Gentamicin-Loaded Hydraulic Calcium Phosphate Bone Cement as Antibiotic Delivery System

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Abstract □ A hydraulic calcium phosphate cement made of β-tricalcium phosphate [β-$Ca_3(PO_4)_2$], monocalcium phosphate monohydrate [$Ca(H_2PO_4)_2 \cdot H_2O$], and water was used as a delivery system for the antibiotic gentamicin sulfate (GS). GS, added as powder or as aqueous solution, was very beneficial to the physicochemical properties of the cement. The setting time increased from 2 to 4.5 min with 3% (w/w) GS and then slowly decreased to 3.75 min with 16% (w/w) GS. The tensile strength increased from 0.4 to 1.6 MPa with 16% (w/w) GS. These effects were attributed to the presence of sulfate ions in GS. The release of GS from the cement was measured in a pH 7.4 phosphate-buffered saline solution at 37 °C by USP paddle method. Factors such as cement porosity, GS content and presence of sulfate ions or polymeric additives were investigated. The amount of GS released was roughly proportional to the square root of time up to ~50% release. Afterwards, the release rate markedly slowed down to zero. In all but two cement formulations, the total dose of GS was released within 7 days, indicating that no irreversible binding occurred between the cement paste and the antibiotic. When small amounts of hydroxypropylcellulose or poly(acrylic acid) were added to the cement, the maximum fraction released was a few percent lower than the total GS dose, suggesting some binding between the polymer and GS. The GS release rate was strongly influenced by the presence of sulfate ions in the cement paste and by the cement porosity. The higher the sulfate ion content of the cement paste, the lower the GS release rate. This influence was attributed to the finer cement microstructure induced by the presence of sulfate ions. Furthermore, when the initial cement porosity was increased from 38 to 69%, the release rate almost tripled (0.16 to 0.45 $h^{-1/2}$). Finally, the biological activity of GS in the cement was maintained, as measured by assaying the release medium.

Introduction

Since their discovery in 1983,[1−2] hydraulic calcium phosphate cements (HCPC) have proved to be excellent bone substitutes because of their resorbability, biocompatibility, and osteoconductivity.[3−8] Recently, several studies have shown that HCPC could also be used as a delivery system for therapeutic peptides,[9] antibiotics,[10−11] anticancer drugs,[12] antiinflammatory drugs,[13−14] and bone morphogenetic protein.[15] The possibility of using a bone substitute as a drug delivery system could provide an attractive and efficient solution for the treatment of bone diseases such as tumors, osteoporosis, or osteomyelitis, which usually require long and painful therapies. For example, therapy of bone infections may easily last 2 years[16] because of the poor accessibility of the infection site by systemically administered antibiotics. To improve therapy, gentamicin sulfate (GS)-loaded poly(methylmethacrylate) (PMMA) beads are sometimes implanted into the infection site.[17] However, because PMMA beads are not resorbable, they have to be surgically removed after a few months and replaced by new beads or a bone substitute to facilitate bone reconstruction. To avoid costly and painful surgery, the use of β-tricalcium phosphate [β-TCP; β-$Ca_3(PO_4)_2$] ceramic blocks loaded with GS has been proposed.[18] Because β-TCP is resorbable and osteoconductive, the blocks do not need to be removed after drug depletion. However, their resorption is rather slow and they cannot be shaped to match the bone defect. To overcome this problem, Plaster of Paris ($CaSO_4 \cdot 1/2H_2O$) loaded with antibiotics was proposed.[19] Plaster of Paris can be shaped to match the bone defect, and its hydrated product, gypsum ($CaSO_4 \cdot 2H_2O$), is biocompatible and resorbable. However, the gypsum resorption rate is too high to provide a good support for new bone. Therefore, because HCPC are made of β-TCP, monocalcium phosphate [MCPM; $Ca(H_2PO_4)_2 \cdot H_2O$], and water have more favorable intermediate resorption rates, they are evaluated in this study as a potential drug delivery system for GS. The reaction end product is dicalcium phosphate dihydrate (DCPD; $CaHPO_4 \cdot 2H_2O$)[20,21]

$$Ca_3(PO_4)_2 + Ca(H_2PO_4)_2 \cdot H_2O + 7\ H_2O \rightarrow 4\ CaHPO_4 \cdot 2H_2O \quad (1)$$

Materials and Methods

Materials—The β-TCP powder (Bioland, Toulouse, France) had a Ca:P molar ratio of 1.456 ± 0.010. The X-ray diffraction (XRD) analysis of the powder showed small amounts of β-calcium pyrophosphate (β-$Ca_2P_2O_7$, β-CPP). The mean diameter was 6.86 μm, with a specific surface area of 1.64 ± 0.08 m^2/g. The plastic and liquid limits were 0.35 ± 0.01 and 0.40 ± 0.01 mL/g, respectively. The MCPM powder was purchased from Aldrich (Buchs, Switzerland; Art. 30'764−5). Even though the powder was sold under the name monocalcium phosphate [MCP; $Ca(H_2PO_4)_2$], XRD analysis indicated that it was, in fact, MCPM. Plaster of Paris, also named calcium sulfate hemihydrate (CSH; $CaSO_4 \cdot 1/2H_2O$), was purchased from Merck (Dietikon, Switzerland; Art. 1.02162). GS was obtained from Selectchemie (Zürich, Switzerland) and from Fluka (Buchs, Switzerland; Art. 48'760). The sulfate content was 35 ± 2%. Four types of hydrogel-forming polymers were used as cement additives and potential release modifiers: hydroxypropylcellulose (HPC, Klucel 99-MF-EP, Aqualon, Wilmington, DE), sodium alginate [SA, Manucol DMF E401 (EP), Kelco, International Tadworth, U.K.], poly(acrylic acid) (PAA, Carbopol 974-P, BFGoodrich, Cleveland, OH), and carboxymethylcellulose (CMC, Blanose CMC Gum 12M31P, Aqualon, Wilmington, DE).

Hydraulic Calcium Phosphate Cement—The cement samples were prepared by briefly mixing 1.2 g of β-TCP and 0.8 g of MCPM, adding this powder mixture to 2 mL of GS solution, and kneading the resulting paste for 30 s. The concentration of GS solution was varied between 0 and 16% (w/w) of the total mass of β-TCP and MCPM. In one series of samples, the GS powder was not dissolved in the mixing liquid, but added to the mixture of β-TCP and MCPM. After kneading, the paste was filled into a syringe (diameter, 12.5 mm) whose tip had been previously cut, and the setting time was measured with a Vicat-type apparatus.[22] Then, the cylindrical sample

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Table 1—Factors and Level Definitions of the Two Multifactorial Experimental Design 2×3

Factor	Symbol	Levels			
		Design	Low	Intermediate	High
Mixing liquid	A	I	0.50 mL/g[a]		0.80 mL/g[a]
		II	0.40 mL/g[a]		0.65 mL/g[a]
Polymer	B	I	0.0%	1.0% CMC	1.0% SA
		II	0.0%	1.0% HPC	0.3% PAA

[a] Per gram of powder.

was pushed out of the syringe and dried in air at room temperature. Finally, the faces were flattened with a no. 220 silicon carbide paper, and the diametral tensile strength was measured at a rate of 0.5 mm/min.[23]

Release Experiments—Three sets of release experiments were made according to three different factorial designs of experiments. In the first design (2^3), the effects of the amounts (factor A) of CSH (levels 0.0 versus 0.4 g), (factor B) of GS (20 versus 100 mg dissolved in the mixing liquid), and (factor C) of mixing liquid (0.80 versus 1.20 mL per gram of powder) were investigated. In the second and third design, which were both 2×3 designs with two replicates, the effects of the amount of mixing liquid and of the addition of four different hydrogel-forming polymers were studied (Table 1). These polymeric additives were used to try to modify the GS release properties. In these two designs, 0.03 g of tetra-sodium pyrophosphate anhydrous (NaPP, $Na_4P_2O_7$ purum; Fluka, Buchs, Switzerland; Art. 71'920) were added to the starting powders to prevent a quick hardening of the cement paste with a low amount of mixing liquid.

The cement samples were prepared as already described. After kneading 1.3 g of β-TCP, 0.7 g of MCPM, and the mixing solution for 30 s, the paste was filled into a syringe (diameter, 12.5 mm) whose tip had been previously cut off. After setting, the samples were unmolded and dipped into 250 mL of phosphate-buffered saline (PBS; pH 7.4; 9.53 g/L $Na_2HPO_4 \cdot 2H_2O$; 1.79 g/L KH_2PO_4; 4.50 g/L NaCl) stirred at 100 rpm (USP Paddle method; instrument: Sotax AT6, Sotax AG, Basle, Switzerland). Then, 2-mL samples were then withdrawn at regular intervals and assayed for GS content by a colorimetric method.[24] Two measurements were done for each sample. At the end of the GS release experiment, the samples were dried in air and their final porosity measured.

The initial porosity of the cement samples was measured on specimens prepared without GS. At the start of the release experiments, the solubility of GS is large enough that all the GS is dissolved in the solution filling up the cement pores. Drying these samples would precipitate GS in the cement pores, hence preventing the measurement of the initial porosity.

Biological Activity—The biological activity of GS was measured before and after mixing with HCPC. The agar diffusion method was chosen as the assay method, using staphylococcus epidermidis ATCC 12228 as the test organism. The GS standard solution was prepared with the pH 7.4 PBS. The GS samples were obtained as follows. The cement sample was prepared by mixing with a spatula 1.3 g of β-TCP, 0.7 g of MCPM, and 2 mL of a 1% (w/w) GS solution in a small beaker. After setting, the cement was scraped off from the beaker surface and added to 250 mL of the pH 7.4 PBS. Cement and buffer were then incubated at 37 °C under constant agitation. After 6 h, the buffer solution was analyzed for biological activity and GS concentration. Three agar plates were used for potency determination. On each of them, six holes of 7 mm in diameter were bored. One hole was filled with 100 μL of the diluted standard (one concentration per plate), and the five other holes were filled with 100 μL of geometric dilutions of the GS sample. After 4 h at 4 °C, and 14 h at 37 °C, the size of the inhibition zones around the holes was measured.

Scanning Electron Microscopy (SEM)—Small pieces of cement were pasted onto an aluminum plate and coated with a ~20-nm thick gold layer. The samples were then observed with a scanning electron microscope JEOL SM6300F (JEOL, Tokyo, Japan).

X-ray Diffraction Analysis—The XRD patterns were obtained on a Siemens Kristalloflex 805 diffractometer (Siemens, Karlsruhe, Germany), using Cu-Kα, Ni-filtered radiation, at an angular sweeping rate of 0.01° (2θ)/s.

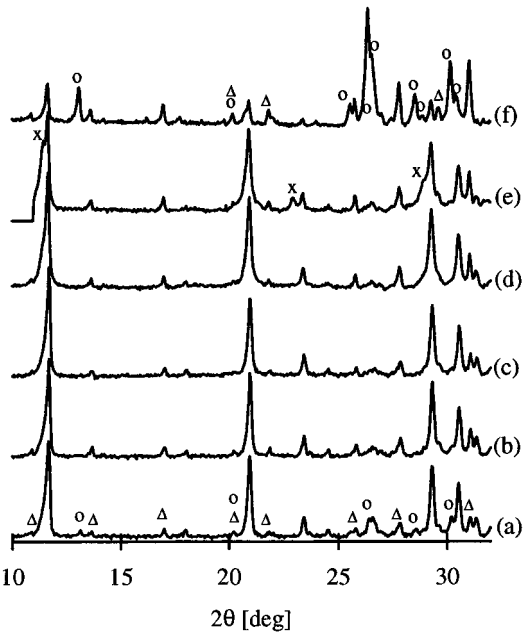

Figure 1—XRD patterns of cements made of 1.2 g of β-TCP, 0.8 g of MCPM, 2 mL of deionized water and (a) 0% GS, (b) 3% GS, (c) 9% GS, and (d) 16%. The last two spectra correspond to a cement sample prepared with 1.3 g of β-TCP, 0.7 g of MCPM, 0.4 g of CSH, 20 mg of GS, and 1.92 mL deionized water (e) before and (f) after release. Peak description: (o) DCP; (Δ) β-TCP; (x) CHPSH. All the unmarked peaks correspond to DCPD. The XRD intensity is given in (counts/s)$^{1/2}$ to ease the observation of small XRD peaks.

Porosity—Porosity measurements were made using Archimedes' principle as described by Bohner et al.[25] Each sample was first impregnated under reduced pressure (0.1 atmosphere) with 2-propanol. The volume of the open porosity was calculated from the difference in weight before and after impregnation. The apparent volume of the sample was calculated from the increase in weight measured when the sample was dipped into a beaker lying on the pan of a balance and filled with 2-propanol. Assuming that the samples had no closed porosity (as evidenced in the work of Bohner et al.[25]), the total porosity can be easily calculated.

Results

According to XRD analysis, the end product of the cement reaction was a well-crystallized DCPD (Figure 1a). A slight excess of β-TCP was used in all cement compositions (stoichiometric amounts: 1.10 g of β-TCP, 0.90 g of MCPM), so the hardened samples contained some residual β-TCP. Some anhydrous dicalcium phosphate (DCP; $CaHPO_4$) could also be observed. Adding GS to the cement paste led to the disappearance of DCP, but did not significantly change the XRD spectra (Figures 1b, 1c, 1d). As GS contains $35 \pm 2\%$ sulfate ions, gypsum (CSD; $CaSO_4 \cdot 2H_2O$) was expected to precipitate in the cements. However, due to both the crystallographic similarities between CSD and DCPD and the presence of β-TCP in the cement, the presence of CSD could not be evidenced. In contrast to GS, the addition of CSH to the cement samples provoked the appearance of three new peaks on the XRD spectrum at $2\theta = 11.47°$, 22.99°, and 29.00° (Figure 1e). All three peaks correspond to calcium hydrogen phosphate sulfate hydrate [CHPSH; $Ca_2H(PO_4)(SO_4) \cdot 4H_2O$]. According to its JCPDS File 30−252, the peaks at 11.44°, 22.97°, and 28.88° correspond to relative intensities of 100, 13, and 8%, respectively. However, the CHPSH peaks at $2\theta = 19.48°$ (40% relative intensity peak) and 22.63° (50%) could not be observed, indicating that CHPSH probably precipitated in the cement sample, but without the habit of the CHPSH crystals used to establish the JCPDS file. After GS release,

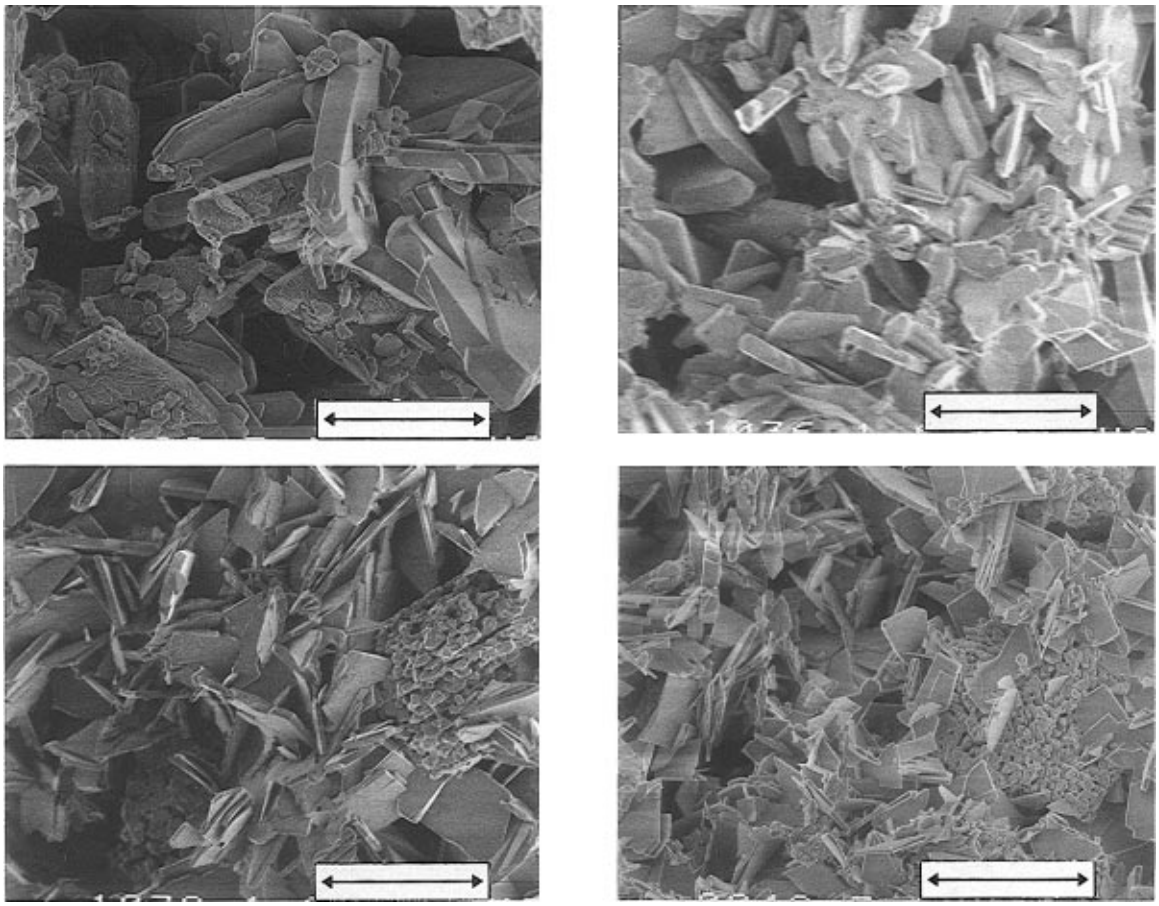

Figure 2—GS effect on the initial microstructure of cements made of 1.2 g of β-TCP, 0.8 g of MCPM, 2 mL of deionized water: (a, top left) 0% GS; (b, top right) 3% GS; (c, bottom left) 9% GS; (d, bottom right) 16% GS. The bars correspond to 10 μm.

all the peaks related to CHPSH had disappeared and a large amount of DCP had precipitated (Figure 1f).

The addition of GS led to a thinner and smaller average crystal size (Figure 2). A similar result was observed when either CSH or less mixing liquid were added to the cement paste (Figure 3). Comparing the cement microstructure before and after GS release showed that the precipitation of DCP crystals did not markedly change the cement microstructure. The main difference was the appearance of layered crystals, possibly resulting from the recrystallization of DCPD crystals into DCP crystals (Figure 3e).[22]

Adding GS as powder or aqueous solution to the cement paste improved the physicochemical properties of the cement. Above 3% (w/w) GS, the setting time was doubled compared with that of cements containing no GS (Figure 4). Moreover, the diametral tensile strength was quadrupled over the investigated range. The difference between the results obtained with GS aqueous solution and those obtained with GS powder was minor.

A typical release curve is shown in Figure 5. All the GS is released within the duration of the release experiment. Apart from a few exceptions discussed later, this behavior has been observed throughout this study. By plotting the accumulated release as a function of the square root of time, the release curve could be linearized up to ~50% and characterized by the slope k. The k values were then statistically analyzed as described by Montgomery.[26]

The results obtained for k in the experimental design 2^3 are shown in Figure 6. The statistical analysis (Table 2) showed that there was <5% chance that factors A, B, and C and the interaction AB had no effect on k. A higher amount of mixing liquid (factor C) resulted in a significant increase in k. Increasing the amount of CSH (factor A) led to a significant decrease in k, but this effect was much smaller when a high amount of GS was already present in the cement paste (interaction AB). A similar effect was also observed with GS (factor B) because the effect of GS on k was much stronger when no CSH was present in the cement paste.

The initial and final porosity (after 8 days release) of the cement samples were increased by the addition of CSH (Factor A) and of mixing liquid (Factor C; Table 2 and Figure 7). For samples free of CSH, the porosity hardly changed during release. However, a few percent increase of the porosity was observed in samples containing CSH.

In the first experimental design (2×3), a higher amount of mixing liquid led to a significant increase in k and the final porosity (Table 3), whereas the presence of CMC and SA in the cement samples significantly decreased k [8.1% decrease with 1% (w/w) CMC, and 16.1% decrease with 1% (w/w) SA]. In the second experimental design (2×3), k and the final porosity increased only with the higher amount of mixing liquid. The presence of HPC and PAA led to a decrease of the maximum amount of GS released [5.6% decrease with 1% (w/w) HPC, and 11.2% decrease with 0.3% (w/w) PAA]. Combining the results of the two experimental designs (2×3) shows that increasing the amount of mixing liquid from 0.4 to 0.8 mL/g doubled k (from 0.164 to 0.314 $h^{-1/2}$) and increased the initial and final porosity by 20% (Figure 8).

The use of HCPC as a drug delivery system for GS necessitates the preservation of full biological activity. As shown in Figure 9, no significant difference of biological activity was observed before and after mixing GS with the cement.

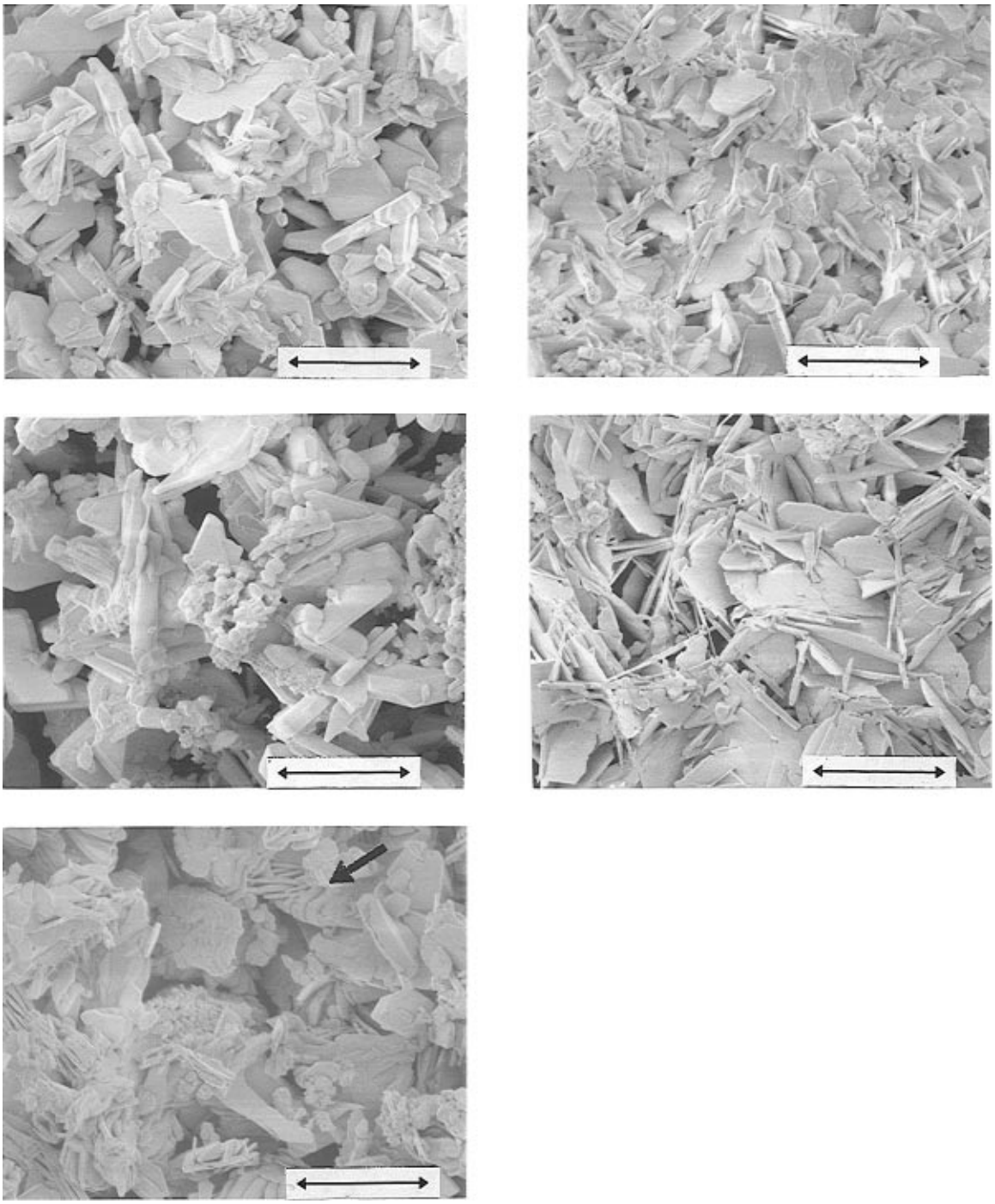

Figure 3—Initial microstructure of cements made of 1.3 g of β-TCP, 0.7 g of MCPM, and (a, top left) 1.6 mL of deionized water and 20 mg of GS; (b, top right) 1.6 mL of deionized water and 100 mg of GS; (c, middle left) 2.4 mL of deionized water and 20 mg of GS; (d, middle right) 1.92 mL of deionized water, 0.4 g of CSH, and 20 mg of GS; (e, bottom right) cement shown in c after release. The bars correspond to 10 μm. The arrow on Figure 3e shows a layered crystal.

Discussion

The effect of GS on the physicochemical properties of the cement is very similar to that previously observed when adding various sulfate concentrations to the cement paste.[25,27] The only difference is that the setting time does not abruptly decrease after reaching a maximum (Figure 4). Because GS contains 35 ± 2% (w/w) sulfate ions, its beneficial effect on the cement properties must be due to the presence of sulfate ions in the mixing solution. From previous experiments, the setting time maximum was expected at 0.1 M sulfate concentration.[25,27] When transforming GS weight fraction into molar sulfate concentration, the maximum setting time indeed appears at 0.1 M (Figure 4). Moreover, no sharp maximum of setting time was observed when additional 0.2 M sulfate ions (Na_2SO_4) were mixed into the cement paste.[28] The increase in tensile strength can be ascribed to the decrease in size and thickness of the DCPD crystals in the cement microstructure (Figure 2), which is due to the presence of sulfate ions.[25,27] The minor difference observed between the results obtained with GS solution and those obtained with GS powder (Figure 4) suggests that the GS powder was dissolved nearly instantaneously upon addition of the reacting water phase.

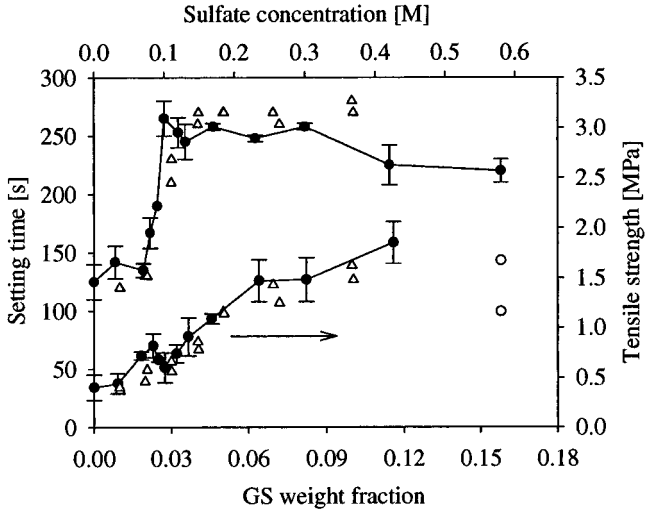

Figure 4—Effect of the amount of GS on the setting time (upper curve) and the tensile strength (lower curve) of the cement. Composition: 1.2 g of β-TCP, 0.8 g of MCPM, 2 mL of solution. Key: (●) GS dissolved in solution, average value ($n = 3$); (△) GS added as powder, data points; (○): GS dissolved in solution, data points (only for tensile strength). The error on the mean is given at a 95% confidence level.

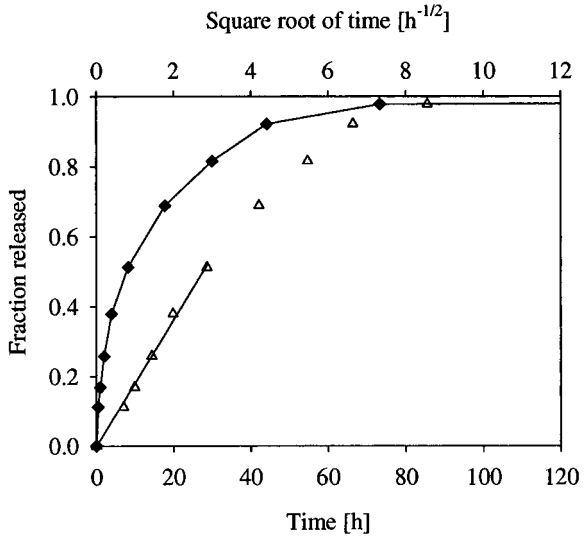

Figure 5—Linearization of a GS release curve. The GS fraction released is plotted as a function of (♦) time or (△) square root of time. The slope of the linear domain is $k = 0.182$ h$^{-1/2}$.

The GS release curves obtained in this study are similar in shape to those previously reported for other HCPC drug systems,[9–13] suggesting that drug release is controlled by diffusion through the pores of the cement. In that case, the drug release from the porous sample can be approximated by eq 2 up to 60% release[29]:

$$m = m_\infty k t^n \qquad (2)$$

where m is the mass of GS released from the cement at time t, m_∞ is the total amount of GS in the cement sample, k is the release rate constant, and n is an exponent varying between 0.42 and 0.50 depending on the cylinder geometry. In this study, the cylinders had a height/radius ratio equal to ~3, corresponding to $n \approx 0.42$.[29] However, for simplicity, the exponent n was taken as 0.50 throughout this study. According to Roseman,[30] 10% or less deviation should be noted for up to 50% release. Considering the small number of data points in the release curves, the experimental values were in good agreement with the square-root-of-time approximation up to ~50% release (Figure 5).

The three experimental designs (2^3 and two 2×3) show that the amount of mixing liquid and the sulfate ion content of the cement exert the most dominant effects on the GS

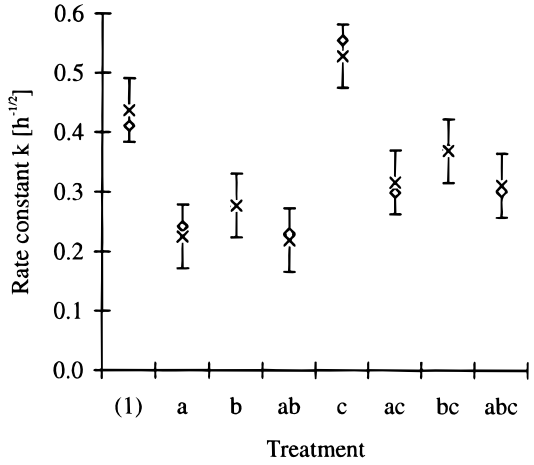

Figure 6—Rate constant results for the multifactorial experimental design 2^3. Factor A: amount of CSH; Factor B: amount of GS; Factor C: amount of mixing liquid. Key: (◊) data points; (×) adjusted values (with the statistical analysis given in Table 2). The size of the error bars corresponds to the 95% confidence interval of the adjusted values.

Table 2—Statistical Analysis of the Multifactorial Experimental Design 2^3[a]

	Rate Constant k [h$^{-1/2}$]			Initial Porosity ϵ_i			Final Porosity ϵ_f		
Source	Effect	F	$F_{5\%}$	Effect	F	$F_{5\%}$	Effect	F	$F_{5\%}$
A	–0.068	48.9	10.1	0.005	18.3	4.4	0.018	25.6	6.6
B	–0.041	18.2	10.1	—[b]	—	—	—[b]	—	—
AB	0.039	15.9	10.1	—[b]	—	—	—[b]	—	—
C	0.046	22.5	10.1	0.053	2466.5	4.4	0.048	182.6	6.6

[a] A, B, and C correspond to the amount of CSH (0.0 – 0.4 g), GS (20 – 100 mg), and mixing liquid (0.8 – 1.2 mL/g), respectively; effect means the average effect of a factor, and F gives its significance as compared with $F_{5\%}$, the F value corresponding to a 5% error risk; average values: $\bar{k} = 0.335$ h$^{-1/2}$; $\bar{\epsilon}_i = 0.640$; $\bar{\epsilon}_f = 0.663$; error on the adjusted values: $\delta(k) = 0.054$ h$^{-1/2}$ (95% confidence interval); $\delta(\epsilon_i) = 0.004$; $\delta(\epsilon_f) = 0.020$. [b] Not significant at the 5% error risk.

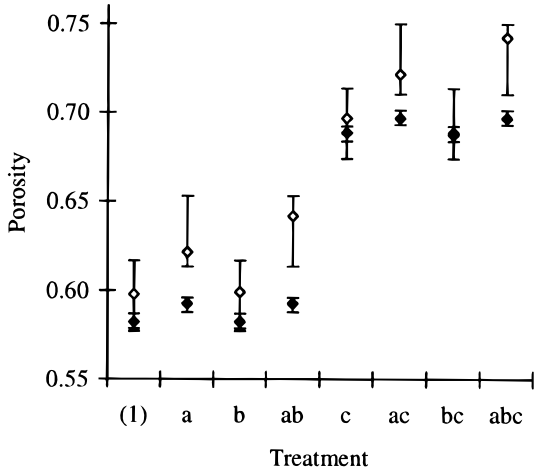

Figure 7—Comparison between the initial and final porosity of the cement samples used in the multifactorial experimental design 2^3. Factor A: amount of CSH; Factor B: amount of GS; Factor C: amount of mixing liquid. Key: (♦) initial porosity; (◊) final porosity. The size of the error bars corresponds to the 95% confidence interval of and around the adjusted values (not shown on the chart for clarity reasons).

Table 3—Statistical Analysis of the Two Multifactorial Experimental Designs 2×3[a]

		Rate Constant k ($h^{-1/2}$)			Final Porosity ϵ_f			Maximum m^b		
Design	Source	Effect	F	$F_{5\%}$	Effect	F	$F_{5\%}$	Effect	F	$F_{5\%}$
I	A	0.049	799	5.3	0.067	574	5.0	—[c]	—	—
I	B_L[d]	–0.019	82	5.3	—[c]	—	—	—[c]	—	—
II	A	0.040	304	5.1	0.068	1171	5.1	—[c]	—	—
II	B_L[d]	—[c]	—	—	—[c]	—	—	—0.055	33.5	5.0

[a] A corresponds to the amount of mixing liquid (I: 0.50 – 0.80 mL/g; II: 0.40 – 0.65 mL/g); B corresponds to the type of polymer (I: control, CMC, SA; II: control, HPC, PAA); effect means the average effect of a factor, and F gives its significance as compared with $F_{5\%}$, the F value corresponding to a 5% error risk; average values: design (I): $\bar{k} = 0.236\ h^{-1/2}$; $\bar{\epsilon}_f = 0.557$; $\bar{m} = 1.006$; design (II): $\bar{k} = 0.204\ h^{-1/2}$; $\bar{\epsilon}_f = 0.489$; $\bar{m} = 0.946$; error on the adjusted values: design (I): $\delta(k) = 0.008\ h^{-1/2}$ (95% confidence interval); $\delta(\epsilon_f) = 0.013$; $\delta(m) = 0.027$; design (II): $\delta(k) = 0.011\ h^{-1/2}$; $\delta(\epsilon_f) = 0.010$; $\delta(m) = 0.038$. [b] Maximum fraction released. [c] Not significant at the 5% error risk. [d] Linear effect of factor B.

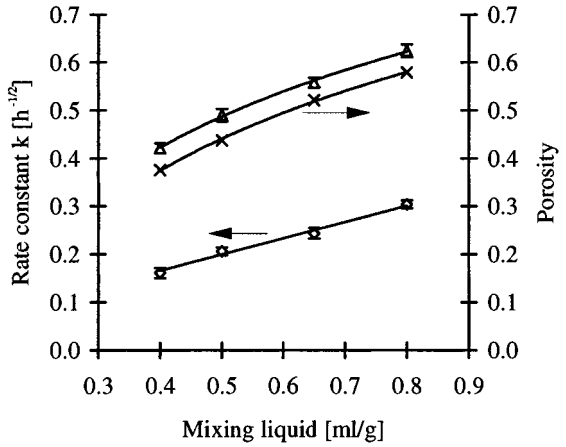

Figure 8—Summary of the effect of the amount of mixing liquid on (◇) k, on (×) the initial porosity, and on (△) the final porosity. The size of the error bars corresponds to ±1.96 standard errors (the error bars of the initial porosities are smaller than the symbols). The regression lines are: $k = 0.338\ V_{mix} + 0.031$ ($r^2 = 0.990$); $\epsilon_i = 0.297\ \ln(V_{mix}) + 0.646$ ($r^2 = 0.999$); and $\epsilon_f = 0.289\ \ln(V_{mix}) + 0.686$ ($r^2 = 0.999$).

release rate. This result may be explained by a porosity change, because both factors greatly affect the cement microstructure (Figures 2 and 3). Applying a relevant diffusion model, the effect of porosity, ϵ, on k can be estimated.[31] According to this model, the flux J (mg/m^{2}s) of a drug through a microporous membrane of thickness l obeys eq 3:

$$J = \frac{DK\epsilon\Delta C}{\tau l} \tag{3}$$

where τ is the membrane tortuosity, ΔC is the difference in concentration between both sides of the membrane, D is the diffusion coefficient in the liquid phase filling the membrane pores, and K is the distribution coefficient of solute between the bulk fluid and the fluid in the membrane pores. Equations 2 and 3 are related by eq 4:

$$\frac{1}{S}\cdot\frac{dm}{dt} = J \tag{4}$$

where S is the apparent surface area of the cement sample. For a cylinder of radius R:

$$S = 2\pi R^2 + \frac{2}{R}\left(\frac{V_c}{1-\epsilon}\right) \tag{5}$$

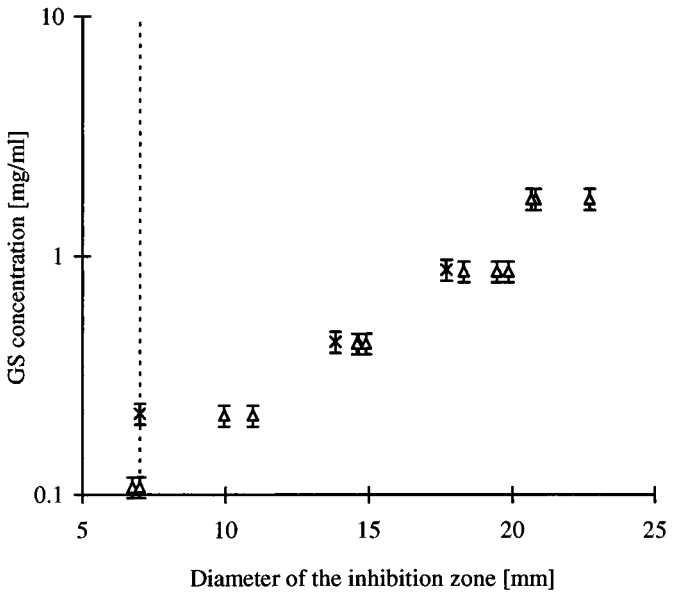

Figure 9—Biological activity of GS (×) before and (△) after mixing with the cement. The size of the error bars corresponds to ±1.96 standard errors. The dotted line corresponds to the bore hole diameter.

where V_c is the volume of solid in the cement sample. Using eqs 2 to 5 and taking $n = 0.5$, k can be expressed as a function of ϵ:

$$k = \frac{2DKt^{1/2}\Delta C}{l m_\infty \tau} S\epsilon \tag{6}$$

In the linear domain of the plot k as a function of $t^{1/2}$, k is constant, as will be the product ($t^{1/2}\Delta C$). When the amount of mixing liquid is varied, all parameters except ϵ should be also constant. In that case, k should vary according to the product (Sϵ). The latter conclusion is valid only if the volume and size distribution of the pores remain constant during release. This is not the case here because the porosity is changed during release (Figure 7). The volume and size distribution of the pores can be modified by the precipitation or the dissolution of crystals, or the recrystallization of DCPD crystals into DCP crystals.[22] The decomposition of DCPD into DCP leads to a net volume loss of 37% and hence provokes an increase of porosity. According to solubility isotherms,[32,33] the solubility of DCPD at pH 7.4 is ~0.8 mmol/L of total calcium ions dissolved and 0.9 mmol/L total phosphate ions dissolved. The release medium is free of calcium ions, but contains 67 mmol/L of total phosphate ions dissolved. Therefore, hardly any dissolution of DCPD is expected. When 0.4 g of CSH are added to the cement paste, 0.47 g of CSD should precipitate in the cement sample. As CSD has a solubility of ~10 mmol/L of total calcium ions dissolved at pH 7.4,[33] 0.4 g of CSD should dissolve in the release medium. The calcium ions liberated by the dissolution of CSD can react with the orthophosphate ions present in the buffer to precipitate a less soluble calcium salt like DCPD, HAp, or octocalcium phosphate [OCP; $Ca_4H(PO_4)_3{\cdot}5H_2O$]. However, no HAp or OCP were detected by XRD. Also, the change of cement porosity during GS release was always in the range 0−5% (Figures 7 and 8). Moreover, SEM micrographs (Figure 3) did not show any significant change of porosity and microstructure apart from the appearance of layered crystals related to the precipitation of DCP crystals. Furthermore, no new crystalline phase could be detected by XRD (Figure 1). Finally, no significant changes of porosity and composition were observed within the first 4 h of GS release.[28] As k is only determined

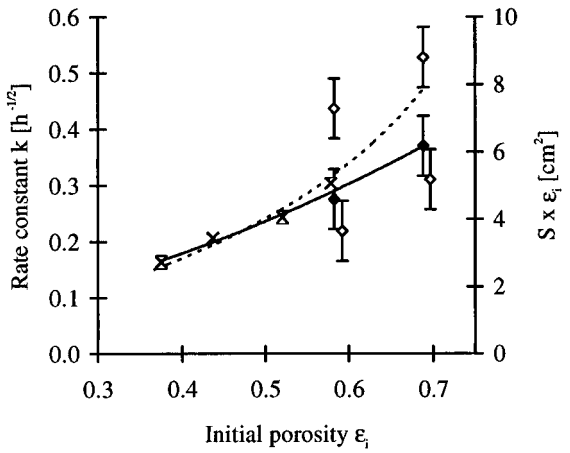

Figure 10—Relationship between the initial porosity ϵ_i and k. The results along the solid line were obtained with cements prepared with 1.3 g of β-TCP, 0.7 g of MCPM, and 5% GS (♦) in the experimental design 2^3, and (×) in the two experimental designs 2 × 3. The other *k* values (◊) were obtained in the experimental design 2^3 with cements prepared with 1.3 g of β-TCP, 0.7 g of MCPM, 0.4 g of CSH, and 5% GS (lower points), and with 1.3 g of β-TCP, 0.7 g of MCPM, and 1% GS (higher points). The size of the error bars corresponds to ±1.96 standard errors. For clarity reasons, the error bars on the x-axis are not shown (smaller than the size of the symbols). The regression line is: $k = 0.487\ \epsilon_i^2 - 0.130\ \epsilon_i + 0.050$; $r^2 = 0.979$. The dashed line corresponds to the product ($S\epsilon_i$).

during the first hours of release (Figure 5), the cement porosity is considered to be constant and equal to the initial cement porosity, ϵ_i. Equation 6 can thus be applied to the results. As shown in Figure 10, the correlation between the product ($S\epsilon_i$) and the experimentally determined *k* values obtained with cements free of CSH is significant, particularly at low porosity. This result suggests that the cement tortuosity is not significantly affected by a change of the amount of mixing liquid, even though the cement microstructure becomes finer with less mixing liquid (Figure 3).[33] This suggestion is in contradiction with observations made in another HCPC-drug delivery system[14] that indicated that the tortuosity was raised 10-fold with a 10% decrease of porosity.

Adding more sulfate ions to the cement paste led to a drop of the release rate (Figure 10). The decrease of *k* cannot be explained by a change of porosity. Neither can it be explained by any interaction between sulfate ions and GS.[28] Therefore, the drop is attributed to a change of cement tortuosity, which could result from a substantial change in cement microstructure when more sulfate ions are added to the cement paste, either as GS or as CSH (Figure 3).[27] The microstructure effect of sulfate ions seems to level off with increasing concentrations. The effect of adding 0.4 g of CSH to samples containing 5% GS is smaller than the effect of adding 4% GS in samples containing 1% GS (Figure 10). The latter effect is observed on *k* (Figure 10) and on the microstructure (Figure 3). The decrease of the maximum GS fraction released in the presence of PAA and HPC (Table 3) should be attributed to a polymer–GS interaction leading to strong binding under the experimental conditions.

The therapeutic concentration of GS is ≥ 4 μg/mL for sensitive microorganisms and ≥ 8 μg/mL for more resistant microorganisms.[34] At the end of a release experiment conducted with 100 mg of GS, the concentration in the release medium is 400 μg/mL. Therefore, the amounts released are large enough for therapeutic applications. Because GS activity drops with a decrease of pH,[35] one problem could arise from the cement pH, which is close to 6 (unpublished results). The answer will be given by the buffer capacity of the body fluids, which should therefore be tested *in vivo*.

In conclusion, the use of HCPC as a drug delivery system for GS appears to be very promising because GS has a very positive effect on the physicochemical properties of the cement, the GS release rate can be easily controlled by varying the porosity or the sulfate content of the cement, and the biological activity of GS is preserved when mixed with the cement.

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