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# Development of a system to adsorb drugs onto calcium phosphate materials

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Several studies were carried out in order to reduce the systemic use of antibiotics due to the high concentration required to provide the minimum inhibitory concentration (MIC) at infected sites. The aim of this study was to develop a system of drug adsorption onto commercial hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) and glass reinforced hydroxyapatite (GR-HA) granules. The drug will then be released for the local treatment of periodontitis. The antibiotics used in this study were metronidazole, a specific antibiotic indicated for the systemic treatment of periodontitis, and ampicillin, a wide spectrum antibiotic. UV spectroscopy was used to measure the amount of drug adsorbed onto HA and GR-HA granules. Results showed that metronidazole was unable to adsorb on the material's surface, as opposed to ampicillin which adsorbed both onto HA and GR-HA. Preliminary release kinetics studies were carried out using a flow through dissolution system. Results are discussed in terms of the influence of the different surface characteristics of the materials on the adsorption processes.


## 1. Introduction

Periodontal disease damages the supporting structures of teeth, namely the periodontal ligament, cementum, alveolar bone and various components of the gingival tissue [1]. This disease is a consequence of an interaction of bacterial plaque and its products with the host's inflammatory responses. Thus, infection and immunological changes are features of periodontal disease. When these changes are confined to the gingival tissues, the condition is referred to as gingivitis. The progression of an established gingivitis to the advanced lesion heralds the onset of periodontitis—an inflammatory disease of the periodontal tissue. This occurs when the inflammatory changes result in the rupture of the connective tissue attachment and apical migration of the junctional epithelium [1–3], which can result in gingival recession or pocket formation, alveolar bone loss, and an increase in tooth mobility. The formation of a periodontal pocket allows plaque to colonize the root surface and the layer of cementum. The pocket environment facilitates the growth of anaerobic microorganisms, and some of those bacterial types have been designated as 'periodontopathogens'. These bacteria have been indicated as being present in the destructive phases of periodontitis.

A specific micro flora has been detected in adult patients with periodontitis. Species commonly identified in such cases include *Porphymonas Gingivalis*, *Bacteria Gingivalis*, *Intermidius* and *Forsythus*, *Actinobacillus Actinomycetemcomitans*, *Selonomous sputigen*, *Eikella Corrodens* and *Spirochetes* [4, 5]. *In vitro* studies have shown that many of these bacteria can produce a variety of enzymes and toxins which can interfere with many cellular functions, notably, inhibiting the normal defence mechanisms in the pocket, inactivating antibodies and preventing phagocytosis [1, 2].

Antibiotics such as metronidazole, tetracycline, amoxicillin and clavulanic acid have been used in the systemic and local treatment of periodontitis [4, 6–9]. Due to the emerging resistance among oral and medial pathogens to common antibiotics, a restrictive and conservative use of systemic antibiotic therapy in periodontitis has been indicated. Thus, new local delivery systems could be alternatives to polymer fibers [3, 10, 11], as the polymer fibers are used for local delivery of antibiotics, as implant materials working as cementum substitutes [3, 10].

There are different approaches to loading drugs onto carrier materials, to be used as local drug delivery systems in periodontitis treatment. Usually in the

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treatment of this pathology there are two regular approaches to local drug delivery. One consists of the usage of hollow fibers filled with the drug and the other consists of polymer strips impregnated with unknown amounts of drug, neither of them providing well controlled delivery conditions. Therefore, there is a need for a system that might well regulate the adsorption and release of specific antibiotics, without affecting the stability of the drug, and enabling its full release, i.e., avoiding chemisorption.

Hydroxyapatite is a well studied ceramic material that is known for its reasonable mechanical behaviour under low compressive load conditions, and excellent biocompatibility, and it is commonly used as coatings for hip prostheses, as well as for artificial roots [12]. The glass reinforced hydroxyapatite used in this work has been extensively studied and has proved to induce osteointegration [13–15] and is being studied for local drug delivery [16].

The aim of this study consisted in attempting to use HA and GR-HA as a material enabling the adsorption of an antibiotic to be used as a local drug delivery system, associating the drug release capability for the periodontitis treatment with the possibility of simultaneously initiating the process of osteoregeneration.

## 2. Materials and methods

### 2.1. Materials preparation

The materials used in this study were commercial HA (from Plasma Biotal, ref. P120 powder) and GR-HA granules. The preparation method for HA [17] and GR-HA [18] has been previously described. A glass of the $P_2O_5$-CaO system ($75P_2O_5$, 15CaO, $10CaF_2$ mol%) was prepared, using reagent grade chemicals ($Ca(H_2PO_4)_2 \cdot H_2O$; $CaF_2$; $P_2O_5$), heated at 1350 °C for 1 h and cast into water. The glass was dried for 24 h in a oven at 100 °C, ball milled and sieved till achieving a particle size distribution of 90% $<75$ $\mu$m. HA was also sieved to the approximately same particle size.

GR-HA was obtained with 2.5, 5.0, 7.5, and 10.0 wt% of glass addition to HA powder. The mixture was wet milled, for 8 h, with methanol as a suspending medium, dried for 24 h in a oven at 100 °C and sieved to a particle size $<75$ $\mu$m, in order to obtain a free flowing powder.

HA and GR-HA were uniaxially pressed to cylindrical samples (0.5 g into 16 mm diameter mould) at 288 MPa. The green compacts of HA were sintered for 1 h at 1150 °C (HA1), 1200 °C (HA2), 1250 °C (HA3) and 1300 °C (HA4), at a heating rate of 4 °C/min, followed by natural cooling inside the furnace. After testing HA samples at different temperatures only one sintering temperature was chosen for GR-HA, i.e., 1200 °C, following the same sintering cycle. Samples were milled and sieved in order to obtain both 75–106 $\mu$m or 250–850 $\mu$m granules, with the latter being easier to manipulate. This range of granule diameter allows for the occurrence of different sizes, improving the capacity to fill the periodontal pocket and maximising the amount of antibiotic to be released.

Fourier transformed infrared (FT-IR) analysis was carried out to chemically characterise HA and GR-HA. Scanning electron microscopy (SEM) was also performed to evaluate grain size and geometry. Porosity of HA samples was measured by the Archimedes method.

### 2.2. Adsorption studies

The adsorption kinetics of a drug onto a material's surface is generally a complex issue. To simplify the system the first drug used in this study was metronidazole, since the other possible therapeutic systems usually contained either two components or tetracycline, which is known to complex with calcium existing in HA [19, 20]. As later explained, due to its simplicity and easy adaptation to the adsorption process, sodium ampicillin was chosen as a model.

In order to study the adsorption kinetics of metronidazole, it was necessary to establish a system capable of assessing the content of metronidazole present either on the material's surface or remaining in solution. The simplest method consisted of measuring the absorbance of metronidazole in solution by UV spectroscopy. A calibration curve was firstly obtained and its use restricted to the linear part, which followed Beer's law (Equation 1), so that the concentration of metronidazole in solution could be determined from the absorbance obtained by UV spectroscopy.

$$A = acl \tag{1}$$

where $A$ is the absorbance, $c$ the concentration, $a$ is a proportionality constant related to the solution being tested (known as absorptivity) and $l$ is the pathlength, which is constant, and in this case equal to 1 [21].

Metronidazole was studied both in aqueous and in hexane solutions (polar and apolar solvent, respectively). The solutions were centrifuged at 4500 rpm for 5 min and the supernatant was removed for analysis. After studying the adsorption of metronidazole, ampicillin adsorption studies were also carried out, following the same technique with aqueous solution, as ampicillin is not soluble in apolar solvents.

Three different set-ups were prepared in order to study the adsorption kinetics of the antibiotics onto the materials. The first consisted of a heater, a contact thermometer, a heated water bath, a magnetic stirrer and a flask with the testing solution and HA or GR-HA sample, assembled as indicated in Fig. 1.

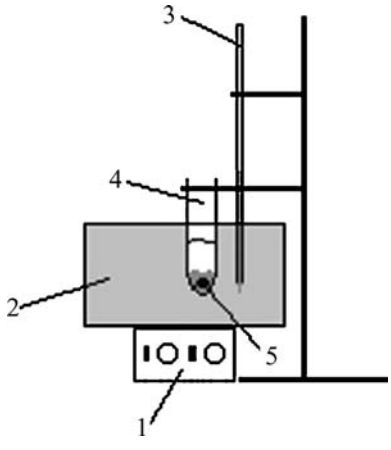


*Figure 1* Adsorption set-up I. 1. Heater; 2. Water bath; 3. Contact thermometer; 4. Flask; 5. Magnetic stirrer.

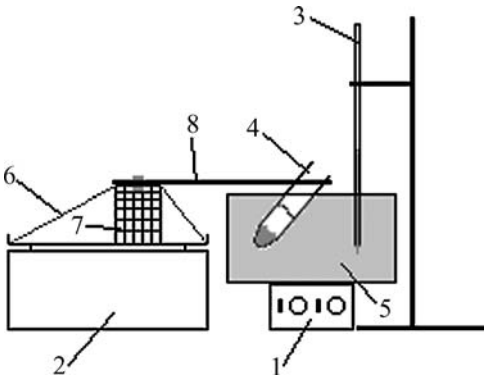


*Figure 2* Adsorption set-up II. 1. Heater; 2. Stirrer; 3. Contact thermometer; 4. Flask; 5. Water bath; 6. Rubber band; 7. Stainless steel support for the cantilever; 8. Cantilever.

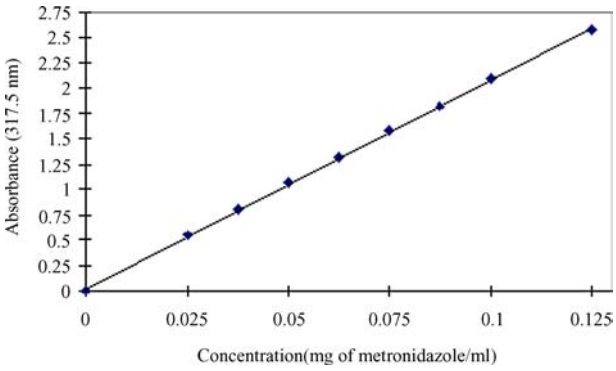


*Figure 3* Metronidazole calibration curve.

The heater and contact thermometer were set to 25 °C, and the solutions were stirred at 200 rpm. Both metronidazole and ampicillin solutions were used at different concentrations (0.10 mg/ml in both cases and also 5.00 mg/ml for metronidazole). The adsorption times for the two antibiotics varied, ranging from 10 to 870 min for metronidazole and from 15 to 1725 min for ampicillin, as the solutions were left in contact with the materials until no change was noticed in the amount of drug in the supernatant. The adsorption studies were performed using HA1 granules (75–106 $\mu$m), as a model, at a ratio of 0.5 g of material to 5 ml of solution. In the second set-up the magnetic stirrer was removed and a new stirring scheme was used, as shown in Fig. 2, including an external vibration stirrer, onto which the flask was fixed through a cantilever.

The thermometer was initially set for 25 °C and later for 37 °C, as it is normal body temperature. More gentle agitation of approximately 50 rpm was used. With this set-up only ampicillin solutions (0.10 mg/ml) were used. The ampicillin was adsorbed onto HA1, HA2 and HA3 in the same ratios as in the first set-up.

In the third set-up, an incubator, thermostated at 37 °C, with continuous agitation at 250 rpm was used. A wider range of ampicillin solutions, from 0.10 to 10.00 mg/ml, were used. In order to limit the amount of samples studied, only HA2 and GR-HA were used in the larger granular form, and similar solid/liquid ratios were used and equal to 0.5 g of material/5 ml of solution.

## 3. Results and discussion

### 3.1. Adsorbed antibiotic measurements

The adsorption of metronidazole was firstly studied in aqueous solution. An UV spectrum of this solution was drawn, from 200 to 1100 nm, in order to determine the absorbance peak, which was found to occur at 317.5 nm. A calibration curve according to Beer-Lambert law was obtained so that the concentration of antibiotic in solution could be determined; for each concentration three samples were collected. The results were fitted to the linear part of the curve (Fig. 3), which is described by the equation

$$A = 20.677c - 0.0188 \qquad (2)$$

and a correlation factor of 0.998 was found. All the concentration results were based on this calibration curve.

After extensive experimentation with metronidazole in aqueous solution, it was realized that it wouldn't adsorb onto neither HA nor GR-HA. Therefore, an organic solvent, hexane, was used, and a new spectrum was drawn (absorbance peak 245 nm). As water is a polar solvent and hexane is an apolar one, the solutions could exhibit different adsorption processes on the material's surface, and thus have different functional groups available for the adsorption process. The maximum concentration of metronidazole in hexane was found to be 1 mg/ml, which corresponds to a maximum absorbance of 0.076. This solution was found to be unstable if stored for more than 24 h, even at 4 °C. Metronidazole studies were discontinued as the adsorption behaviour was not the expected one, once that metronidazole did not adsorb to HA, both in the presence of polar or apolar solvents. The functional groups available from metronidazole were the hydroxyl groups ($OH^-$), and the positive bridging sites of HA and GR-HA ($Ca^{2+}$) are more difficult to obtain than the negative ones as ($OH^-$) and ($PO_4^{3-}$). There would also be a competition of the hydroxyl groups of metronidazole and hydroxyapatite and this would induce an exchange of the total groups and originate a chemical change of metronidazole, losing it's antibiotic activity.

A new UV spectrum was drawn for sodium ampicillin aqueous solution (1 mg of ampicillin/1 ml deionised water) and the absorbance peak was found to be at 230 nm. A calibration curve was made and adjusted to Beer-Lambert law with a correlation factor of 0.9998, with a range of sodium ampicillin concentrations from 0 to 0.1 mg/ml [16]. As for metronidazole, three samples were used for each concentration. In order to understand if sodium ampicillin formed complexes with HA or GR-HA, FT-IR studies were carried out (Fig. 4). No such behaviour was found as the transmittance characteristic peaks for HA overlap those shown for HA with adsorbed sodium ampicillin. If complexes were to appear, either a change in the hydroxyl group (3571 and 632 $cm^{-1}$) vibrations, or a slight dislocation of the phosphate groups (1090, 1043, 962, 602, 570 and 473 $cm^{-1}$) should be observed. This procedure was carried out to make sure that, as opposed to tetracycline, sodium ampicillin did not complex with HA.

A number of different adsorption set-ups were tried, deriving from the attempt to minimize experimental

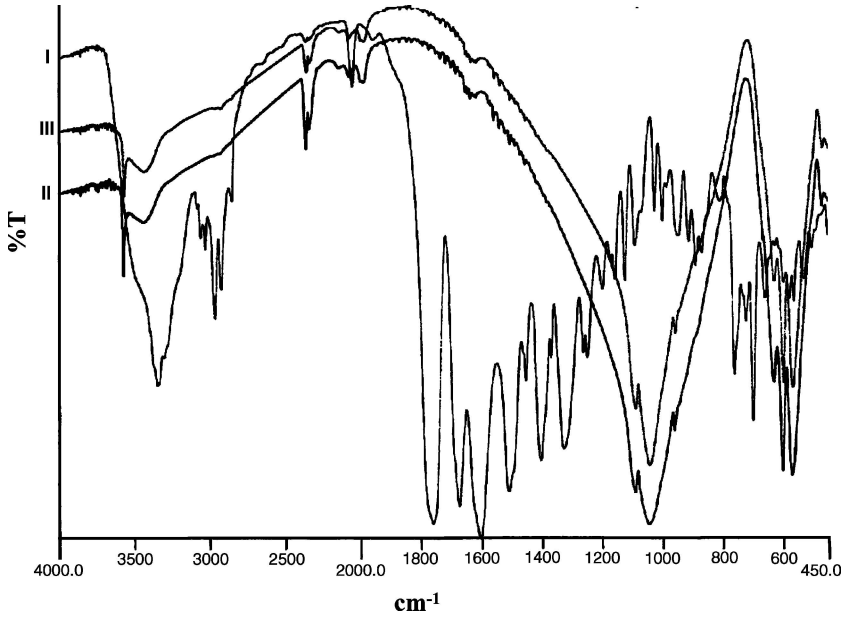


*Figure 4* FTIR Spectra of Sodium ampicillin (I); HA (II) and HA after adsorbing sodium ampicillin (III).

errors in antibiotics adsorption onto HA and GR-HA. A non-agitated medium was not an option, as the concentration gradients generated in this situation would undoubtly introduce errors in the adsorption process and analysis.

The first set-up used included a magnetic stirrer that was in contact both with the adsorbing material and the solution (as seen in Fig. 1). The results obtained by this method were not very encouraging; in fact, there seemed to exist no adsorption of metronidazole onto HA1 (Fig. 5(a)). The results with methanol solution were similar to those obtained with aqueous solutions. Several other tests were performed in order to explain the reason for the non-linear behaviour of metronidazole adsorption curve, as the amount of metronidazole in solution was constantly varying. To understand this behaviour and test if either the stirrer or the flasks were adsorbing metronidazole, a metronidazole solution was put in contact with the magnetic stirrer, polyethylene (PE) flasks and glass flasks for 24 h, which was the maximum time for the adsorption tests. The stirrer was then removed and placed in a glass flask with distilled water for 2 h. For the PE flasks, after the 24 h, the metronidazole solution was replaced by distilled water and stood in contact for the same time length. From these tests it was noticed that metronidazole was adsorbing to the stirrer and to the PE flasks, and not to HA.

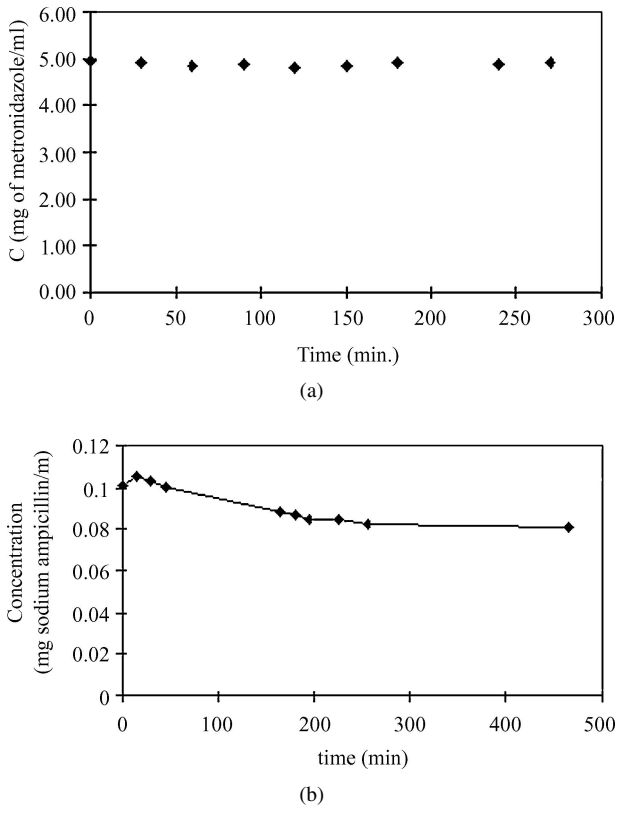


*Figure 5* Adsorption results. (a) metronidazole; (b) sodium ampicillin in set-up I.

An alternative drug was then chosen, to be able to be adsorbed onto both HA and GR-HA materials. Sodium ampicillin was chosen as a model, since it is a simple drug, with a comparable molecular structure to that of amoxicillin. It is a wide spectrum antibiotic, although not specific for the periodontitis treatment. It was, however, a good candidate for the studies on the adsorption behaviour, as its structure, with the sodium and hydrogen atoms placed in potentially bridging sites, would indicate eventual ability to adsorb onto the desired materials, in which the groups that could potentially bind would be $OH^-$. The adsorption tests onto the stirrer and to the PE and glass flasks were performed indicating that the magnetic stirrer adsorbed ampicillin (Fig. 5(b)) in a total amount of 0.013 mg/ml and that the PE flasks also adsorbed it (0.010 mg/ml). These facts led to the evolution towards adsorption set-up II, where 30 mm diameter glass flasks and external stirring system (cantilever) were used. The adsorption behaviour of the samples in this set-up was as expected, but the fact that only one sample could be adsorbing at the time, led to the search for a new, more stable set-up, where more samples could be tested simultaneously.

An incubator with continuous agitation was used as adsorption set-up III. In this case, 30 samples could be used at the same time, with continuous agitation rate of 250 rpm (sufficient for intense non-turbulent agitation), and at a stabilized temperature of 37 ± 0.5 °C (body temperature).

### 3.2. Adsorption results

The first results discussed in this section are the ones referring to the adsorption set-up II, as the results obtained by set-up I, as described previously, were not reproducible. The results from this set-up were used to estimate the time length needed for the solution to be in contact with the material until an equilibrium of adsorbed sodium ampicillin/sodium ampicillin in solution was reached. Different equilibrium times were obtained for different material sintering temperatures. That can be explained by the higher microporosity of the materials sintered at lower temperature (Table I) and thus a greater adsorption time induced a higher amount of adsorbed sodium ampicillin for lower sintering temperature (Fig. 6).

In order to standardize the assays, the highest adsorption time was used to test the materials adsorption with set-up III.

As in set-up III several tests could be carried out at the same time, new sodium ampicillin/material ratios were used. GR-HA was also used. Thus, it was necessary to repeat the equilibrium tests, and a 7 h adsorption time was found to be convenient for all the materials.

The amount of sodium ampicillin adsorbed varied with the initial solution concentration, indicating that perhaps the adsorption sites available at the material's surface were not being totally occupied and that the adsorption equilibrium was the controlling step. This assumption was adequate for solutions up to 10 mg/ml. Above this concentration the amount of sodium ampicillin adsorbed was the same for all the solutions used (up to 20 mg/ml), proving that for the 10 mg/ml solution all the potential material adsorption sites had been occupied and, consequently 10 mg/ml was the highest solution concentration used in these tests.

It was also noticed that HA adsorbed more than GR-HA and that, for the GR-HA, the higher the amount of glass present, the higher was the adsorption, as previously discussed [16].

TABLE I Porosity HA samples obtained by the Arquimedes method

| | HA1 | HA2 | HA3 | HA4 |
|---|---|---|---|---|
| Porosity | 24.11 ± 0.51 | 16.99 ± 0.78 | 6.12 ± 0.38 | 6.07 ± 0.43 |

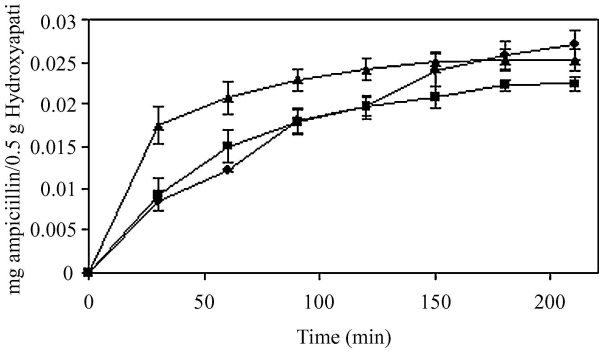


*Figure 6* Adsorption of ampicillin to hydroxyapatite sintered at 1200 °C (■), 1150 °C (▲), and 1100 °C (♦), for set-up II.

## 4. Conclusion

Effort has been carried out to develop a system that enables the adsorption and release of drugs, applicable to periodontitis treatment, based on a novel ceramic carrier that would be able to physically adsorb the drug. This should promote drug release in sufficient amounts at the implantation site to destroy the anaerobic bacterial population characteristic of this disease, and that might last further to enable osteoconduction, promote bone formation and site recovery.

From all the experimental set-ups studied the one with more stability and better adsorption results was adsorption set-up III. Thus, set-up has been carried out I is the one to be used in future for further adsorption studies. Although sodium ampicillin, by itself cannot be used for the treatment of periodontitis, unlike metronidazole, it may be used as an adequate model. Due to its resemblance with amoxicillin, it gives a good indication as to what the behaviour of the latter would be.

The adsorption behaviour of sodium ampicillin onto the material samples varies according to the composition and morphology of the adsorbing material (HA or GR-HA), to the concentration of ampicillin in the initial solution and to the time of contact with the solution, until equilibrium or a total occupation of the adsorption sites is achieved. These results indicate that, the amount of adsorbed sodium ampicillin onto HA or GR-HA samples can be controlled by applying different test conditions.

Ongoing work includes *in vitro* studies to simulate the oral fluid renewal rate in periodontal pockets.

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## References

1. R. A. SEYMOUR, P. A. HEASMAN and I. D. M. MACGREGOR, in "Drugs, Diseases, and the Periodontium," edited by R. A. Seymour and P. A. Heasman (Oxford University Press, Oxford, 1992) p. 1.
2. R. A. CARNEVALE, G. W. GRECO, C. M. BULLARD and J. S. HANKER, in "Biomedical Materials and Devices," edited by J. S. Hanker and B. L. Giammara (Materials Research Society, Pittsburgh, 1987) p. 247.
3. M. FRIEDMAN and D. STEINBERG, *Pharm. Res.* **7** (1990) 313.
4. S. HAGIWARA, N. TAKAMATSU, Y. TOMINAGA and M. UMEDA, *J. Period.* **69** (1998) 62.
5. T. L. WRIGHT, R. P. ELLEN, J. M. LACROIX, S. SINNADURAI and M. W. MITTELMAN, *J. Period. Res.* **32** (1997) 473.

6. G. GOLOMB, M. FRIEDMAN, A. SOSKOLNE, A. STABHOLZ and M. N SELA, *J. Dent. Res.* **63** (1984) 1149.
7. J. M. GOODSON, P. E. HOGAN and S. L. DUNHAM, *J. Period.* **55** (1984) 81.
8. B. V. SOMAYAJI, U. JARIWALA, P. JAYACHANDRAN, K. VIDYALAKSHIM and R. V. DUDHANI, *ibid.* **69** (1998) 409.
9. K. STOLTZE, *J. Clin. Period.* **19** (1992) 698.
10. J. M. GOODSON, D. HOLBOROW, R. L. DUNN, P. HOGAN and S. DUNHAM, *J. Period.* **54** (1983) 575.
11. D. YU, J. WONG, Y. MATSUDA, J. L. FOX, W. I. HIGUCHI and M. OTSUKA, *J. Pharm. Sci.* **81** (1992) 529.
12. K. NISHIHARA, *Clin. Mater.* **12** (1993) 159.
13. M. P. FERRAZ, F. J. MONTEIRO and J. D. SANTOS, *J. Biomed. Mat. Res.* **45** (1999) 376.
14. M. P. FERRAZ, J. C. KNOWLES, I. OLSEN, F. J. MONTEIRO and J. D. SANTOS, *ibid.* **47** (1999) 603.
15. M. A. LOPES, J. D. SANTOS, F. J. MONTEIRO, C. OHTSUKI, A. OSAKA, S. KANEKO and H. INOUE, *ibid.* **54** (2001) 463.
16. A. C. QUEIROZ, J. D. SANTOS, F. J. MONTEIRO, I. R. GIBSON and J. C. KNOWLES, *Biomaterials* **22** (2001) 1393.
17. J. D. SANTOS, J. C. KNOWLES, R. L. REIS, F. J. MONTEIRO and G. W. HASTINGS, *ibid.* **15** (1994) 5.
18. J. C. KNOWLES, J. D. SANTOS and G. W. HASTINGS, World Patent, WO0068164 (1994).
19. H. M. MYERS, H. J. TOCHON-DANGUY and C. A. BAUD, *Calcif. Tissue Int.* **35** (1983) 745.
20. A. RATIER, I. R. GIBSON, S. M. BEST, M. FRECHE, J. L. LACOUT and F. RODRIGUES, *Biomaterials* **22** (2001) 897.
21. D. J. ANDO, in "Ultraviolet and Visible Spectroscopy" (John Wiley & Sons, West Sussex, 1996) p. 16.

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