J Mater Sci: Mater Med (2006) 17: 413–420
DOI 10.1007/s10856-006-8468-7

Characterization, bioactivity and ampicillin release kinetics of TiO_2 and TiO_24SiO_2 synthesized by sol-gel processing

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Received: 16 September 2004 / Accepted: 7 July 2005

Abstract Local drug delivery of antimicrobics by sustained release delivery system can be used to treat periodontal disease. Advantages of these systems may include maintaining high levels of antibiotic in the gingival crevicular fluid for a sustained period of time and ease of use with high patient acceptance. The materials used are TiO_2 and TiO_24SiO_2, mixed with sodium ampicillin, a broad-spectrum antibiotic, have been synthesized by sol-gel method. The amorphous nature of the gels was ascertained by X-ray diffraction analysis. Release kinetics in a simulated body fluid (SBF) have been subsequently investigated. The amount of sodium ampicillin released has been detected by UV-VIS spectroscopy and SEM. The release kinetics seems to occur in more than one stage. HPLC analysis has also been taken to ensure the integrity of ampicillin after the synthetic treatment. Finally, SEM micrographs and EDS analysis showed the formation of a hydroxyapatite layer on the surface of the samples soaked in SBF. Both the materials showed good release and could be used as drug delivery bioactive systems. High antimicrobial effects of samples against *Escherichia coli* and *Streptococcus mutants* were found.

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1. Introduction

Periodontitis is a group of dentoalveolar infection that remains one of the major causes of adult tooth loss. About 36.8% of adult Americans are estimated to have this disease [1]. These infections are caused by a pathogenic flora established within the gingival sulcus, which later deepens to become a periodontal pocket. The bacterial microflora deep within the periodontal pocket differs significantly from that of the supragingival environment in that it contains more anaerobes, more Gram-negative organisms and a greater proportion of motile species [2–9]. Because these bacteria are indigenous to the oral microbiota, their elimination may be difficult, and the likelihood of repopulation of the periodontal pockets and recurrence of infection following the administration of therapy is high [10]. However, the systemic route of antibiotic administration may not be ideal because of the concern over the development of bacterial resilience that may be induced over long periods of time [11, 12]. Systemic antibiotic therapy over a long period of time also raises the risk of undesirable side effects such as nausea, diarrhea, fever, abdominal pain and pseudomembranous colitis. The local delivery of antimicrobial therapy to periodontal pockets has the benefit of putting more drug at the target site while minimizing exposure of the total body to the drug [13]. Controlled, localized drug release offers several advantages over other delivery options. Plasma concentrations of drugs administered via injection, inhalation or ingestion, methods which can require repeated and relatively greater dosing and patient compliance, reflect the typical kinetics of an inefficient bolus delivery. Controlled local release systems provide the desired constant drug concentrations at the delivery site, lower systemic drug levels and a reduced potential for deleterious side effects. In this study TiO_2 and TiO_24SiO_2materialsare used as support matrices for controlled drug release,which could

be used for implantation in bone tissue, in particular for dental applications. Local drug release in the implant material for medical applications appears to be a very interesting alternative to the systemic therapy. The possibility of introducing drug release systems into the implant site has been widely studied and used. These include localized delivery of the drug to a particular part of the body, assurance of treatment continuity in the nocturnal phase, drug stability, reduced need for follow-up care and optimized drug absorption.

Titanium has been extensively used as an implant material in different medical applications for over 30 years due to their excellent mechanical properties, high corrosion resistance and chemical stability. However, it is still thought as a bio-inert material. The combination of good biocompatibility of hydroxyapatite and excellent mechanical properties of titanium is a candidate way to expand the biomedical application. The materials are mixed with Ampicillin, a β-lactam antibiotic that is active against both gram-positive and gram-negative bacteria and is widely used for the treatment of infections [14]. Most drugs can be administrated by a variety of routes, broadly defined as local and systemic [15].

As the systemic use of antibiotics may cause several side effects (sensitivity, resistant strains, super infections) the local administration of antibiotics has received considerable attention [16].

The effective use of antibacterial agent for treatment of periodontal diseases requires an adequate drug concentration at the site of action, and a means of maintaining that level for a long enough period to allow the agent to act [17]. Placing ampicillin directly into $TiO_2$and TiO_24SiO_2, which are used as biomedical materials, leads the local antibiotic administration to prevent and treat bone infection in orthopedic surgery [18]. The use of ceramic materials as carriers for drug release has been also extensively reported in the literature for many biomedical applications [18, 19]. However, there are only a few studies about filling bone materials showing simultaneously controlled drug release and bioactive behaviour [20]. Actually, it seems to be a very attractive idea to look for materials that could release an antibiotic in a local and controlled way while showing bioactive properties [21–23]. These materials would prevent infections and also would ensure the bone integration and regeneration [24, 25].

The aim of this work is to study the antibiotic release kinetics from amorphous bioactive materials containing sodium ampicillin that have been synthesized by a sol-gel method.

2. Experimental

2.1. Sol-gel synthesis

TiO_2 and TiO_24SiO_2, amorphous materials, all mixed with sodium ampicillin (Sigma-Aldrich) were prepared by means of a sol-gel method, using analytical reagent grade Titanium(IV)butoxide (TBT) (Sigma-Aldrich) and tetramethylortosilicate (TMOS) (Sigma-Aldrich), as precursors materials. Figure 1 shows the flow chart of TiO_24SiO_2, gel synthesis. The flow chart of TiO_2gel, is similar. TBT and TMOS hydrolisis were carried out by using CH_3COOH as catalyst, which were mixed with a solution of H_2O/EtOH/AMP5%. After the addition of each reactant the solution was stirred for 2 min for TiO_2 and 2h for TiO_24SiO_2 and the resulting sol were uniform and homogeneous. The time of gelification was a few minutes for TiO_2 and 1 day for TiO_24SiO_2. After gelation, the gels were dried at room temperature or at 60°C in air for 24 h to remove the residual solvent; this treatment does not modify the stability of ampicillin and small glassy pieces were obtained. Discs with a diameter of 13 mm and a thickness of 2 mm were obtained by pressing fine (<125 μm) gel powders into a cylindrical holder.

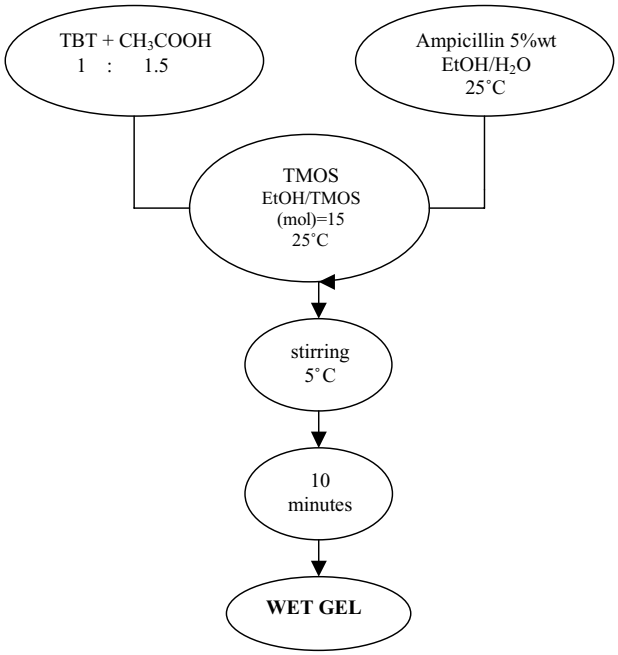


Fig. 1 Flow chart of TiO_24SiO_2 production with ampicillin incorporation.

2.2. Study of *in vitro* release

For the study of ampicillin release, the discs of the investigate materials were soaked in 15 ml of SBF, continuously stirred, at 37°C. The SBF was previously filtered with a Millipore (0.22 μm) system, avoiding bacterial contamination. Ampicillin release measurements were carried out by means of UV-VIS spectroscopy with a Shimadzu UV mini-1240. Absorbance values were taken at a wavelength λ = 197 nm, at which sodium ampicillin, in SBF, shows an absorbance maximum.

Calibration curve, was determined by taking absorbance vs sodium ampicillin concentration between 0 and 3 mM as parameters. For this interval, the calibration curve fits the Lambert and Beers' law [26]:

$$A = 1.265 \times C,$$

where A is the absorbance and C is the concentration (mM).

2.3. HPLC apparatus

Chromatographic experiments were carried out on a Shimadzu HPLC system, equipped with a Class-VP 5.0 software, an UV spectrophotometric detector SPD-10AVvp and two pumps LC-10ADvp, with low-pressure gradient systems. Samples of solutions were injected by a syringe via a Rheodyne loop injector. The loop volume was 20μl. The analytical column was a Phenomenex C_{18} (150 × 4.60 mm; 5μ). Flow rate of the mobile phase A (water) was set at 0.8 ml/min and that of the mobile phase B (methanol) was set at 0.2 ml/min. The total run time was 10 minutes.

HPLC grade methanol was obtained by Sigma-Aldrich. HPLC grade water was prepared using a Millipore (0.22μm) system.

A standard solution of ampicillin 3 mM in SBF is prepared and the samples were taken at the end of the release from the materials.

2.4. Study of *in vitro* bioactivity

In order to study their bioactivity, the discs of the studied materials, were soaked in an SBF with ion concentrations (Na^+ 142.0, K^+ 5.0, Ca^{2+} 2.5, Mg^{2+} 1.5, Cl^- 147.8, HCO_3^- 4.2, HPO_4^{2-} 1.0, SO_4^{2-} 0.5mM) nearly equal to those in human blood plasma at 37°C [23]. The SBF was prepared by dissolving reagent grade chemicals NaCl, $NaHCO_3$, KCl, $MgCl_2$, HCl 1M, $CaCl_2 \bullet 6H_2O$, Na_2SO_4 (Sigma-Aldrich) in ultra-pure water and buffered at pH 7.4 using tris(hydroxymethyl)-aminoethane (Sigma-Aldrich) and 1M HCl at 37°C. After an immersion period of 7, 14 and 21 days, the materials were removed from the SBF, gently washed with ultra-pure water, and dried at 40°C. The ability to form an apatite layer was studied by submitting reacted samples to scanning electron microscopy (SEM) and EDS microscopy.

3. Results and discussion

3.1. X-ray diffraction analysis

After gelation the gels were dried by air and small glassy pieces were obtained, Fig. 2. The X-ray diffraction patterns, TiO_2+ampicillin 5% wt or $TiO_2 4SiO_2$+ ampicillin 5% wt, dried gels showed in Figs. 3(a) and 3(b) respectively, exhibit broad humps characteristic of their amorphous nature, while the X-ray diffraction pattern, Fig. 4, of the ampicillin is crystalline. The absence in both systems of diffraction peaks due to ampicillin indicates that this component appears to be in an amorphous form or in the form of very small crystallites not detectable by XRD.

3.2. Release kinetics

Kinetic measurements of ampicillin release from the studied materials were carried out in 15.0 ml of SBF at 37°C. In order to establish the relationship between the UV

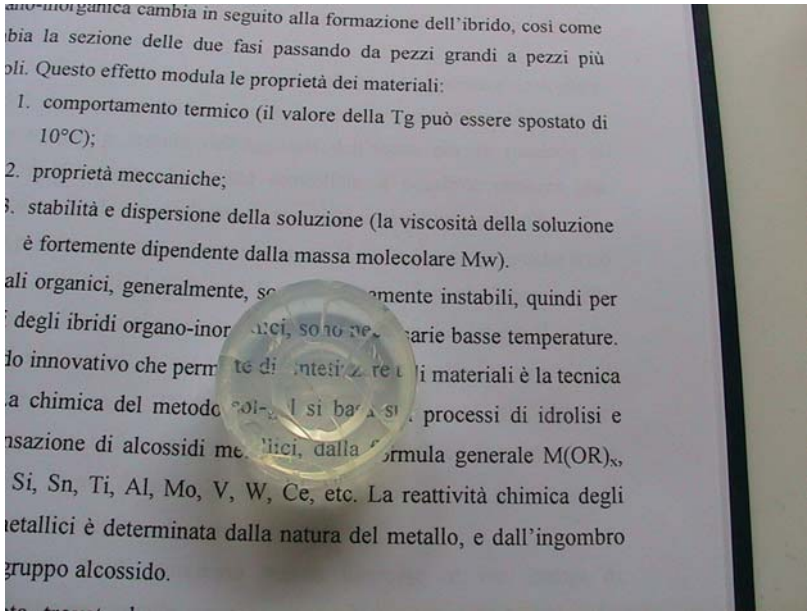


Fig. 2 Samples of $TiO_2 4SiO_2$ with ampicillin incorporated after drying.

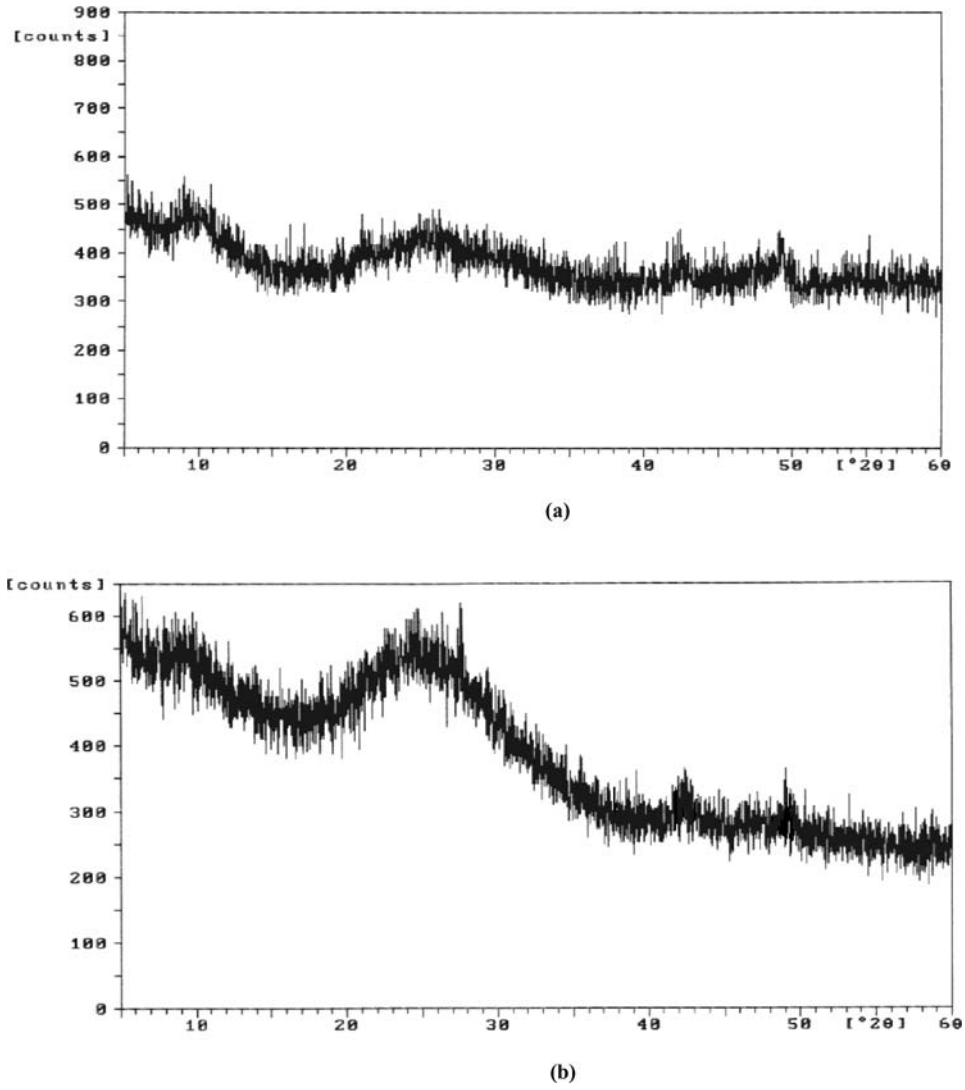


Fig. 3 X-ray diffraction pattern showing amorphous nature of samples of (a) TiO_2, and (b) TiO_24SiO_2.

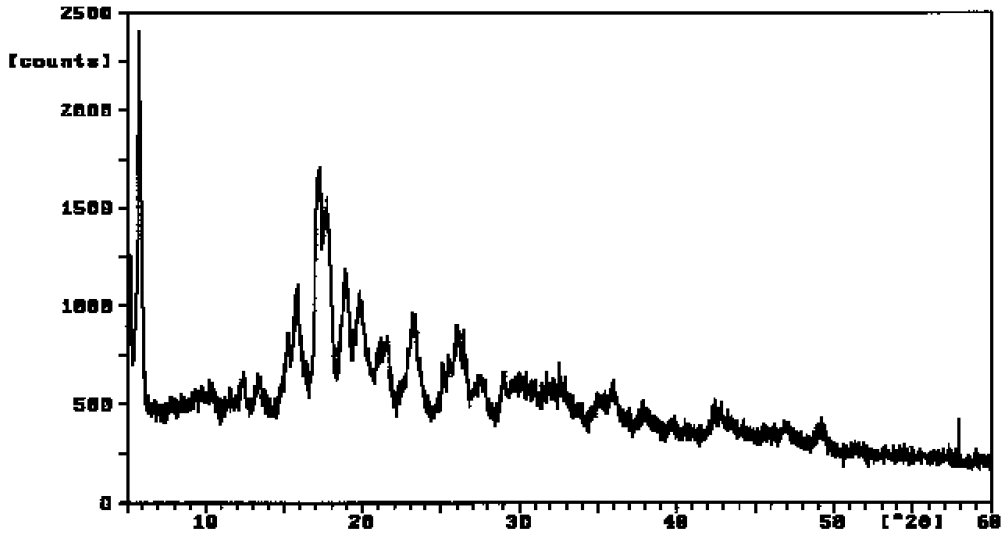


Fig. 4 X-ray diffraction pattern showing the crystalline nature of ampicillin.

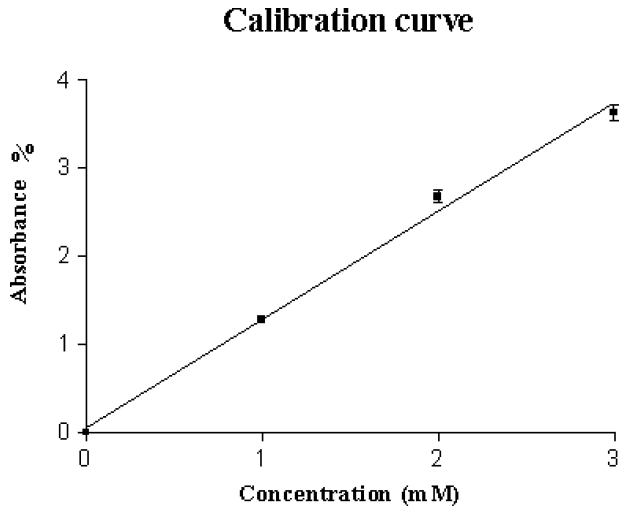


Fig. 5 Calibration curve UV absorbance of sodium ampicillin (r^2 = 0.9907)

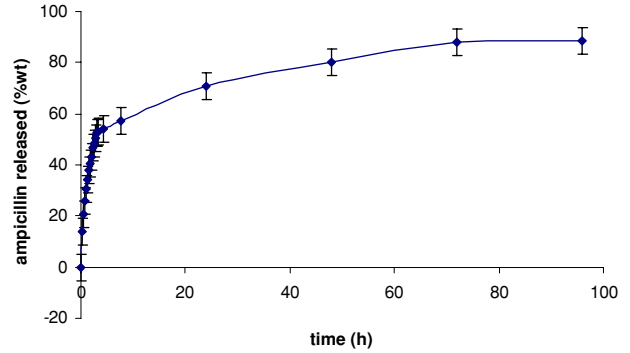


Fig. 6 Time-dependent drug release plot for TiO_2 at 37°C in SBF solution.

absorbance of sodium ampicillin, at 197 nm, and the concentration of the sodium ampicillin solution, a calibration curve ($r^2 = 0.9907$), Fig. 5, was drawn for standard solutions of sodium ampicillin with 4 levels of concentration: 0.0 mM, 1.0 mM, 2.0 mM and 3.0 mM. All standard solutions were prepared in SBF.

Figures 6, 8 show the cumulative percentage of ampicillin released over time from TiO_2 and TiO_24SiO_2 gels. It was observed that from TiO_2 gel, the ampicillin was released in a relatively fast manner during the initial 6 h (ca 50%wt) and then in a controlled manner during the rest of the experimental period, while ampicillin release from $TiO_24SiO_2$is slower and it is complete in about a week. The differences observed in the release behaviour of the ampicillin from TiO_2 and TiO_24SiO_2 can be probably due to the different networks of the two gels.

The two stages release observed in all cases suggests that the initial stage of release to occurs mainly by dissolution and diffusion of the drug entrapped close to or at the surface of the

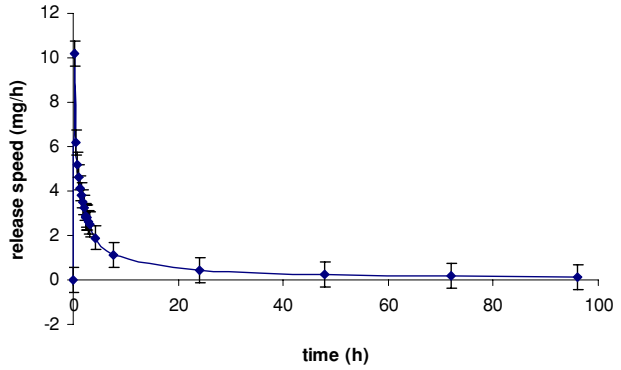


Fig. 7 Time-dependent drug release rate plot for TiO_2 at 37°C in SBF solution.

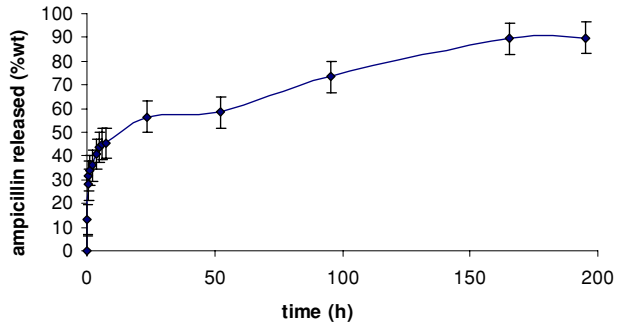


Fig. 8 Time-dependent drug release plot for TiO_24SiO_2at 37°C in SBF solution.

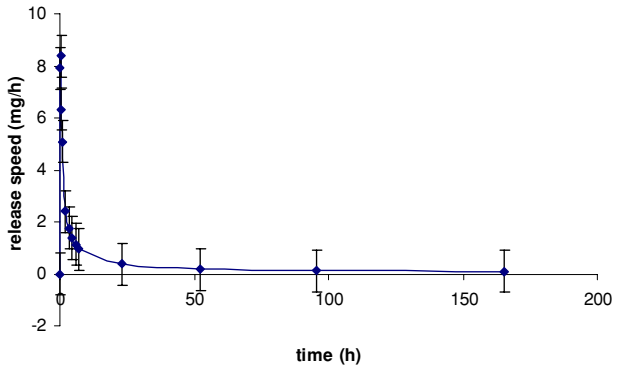
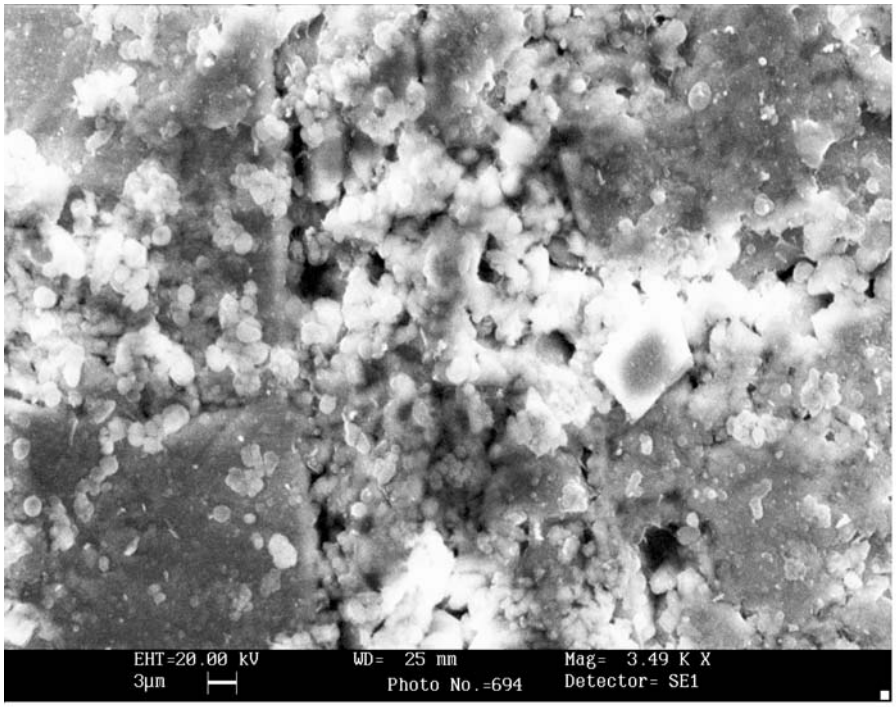


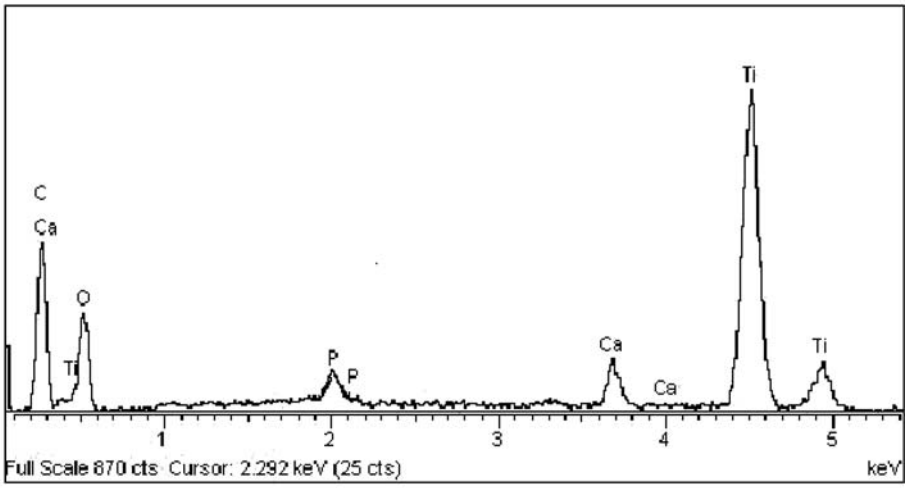
Fig. 9 Time-dependent drug release rate plot for TiO_24SiO_2at 37°C in SBF solution.

samples. The second and slower stage release was thought to involve the diffusion of drug entrapped within the inner part of clusters. An interesting observation is the general presence of a lag period, which is indicative of the need for solvent penetration into the structure.

Figures 7, 9 show this particular kinetic describing the changes of the release speed during the two stages. For the materials, TiO_2 and TiO_24SiO_2,no appreciable differences were found between room temperature or at 60°C in air.



(a)



(b)

Fig. 10 SEM micrograph (a) and EDS (b) of TiO_2 soaked for 14 days in SBF.

3.3. HPLC assay

A chromatographic analysis was carried out to ensure the integrity of the β-lactam ring of ampicillin, after the synthetic treatment. The conditions described in the experimental section allow to separate the two isomers of the antibiotic with retention times of 1.6 min and 2.2 min. Both the materials, TiO_2 and TiO_24SiO_2, release ampicillin without any sign of decomposition, as demonstrated by the perfect superimposition of the samples chromatograms with that of a standard solution of ampicillin (data not shown).

3.4. SEM micrograph and EDS analysis

Moreover an evaluation of the morphology of the apatite deposition and a qualitative elemental analysis were carried out by electron microscopy observations on pelletised discs previously coated with a thin graphite film. Figs. 10(a) and 11(a) show the SEM micrographs of TiO_2, TiO_24SiO_2 respectively samples soaked in SBF for 14 days. The characteristic apatite globular crystals are clearly visible. As it can be seen, the EDS reported in Figs. 10(b) and 11(b) confirm that the surface layer observed in the SEM micrographs is composed of calcium phosphate (see table 1).

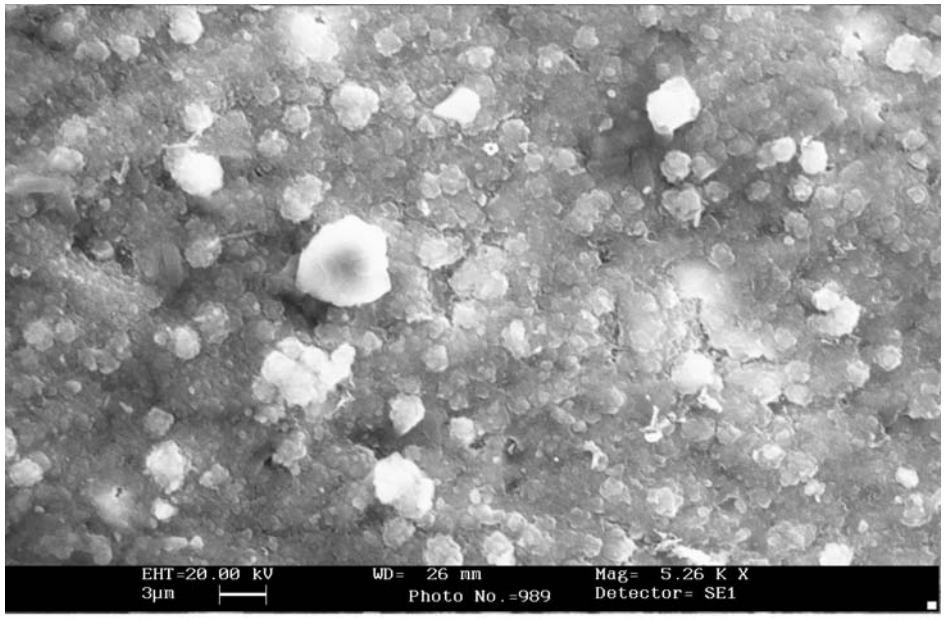


Fig. 11 SEM micrograph (a) and EDS (b) of $TiO_2 4SiO_2$ soaked for 14 days in SBF solution.

Table 1

14 days after immersion in SBF	Contents of Ca Atomic %	Contents of P Atomic %
TiO_2 Amp 5%	2.35	3.40
TiO_2 $4SiO_2$ Amp 5%	3.45	4.55

3.5. Antibacterial tests

Antibacterial property was examined by mixing 0.5 g of gel with glass powder with 5 ml of Tryptone Water Dehydrated physiologic solution. The same physiologic solution was used for bacteria. 500 μl of containing bacteria solution were inoculated in 1 ml of sample solution. Sample and bacteria were in contact in plate at room temperature for 15 min, 30 min, 1 and 2 h. After each sampling time, EC X-GLUC agar for E. coli and AZIDE MALTOSE agar (KF) for S. mutans were added to sample plates. The plates were aerobically incubated 24 h at 44°C for E. coli, but anaerobically 48 h at 37°C for S. mutans. The number of the colonies was counted. The comparison of cell-forming unit with a control with physiologic solution, bacteria and agar, without any foreign substances were made.

Table 2 shows the complete inhibition of the growth of E. coli after only 2 h of contact with the studied materials TiO_2 and $TiO_2 4SiO_2$, mixed with sodium ampicillin. The same results were observed with S. mutans.

These results indicate that the samples show a high antibacterial activity against E. coli and S. mutans and are consistent with the antimicrobial effect of ampicillin.

Table 2 Species bacteria

Incubation time (min)	*Escherichia coli* (UFC)	*Streptococus mutans* (UFC)
0	8000	1100
15	320	150
30	150	75
60	15	10
120	0	0

Conclusions

In this paper the synthesis of amorphous materials containing ampicillin, by sol-gel method, has been characterized, to employ new strategies for controlled release dosage forms.

The release kinetics demonstrates that the investigated materials supply high doses of the antibiotic during the first hours when soaked in SBF and then a slower drug release supplies a maintenance doses until the end of the experiment.

The bioactivity of the studied materials has been demonstrated by the formation of a hydroxyapatite layer on the samples surfaces when they are in contact with SBF. The presence of ampicillin does not inhibit this bioactive behaviour.

The high antibacterial activity of TiO_2 and TiO_24SiO_2 mixed with sodium ampicillin against E. Coli and S. mutans suggests the use of these materials for medical application such as dental restoration.

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