Time-Delayed Release of Bioencapsulates: A Novel Controlled Delivery Concept for Bone Implant Technologies

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Vancomycin has been successfully entrapped into amino-polysiloxane matrixes via a one-step room-temperature sol−gel process, resulting in biodoped monolithic hybrid materials. The vancomycin-containing matrixes have been characterized by means of Fourier transform infrared (FTIR) and ^{29}Si magic angle spinning nuclear magnetic resonance (^{29}Si MAS NMR) solid-state spectroscopies, evidencing the effective encapsulation of the drug. In vitro swelling behavior and delivery tests carried out under physiological conditions (37 °C, pH 7.4) showed not only the absence of burst effect but also, and remarkably, a zero-release period, or lag time, where no vancomycin was released to the medium during the first hours of assay. Subsequently, there was a sustained release of vancomycin over a prolonged period of days. This lag time is an essential requirement for implantable bioceramics that would allow the surgeon to perform the surgical procedure with zero drug release. The delivery behavior has been also tested mimicking bone infection conditions (37 °C, pH 6.5), and the lag time and subsequent sustained release are comparable to those obtained at physiological conditions, despite of the decrease of pH. This research work opens new possibilities for the design of novel time-delayed controlled delivery systems for bioencapsulates, useful in bone implant technologies.

Introduction

The design of new materials applicable in bone implant technologies has received great attention during the last years.[1–3] Several bioceramics have been reported as good candidates in bone tissue regeneration acting as local controlled delivery systems of drugs and other biologically active molecules that promote new bone formation.[4,5] Therefore, several biologically active molecules such as antibiotics and/or anti-inflamatories have been confined into ceramic carriers to treat adverse phenomena, such as infection and/or inflammation, which normally take place after the implantation process.[6–15] Also the confinement of drugs employed for osteoporosis treatments, such as bisphosphonates, into bioceramics has been recently described.[16] Moreover, the confinement and release of biologically active molecules, such as amino acids, peptides, proteins, and growth factors into ceramic matrixes have been reported as well.[17–20] Molecules are commonly loaded into the ceramic matrixes by adsorption mechanisms. This represents a great limitation because these drug delivery systems frequently exhibit a burst release effect, where most of the drug loaded is rapidly released to the delivery medium during the first hours of assay. Furthermore, when dealing with bone implants a time-delayed delivery with an initial zero-release or lag time period would be desirable to allow the surgeon to carry out the surgical procedure.

Several strategies have been developed trying to minimize the fast delivery of molecules to the medium, such as organically modifying the ceramic carrier with functions that undergo attracting interactions with the functional groups of the targeted molecule, then minimizing the initial burst

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(1) Vallet-Regí, M. *J. Chem. Soc., Dalton Trans.* **2001**, *2*, 97.
(2) Vallet-Regí, M. *Dalton Trans.* **2006**, 5211.
(3) Hench, L. L.; Polar, J. M. *Science* **2002**, *295*, 1014.
(4) Vallet-Regí, M. *Chem. Eur. J.* **2006**, *12*, 5934.
(5) Vallet-Regí, M.; Balas, F.; Colilla, M.; Manzano, M. *Solid State Sci.* **2007**, *9*, 768.
(6) Vallet-Regí, M.; Rámila, A.; del Real, R. P.; Pérez-Pariente, J. *Chem. Mater.* **2001**, *13*, 308.
(7) Mal, N. K.; Fujiwara, M.; Tanaka, Y. *Nature* **2003**, *421*, 350.
(8) Andersson, J.; Rosenholm, J.; Areva, S.; Linden, M. *Chem. Mater.* **2004**, *16*, 4160.
(9) Lin, Y. S.; Tsai, C. P.; Huang, H. Y.; Kuo, C. T.; Hung, Y.; Huang, D. M.; Chen, Y. C.; Mou, C. Y. *Chem. Mater.* **2005**, *17*, 4570.
(10) Zhu, Y. F.; Shi, J. L.; Shen, W. H.; Dong, X. P.; Feng, J. W.; Ruan, M. L.; Li, Y. S. *Angew. Chem., Int. Ed.* **2005**, *44*, 5083.
(11) Kim, H.-J.; Matsuda, H.; Zhou, H. S.; Honma, I. *Adv. Mater.* **2006**, *18*, 3083.
(12) Horcajada, P.; Serre, C.; Vallet-Regí, M.; Sebban, M.; Taulelle, F.; Ferey, G. *Angew. Chem., Int. Ed.* **2006**, *45*, 5974.
(13) Avnir, D.; Coradin, T.; Lev, O.; Livage, J. *J. Mater. Chem.* **2006**, *16*, 1013.
(14) Vallet-Regí, M.; Balas, F.; Arcos, D. *Angew. Chem., Int. Ed.* **2007**, *46*, 7548.
(15) Slowing, I. I.; Trewyn, B. G.; Giri, S.; Lin, V. S.-Y. *Adv. Funct. Mater.* **2007**, *17*, 1225.
(16) Balas, F.; Manzano, M.; Horcajada, P.; Vallet-Regí, M. *J. Am. Chem. Soc.* **2006**, *128*, 8116.
(17) Song, S.-W.; Hidajat, K.; Kawi, S. *Langmuir* **2005**, *21*, 9568.
(18) Vallet-Regí, M.; Colilla, M.; Izquierdo-Barba, I. *J. Biomed. Nanotechnol.* **2008**, *4*, 1.
(19) Vallet-Regí, M.; Balas, F.; Colilla, M.; Manzano, M. *Prog. Solid State Chem.* [Online early access]. DOI: 10.1016/j.progsolidstchem.2007.10.002. Published Online: October 30, 2007.
(20) Balas, F.; Manzano, M.; Colilla, M.; Vallet-Regí, M. *Acta Biomater.* **2008**, *4*, 514.

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effect.[14,21] Thus, for instance, ca. 55% alendronate loaded into unmodified SBA-15 mesoporous material is released to the medium during the first 24 h of assay. This burst effect drops to ca. 25% of alendronate delivered when the silica walls of SBA-15 are organically modified using amino groups.[16] However, this problem is still a matter of discussion and requires the development of new advanced materials that allow one to solve the clinical situation.

The burst effect drawback could be overridden by using one-step procedures, in which the synthesis of the ceramic material would take place at the same time that the drug is physically entrapped. Therefore, the drug release would be controlled by the slow degradation of the matrix and/or slow diffusion of the drug through the matrix.

The one-step incorporation of biologically active molecules into ceramic carriers requires synthesis procedures that employ mild conditions, such as room temperature and aqueous media. This can be easily achieved by means of the sol−gel chemistry.[22,23] One of the main advantages of sol−gel technology is that it allows not only the entrapment of several kind of molecules within the resulting xerogel but also preserves their functionality when dealing with biologically active molecules, in the so-called bioencapsulation process.[24–28] This is especially important when the confinement of drugs with a limited shelf life is aimed. Thus, molecules bioencapsulated into sol−gel matrixes are protected from biological degradation and are often considerably stabilized to chemical and thermal activation. This would result in an improved storage stability, and therefore, drug release would last over a clinically relevant period of time in an active form.

The release of entrapped molecules from sol−gel matrixes can be attained by the design of hybrids containing amino groups, due to the high solubility of these materials in aqueous media.[29] However, the extremely fast dissolution rate of amino-polysiloxane matrixes would lead to a rapid release of the entrapped molecules. This is an effective strategy for the storage and fast release of actives in applications such as fabric care[30] but limits their use in the clinical practice in which a time-delayed release followed by a sustained delivery of molecules over a prolonged period of time at the targeted site is needed.

Previous studies carried out by our research group have demonstrated that the problem of aqueous instability of amino-polysiloxane matrixes can be successfully overcome by using an aminosilane precursor together with another alkoxysilane containing a more hydrophobic group.[31] Organic−inorganic hybrid materials were prepared from different molar ratios of [*N*-(2-aminoethyl)-3-aminopropyl-]trimethoxysilane (DAMO) and (γ-methacryloxypropyl)trimethoxysilane (MPS), and the results clearly established that the MPS/DAMO molar ratio governed the degradation rate of the resulting hybrid matrixes. Moreover, with the addition of small amounts of calcium salts the materials exhibited in vitro bioactive behavior.

We attempt here to develop new controlled delivery systems by entrapping biologically active molecules into amino-polysiloxane matrixes starting from MPS and DAMO and using a one-step sol−gel process at room temperature. Vancomycin, the most effective antibiotic against Gram-positive bacteria used clinically to treat osteomyelitis infections,[32] was chosen as model antibiotic. In addition, bacterial inhibition assays previously reported revealed that the bactericidal efficacy of released vancomycin from silica xerogels is retained.[27] The MPS/DAMO molar ratio selected was that resulting in a slow dissolution of the matrix in the simulated body fluid, in order to obtain a lag time in the drug release and a subsequent sustained delivery of the entrapped drug. The evolution of the release rates and drug delivery profiles were studied as a function of the amount of vancomycin incorporated into the polysiloxane network. Moreover, with the aim of testing the behavior of these systems in bone infection situations, the in vitro drug delivery assays were performed at two different values, pH 7.4, i.e., physiological pH in healthy bone tissues, and pH 6.5, since a decrease of pH occurs in the surroundings of infected bone tissue.[33–35] Scheme 1 shows the experimental strategy followed throughout this work. This research is an effort to establish novel strategies for the development of time-delayed controlled release of biologically active molecules applicable in bone implant technologies.

Experimental Section

Reagents. The sol−gel precursors [*N*-(2-aminoethyl)-3-aminopropyl]trimethoxysilane and (γ-methacryloxypropyl)trimethoxysilane (Chart 1) were purchased from Sigma-Aldrich, Inc. Vancomycin hydrochloride was a generous gift from Laboratorios Normon S.A. Deionized water was further purified by passage through a Milli-Q Advantage A-10 purification system (Millipore Corporation) to a final resistivity of 18 MΩ cm or higher. All other chemicals (methanol, acetonitrile, $K_2HPO_4 \cdot 3H_2O$, NaCl, etc.) were of the best quality commercially available and used as received.

Preparation of Vancomycin-Containing Hybrid Materials. The vancomycin-containing hybrid materials were synthesized via sol−gel by dissolving appropriate amounts of DAMO and MPS in

(21) Muñoz, B.; Rámila, A.; Pérez-Pariente, J.; Díaz, I.; Vallet-Regí, M. *Chem. Mater.* **2003**, *15*, 500.
(22) Brinker, C. J.; Scherer, G. W. *Sol−Gel Science, The Physiscs and Chemistry of Sol−Gel Processing*; Academic Press, Inc.: San Diego, CA, 1990.
(23) Sanchez, C.; Julián, B.; Belleville, P.; Popall, M. *J. Mater. Chem.* **2005**, *15*, 3559.
(24) Gill, I. *Chem. Mater.* **2001**, *13*, 3404, and references therein.
(25) Livage, J.; Coradin, T.; Roux, C. Bioactive sol−gel hybrids In *Functional Hybrid Materials*; Gómez-Romero, P., Sanchez, C., Eds.; Wiley-VCH: Weinheim, Germany, 2004; pp 387−404.
(26) Radin, S.; Ducheyne, P. *Biomaterials* **2007**, *28*, 1721.
(27) Radin, S.; Ducheyne, P.; Kamplain, T.; Tan, B. H. *J. Biomed. Mater. Res.* **2001**, *57*, 313.
(28) Aughenbaugh, W.; Radin, S.; Ducheyne, P. *J. Biomed. Mater. Res.* **2001**, *57*, 321.
(29) Zhmud, B. V.; Sonnefeld, J. *J. Non-Cryst. Solids* **1996**, *195*, 16.
(30) Deshpande, K.; Dave, B. C.; Gebert, M. S. *Chem. Mater.* **2006**, *18*, 4055.
(31) Colilla, M.; Salinas, A. J.; Vallet-Regí, M. *Chem. Mater.* **2006**, *18*, 5676.
(32) Fass, R. Bone and joint infections In *Antibiotics and Chemotherapy*; O'Grady, F., Lambert, H., Greenwood, D., Eds.; Churchill Livingstone: New York, 1997; pp 760−767.
(33) Kobayashi, K.; Kobayashi, K.; Soeda, W.; Watanabe, T. *J. Periodontol.* **1998**, *69*, 1036.
(34) Hays, R. C.; Mandell, G. L. *Proc. Soc. Exp. Biol. Med.* **1974**, *147*, 29.
(35) Lardner, A. *J. Leukocyte Biol.* **2001**, *69*, 522.

Scheme 1. Synthesis of Vancomycin-Containing Hybrid Materials and Experimental Approach for Investigating the in Vitro Drug Delivery

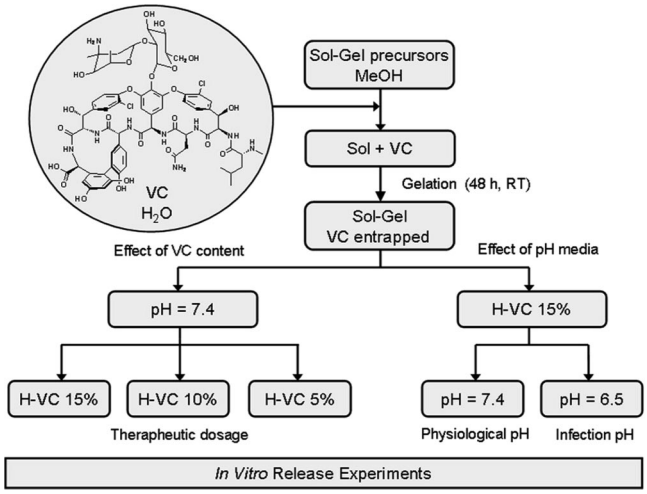
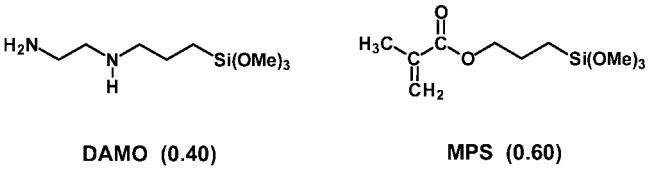


Chart 1. Sol−Gel Precursors [*N*-(2-Aminoethyl)-3-aminopropyl]trimethoxysilane and (γ-Methacryloxypropyl)trimethoxysilane, along with Their Molar Ratios (DAMO/MPS 0.40:0.60)



DAMO (0.40) MPS (0.60)

Table 1. Sample Notation and Nominal Compositions in Molar Ratios of the Vancomycin-Containing Hybrids Prepared[a]

sample	DAMO/MPS (0.40:0.60)	MeOH	H_2O	VC (wt %)
H-VC 0%	1	3	3	0
H-VC 5%	1	3	4	5
H-VC 10%	1	3	6	10
H-VC 15%	1	3	7	15

[a] The amount of vancomycin is expressed by weight percent with respect to the initial sol−gel precursors.

molar ratio 0.40:0.60 in methanol. In a typical synthesis a total amount of alkoxysilanes of 5.6 mmol was used. The corresponding amount of vancomycin was dissolved in deionized water. Sols with nominal vancomycin concentrations of 0%, 5%, 10%, and 15% by weight (percent of drug to total alkoxysilane precursors) were prepared by this method. For the blank sample (H-Vc 0%), the water amount was the stoichiometric water/alkoxide ratio needed to produce alkoxysilane hydrolysis. When adding vancomycin the water amount was raised up as the vancomycin content was increased, in order to avoid precipitation of the drug during the sols preparation. With this procedure, all the sols remained clear when adding the drug and the addition of vancomycin did not measurably affect the time to gelation. The nominal composition in molar ratios of the materials prepared in this work is shown in Table 1. The vancomycin/water solution was then added dropwise under stirring, and the mixture was stirred for 10 min at room temperature. The sol was cast in appropriate polyethylene molds and left to stand overnight at room temperature to reach the sol−gel transition. The resulting gel was dried at room temperature in air for 48 h and then at 30 °C under vacuum for 12 h to obtain stable disk-shaped monolithic xerogels of 8 mm diameter × 3 mm height dimensions.

Characterization of Hybrid Materials before and after In Vitro Delivery Assays. To perform the materials characterization vancomycin-containing hybrid samples were finely grounded to powder. Fourier transform infrared (FTIR) spectra were recorded in a Nicolet Nexus spectrometer using the KBr pellet method. Solid-state ^{29}Si magic angle spinning nuclear magnetic resonance (^{29}Si MAS NMR) spectra were obtained on a Bruker Avance AV-400WB spectrometer (400 MHz). The spectrometer frequency was set to 79.49 MHz, and the chemical shift values were referenced at 0 ppm to 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS sodium salt). The samples were packed into a zirconia rotor spinning at 10 kHz, and the spectra were recorded using a 10 s recycle delay, 3.5 ms of contact time, a pulse wide of 4.5 μs, and the number of scans were 10 000. The surface characterization of dry monolithic samples was performed by using scanning electron microscopy (SEM). Monoliths were mounted into an aluminum stud and gold-coated by plasma vapor deposition. The surface and cross-sectional SEM micrographs of monoliths were recorded by a field emission scanning electron microscope (JEOL model JSM-6335 F) with an acceleration voltage of 10 kV. Surface area measurements of hybrid materials were carried out by nitrogen adsorption/desorption analyses performed at 77 K using an ASAP 2020 porosimeter (Micromeritics Co., Norcross GA). Prior to the analysis monolithic samples were degassed at 313 K for 24 h under a vacuum lower than 10^{-5} Torr.

In Vitro Swelling Behavior and Delivery Assays. Swelling behavior experiments of the hybrid materials were performed in parallel to the in vitro delivery assays, following the same experimental conditions at pH = 7.4, as described below. Monolith swelling was monitored by gravimetrically measuring water intake as a function of time. Monolith weights were recorded by periodically removing them from the swelling media, blotting free water on the surface with adsorbent tissue, and weighing. Two monolith samples of each hybrid material were measured to calculate the average value. In vitro tests of vancomycin release from the hybrid matrixes were performed as follows. Solutions of 50 mM phosphate-buffered saline pH = 7.4 or 6.5 (ionic strength 250 mM adjusted with NaCl) were used as delivery medium. The monoliths were hung on a platinum wire and soaked into 25 mL of phosphate buffer. The solution was kept at 37 °C, and to avoid limitation of the delivery rate by external diffusion constraints, continuous stirring (200 rpm) was maintained during the assays. The concentration of vancomycin released was monitored by using high-performance liquid chromatography (HPLC). HPLC assays were performed using a liquid chromatographic system equipped with a Waters Alliance 2695 separation module and a Waters 2996 variable-wavelength diode array detector and controlled by Millenium[32] software (Waters, Milford, MA). A Zorbax Eclipse XDB-C18 column (5 μm, 4.6 × 150 mm) supplied by Agilent Technologies was used as stationary phase at 37 °C oven temperature. The mobile phase consisted on 12% acetonitrile and 88% 10 mM K_2HPO_4 buffer solution at pH = 6.9 (v/v). The flow rate was 1 mL/min, and the injection volume 10 μL. The absorbance was monitored at 280 nm, and vancomycin exhibited a narrow and well-defined peak at a retention time of 3.9 min. Vancomycin solutions at pH values of 7.4 and 6.5 with concentrations in the range of 0.01−1.00 mg/mL were used for calibration. For both pH values the curve was linear with a relationship of Area = 2×10^6[VC] (correlation coefficient >0.99). With the use of this relationship the area measured in the chromatogram was directly converted to an apparent drug release. The apparent drug release was normalized to the amount of drug loaded within the samples to observe the relative amount of drug release. Vancomycin concentration was determined from the average of the readings from

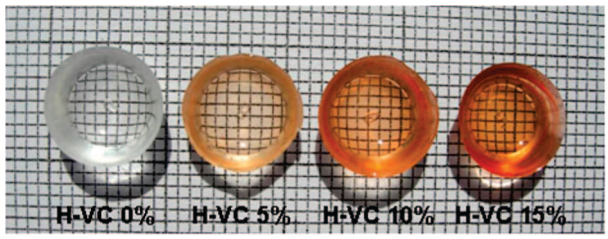


Figure 1. Disks of hybrid xerogels with different nominal concentrations of vancomycin.

four different samples ($N = 4$), and data were presented as mean ± standard deviation.

Results and Discussion

Characterization of Vancomycin-Containing Hybrid Materials. The hydrolysis and polycondensation reactions of DAMO and MPS alkoxysilane precursors produce a polyorganosiloxane matrix following a sol−gel process. The synthesis takes place without thermal activation, at room temperature, in the presence of water molecules. The sol−gel process is catalyzed by the basic amino functional groups of DAMO.[36] Almost complete hydrolysis and polycondensation of the alkoxysilanes are achieved within 24 h. The addition of vancomycin during the synthesis allows its entrapment in the resulting xerogels. The nominal amount of vancomycin was varied from 0% to 15%, in order to obtain a different released amount of the drug according to the therapeutic dosage required.

Disk-shaped vancomycin-doped xerogels were obtained as glassy, optically transparent, and crack-free monoliths (Figure 1). The intensity of orange color increased with the vancomycin concentration, and the transparency and the absence of any cloudiness indicated that precipitation of vancomycin did not take place during sols preparation. Incorporation of up to 15% of vancomycin did not visibly affect the appearance of the monoliths; however, higher percentage of incorporated vancomycin generated a nontransparent monolithic sample that was not used for further experimentation.

Figure 2 displays FTIR spectra of vancomycin-free hybrid (H-VC 0%) and xerogel with the highest vancomycin content (H-VC 15%). The vancomycin FTIR spectrum is also displayed in order to clarify the assignment. FTIR spectra of H-VC 5% and H-VC 10% samples were similar to that of H-VC 15%. Incorporation of vancomycin up to 15% did not visibly affect the general spectral features of the xerogels, compared to the blank sample without vancomycin.

Absorption bands in the spectrum of the hybrid without vancomycin include the bands that arise from the organic fragments of DAMO and MPS precursors: 3372, 3306 cm^{-1} (ν_{N-H}) from primary (NH_2) and secondary (NH) amine groups and (ν_{O-H}) from silanol groups (Si−OH) and water adsorbed, which presence has been verified by means of thermal analysis; 2937, 2890, and 2833 cm^{-1} (ν_{asC-H}) and (ν_{sC-H}) from methylene groups (CH_2); 1729 cm^{-1} ($\nu_{C=O}$), 1639 cm^{-1} ($\nu_{C=C}$), and 1301 cm^{-1} (ν_{C-O}) from MPS; 1574

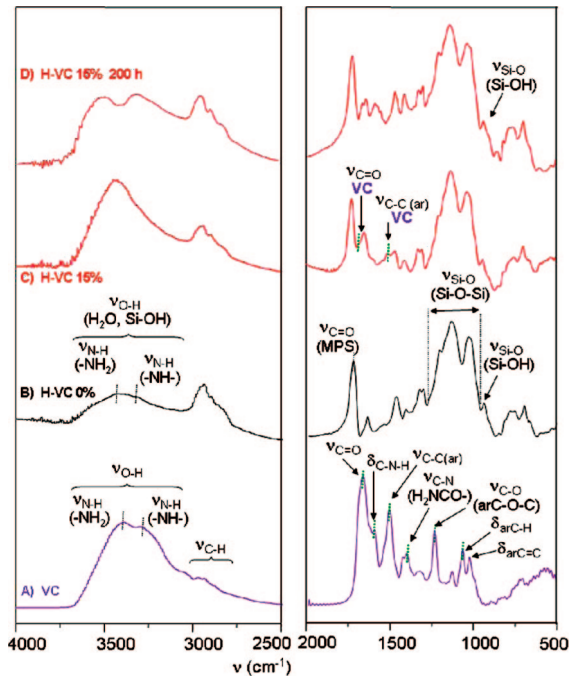


Figure 2. FTIR spectra of (A) vancomycin (VC) and (B) hybrid materials without vancomycin (H-VC 0%) and (C) with 15% of vancomycin nominal composition (H-VC 15%). (D) FTIR spectrum of H-VC 15% after 200 h of immersion time in the delivery medium is also displayed.

cm^{-1} (δ_{C-N-H}) from DAMO. In addition to the bands attributed to the organic part of the precursors DAMO and MPS, in the lower energy region the characteristic bands of silica network vibrations appear. High intensity bands assigned to asymmetric and symmetric stretching (ν_{Si-O}) of siloxane groups (Si−O−Si) are observed at 1200, 1132, and 1033 cm^{-1}, and the 692 cm^{-1} band due to Si−O−Si bending mode ($\delta_{Si-O-Si}$) is also observed. The band at 935 cm^{-1} (ν_{Si-O}) assigned to stretching of silanol groups (Si−OH) also appears, but the relative high intensity of siloxane bands suggests a high polymerization degree and the formation of a three-dimensional network.[37] These spectral features are in agreement with well-hydrolyzed and polymerized silica-based networks.[22]

It is noteworthy that the entrapment of vancomycin in the hybrid matrixes did not cause any noticeable shift of the silica sol−gel bands that now appear at 1203, 1134, 1031, and 692 cm^{-1}. Thus, incorporation up to 15% of vancomycin does not seem to produce any detectable change in the silica gel network. Characteristic bands of vancomycin functional groups, mostly amide and alcohol and one acid group, overlap with those vibrations in MPS and DAMO and consequently are not observed in a pronounced manner. Bands due to vancomycin clearly identified in the H-VC 15% spectrum are 1652 cm^{-1} ($\nu_{C=O}$) amide I, 1588 cm^{-1} (δ_{C-N-H}) amide II, 1506 cm^{-1} (ν_{C-C}) of the aromatic rings, and a shoulder at 1231 cm^{-1} (ν_{C-O}) of the C−O−C_{ar} groups.

The overall appearance of the spectra suggests that the xerogels, either with or without vancomycin, are well-hydrolyzed and polymerized as has been corroborated using the ^{29}Si MAS NMR technique.

(36) Innocenzi, P.; Brusatin, G. *J. Non-Cryst. Solids* **2004**, *333*, 137.

(37) Bharathi, S.; Fishelson, N.; Lev, O. *Langmuir* **1999**, *15*, 1929.

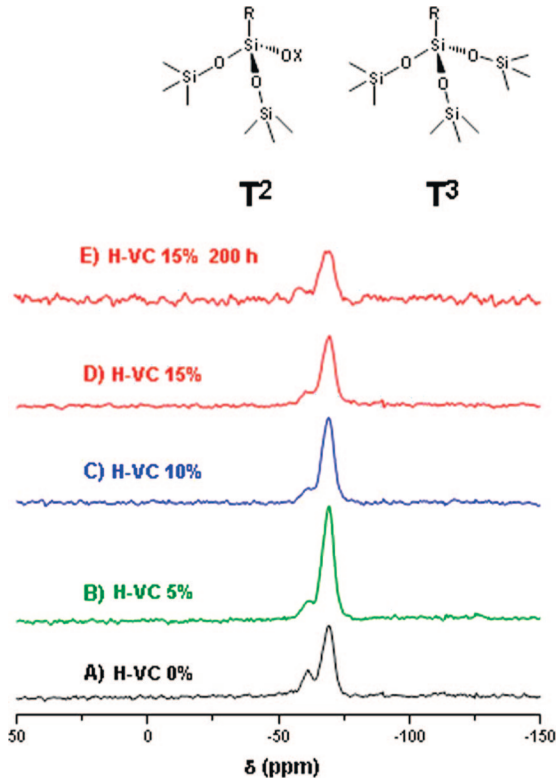


Figure 3. ^{29}Si MAS NMR spectra of (A) H-VC 0%, (B) H-VC 5%, (C) H-VC 10%, and (D) H-VC 15% hybrid materials and (E) H-VC 15% after 200 h of immersion time in the delivery medium.

Table 2. Chemical Shifts δ (ppm) of Each T^n Unit, and Peak Area (%) from Deconvoluting the ^{29}Si MAS NMR Spectra of H-VC Hybrid Materials and H-VC 15% after 200 h of Immersion Time in the Delivery Medium (pH 7.4, Phosphate Buffer)

	δ (ppm) and peak area (%)		
sample	T^2	T^3	T^3/T^2
H-VC 0%	− 61.3 (19)	− 69.3 (81)	81/19
H-VC 5%	− 61.5 (17)	− 69.4 (83)	83/17
H-VC 10%	− 61.4 (16)	− 69.2 (84)	84/16
H-VC 15%	− 60.3 (19)	− 69.5 (81)	81/19
H-VC 15% 200 h	− 58.9 (16)	− 68.9 (84)	84/16

^{29}Si MAS NMR spectra of the vancomycin-free and vancomycin-containing hybrid materials are given in Figure 3. As expected, only T^n signals appear since silicon atoms of DAMO and MPS precursors are covalently bonded to a carbon atom of an organic group. Chemical shifts and relative peak areas are presented in Table 2. From all possible T^n signals [R−Si(OSi)$_n$(OX)$_{3-n}$] only T^2 and T^3 turn up, at ca. 61 and 69 ppm, respectively, indicating that most of the Si−OH groups generated during the hydrolysis step condensed to Si−O−Si units.[38,39] The absence of T^0 and T^1 signals points to a high cross-linking degree in the polysiloxane matrix. The ratio of the relative peak areas of T^3/T^2 signals is ca. 80/20, i.e., there is a small proportion of T^2, and this is also indicative of a high cross-linking degree, giving rise to a dense polysiloxane matrix, with a small proportion of free silanols (T^2). From the ^{29}Si MAS NMR spectra it can be observed that all vancomycin-containing sol−gel matrixes present a similar T^3/T^2 relative peak area relationship which indicates that the increase of vancomycin proportion incorporated to the sol does not significantly affect the cross-linking degree of the polysiloxane matrixes. As has been explained above, these findings are also in agreement with the FTIR results.

In Vitro Swelling and Drug Delivery Assays. Moderately hydrophilic matrixes entrapping drugs in which drug release is controlled by the inward flux of solvent molecules and subsequent swelling of the polymer matrix are considered swelling-controlled systems.[40] The amino-polysiloxane matrixes encapsulating vancomycin prepared in this work behave like a hydrogel. When a dry hydrogel begins to absorb water, the first water molecules entering the matrix will hydrate the most polar, hydrophilic groups, then the network swells and exposes hydrophobic groups, which also interact with water molecules. After the polar and hydrophobic sites have interacted with water molecules, the network will imbibe additional water due to the osmotic driving force of the network chains toward infinite dilution. This additional swelling is opposed by the covalent or physical cross-links, leading to an elastic network retraction force. Thus, the hydrogel will reach an equilibrium swelling level. As the network swells, if the network chains or cross-links are degradable, the gel will begin to disintegrate and dissolve, at a rate depending on its composition.[41] When the amino-polysiloxane network is in contact with aqueous solutions swelling occurs to achieve thermodynamic equilibrium due to water concentration gradients. The embedding of water into the amino-polysiloxane hybrid originates transformation of the material from a glassy to a rubber state, and the matrix undergoes slow dissolution promoting enhanced diffusion of drug out of the polysiloxane network. Scheme 2 illustrates the vancomycin bioencapsulation and the in vitro drug release processes.

In order to clarify the dissolution and release processes, the swelling behavior of the vancomycin-free and vancomycin-containing hybrid matrixes in the delivery medium (pH = 7.4) was investigated by gravimetrically monitoring the weight variation of monoliths with the immersion time. The swelling ratio (S), which corresponds to the average hydration degree (or water uptake), can be determined according to the following equation:[42]

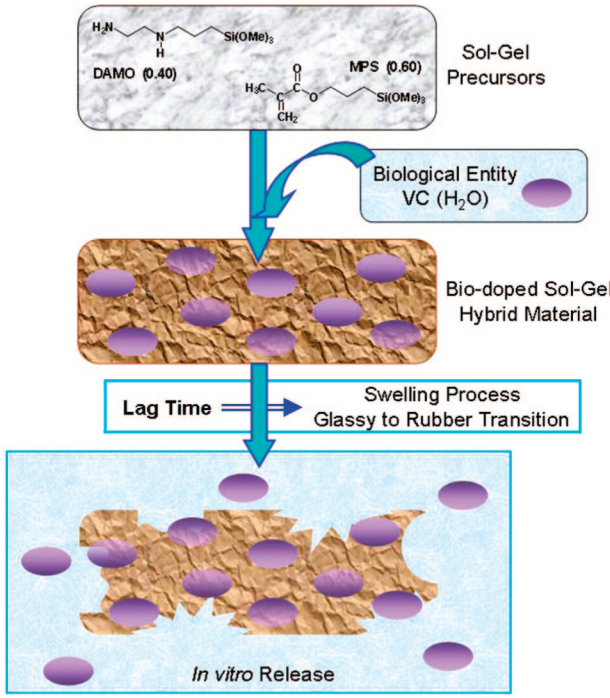
$$S = \frac{(W_t - W_0)}{W_0} \quad (1)$$

where W_t is the weight of the monolith after immersion time t, and W_0 is the weight of the dry monolith.

Figure 4 shows the variation of the swelling ratio with the immersion time of monoliths in the delivery medium. For all samples the weight stabilized at ca. 24 h, reaching an equilibrium swelling ratio. The values of maximum swelling ratios exhibited the same trend than vancomycin content, i.e., the greater the vancomycin content is, the higher

(38) Crandall, J. K.; Morel-Fourrier, C. *J. Organomet. Chem.* **1995**, *489*, 5.
(39) Peeters, M. Pm. J.; Wakelkamp, W. J. J.; Ketgens, A. P. M. *J. Non-Cryst. Solids* **1995**, *189*, 77.
(40) Peppas, N. A.; Bures, P.; Leobandung, W.; Ichikawa, H. *Eur. J. Pharm. Biopharm* **2000**, *50*, 27.
(41) Hoffman, A. S. *Adv. Drug Delivery Rev.* **2002**, *54*, 3.
(42) Román, J.; Cabañas, M. V.; Peña, J.; Doadrio, J. C.; Vallet-Regí, M. *J. Biomed. Mater. Res.* **2008**, *84A*, 99.

Scheme 2. Schematic Picture Illustrating the Vancomycin Bioencapsulation into Amino-polysiloxane Matrixes and the in Vitro Drug Release Process



is the maximum swelling ratio. All swelling profiles are similar to that of the vancomycin-free sample and, since the molar ratio composition for the alkoxysilane precursors, MPS/DAMO, is maintained in all matrixes, the increase in the water uptake for drug-containing matrixes must be due to the hydrophilic or polar nature of the vancomycin incorporated.[43,44] Thus, the swelling process would be highly influenced by the vancomycin content.

Drug release from simple swellable delivery systems can be described by the following expression, also known as the power law:[45]

$$\frac{M_t}{M_\infty} = kt^n \qquad (2)$$

where M_t/M_∞ is the fractional drug released at time t; k is a constant incorporating structural and geometric characteristics of the macromolecular network systems and the drug, which can be related to the kinetics release rate, and n is a release diffusional exponent indicative of drug transport mechanism.[46] Equation 2 is valid as long as the drug carrier swells only moderately in the penetrating fluid. Moreover, to the determination of the exponent n only the portion of the release curve where $M_t/M_\infty < 0.6$ should be used.

In addition, if there is a delay in drug release to the medium, according to Fassihi and co-workers,[47,48] eq 2 can be rewritten as follows:

$$\frac{M_t}{M_\infty} = k(t - t_L)^n \qquad (3)$$

where t_L is the lag time. This lag phase in amino-polysiloxane matrixes would be related to the swelling ratio, i.e., the time required to achieve the glassy to rubber state transition.

Vancomycin Release from Hybrids with Different Drug Contents. The fractional cumulative release profiles of vancomycin from samples containing different amounts of drug entrapped as a function of soaking time are displayed in Figure 5. As stated in the Experimental Section, release tests were carried out by soaking samples into a stirred 50 mM phosphate-buffered solution at pH 7.4 and 37 °C. Table 3 summarizes the kinetic parameters resulting from the fit to eq 3 of vancomycin release profiles from H-VC 5%, H-VC 10%, and H-VC 15% samples. The confidence limits presented in this work for any parameter are the 95% confidence limits. These parameters were optimized using unweighted least-squares analysis. The values of correlation

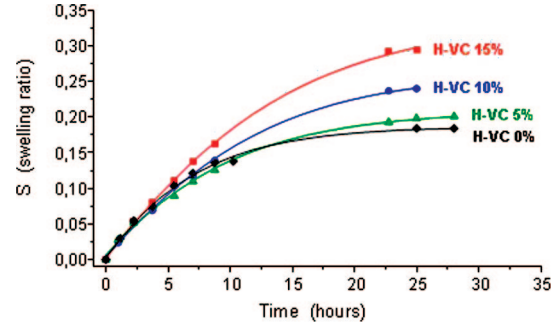


Figure 4. Swelling ratio of vancomycin-free (H-VC 0%) and vancomycin-containing (H-VC 5%, H-VC 10%, and H-VC 15%) hybrid materials as a function of immersion time in the delivery medium (pH 7.4, phosphate buffer).

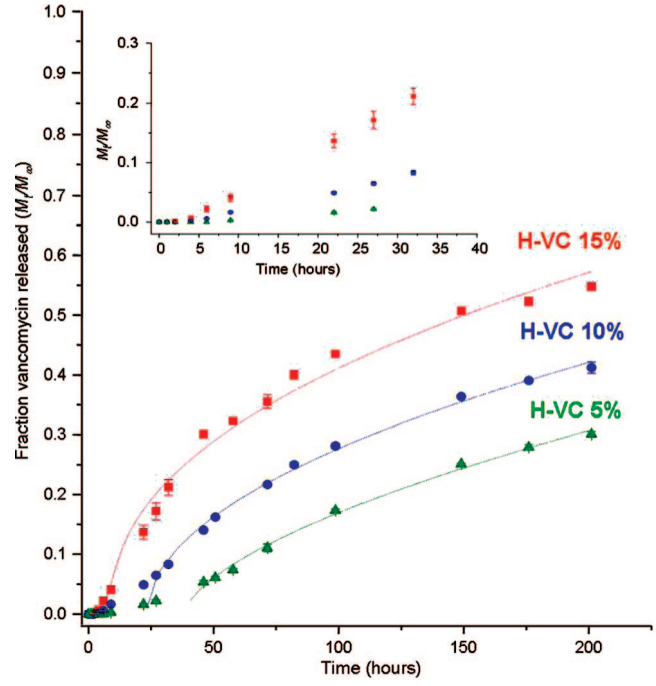


Figure 5. Cumulative fraction of vancomycin released (M_t/M_∞) vs time at pH 7.4 (phosphate buffer) for H-VC 5%, H-VC 10%, and H-VC 15% samples. Error bars represent the standard deviation for four measurements ($N = 4$).

(43) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615.
(44) Hamamoto, H.; Kamura, K.; Razanajatovo, I. M.; Murakami, K.; Santa, T.; Sekimizu, K. *Int. J. Antimicrob. Agents* **2005**, *26*, 38.
(45) Korsmeyer, R. W.; Gurny, R.; Doelker, E. M.; Buri, P.; Peppas, N. A. *Int. J. Pharm.* **1983**, *15*, 25.
(46) Ritger, P. L.; Peppas, N. A. *J. Controlled Release* **1987**, *5*, 37.
(47) Kim, H.; Fassihi, R. *J. Pharm. Sci.* **1997**, *86*, 323.
(48) Pillay, V.; Fassihi, R. *J. Controlled Release* **1999**, *59*, 229.

Table 3. Release Kinetic Parameters of Vancomycin from Different Hybrids in Phosphate Buffer at pH 7.4[a]

sample	lag time t_L (h)[b]	kinetics constant $k \times 10^3$ (h^{-n})[b]	release exponent n[b]	r^2	χ^2
H-VC 5%	38.3 (± 3.9)	13.3 (± 4.3)	0.617 (± 0.064)	0.994	0.00044
H-VC 10%	23.6 (± 2.3)	31.4 (± 6.4)	0.502 (± 0.041)	0.991	0.00023
H-VC 15%	8.6 (± 0.5)	55.0 (± 6.5)	0.445 (± 0.025)	0.991	0.00008

[a] The confidence limits for any parameter are 95%. [b] Standard deviations for t_L, k, and n are given in parentheses.

coefficient, r^2, are higher than 0.99, which is indicative of the satisfactory fit of experimental data to the proposed model. Moreover, the goodness of fit to eq 3, expressed as χ^2, points to an accurate approximation to the mathematical model, with values lower than 0.0005 for all studied samples.

All release profiles exhibit a lag time, in which no vancomycin is released to the medium, followed by a slow and sustained release. It should be highlighted that the lag time decreases with the increase of vancomycin content. Hence, lag time is ca. 38, 24, and 9 h for H-VC 5%, H-VC 10%, and H-VC 15% samples, respectively. In these systems the aqueous dissolution medium surrounding penetrates the glassy siloxane network in the swelling process thus originating a transition to the rubber state. According to the experimental results the lag time, i.e., the time necessary to reach the glassy to rubber state transition, decreases with the increasing of vancomycin content. The previously commented ^{29}Si NMR results indicated that the cross-linking degree of the starting polysiloxane matrixes is not significantly affected by the vancomycin content. Consequently, the decrease in the lag time would be related to the increasing of the hydrophilic character of the resulting vancomycin-containing glassy solid at higher drug contents, which leads to an increase in the swelling ratio and thus to a higher rate of glassy to rubber transition.

After the lag period the entrapped vancomycin is released to the aqueous medium at a rate that is tightly related to the kinetics constant, k. The values of k obtained from the adjust of release profiles to eq 3 are 13.3×10^{-3}, 31.4×10^{-3}, and 55.0×10^{-3} h^{-n} for H-VC 5%, H-VC 10%, and H-VC 15% hybrids, respectively. Hence, the greater the vancomycin content is the higher is the kinetic constant value. This fact agrees with the swelling moving fronts model proposed by Lee and Peppas.[49] This model involves that during the whole swelling process, two different states, the glassy core and gel layer (rubbery), exist. Hence, there are also two moving fronts, the glass−rubbery front and the rubbery−solvent front, which would be governing the drug delivery to the medium. As soon as the network at rubbery−solvent front interface reaches its thermodynamic equilibrium with the surrounding medium, such interface starts to dissolve. Thus, both the fronts move inward until the glass−rubbery and glassy core disappear and the rubber matrix starts to solve. According to the k values resulting from the fit of experimental data to eq 3, such processes are faster when greater vancomycin contents are present into the polysiloxane networks.

The release exponent n is indicative of the release mechanism and depends on the matrix carrier geometry.[46] Classically, analysis of controlled release data from cylinder-shaped devices give values of $n = 0.45$, $0.45 < n < 0.89$, or $n = 0.89$ indicating Fickian release, anomalous (non-Fickian) transport, and case-II release, respectively. Finally, values of $n = 1$ (zero-order release) mean that the drug release is independent of time. The mean n values resulting from the fitting of experimental release data to eq 3 are 0.617, 0.502, and 0.445 for H-VC 5%, H-VC 10%, and H-VC 15% samples, respectively. These results point to anomalous diffusion for hybrids containing the lowest vancomycin contents, i.e., H-VC 5% and H-VC 10% samples, and pure Fickian diffusion for H-VC 15% material. The vancomycin pure or normal Fickian diffusion from the H-VC 15% material involves that the system has reached equilibrium between the solvent molecules moving into the glassy network with a well-defined velocity front and the simultaneous increase in the thickness of the swollen (rubbery region) with time in the opposite direction. In such case, the polysiloxane matrix relaxation is negligible, i.e., there are no changes in the amino-polysiloxane matrix structure as a result of interactions with the drug molecules, and consequently drug release is controlled by Fickian diffusion. On the contrary, anomalous diffusion occurs when the matrix relaxation rate after swelling, which promotes strong drug−matrix interactions, is on the same time scale as the diffusion rate. This is the case of drug transport observed for the H-VC 5% and H-VC 10% systems, which could point to the coexistence of Fickian diffusion and slow matrix relaxation of the polysiloxane network processes in the swollen region.[50] This restriction of matrix relaxation is believed to affect drug diffusion within the polysiloxane network which leads to an increase in exponent n, indicating that drug release shifts from a diffusion-controlled toward a swelling-controlled mechanism.

All these findings point to a drug-content-dependent release of vancomycin from hybrid matrixes. Thus, the fractional cumulative vancomycin released after 8 days of assay is 0.30, 0.41, and 0.55, i.e., the amount of drug released is 20.8, 55.6, and 109.0 mg/g for H-VC 5%, H-VC 10%, and H-VC 15%, respectively. This fact suggests that drug dosage can be successfully modulated by varying the vancomycin content in the hybrids.

With the aim of further characterizing the matrix degradation process IR, ^{29}Si NMR, and SEM analyses of the hybrid material with the highest vancomycin content (H-VC 15%) were performed after different immersion times in the delivery medium and compared to those of the sample before soaking.

The FTIR spectrum of the H-VC 15% hybrid material after 200 h in the delivery medium is shown in Figure 2D. No significant differences with the original sample are observed

(49) Lee, P. I.; Peppas, N. A. *J. Controlled Release* **1987**, *6*, 207.

(50) Lee, P. I.; Kim, C. J. *J. Controlled Release* **1991**, *16*, 229.

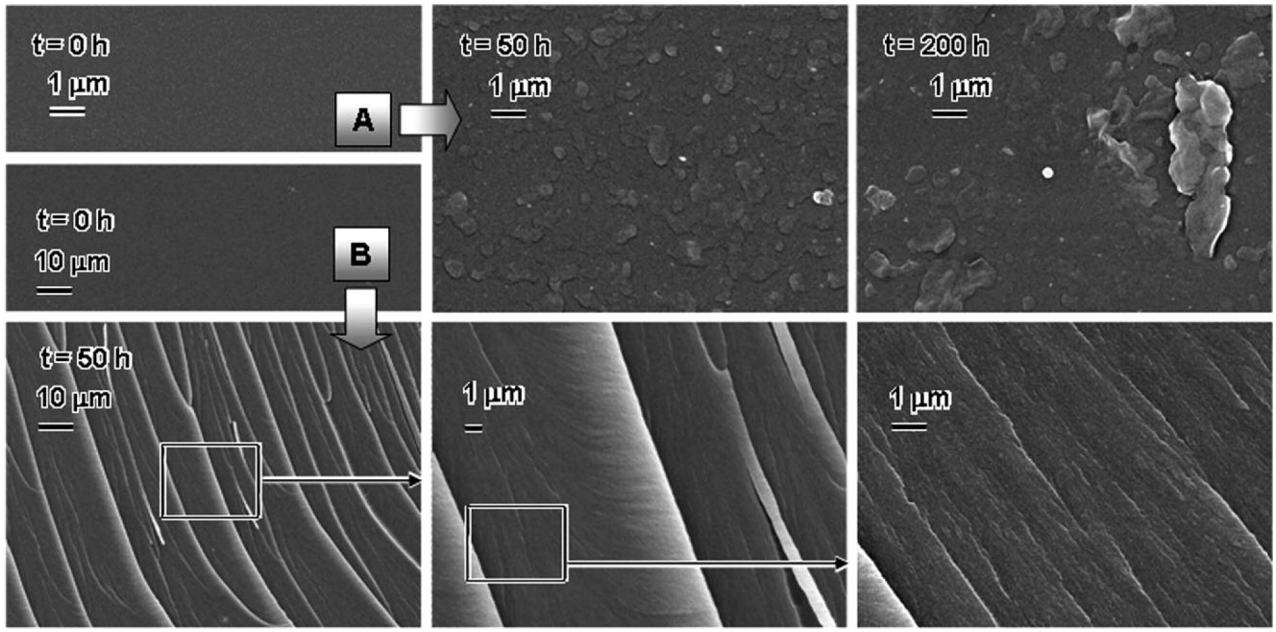


Figure 6. (A) SEM micrographs of the H-VC 15% hybrid material surface before ($t = 0$ h) and after different immersion times in the delivery medium ($t = 50$ and 200 h). (B) SEM micrograph of the H-VC 15% hybrid material cross section at time 0 and after 50 h of immersion time in the delivery medium (details at higher magnifications are also shown).

in the energy region where the characteristic bands of the silica network vibrations appear, and the relative intensity of the band assigned to stretching of the silanol group (Si−OH) is similar in both spectra, indicating a similar degree of hydrolyzed siloxane bonds. To confirm this fact and to determine the cross-linking degree of the remaining matrix, ^{29}Si NMR was carried out. In Figure 3E the ^{29}Si MAS NMR spectrum of H-VC 15% hybrid material after 200 h in the delivery medium is displayed. The peaks that appeared are only for T^2 and T^3 signals, in a ratio of relative peak areas of $T^3/T^2 = 84/16$. These data point to a high cross-linking degree in the remaining polysiloxane matrix, similar to that of the original hybrid material.

Figure 6A shows SEM images from the surface of the H-VC 15% hybrid material before and after being soaked for different time periods in the delivery medium. At time 0 it can be observed that original amino-polysiloxane matrixes are nonporous materials with a smooth surface. After 50 h of degradation a change in the appearance of the matrix surface has taken place, and small fragments start to come unstuck, probably due to partial solubilization of the amino-polysiloxane matrix. No noticeable changes in surface morphology are observed for longer immersion times. For instance, after 200 h of assay the surface presents a similar appearance as after 50 h, although erosion is more pronounced.

SEM micrographs of the H-VC 15% hybrid material cross section at time 0 and after 50 h of immersion time in the delivery medium are shown in Figure 6B. After 50 h the morphology presented cracks arranged in a parallel manner to the hybrid surface that could be indicative of layer formation. Neither in SEM surface images nor in the cross-sectional ones could the appearance of pores or voids be observed.

Surface area measurements of the vancomycin-free sample and of the H-VC 15% sample before and after in vitro delivery assays were made by means of nitrogen adsorption analyses. Hybrid materials exhibited gas adsorption isotherms corresponding to nonporous materials,[51] and the BET specific surface areas were lower than 16 m^2/g for the hybrid samples. These features were maintained for the H-VC 15% sample after soaking it for the delivery assay, so generation of porosity during the dissolution process could be ruled out.

Two types of degradation mechanisms have been reported in biodegradable polymers: bulk and surface mechanisms.[52,53] In light of the SEM results after different soaking times in the delivery medium, together with nitrogen adsorption isotherms and ^{29}Si NMR and IR spectra of samples after 200 h of immersion time, a surface mechanism could be established for the degradation process, in which dissolution occurs layer by layer. Hydrolysis of siloxane bonds on the surface, more exposed to the delivery medium, would take place, and subsequent dissolution would expose next layer. Then surface degradation would continue layer by layer, in a sustained manner, modulated by the molar ratio of the alkoxysilane precursors.[31]

Vancomycin Release Simulating Conditions of Bone Infection. The fractional cumulative release profiles of vancomycin from H-VC 15% samples at two different pH values of 7.4 (physiological pH) and pH 6.5 (pH surrounding bone infection) are displayed in Figure 7. The release tests were performed by soaking samples into stirred 50 mM phosphate-buffered solutions at pH values of 7.4 and 6.5. Table 4 summarizes the kinetic parameters resulting from the fit to eq 3 of the corresponding vancomycin release profiles. As mentioned above, the confidence limits presented in this work for any parameter are 95%. The optimization

(51) Gregg, S. J.; Sing, K. S. W. *Adsorption, Surface Area and Porosity*, 2nd ed.; Academic Press: New York, 1982.
(52) He, B.; Wan, E.; Chan-Park, M. B. *Chem. Mater.* **2006**, *18*, 3946.
(53) Li, S. M.; Vert, M. Biodegradation of aliphatic polyesters In *Biodegradation of Aliphatic Polyesters in Degradable Polymers, Principles and Applications*; Scott, G., Gilead, D., Eds.; Chapman & Hall: New York, 1995.

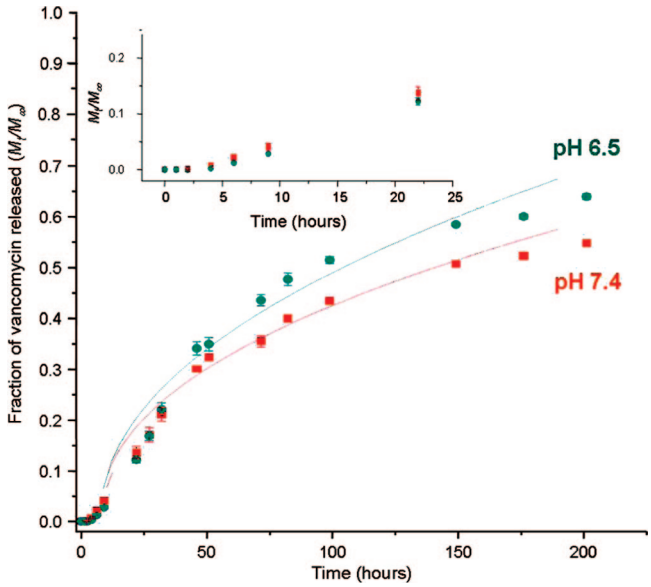


Figure 7. Cumulative fraction of vancomycin released vs time for the H-VC 15% sample at pH values of 7.4 and 6.5 (phosphate buffer). Error bars represent the standard deviation for four experiments ($N = 4$).

Table 4. Release Kinetic Parameters of Vancomycin from H-VC 15% Samples in Phosphate Buffer at Two pH Values: 7.4 and 6.5[a]

pH	lag time t_L (h)[b]	kinetics constant $k \times 10^3$ (h^{-n})[b]	release exponent n[b]	r^2	χ^2
7.4	8.6 (±0.5)	55.0 (±6.5)	0.445 (±0.025)	0.991	0.00008
6.5	8.9 (±0.4)	56.8 (±9.6)	0.471 (±0.036)	0.981	0.00126

[a] The confidence limits for any parameter are 95%. [b] Standard deviations for t_L, k, and n are given between parentheses.

of such parameters was performed as previously stated. The r^2 coefficient values at pH 7.4 and 6.5 are 0.991 and 0.981, pointing to a good fit of data to the purposed model. This fact is also noticeable from χ^2 values, being in any case lower than 0.001 for both tests.

From kinetic parameters summarized in Table 4 it can be deduced that the lag time is not affected by the decrease in pH, i.e., lag time values are ca. 9 h in both experiments. Moreover, the values of kinetic constants and release exponent are not statistically different (Student t test, $p > 0.05$). In both cases drug transport is governed by Fickian diffusion mechanism with mean n and k values of ca. 0.45 and ca. 56 h^{-n}.

The results indicate that these hybrid systems encapsulating vancomycin could be useful for bone implant technologies even if slight variations in the pH values associated to bone infection processes take place.

Conclusions

Amino-polysiloxane hybrids bioencapsulating vancomycin were successfully obtained as monoliths through a one-step sol−gel process at room temperature. The in vitro delivery assays at physiological conditions (37 °C, pH 7.4) revealed that no vancomycin was released to the medium during the first hours of assay. Subsequently, the drug was released in a sustained manner for more than 1 week. The release profiles depended on the amount of vancomycin entrapped, allowing one to modulate drug dosage. The zero-release period ranged from ca. 9 to 38 h depending on the amount of vancomycin incorporated in the polysiloxane matrix. This lag time is an essential requirement for implantable bioceramics that would allow the surgeon the implant fixation with zero drug release during the surgical procedure. Therefore, these drug delivery systems exhibit enhanced properties compared with conventional ones, which normally exhibit a burst effect with ca. 50% of the drug quickly released to the surrounding body fluids.

In vitro drug delivery tests carried out simulating conditions of bone infection (37 °C, pH 6.5), demonstrated that despite of the decrease of pH, the lag time and later sustained release of vancomycin were comparable to those obtained at pH 7.4. Therefore, drug release behavior of these systems was preserved even in bone infection conditions.

The results presented here are a first approach toward the design of new controlled drug delivery devices for bone implant technologies. This research opens many paths for the local delayed and sustained release of biologically active molecules, such as drugs, amino acids, peptides, proteins, and growth factors, from bioceramic implants that promote new bone formation.

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