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Study of a hydraulic calcium phosphate cement for dental applications

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Calcium phosphate-based cements (CPCs) have attracted much interest because of their good osteoconductivity for bone reconstruction. We obtained CPCs by mixing calcium bis-dihydrogenophosphate monohydrate (MCPM) and calcium oxide with water or sodium phosphate buffers (NaP) as liquid phase. Cement samples with different calcium-to-phosphate ratios (Ca/P), liquid-to-powder ratios (L/P) and liquid phases were analyzed by X-rays diffraction (XRD), pH-metry, extensometry and calorimetry. Antibacterial activity on two bacterial strains (*Streptococcus mutans, Lactobacillus acidophilus*) and a polycontaminated bacterial inoculum was also studied using the agar diffusion method. The best mechanical properties ($\approx$ 25 MPa) corresponded to Ca/P ratios between 1.67 and 2.5, a 1 M sodium phosphate buffer pH 7, as liquid phase and a L/P ratio of 0.6 ml g^{-1}. The final setting time increased with the Ca/P ratio. The setting expansion, around 1–2%, depended on the Ca/P and L/P ratios. The inner temperature of the cements rose to 45° during setting then decreased rapidly. The injectability was 100% up to 3.5 min and then decreased. It increased with increasing the L/P ratio but to the detriment of the compressive strength and setting time. XRD analysis indicated that the setting reaction led to a mixture of calcium hydroxide and calcium-deficient hydroxyapatite even for a Ca/P ratio of 1.67. Consequently, the pH of the surrounding fluids rose to 11.5–12 during their dissolution. Bacterial growth inhibition was only clearly observed for Ca/P $\geq$ 2. This bioactive calcium phosphate cement can potentially be employed for pulp capping and cavity lining as classical calcium hydroxide-based cements, but it is not usable, in the present formulation, for root canal filling because of its short setting time.

1. Introduction

Since the studies of Brown and Chow [1], calcium phosphate-based cements (CPCs) have attracted much interest because of their good osteoconductivity for bone reconstruction. By mixing calcium bis-dihydrogenophosphate monohydrate (MCPM) and calcium oxide with water or sodium phosphate buffers as liquid phase, we obtained CPCs [2, 3]. When the calcium-to-phosphate ratio (Ca/P) was 1.67 and the liquid phase was water, the final product was stoichiometric hydroxyapatite (HAp) [2]. But as we will see, when the liquid phase was a pH 7 phosphate buffer, the final product was, in fact, a mixture of calcium-deficient hydroxyapatite (CDHAp) and calcium hydroxide. Consequently, its dissolution led to an increase in pH of the surrounding solution to 11.5, making this cement *a priori* more efficient for dental applications than for orthopedic ones.

Indeed, calcium hydroxide-based cements are currently used in dentistry for direct or indirect pulp capping, apexification, apexogenesis and root canal filling [4–7]. Calcium hydroxide presents some advantages but also some drawbacks.

The main advantage of calcium hydroxide is its biological activity. It presents antibacterial and anti-inflammatory activities principally due to the high pH value of the surrounding environment (around 12.5) following its dissolution. Most bacteria do not resist a pH above 9.5, and the alkalinity allows the resolution of the exudates which maintain the inflammatory state. Calcium hydroxide acts as a chemical buffer *vis-à-vis* acidic dental cements because of this alkalinity and as a thermal buffer towards metallic materials because of its low thermal conductivity. Finally, it does not inhibit the polymerization of composite or acrylic reconstitutions unlike eugenol-based cements.

Calcium hydroxide also presents some drawbacks: (i)

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it provokes pulp necrosis [7, 8] during the first days, then the pulp reacts by establishing an atubular tertiary dentine bridge, but this dentine formation is made to the detriment of the pulpal volume with long-term biological consequences [8]; (ii) when the paste is only calcium hydroxide, its application in the root canal system is easy but the low hardening and the retraction by drying do not allow tight fillings, consequently it is only used as temporary material in this indication for which hermeticity is a priority; (iii) to get round this disadvantage, i.e., to increase the crushing strength and to decrease the setting time, non-bioresorbable polymeric bases were added (Dycal®, Life Kerr®, Cav-Hycal®, etc.), but under these conditions the setting time is too short to use these materials as root canal filling.

Given the final composition of our calcium phosphate cement, we investigated its potential use for the same indications as calcium hydroxide-based materials. This material will have to keep the advantages of calcium hydroxide while minimizing its drawbacks.

2. Materials and methods

2.1. Chemicals and materials

All chemicals were analytical reagent grade from Aldrich (CaO, HAp), Fluka (MCPM, NaH_2PO_4, Na_2HPO_4, $12(H_2O)$, $Ca(OH)_2$) or Prolabo (H_3PO_4). Commercial CaO was heated at 900 °C for 2 h to remove H_2O and CO_2 and stored in a vacuum desiccator. The CaO particle size was around 7 μm (2–40; specific area, $1.3\,m^2/g$, Mastersizer, Malvern Instruments). Commercial MCPM contained 3% moisture (determined by thermogravimetry) and 5% free H_3PO_4 (determined by acidimetry); particles size was around 230 μm (50–700; specific area, $0.06\,m^2/g$). The commercial powder was pulverized for 5 h in a rotating micromill (Retsch Instruments) before use to obtain a final particle size of around 20 μm (5–130; specific area, $0.60\,m^2/g$) and was used without further treatment. Water was doubly distilled on quartz after deionization on an ion-exchange resin. Trypticase soy agar (bacterial culture medium) was from bioMèrieux and sucrose from Merck.

Powder XRD patterns were obtained with an automatic Philips Diffractometer controled by an IBM PC (36 acquisitions, 3–35 °θ, 3200 points or 5 or 36 acquisitions, 2–20 °θ, 900 points, acquisition delay 500 ms) using an anti-cathode Cu K_α (0.1542 nm) with a nickel filter.

2.2. Preparation of calcium phosphate cement (CPC)

Cement powder was prepared by weighing appropriate amounts of the two components to obtain the desired calcium-to-phosphate ratio (Ca/P) and mixed just before use by crushing in an agate mortar. At this stage, when phosphate buffer was used as the liquid phase, the amount of phosphate in the buffer was taken into account. The liquid phase was either pure water, 0.1 M or 1 M H_3PO_4 or 0.1 M, 0.25 M, 0.45 M, 0.75 M or 1 M phosphate buffers (pH 7) prepared from NaH_2PO_4 and Na_2HPO_4, $12(H_2O)$. The powder was generally incorporated into the liquid phase by successive fractions as for dental zinc phosphate cements (1/6 of the powder was added every 15 s) and kneaded with the aid of a spatula between each addition to produce a paste of workable consistency. After a mixing time of two minutes, carried out on a glass plate at 20 ± 1 °C, the paste was loaded into the molds, clamped and stored at 37 °C and 100% relative humidity (RH). The different calcium-to-phosphate ratios tested ranged from 1.1 to 3 and liquid-to-powder (L/P) ratios from 0.5 to 1.5.

2.3. pH measurements

Two methods were used to measure the pH of the cements. In the first method (method 1), cement samples with $Ca/P = 1.67$ and different liquid phases were prepared and stored at 37 °C, 100% RH. At regular intervals, up to 24 h, samples were removed, crushed and the resulting powder was dispersed under stirring in CO_2-free water. The pH was noted after 10 min. In the second one (method 2), initial materials were immersed under stirring in CO_2-free water, with a final liquid-to-powder ratio of 2 and the pH was recorded over a period of 7 h.

2.4. Mechanical strength and setting time measurements

Compressive strength (CS) was measured with an Instron 4444 testing machine on samples (4 mm diameter × 6 mm height) clamped and stored at 37 °C and 100% RH as already described [2]. Initial and final setting times and swelling time were measured as described by Driessens *et al*. [9, 10].

2.5. Dimensional and thermal behavior during setting

The expansion and the heat dissipated during setting were measured with a self-made apparatus. A hole of 8.2 mm in diameter was made in a Plexiglas cylinder (4 cm diameter × 4 cm height) thermostated at 37 °C. The cement was injected into the hole, covering a previously inserted thermocouple. Then a Plexiglas plug (8.0 mm diameter) was inserted, packing down the cement, and an extensometer was placed at the top of the plug. The inner temperature of the cement and its expansion were recorded until they became stabilized.

2.6. Injectability

The injectability of cements was estimated by the weight percentage of the cement paste that could be injected with a 2.5 ml syringe (Plastipak®), inner diameter of 8.5 mm and an opening of 2 mm [11]. Two grams of cement were prepared, loaded into the syringe and manually injected at times 2.5, 3, 3.5 min, etc., up to 10 min after the start of mixing. The mass of cement that was injected and the mass remaining in the syringe were accurately weighed and the percentage calculated.

2.7. Antibacterial activity

The antibacterial activity of the different cement formulations was investigated using the agar diffusion

method. Petri dishes (5 cm in diameter) were filled with 10 ml trypticase soy agar supplemented with 1% w/w sucrose and buffered at pH 7. Then 20 µl aliquots of the bacterial suspensions (approx. 300×10^6cells ml^{-1}) were inoculated onto the surface of the agar plates. Cement samples (8.5 mm diameter × 5 mm height) were placed at the center of the plates, 5 min after mixing. The plates were cultured aerobically in an closed jar at 37 °C. A container with a 10 M sodium hydroxide solution was placed in the jar to absorb the carbon dioxide which could react with hydroxyl ions. We tested the susceptibility to the CPCs of *Streptococcus mutans, Lactobacillus acidophilus* (clinical isolates) and a polycontaminated bacterial inoculum isolated from dental plaque. The zone of inhibition was measured at 1, 2, 4 and 6 days because of the slow growth of *L. acidophilus* and also to allow for an eventual variation of inhibition with time. At least six experiments were performed for each tested cement as well as for calcium hydroxide taken as an internal reference.

3. Results

3.1. Final composition of the cements

With a calcium-to-phosphate ratio of 1.67, the setting reaction proceeded in two steps [2]. The first step was the fast formation of dicalcium phosphate dihydrate DCPD (during mixing) from MCPM and an equivalent part of CaO, whatever the liquid phase (Equation 1).

$$3Ca(H_2PO_4)_2 \cdot H_2O + 3CaO + 6H_2O \rightarrow 6CaHPO_4 \cdot 2(H_2O) \quad (1)$$

The second step was slower and was dependent on the liquid phase. With water or 1 M H_3PO_4 solution as liquid phase, DCPD reacted completely under 24 h with the remaining CaO forming stoichiometric hydroxyapatite according to Equation 2 (first XRD pattern in Fig. 1).

$$6CaHPO_4 \cdot 2(H_2O) + 4CaO \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 14H_2O \quad (2)$$

With a pH 7 sodium phosphate buffer (NaP) as liquid phase, the reaction was very slow; 30% DCPD remained after three days [2] and it did not completely disappear even after three months with 0.1 and 0.25 M NaP as shown by the small diffraction peaks at 5.86 °θ and 10.50 °θ (Fig. 1) corresponding to DCPD. After three months, whatever the sodium phosphate buffer concentration, the final composition of the cement samples (Fig. 1) was a mixture of an apatitic product (main diffraction peaks at 13.02, 14.18 14.50, 15.98 16.18 and 16.48 °θ) and calcium hydroxide (diffraction peaks at 9.08 and 17.12 °θ).

Because the initial Ca/P ratio, which took into account the phosphate provided by the buffer, was 1.67 and the presence of a non-negligible amount of calcium hydroxide, we concluded that the apatitic product was calcium-deficient hydroxyapatite (CDHAp) in which sodium ions probably partially replaced the calcium ions (Equation 3). Work is in progress to determine the exact composition of this product.

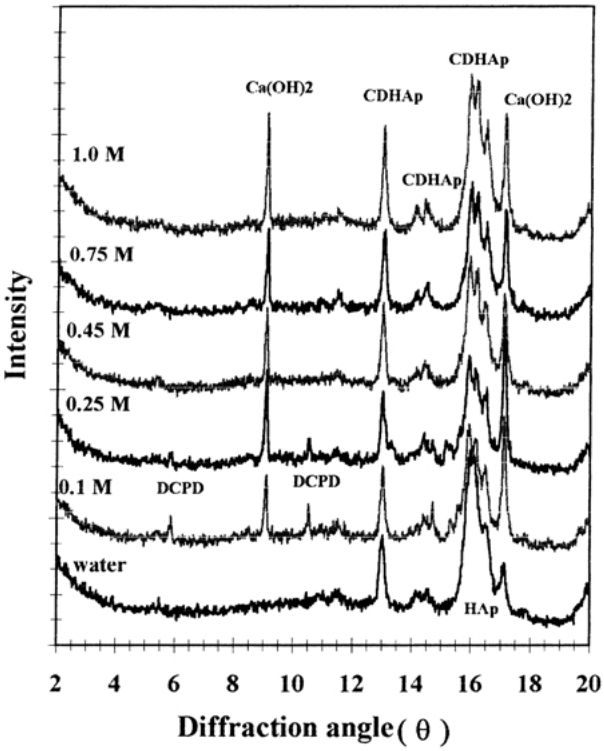

Figure 1 XRD patterns of cements prepared with different sodium phosphate buffer concentrations after three months of storage at 37 °C, 100% RH; Ca/P = 1.67, L/P = 0.6 ml g^{-1}.

$$(6-x)CaHPO_4 \cdot 2(H_2O) + 4CaO + x\ NaP \rightarrow CDHap + y\ Ca(OH)_2 + z\ H_2O \quad (3)$$

It remains that, the amount of calcium hydroxide seemed to be independent on the NaP buffer concentration as shown by the invariance of the ratio of the intensities of the $Ca(OH)_2$ peak at 9.08°θ and of the CDHAp peak at 13.02°θ (Fig. 2).

3.2. pH evolution

Given the initial presence of calcium oxide and its transformation to calcium hydroxide in the cements, we studied the change in pH during dissolution (method 1) and during setting (method 2) for cements with Ca/P = 1.67. During dissolution (Fig. 3), the pH of the solution rose to a value of 12.5 from the first minutes up to one hour, whatever the liquid phase; then it more or less decreased depending on the liquid phase. With

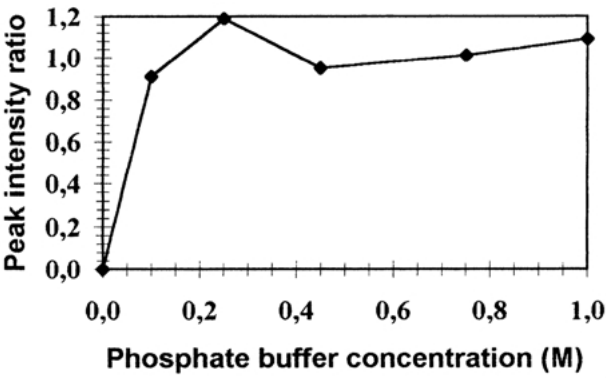

Figure 2 Variation in the ratio of the intensities of the $Ca(OH)_2$ peak at 9.08°θ and of the CDHAp peak at 13.02°θ (observed on XRD patterns in Fig. 1) as a function of the sodium phosphate buffer concentration.

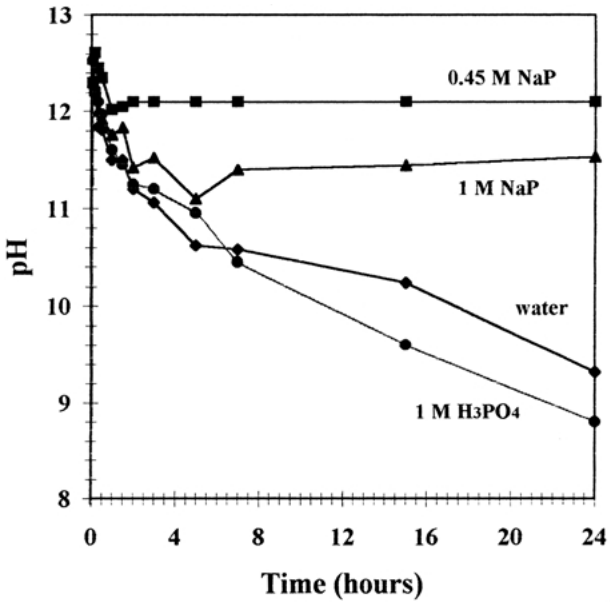

Figure 3 Variation in the pH of the suspension obtained by dispersion of crushed cement samples as a function of the time of crushing after mixing (method 1) and prepared with different liquid phases.

orthophosphoric acid or water as the liquid phase, the pH value decreased towards neutrality because of the fast and complete transformation of the starting materials to give stoichiometric hydroxyapatite. But with sodium phosphate buffer, the pH value became stabilized around 11.5–12, the reaction being slow and leading to a mixture of CDHAp and $Ca(OH)_2$.

During setting, the pH followed a reverse change depending on the liquid phase used (Fig. 4): (i) with water the pH rapidly rose to 12.2 then very slowly decreased (pH = 12.0 after 7 h and 11.1 after 24 h); (ii) with 1 M sodium phosphate buffer, the pH did not exceed a value of 9 and then decreased to become stabilized at a pH value of 7.3 after 50 min, but at this time the cement set. This difference in behavior, which was probably due to the experimental conditions used in the two methods, will be presented and discussed in a future paper.

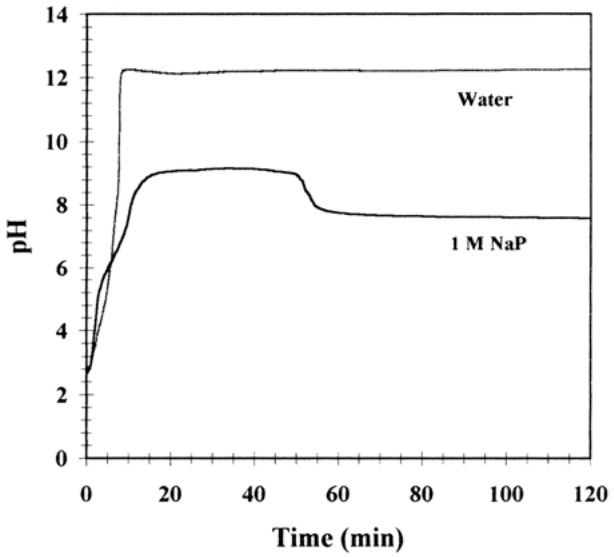

Figure 4 Variation in the pH of the suspension obtained by dispersion of starting materials as a function of time (method 2) and prepared with different liquid phases.

3.3. Mechanical properties

3.3.1. Compressive strength

The compressive strength (CS) of cement samples with Ca/P = 1.67, a liquid-to-powder ratio L/P = 0.6 and different sodium phosphate buffer concentrations was measured after 1, 45 and 90 days of storage at 37 °C and 100% RH (Fig. 5) on at least six samples. At day one, the CS increased with the NaP buffer concentration but after three months it was independent and stabilized around 30 MPa. With water as liquid phase we did not observe any variation of CS with time. Compressive strength at day one also varied with the Ca/P ratio for a given L/P ratio and with the L/P ratio for a given Ca/P ratio (Fig. 6). The best CS values (> 20 MPa) were obtained for Ca/P ratios between 1.67 and 2.5 and a L/P ratio of 0.6 ml g^{-1}, but the CS strongly decreased when the L/P ratio increased (Fig. 6).

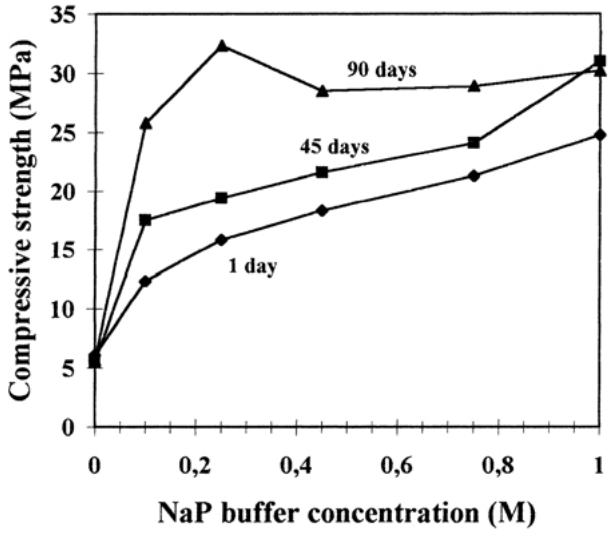

Figure 5 Variation in the compressive strength of cement samples as a function of the sodium phosphate buffer concentration after 1, 45 and 90 days of storage at 37 °C and 100% RH; Ca/P = 1.67, L/P = 0.6 ml g^{-1}.

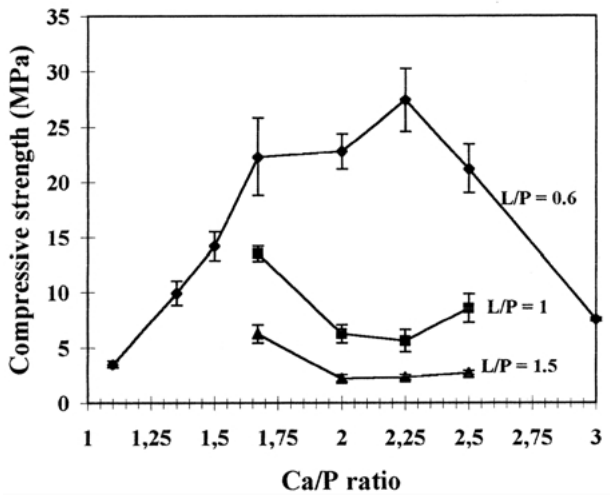

Figure 6 Variation in the compressive strength of cement samples prepared from different calcium-to-phosphate ratios and with different liquid-to-powder ratios; liquid phase was 1 M NaP and the time of storage was 24 h.

TABLE I Initial and final setting times and swelling time for cements with different Ca/P and L/P ratios and 1 M sodium phosphate buffer as liquid phase

Ca/P	Times (min)			
	L/P	Initial setting	Final setting	Swelling
1.67	0.6	4.0 ± 0.1	9 ± 1	4.0 ± 0.5
	1.0		16.5 ± 0.5	
2.0	1.0	3.0 ± 0.5	15 ± 2	> 30
	1.5	4.0 ± 0.5	30 ± 5	> 60
2.25	1.0	< 3.0	20 ± 3	> 60
	1.5	3.5 ± 0.5	> 30	> 60

3.3.2. Setting and swelling times

Initial and final setting time and swelling time were measured for different Ca/P ratios, L/P ratios and phosphate buffer concentrations. Some of the results are given in Table I. Initial and final setting times decreased when the buffer concentration increased [2]. The initial setting time decreased when the Ca/P ratio increased and inversely the final setting time increased (Table II). The use of a greater amount of liquid phase by increasing the L/P ratio did not improve the initial setting time; only the workability with the spatula was better but 30 s after the spatulation was stopped, initial setting took place. With Ca/P = 1.67, the swelling time was correct ($\approx$ 4 min); but when CaO was in excess (Ca/P > 1.67), it became markedly longer. Exact swelling times for Ca/P > 1.67 were not determined.

3.3.3. Dimensional and thermal behavior

Whatever the Ca/P ratio, during setting we observed a slight expansion (1–2%) of the cements (Fig. 7). This expansion seemed to be dependent on the Ca/P ratio and the L/P ratio, but additional experiments will be necessary to clarify this point. Similarly, cement temperature rose during setting (Fig. 8), but the maximum temperature never exceeded 50 °C and was generally below 45 °C.

3.3.4. Injectability

The variations of injectability with time for cements with Ca/P = 1.67 and different L/P ratios are represented in Fig. 9. The time of 100% injectability increased with the increase in the L/P ratio. For L/P = 1, it was around 6 min but it decreased when the Ca/P ratio was increased as a consequence of the diminution of the initial setting time. With Ca/P = 2.25 and L/P = 1.5, the time of 100% injectability was brought down to 3 min.

3.4. Antibacterial activity

Because small differences can occur during the preparation of the culture media, the Petri dish filling and the bacterial inoculum, calcium hydroxide was systematically used as internal reference. For each set of experiments, a relative zone of inhibition (ZI_{rel}) was calculated by dividing the zone of inhibition of the test cement (after substracting of the cement sample diameter) by the mean value of the zone of inhibition (23–40 mm) of the internal reference (after substracting of its diameter). The growth inhibition of a polybacterial inoculum by calcium phosphate cements prepared with a L/P ratio of 1.5 and different Ca/P ratios is presented in Fig. 10. In this figure, the *x*-axis corresponds to a P/Ca ratio instead of a Ca/P ratio; in this way, for $Ca(OH)_2$, P/Ca = 0 instead of Ca/P = ∞. The inhibition curve was S-shaped. For cements with Ca/P values of 1.67 and 1.85

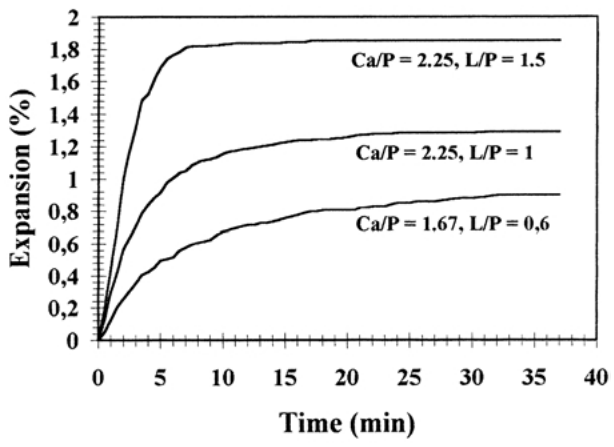

Figure 7 Variation in the cement expansion during setting as a function of time for different calcium-to-phosphate and liquid-to-powder ratios; liquid phase was 1 M NaP.

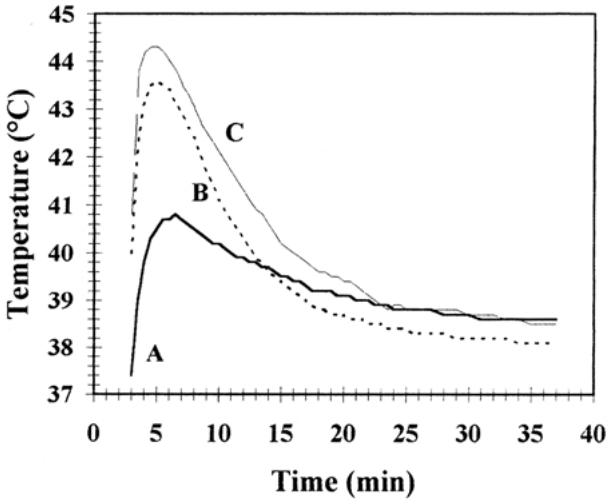

Figure 8 Variation in the cement temperature during setting with the time for different calcium-to-phosphate and liquid-to-powder ratios; the liquid phase was 1 M NaP. A: Ca/P = 1.67, L/P = 0.6 ml g^{-1}; B: Ca/P = 2.25, L/P = 1 ml g^{-1}; C: Ca/P = 2.25, L/P = 1.5 ml g^{-1}.

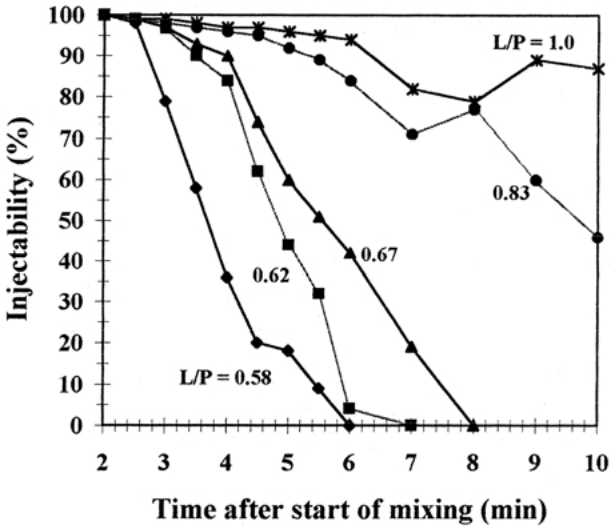

Figure 9 Variation in the cement injectability as a function of time after mixing for different liquid-to-powder ratios; Ca/P = 1.67, the liquid phase was 1 M NaP.

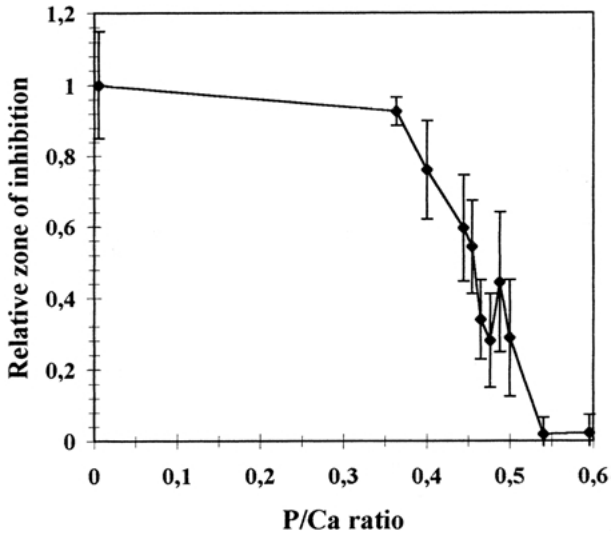

Figure 10 Variation in the growth inhibitory effect of CPCs after five days as a function of the phosphate-to-calcium ratio (P/Ca); L/P = 1.5, the liquid phase was 1 M NaP; the test strain was a polycontaminated bacterial inoculum. The results are expressed in terms of relative zone of inhibition (see text).

(P/Ca of 0.6 and 0.54) a clear inhibition was only observed with some samples. Effective growth inhibition was only reproducible with Ca/P $\geq$ 2, whatever the bacterial strain, the bacterial population of the inoculum (up to 30×10^9 cells ml^{-1}) and the L/P ratio used to prepare cement samples.

4. Discussion

Despite the alkaline pH that accompanies the dissolution of cement samples prepared with a calcium-to-phosphate ratio of 1.67, due to the presence of calcium hydroxide even after three months, we did not observe a real antibacterial activity for this cement. This is the reason for which we studied cements with Ca/P ratios above 1.67.

The use of the agar diffusion method to study the antibacterial activity of calcium hydroxide is a subject of controversy [12–14]. Although the antibacterial activity of calcium hydroxide has been clinically proved, Barbosa *et al*. [13] reported that calcium hydroxide did not show any antibacterial effect against most of the bacteria used in their study. For this reason, Suzuki *et al*. [12] preferred to use the agar dilution test. In this method, the calcium hydroxide powder is diluted in the agar, but with hardened cement samples this method is not applicable. Moreover, the agar diffusion test seems to us more representative of the situation occurring in treated teeth in which the hydroxyl ions (the effective drug) have to diffuse from the filling material surface into the dentin. The origin of this controversy is principally the buffering effect of the agar but also the presence of carbon dioxide particularly under anaerobic conditions under which hydroxyl ions are partly neutralized. Considering this latter observation, an antibacterial activity of the cement with a Ca/P ratio of 1.67 cannot be dismissed. Only *ex vivo* or *in vivo* experiments will allow us to verify this hypothesis because biological fluids are also buffered. However that may be, in this work, pronounced growth inhibition of the bacterial strains tested, and even of some fungal strains (not identified), by calcium hydroxide was observed. We also tested the antibacterial activity of two commercial calcium hydroxide-based cements (Dycal® and Life Kerr®). Their growth inhibition was in the order of: Ca/P = 2 > Dycal > Life.

The rapid setting of the cements (3–4 min) is convenient for pulp capping but not for root canal filling. For this latter application, the rheological properties of the cements will have to be improved. The expansion during setting is well oriented to avoiding marginal leakage.

The swelling observed during contact with water for cements prepared with a Ca/P ratio above 1.67 is due to the presence of CaO in excess. This is an ocalexic-like phenomenon [15, 16]. This phenomenon should facilitate the intratubular penetration of the material by contact with biological fluids present in the tubules and thus its antibacterial activity [17].

The temperature reached by the calcium phosphate cements during setting remains under 54 °C, above which the collagen becomes denatured [18]. The period during which the cement is above body temperature is short ($\approx$ 10 min) and should not be an inconvenience.

Lastly, the presence of precipitated DCPD followed by that of HAp confers on this material a hardness markedly greater than that of pure calcium hydroxide pastes. When these cements are prepared with a L/P ratio of 1, they are slightly less resistant to compression than commercial calcium hydroxide-polymer cements (10–15 MPa) used for pulp capping; they are also less adhesive but less brittle (frequently Dycal and Life samples break when they are removed from the molds). However, they are sufficiently hard to be rapidly covered with a definitive restorative material.

5. Conclusion

A cement prepared from a mixture of calcium bis-dihydrogenophosphate monohydrate and calcium oxide with a calcium-to-phosphate ratio of 2, a liquid-to-

powder ratio of 1 and a 1 M sodium phosphate buffer, pH 7, as liquid phase can potentially be employed for indirect and direct pulp capping and cavity lining as classic calcium hydroxide-based cements. But it is not usable, in the present formulation, for root canal filling because of its short setting time and low injectability. The fast precipitation of dicalcium phosphate dihydrate followed by that of hydroxyapatite ensures rapid setting and sufficient hardness to this material, allowing its immediate covering with a definitive restorative material. The slight expansion diminishes the marginal leakage risk.

The DCPD-HAp network acts as a drug delivery system, releasing hydroxyl ions that have the antibacterial activity. This release is five times less than with pure calcium hydroxide pastes. Consequently, pulpal necrosis would be expected to be less severe, in this way preserving the vitality and the integrity of the pulp. A living and healthy pulp remains the best barrier against bacterial invasion.

Finally, the osteoconduction of hydroxyapatite is now well established [19, 20] and recent studies show that matrix proteins and growth factors play a role in dentinogenesis [21–23]. Because our material, after setting, is mainly hydroxyapatite we can envisage, that, under the influence of these biological factors, functional dentin will be formed inside the restorative material instead of formation of a dentinal bridge to the detriment of pulpal volume.

Acknowledgments

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