppas model were obtained for the linear range of applicability[22] in this work until the 5th day

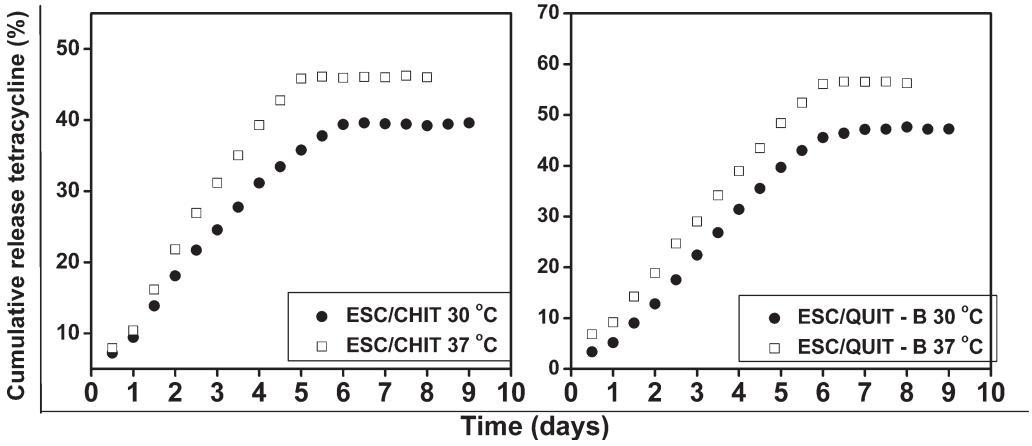

Figure 4. Profiles of the tetracycline cumulative release (%) for ESC/CHIT and ESC/CHIT-B in 10 mL of pH 7.4 PBS at 30 and 37°C.

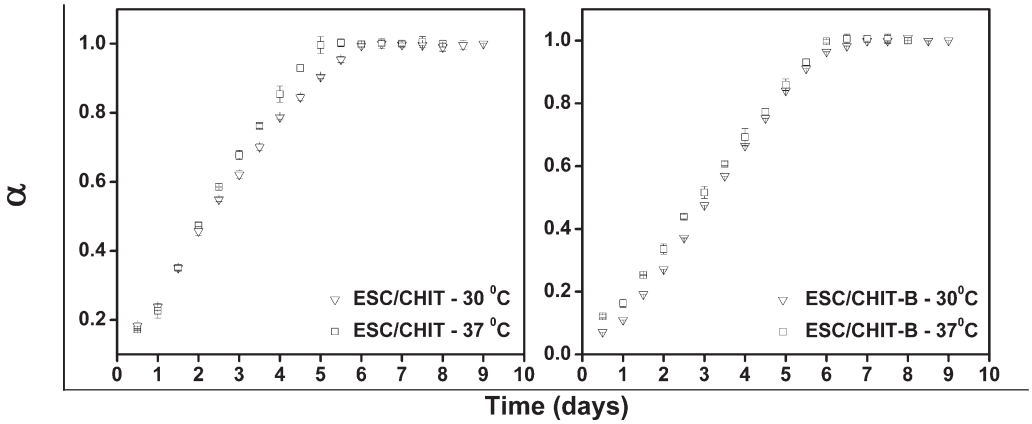

Figure 5. Plots of the tetracycline cumulative release (α) versus the time for ESC/CHIT and ESC/CHIT-B in 10 mL of pH 7.4 PBS at 30 and 37°C.

of drug release for each system studied. For the range of applicability, we observed that the release data fit the Peppas model well because the r^2's were greater than 0.99.

The Higuchi model describes the release of drugs as the square root of time based on the Fickian diffusion, according to eq. (3):[39,40]

$$\alpha = K_H \sqrt{t} \tag{3}$$

where K_H is a constant reflecting the design variables of the system and that is obtained from the slope of α versus $t^{1/2}$ plots (details not shown).

The drug-release data fitted to the Peppas and Higuchi models are shown in Table I. Different release mechanisms could be inferred from the values of *n*. The *n* values of 0.78 at 30°C and 0.86 at 37°C for ESC/CHIT were regarded as an indication of the non-Fickian release type or anomalous transport. Non-Fickian kinetics are regarded as coupled diffusion/polymer relaxation.[41,42] On the other hand, from the *n* values of 1.2 and 1.1 for ESC/CHIT-B, it was tempting to conclude that super case II kinetics were operating, in which drug release seemed to be controlled by polymer relaxation.[41,42] Furthermore, the values of the kinetic constant ranged from 0.258 to 0.268 min^{-1} for ESC/CHIT and from 0.119 to 0.169 min^{-1} for ESC/CHIT/B at 30 and 37°C, respectively; this indicated that the tetracycline release was dependent on the treatment of the membranes. Through evaluation of the values of the k_H parameter from the Higuchi model, the same conclusions were also reached.[40]

Because the OH groups in HA in fish scales can act as active sites to adsorb bioactive molecules by hydrogen bonds,[43] we assumed that the drug in the ESC/CHIT and ESC/CHIT-B was only held by hydrogen bonds and hydrophobic interactions. Because the tetracycline was not chemically attached to the membranes, it remained in a biologically active form and would be therapeutically effective in the body as soon as it is released.[44] Taking into account this aspect, we hope that inorganic–organic hybrid membranes loaded with tetracycline, particularly one crosslinked with sodium tetraborate (ESC/CHIT-B), may enable safe, straightforward encapsulation and sustained release of therapeutic agents for use in the treatment of periodontitis.

CONCLUSIONS

Because of the excellent biocompatibility characteristics of CHIT and HA, they are likely to be of importance in the design of biocompatible materials. In this study, we made an attempt

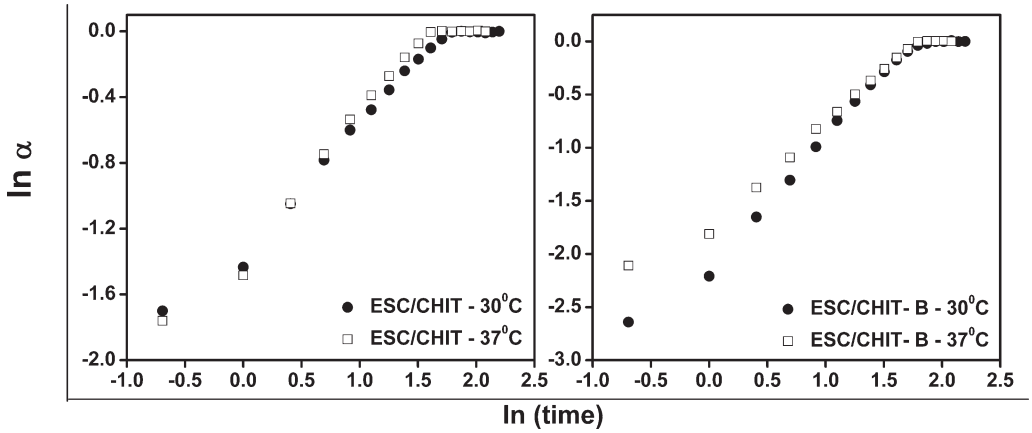

Figure 6. Plots of ln α versus ln *n* for tetracycline release from ESC/CHIT and ESC/CHIT-B at 30 and 37°C.

Table I. Parameters of the Peppas and Higuchi Kinetic Models for Tetracycline Release from ESC/CHIT and ESC/CHIT-B in 10 mL of pH 7.4 PBS at 30 and 37°C

		Higuchi		Peppas		
Material	Temperature (°C)	K_H (min^{-1})	r^2	n	k (min^{-1})	r^2
ESC/CHIT	30	0.534	0.998	0.783	0.268	0.994
	37	0.631	0.999	0.857	0.258	0.994
ESC/CHIT-B	30	0.689	0.998	1.220	0.119	0.993
	37	0.645	0.997	1.110	0.169	0.998

to develop membranes based on fish scales and CHIT for the treatment of periodontitis.

It was shown that the release rates of tetracycline were dependent on the treatment to which the membranes were submitted. We observed that 45.9 and 56.2% of the drug were released at 30 and 37°C, respectively, from the crosslinked membrane, ESC/CHIT-B. For ESC/CHIT, 39.6% of tetracycline was released at 30°C, whereas 47.2% was released from ESC/CHIT at 37°C. The relatively low efficiency of releases may have been due to the low solubility of tetracycline in PBS (pH 7.4). The cumulative tetracycline release was sustained for up to 6 and 7 days for the uncrosslinked and crosslinked membranes, respectively.

From the evaluation of the results with the Peppas and the Higuchi kinetic models, we observed that for ESC/CHIT, a coupled diffusion/polymer relaxation mechanism was responsible for the tetracycline release, whereas for the crosslinked membrane, ESC/CHT-B, drug release seemed to be controlled by polymer relaxation. Evidently, other pharmacological and *in vivo* studies must be performed before use of the materials from this study in the treatment of periodontitis. However, from the tetracycline release results and the kinetic modeling, we think that this CHIT/fish scale-based material will be useful for this specific purpose.

ACKNOWLEDGMENTS

Financial support and fellowships from Brazilian agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) to E.F.S.V., R.A.C., and A.R.C. and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) to G.K.R.C. are gratefully acknowledged.

REFERENCES

1. Sousa, F. O.; Blanco-Méndez, J.; Pérez-Estévez, A.; Seoane-Prado, R.; Luzardo-Álvarez, A. *J. Biomater. Appl.* **2011**, *27*, 187.
2. Perno, M. Pharmacotherapy in Periodontal Therapy Special Supplemental. American Dental Hygienists' Association Web site; September–October 2001. http://204.232.157.152/downloads/sup_antimicrobials.pdf (accessed August **2013**).
3. Walker, C.; Karpin, K. *J. Periodontol.* **2002**, *73*, 1188.
4. Betsiou, M.; Bantsis, G.; Zoi, I.; Sikalidis, C. *Ceram. Int.* **2012**, *38*, 2719.
5. Moioli, E. K.; Clark, P. A.; Xin, X.; Lal, S.; Mao, J. *J. Adv. Drug Delivery Rev.* **2007**, *59*, 308.
6. Abha, D.; Sheeja, K.; Bhagyashri, *J. Int. J. Pharm. Biol. Sci.* **2011**, *1*, 17.
7. Ahmed, M. G.; Harish, N. M.; Narayana, C. R.; Prabhu, P. *Trop. J. Pharm. Res.* **2009**, *8*, 33.
8. Zhang, C. Y.; Zhang, C. L.; Wang, J. F.; Lu, C. H.; Zhuang, Z.; Wang, X. P.; Fang, Q. F. *J. Appl. Polym. Sci.*, to appear.
9. Zhang, C. Y.; Chen, J.; Zhuang, Z.; Zhang, T.; Wang, X. P.; Fang, Q. F. *J. Appl. Polym. Sci.* **2012**, *124*, 397.
10. Chang, H.; Lin, Y.; Tsai, Y.; Tsai, M. *J. Appl. Polym. Sci.*, to appear.
11. Yin, Y.; Zhao, F.; Song, X. F.; Yao, K.; Lu, W. W.; Leong, J. C. *J. Appl. Polym. Sci.* **2000**, *77*, 2929.
12. Vieira, E. F. S.; Cestari, A. R.; Airoldi, C.; Loh, W. *Biomacromolecules* **2008**, *9*, 1195.
13. Kosaraju, S. L. *Crit. Rev. Food Sci. Nutr.* **2005**, *45*, 251.
14. Álvarez, A. L.; Espinar, F. O.; Méndez, J. B. *Pharmaceutics* **2011**, *3*, 538.
15. Yang, L.; Yin, J.; Wang, L.; Xing, G.; Yin, P.; Liu, Q. *Ceram. Int.* **2012**, *38*, 495.
16. Vieira, E. F. S.; Cestari, A. R.; Carvalho, W. A.; Oliveira, C. S.; Chagas, R. A. *J. Therm. Anal. Calorim.* **2012**, *109*, 1407.
17. Miyake, Y.; Tsuruda, K.; Okuda, K.; Widowati, I. Y.; Suginaka, H. *J. Periodontal Res.* **1995**, *30*, 290.
18. Fernandes, L. A.; Martins, T. M.; Almeida, J. M.; Nagata, M. J. H.; Theodoro, L. H.; Garcia, V. G.; Bosco, A. F. *J. Appl. Oral Sci.* **2010**, *18*, 635.
19. Obaidat, A. A.; Hammad, M. M. *J. Appl. Polym. Sci.* **2010**, *116*, 333.
20. Vieira, E. F. S.; Costa, L. P.; Cestari, A. R. *J. Appl. Polym. Sci.* **2010**, *118*, 857.
21. Cruz, A. B.; Surdi, M.; Cenci, M. M. P.; Rodrigues, C. A. *Lat. Am. J. Pharm.* **2008**, *27*, 360.
22. Adnadjevic, B.; Jovanovic, J.; Drakulic, B. *Thermochim. Acta* **2007**, *466*, 38.
23. Lévêque, I.; Rhodes, K. H.; Mann, S. *J. Mater. Chem.* **2002**, *12*, 2178.
24. Fan, J.; Bi, L.; Wu, T.; Cao, L.; Wang, D.; Nan, K.; Chen, J.; Jin, D.; Jiang, S.; Pei, G. *J. Mater. Sci.: Mater. Med.* **2012**, *23*, 399.

25. Liuyun, J.; Yubao, L.; Chengdong, X. *J. Biomed. Sci.* **2009**, *14*, 1.
26. Li, J.; Dou, Y.; Yang, J.; Yin, Y.; Zhang, H.; Yao, F.; Wang, H.; Yao, K. *Mater. Sci. Eng. C* **2009**, *29*, 1207.
27. Balakrishnan, B.; Jayakrishnan, A. *Biomaterials* **2005**, *26*, 3941.
28. Tian, F.; Liu, Y.; Hu, K.; Zhao, B. *J. Mater. Sci.* **2003**, *38*, 4709.
29. Wang, X.; Wang, X.; Tan, Y.; Zhang, B.; Gu, Z.; Li, X. *J. Biomed. Mater. Res. Part A* **2008**, *89*, 1079.
30. Huang, Y.; Hsiao, P.; Chai, H. *Ceram. Int.* **2011**, *37*, 1825.
31. Zhang, H. Y.; Tehrany, E. A.; Kahn, C. J. F.; Ponçot, M.; Linder, M.; Cleymand, F. *Carbohydr. Polym.* **2012**, *88*, 618.
32. Tuhin, M. O.; Rahman, N.; Haque, M. E.; Khan, R. A.; Dafader, N. C.; Islam, R.; Nurnabi, M.; Tonny, W. *Radiat. Phys. Chem.* **2003**, *81*, 1659.
33. Xia, Z.; Calderon-Colona, X.; Trexlera, M.; Elisseeff, J.; Guo, Q. *Thermochim. Acta* **2006**, *527*, 172.
34. Costa, P.; Lobo, J. M. S. *Eur. J. Pharm. Sci.* **2001**, *13*, 123.
35. Siepmann, J.; Peppas, N. A. *Adv. Drug Delivery Rev.* **2001**, *48*, 139.
36. Peppas, N. A. *Pharm. Acta Helvetiae* **1985**, *60*, 110.
37. Wang, Q.; Wang, W.; Wu, J.; Wang, A. *J. Appl. Polym. Sci.* **2012**, *124*, 4424.
38. Domaratzki, R. E.; Ghanem, A. *J. Appl. Polym. Sci.*, to appear..
39. Jalali, M. B.; Adibkia, K.; Valizadeh, H.; Shadbad, M. R. S.; Nokhodchi, A.; Omidi, Y.; Mohammadi, G.; Nezhadi, S. H.; Hasan, M. *J. Pharm. Pharm. Sci.* **2010**, *1*, 167.
40. Jose, S.; Fangueiro, J. F.; Smitha, J.; Cinu, T. A.; Chacko, A. J.; Premaletha, K.; Souto, E. B. *Eur. J. Med. Chem.* **2013**, *60*, 249.
41. Li-Fang, F.; Wei, H.; Yong-Zhen, C.; Bai, X.; Qing, D.; Feng, W.; Min, Q.; De-Ying, C. *J. Pharm.* **2009**, *375*, 8.
42. Wei, X.; Suna, N.; Wu, B.; Yin, C.; Wu, W. *Int. J. Pharm.* **2006**, *318*, 132.
43. Guo, Y.; Yao, Y.; Guo, Y.; Ning, C. *Micropor. Mesopor. Mater.* **2012**, *155*, 245.
44. Jayakumar, R.; Reis, R. L.; Mano, J. F. *J. Bioact. Compat. Polym.* **2006**, *21*, 327.