Acrylic-phosphate glasses composites as self-curing controlled delivery systems of antibiotics

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New antibiotic delivery systems based on self-hardening methyl methacrylate (MMA)/polymethyl methacrylate (PMMA) systems and phosphate glasses (PG) in the system P_2O_5–CaO–Na_2O have been developed. Self-curing formulations were prepared by mixing the solid component containing PMMA beads, different proportions of PG (30–70 wt %) and vancomycin (5 wt %) as antibiotic, with the liquid component made of MMA monomer. Dough and setting times increased with the content of PG but peak temperature decreased to values well below to guarantee the chemical stability of the antibiotic drug, gentamicin or vancomycin. Mechanical properties of the PMMA/PG composites were evaluated in compression test giving rise to values of compressive strength in the range of 100 MPa. The release of vancomycin was analyzed *in vitro* by immersion of samples in phosphate buffer of pH = 7.4. Release profiles were influenced by the content of PG present in the cement. An initial burst of drug release was observed in all cases. The composites with 70 wt % PG released nearly the total amount of drug loaded in a period of 45 days, and those containing 60 wt % PG released the 70% of the vancomycin in the same period of time. However, either the control of the composite with 30 wt % PG released only the 30% of the drug in 10–15 days. The surface of the drug-loaded composites before and after release experiments was analyzed by ESEM. The deposition of some aggregates at certain points of the surface was detected for the specimens immersed in buffer phosphate after 45 days. This material was characterized by FTIR and Raman spectroscopy as an amorphous phosphate formed by calcium ortho and pyrophosphates, and indicates an interaction between the hydrated layer at the place of the glass and the surrounding medium.

Introduction

Current clinically used self-curing acrylic formulations for drug delivery systems are mainly based on poly methylmethacrylate (PMMA) [1, 2]. This type of systems are made by *in situ* polymerization of methyl methacrylate (MMA) in presence of PMMA powder. The polymerization reaction is initiated by a redox system constituted by benzoyl peroxide as the initiator and a tertiary aromatic amine (4-*N*,*N*-dimethyltoluidine) as the activator. Gentamicin [3] and other antibiotics [4] are added for both local and systemic treatment. The additives diffuse out of the PMMA in measurable quantities. PMMA beads are also used as a carrier for local implantation of antibiotics [5]. The use of antibiotic impregnated cement and antibiotic-impregnated PMMA beads have been found effective in the treatment of chronic osteomyelitis and acute musculoskeletal infections, as well as in soft-tissue infections of the neck, abdomen and rectum. Research has been focused on the development of novel delivery systems due to the disadvantages of PMMA as an antibiotic carrier, e.g. the need for a second operation of the non-resorbable cement as well as the thermal damage caused by the exothermic polymerization reaction. Gerhart *et al*. [6] prepared biodegradable bone cement based on poly (propylene fumarate) (PPF) and MMA monomer. These cements loaded with vancomycin have been effective in the prevention of infections in the tibia of rats [7]. Other biodegradable composites for antibiotic delivery systems have been prepared from poly(lactide/glycolide), copolymers [8] or microspheres [9], anhydrate microcapsules [10] or bioerodible polyanhydrides [11]. Partially biodegradable composites have been prepared from polymethyl methacrylate/poly (ε-caprolactone) (PMMA/PCL) [12] as an alternative to the drug delivery systems which can be polymerized *in vivo* and also supply some structural support before degradation. Other formulations of self-curing cements for use as drug delivery systems have been prepared from acrylic resins polymerized in presence of a bioactive component. A new bioactive bone cement consisting of *bis*-GMA/triethylene-glycol dimethacrylate (TEGDMA) and apatite or wollastonite-

containing glass-ceramic powder has been developed and used as the basis of drug delivery systems [13]. Likewise, the controlled release of gentamicin has been studied from polyethylmethacrylate–polymethyl methacrylate)–hydroxyapatite [14] and from materials prepared from poly(L-lactic acid)–PMMA using bioactive glasses as the SiO_2-70, CaO-26, P_2O_5-4 (mol %) system [15].

Considering the physico-chemical characteristics of self-curing PMMA based acrylic formulations, when bulk filling mass or PMMA balls are charged with different kind of drugs like antibiotics, anti-inflammatory drugs, analgesics, etc., the release of these compounds is restricted relatively by the low permeability of the PMMA-based formulations to the physiological fluids. This phenomenon justifies the design and application of new formulations with components that are not chemically stable in physiological conditions, i.e. biodegradable poly(hydroxyalkanoates) such as poly(lactic acid) or poly(ε-caprolactone), or an alternative way based on components that are slowly solubilized in the physiological medium such as hydrophilic polymer microdomains or bioglass particles dispersed in the PMMA bulk matrix.

In this paper, we report the preparation of composites based on PMMA and soluble phosphate glasses (PG) in the system P_2O_5-44.5, CaO-44.5, Na_2O-11 (mol %), for use as carriers of antibiotic controlled delivery. These glasses have a chemical composition similar to that of the inorganic phase of bone and present the unique property of being completely soluble in aqueous media [16, 17]. Vancomycin was the selected antibiotic due to its broad spectrum [18], its effectiveness in treating osteomyelitis and in preventing osseous staphylococcal infections [19, 20].

Experimental

Materials

PMMA beads (33 μm of average diameter) were supplied by Industrias Quirurgicas de Levante (IQL beads) and have earlier been characterized [21]. MMA (Acros Organics) and vancomycin hydrochloride (Combinopharm) were used as received. Benzoyl peroxide (BPO) (Fluka) was purified from fractional recrystallization from methanol, mp = 104 °C. 4-N, N-Dimethylaminobenzyl alcohol (DMOH), was prepared as reported previously [22]. $NH_4H_2PO_4$, $NaCO_3$ and $CaCO_3$ were used as received in the elaboration of the PG.

Preparation of PG

Phosphate glasses in the system $P_2O_5 - CaO - Na_2O$ were obtained as reported previously [23] by melting the raw materials, $NH_4H_2PO_4$, $NaCO_3$ and $CaCO_3$, on a platinum crucible at a temperature of 1200 °C followed by rapid quenching on a metallic plate preheated at 350 °C. The materials were finally annealed during 30 min at 445 °C and then left for 8 h under gradual cooling conditions. The chemical composition of the resulting glasses (BV11) was in the metaphosphate range (44.5 mol % P_2O_5 – 44.5 mol % CaO – 11.0 mol% Na_2O).

Formulation and characterization of PMMA/PG composites

Composites for drug delivery systems of vancomycin were prepared according to the traditional protocol by mixing a solid component with a liquid component in a s:l ratio of 2:1. The solid component consisted of PMMA beads, BV11 PG, PG, (30, 60 or 70 wt %), vancomycin hydrochloride (5 wt %) and BPO (1.5 wt %) as initiator. The liquid component consisted of MMA monomer and N,N-dimethylaminobenzyl alcohol (DMOH) (1 wt %) as a low toxicity activator.

Curing parameters

The cement in the dough state was introduced inside a cylindrical Teflon mold equipped with a thermocouple connected to a temperature recorder and positioned in the center of the mold at a height of 3.0 mm in the internal cavity [21]. Time was measured from onset of mixing the powder with the liquid and the temperature recorded. The polymerization was carried out at a temperature of 25 °C in all cases. Curing parameters, e.g. setting time and peak temperature of the reaction mass, were determined according to the ISO 5833 standard specification [24].

Mechanical properties of PMMA/PG composites

Appropriated samples of composites containing 20, 40 or 60 wt % of BV11 PG, were evaluated in compression test at room temperature on an Instron 4301 universal testing machine. The test was performed at a crosshead rate of 20 mm/min according to ISO 5833 standard. The deformation was calculated directly from the crosshead displacement and the compressive strength was determined for each specimen at the maximum stress. A minimum of five specimens from each batch were mechanically tested. One-way ANOVA was performed for mechanical properties results at 0.05 level of significance ($p = 0.05$).

Microscopic characterization

The surface of the composites was analyzed by environmental scanning electron microscopy using a ESEM XL30 (Philips) microscope. Likewise, the surfaces of unloaded PMMA/PG composites and drug-loaded PMMA control were observed for comparison purposes.

Preparation of drug-loaded samples and drug release measurements

Rectangular-shaped samples (10 × 100 mm) and 1 mm thickness were prepared for release experiments. The samples were immersed in vials containing 15 ml phosphate buffer (Titrisol, Merck) (pH = 7.4) and incubated at 37 °C without stirring. The dissolution medium was collected at different periods of time and analyzed. Fresh phosphate buffer (15 ml) was then added for the next period. The release measurements were determined by means of high-performance liquid chromatography (HPLC) Perkin Elmer LC-250 pump, a

UV-Vis detector Perkin-Elmer LC-95 and a Waters μBoundapack C-18 column of 3.9 × 300 mm. The wavelength used was 215 nm. A methanol/aqueous solution of PIC-A (Waters) (60 : 40) was used as the mobile phase and flow rate of 1 ml/min. All samples were assayed in triplicate. The retention time of the vancomycin peak in samples relative to the standard was 2.95 ± 0.05 (mean ± S.D., $n = 200$). The calibration curve was made for the complete set of the measurements, displaying a correlation coefficient of 0.9957.

In vitro behavior

In order to evaluate the *in vitro* behavior of the formulations, composite samples prepared in presence of vancomycin were immersed in 15 ml phosphate buffer (Titrisol, Merck, 0.026 mol/l KH_2PO_4, 0.041 mol/l Na_2HPO_4) and maintained at 37 °C. Variation of weight was evaluated gravimetrically at different periods of time. At appropriate times, the samples were removed, blotted quickly with absorbent paper to remove the water attached on its surface and weighed. In all the experiments a minimum of three samples were measured and averaged. The variation of weight was calculated from the following relationship:

$$\% \text{ Variation of weight} = [(W_t - W_0)/W_0] \times 100,$$

where W_t is the weight of specimen at time t and W_0 is the initial mass of the dry specimen.

The morphology of the surface of samples after immersion in phosphate buffer was examined by ESEM using a ESEM XL30, Philips, microscope. The composition of the surface was analyzed by ATR–FTIR spectroscopy (Perkin-Elmer, spectrum one) and by Fourier Transform Raman (FT–Raman) spectroscopy.

Results

Preparation and characterization of drug-loaded PMMA/PG composites

Table I shows the values of curing parameters of antibiotic-loaded acrylic formulations prepared in presence of different amounts of PG. Values of dough, setting and working times increased with the content of PG in the formulation. On the contrary, the maximum temperature reached during the polymerization reaction of the monomer methyl methacrylate decreased with increasing concentration of PG. The morphology of the drug-loaded composites was analyzed by ESEM. Fig. 1 shows the ESEM micrographs of a drug-loaded composite prepared with 70 wt % PG in the solid phase, along with the corresponding unloaded composite and the drug-loaded control of PMMA. In all cases, the particles of PG and vancomycin were immersed in the polymerized matrix of PMMA. The surface of the drug-loaded PMMA control showed a heterogeneous distribution of discrete vancomycin particles, which were located forming groups inside pores. The surface of the PMMA/PG composites showed a rather homogeneous distribution of the PG particles although some round aggregates throughout the surface could be detected. The surface of the drug-loaded PMMA/PG composites was the most homogeneous and the pores containing the vancomycin particles, as were observed in the control, disappeared.

Mechanical properties were evaluated by means of compression test from samples prepared in absence of vancomycin in order to assess the influence of the PG separately. Results are collected in Table II. A significant increase of compressive stregth was obtained for the composites prepared with 20 and 40 wt % PG but no significant changes were observed for a content of 60 wt % PG in the solid phase. Young's modulus, on the other hand, increased significantly with the content of PG for all concentrations compared to that of the PMMA control.

Vancomycin release from PMMA/PG composites

Fig. 2(b) shows the vancomycin release with time for composites containing different proportions of PG. Release profiles were influenced by the content of PG present in the cement. In all cases an initial but moderate burst of drug release was observed within the first 30 min, in which some of the drug was released at an initially high rate (Fig. 2(a)). The cement containing 70% PG produced an initial release of 30% of the total amount in the first half an hour, in contrast to the PMMA control in which the amount released in this period of time was

TABLE I Values of curing parameters of vancomycin loaded acrylic formulations based on PMMA and different proportions of PG (BV11) measured at 25 °C

Formulation	t_{dough} (min) (S.D.)	$t_{setting}$ (min) (S.D.)	$t_{working}$ (min) (S.D.)	T_{max} (°C) (S.D.)
PMMA	3.20 (0.1)	12.8 (1.4)	9.7 (1.4)	72.8 (3.4)
30 wt % PG	8.7 (1.5)	21.5 (0.5)	12.8 (2.0)	64.7 (1.2)
60 wt % PG	15.0 (1.5)	28.0 (1.5)	13.0 (1.5)	54.3 (0.5)
70 wt % PG	20.3 (0.8)	33.0 (3.0)	13.0 (2.2)	40.8 (2.7)

TABLE II Values of compression test, compressive strength (σ_c) and elastic modulus (E_c), of PMMA/PG composites

Formulation	σ_c (MPa) (S.D.)	E_c (MPa) (S.D.)
PMMA	100 (10)	1.3 (0.1)
20 wt % PG	110 (5)	1.6 (0.0)
40 wt % PG	118 (7)	1.8 (0.1)
60 wt % PG	100 (6)	2.0 (0.2)

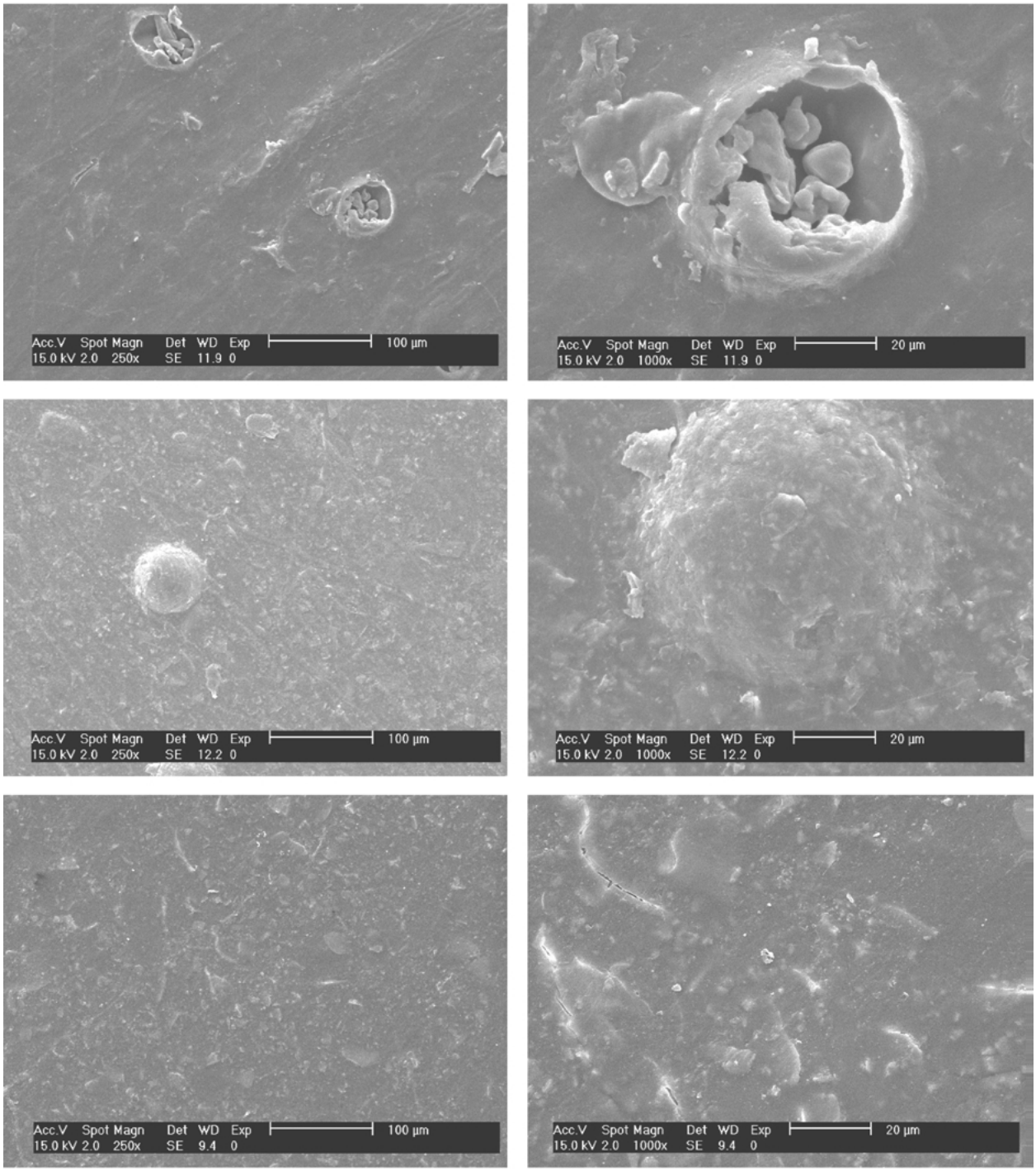

Figure 1 ESEM micrographs of the surface of the drug-loaded PMMA control (upper), the PMMA/70 wt % PG composite without vancomycin (middle) and the drug-loaded PMMA/70 wt % PG composite (lower).

close to the 17% of the initial amount. The cements prepared with 60% and 30% PG released a lower amount of drug, 8% and 6% respectively, during this period of time. After the initial burst, the release of the vancomycin was linear with time during the first 9 h, also in all cases. The equilibrium drug release content and time to reach equilibrium drug release increased with increasing PG content in the composite (Figure 2(b)). The composites with the higher amount of PG released nearly the total amount of the initial drug in a period of 45 days, and the composite with 60 wt % PG released the 70% of the vancomycin in the same period of time. On the contrary, either the control or the composite with 30 wt % PG released only the 30% of the drug in a period of 10–15 days.

In vitro behavior

In order to know the weight changes produced in the composites prepared with the PG, they were soaked in phosphate buffer at pH = 7.4. The profiles obtained are plotted in Fig. 3. An increase of weight was observed at the beginning of the experiment for all samples due to water absorption, with higher values of gained weight for samples containing PG in comparison to the PMMA control. The curves of the composites containing PG displayed a maximum in the range 14–16% in a period of 5 days whereas the control reached a constant value of gained weight of 4% in a period of 35 days. A sudden weight loss was measured for the composites prepared with 60 and 70 wt % PG in the solid phase due to the dissolution of the PG in the medium, the gained weight

decreasing up to a value ranging from 2–4% after 25 days.

The composite containing 30 wt % PG in the solid phase behaved differently. A moderate weight loss was observed, the gained weight decreasing up to 11% after 45 days. Fig. 4 shows the ESEM micrographs of the surface of the drug-loaded composite prepared with 70 wt % PG in the solid phase soaked in phosphate buffer for 45 days. The deposition of some particles forming aggregates of 1–2 mm on some areas of the surface of the composite could be observed, whereas the rest of the surface remained without structural changes. These aggregates were characterized by ATR–FTIR and FT–Raman spectroscopy (Fig. 5). In the ATR–FTIR spectrum two absorption wide bands at 1101 and 907 cm^{-1} were observed, and their frequencies were assigned to stretching vibrations of the P–O and P–O–P groups respectively. The presence of wide bands is indicative of an amorphous material in comparison with the more defined peaks that are obtained in the spectrum of crystalline compounds. The FT–Raman spectrum presented three well-defined bands at 355, 751 and 1042 cm^{-1}. The band that appeared at 751 cm^{-1} could be assigned to the vibration of the POP bond in a Q_1 tetrahedron, i.e., pyrophosphate group (P_2O_7) [25] and the band at 1042 cm^{-1} could be assigned to the vibration of the group $(PO_3)_{sym}$ in the Q_1 tetrahedron. In addition to this, a new small band at approximately 960 cm^{-1} appeared. This band was detected in the spectrum of the surface of the BV11 PG after 4 weeks of dissolution in SBF and was assigned to the symmetric stretch of the orthophosphate group PO_4^{3-} [23]. Then the Raman analysis indicates the presence of pyrophosphate and orthophosphate groups in the structure of the calcium phosphate deposited on the surface of the material, which is formed after the dissolution of the initial glasses.

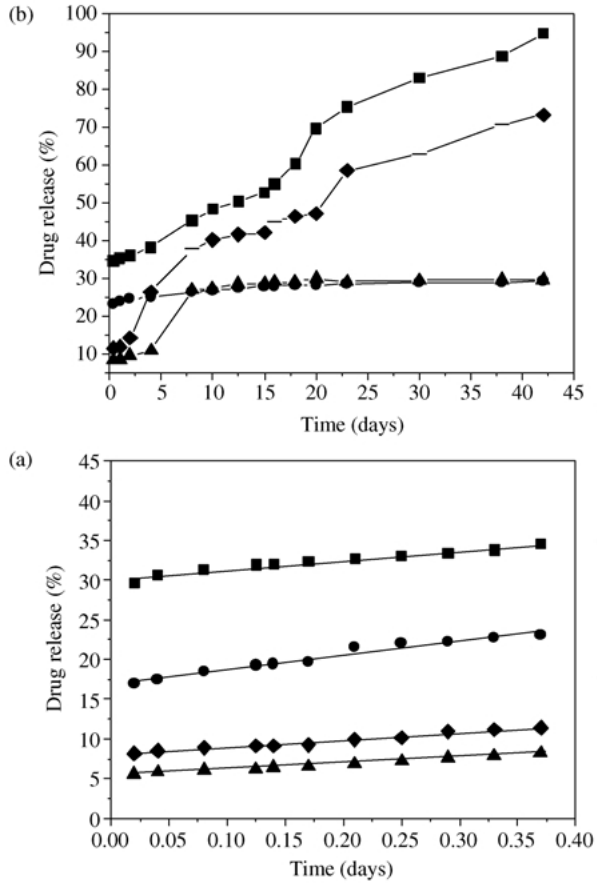

Figure 2 Release curves of vancomycin in phosphate buffer (pH = 7.0) from composites based on PMMA/PG, 30 wt % PG (▲), 60 wt % PG (◆) and 70 wt % PG (■), and from the control of PMMA (●). (a) Enlargement of initial release period. (b) Total release period.

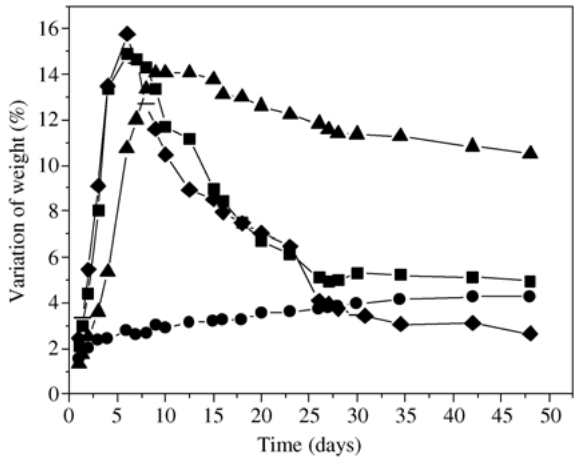

Figure 3 Variation of weight with time of immersion of samples of drug-loaded acrylic composites containing 30 wt % PG (▲), 60 wt % PG (◆) and 70 wt % PG (■), along with the control of PMMA (●).

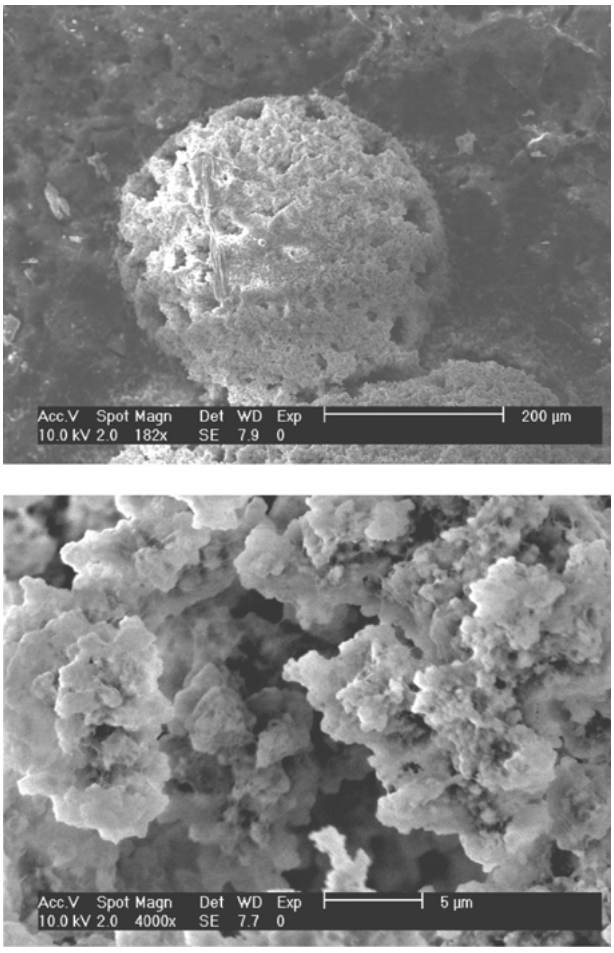

Figure 4 ESEM micrographs of the surface of the vancomycin-loaded composite prepared with 70 wt % PG after 45 days of immersion in phosphate buffer (pH = 7.4).

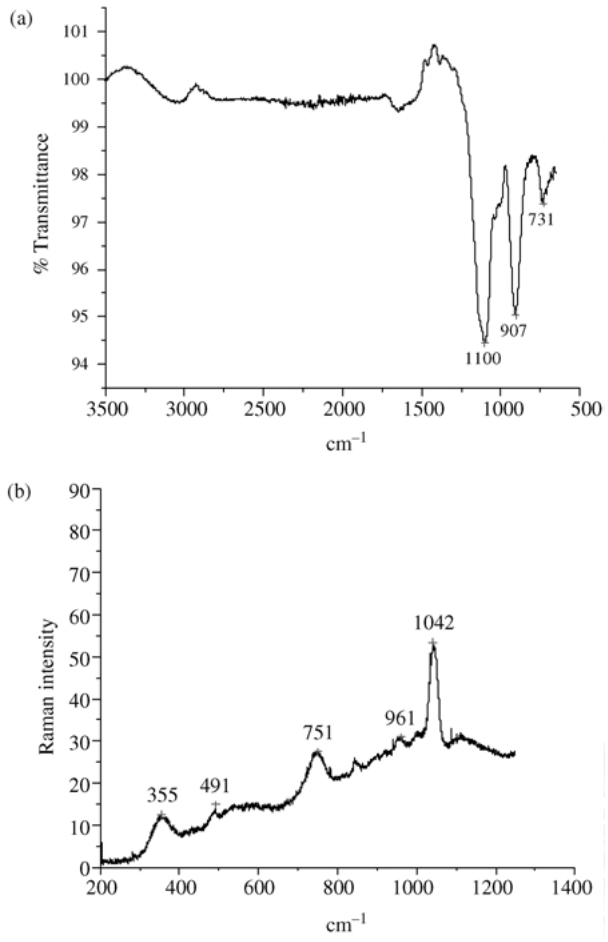

Figure 5 ATR–FTIR spectrum (a) and FT–Raman spectrum (b) of the depositions formed on the surface of a drug-loaded composite prepared with 70 wt % PG after 45 days of immersion in phosphate buffer (pH = 7.4).

Discussion

We have developed a new antibiotic delivery system that is a composite consisting of soluble PG and PMMA beads in a matrix of PMMA. We have varied the proportion of the bioactive component (30, 60 or 70 wt % with respect to the solid phase) but maintained constant the content of the antibiotic, 5 wt % with respect to the solid phase, that is the usual concentration employed in the commercial acrylic bone cement formulations [26]. The aim of adding PGs was on the one hand, to confer osteoconductive properties to the acrylic formulation, and on the other hand, to contribute to the diffusion of the drug as the dissolution of the glasses in the physiological medium takes place. The curing of these formulations provided exotherms of decreasing peak temperature with values in the range 65–40 °C. These low temperatures would diminish the risk of thermal and chemical trauma at the site of implantation and guarantee the chemical stability of the drug, vancomycin, which could be affected by the temperature reached for pure PMMA formulations. Setting time increased with the content of PG in the composite, mainly due to the increase of the dough time. The large values of dough time can be considered interesting for the application of these formulations as injectable formulations. However, the values of working time, which is defined as the difference between dough and setting, are in the range accepted for standard specifications [24]. Regarding the mechanical properties of these composites, the effect of adding different proportions of PG to the PMMA powder was studied. The compressive strengths of all the formulations were comparable to that of the control PMMA, (100 $\pm$ 10 MPa) and higher than that requested by the standard ISO. This indicates that these composites would provide initial mechanical support at the site of implantation. The effect of vancomycin on the mechanical properties of bone cements is documented in the literature. Argenson *et al*. [27] reported that the addition of 2 g of vancomycin in 40 g of cement powder did not significantly affect the tensile properties of the studied cement, and also, Gerhart *et al*. [6] published that the incorporation of the same amount of vancomycin did not affect significantly the compressive strength on their biodegradable cements.

Regarding elution experiments, substantial differences were observed in the kinetics release for the composites in comparison with the control of plain PMMA. The release of vancomycin can be divided into three different stages. The first stage was characterized by an initial but moderate burst release of the antibiotic within the first 30 min, which was higher for the composite prepared with 70 wt % PG, followed by the PMMA control and lower for the composites prepared either with 60 or 30 wt % PG. The second stage was characterized by a linear elution of the antibiotic with time, and lasted for 9 h. The data can be fitted to an equation: % drug release $= a + b\,t$. The results obtained for each sample are as follows:

PMMA control:	$a = 16.92 \pm 0.2457$	$b = 18.13 \pm 1.1422$ $\quad R = 0.9826$
30 wt % PG:	$a = 5.41 \pm 0.0545$	$b = 7.35 \pm 0.2534$ $\quad R = 0.9947$
60 wt % PG:	$a = 7.98 \pm 0.1004$	$b = 9.35 \pm 0.4666$ $\quad R = 0.9890$
70 wt % PG:	$a = 30.16 \pm 0.1814$	$b = 11.69 \pm 0.8435$ $\quad R = 0.9774$

The constant a refers to the initial burst and the constant b to the release rate during this time. This rate was rather similar for the composites containing PG, with a trend to increase with the content of PG, but in all cases inferior to that of the control which could be accounted for by the different distribution of the vancomycin particles on the surface of the corresponding systems as shown in Fig. 1. The relatively low burst effect observed for the systems charged with 30 and 60 wt % of the PG, is the result of a strong interaction of vancomycin and the PG. These components are distributed mainly in homogeneous microdomains coated or isolated by a layer of PMMA (see Fig. 1) characterized by its low permeability. Therefore, the drug is initially retained in the composite system by ionic interactions with the PG. When the content in PG is higher, the permeability is much higher and therefore, the initial contact with the hydrated medium is favored with respect to the control PMMA.

Afterwards, the third stage begins in which the release rate decreases. In this stage the control, which eluted the majority of the vancomycin during the initial burst, delivered the rest of drug very slowly, releasing only

30% of the initial amount in 15 days. However, the composites with 60 and 70 wt % PG continued releasing the drug at a uniform rate until about 94% and 60% was released in a period of 50 days. These differences can be attributed to the dissolution of the PG in the medium. Taking into consideration the variation of weight of the composites when immersed in phosphate buffer (Fig. 3), the release after 5 days can be correlated with the drastic weight loss for the composites containing either 60 or 70 wt % PG. However, the control of PMMA and the composite prepared with 30 wt % PG did not experience such substantial weight loss, coinciding in the decrease, and further stabilization, of the release after the first 5 days for these systems.

The morphology of the surface of the composites after immersion in saline solution was analyzed by ESEM. The surface of the composites prepared with 60 or 70 wt % PG presented the precipitation of some aggregates on some areas, which can be attributed to the solubility of the PG. Previous studies on the dissolution of the current PG [28] revealed structural changes at the surface of the glass at long periods of time (4 weeks of dissolution), which were identified by FT-Raman spectroscopy as orthophosphate glasses. Accordingly, the aggregates formed on the composites prepared in this work were characterized by different spectroscopic techniques such as ATR–FTIR and FT–Raman. Both techniques conclude that the inorganic material deposited on the surface of the composite is an amorphous phosphate formed by calcium orto (PO_4^{3-}) and pyrophosphates ($P_2O_7^{2-}$) after characterisation of the corresponding absorption and dispersion bands. This fact shows that an interaction between the hydrated layer at the place of the glass and the surrounding medium occurs.

Conclusions

Controlled delivery systems consisting of self-curing formulations of PMMA beads and PG in a matrix of PMMA have been developed. The composites containing 60 and 70 wt % of glasses with respect to the solid phase were the most effective in releasing the drug, giving rise to values of release of 60% and 94% of the initial amount of drug, respectively, in a period of 45 days. The release has been associated with the dissolution of the glasses in the medium. In addition, the deposition of amorphous orto and pyrophosphates at the place of the glasses was observed indicating an interaction with the surrounding medium.

Acknowledgment

Financial support from the Comision Interministerial de Ciencia y Tecnologia, (CICYT) (MAT99-1064-C02-01) is gratefully acknowledged.

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Received 24 May
and accepted 29 May 2002