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Crystallization mechanisms of calcium phosphate cement for biological uses

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Self-setting calcium phosphate cement for dental or surgical applications can be prepared by the addition of a liquid to a mixture of acidic and basic calcium phosphate. After hardening, the final compound becomes hydroxyapatite. Using an orthogonal central composite plan, the main factors which control the setting and the final hardness of the cement were defined and models are proposed. The mechanisms of crystallization, the role of free and linked water, and the nature of the final and intermediate compounds are described.

1. Introduction

Calcium phosphates are materials used in orthopaedic and dental applications for bone defect filling, hip coating, oral surgery, etc. A new development has recently appeared: self-setting cements. These cements are the subject of considerable interest for various applications. Their structure and composition, close to that of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, make them biocompatible materials [1, 2].

Calcium phosphate cements are prepared by the addition of water to a mixture of acidic and basic calcium phosphate compounds. First, a paste is formed which, when sufficiently stiff, can be placed in the surgical site where it hardens.

All the tests on pure calcium phosphate cements have proved that these materials are perfectly biocompatible. However, other properties of these cements have to be controlled: setting time, porosity and hardness. These properties are correlated with the various crystallization mechanisms which occur during the formation of hydroxyapatite cement. In this paper we describe these mechanisms and their effects on the setting time and hardness.

2. Materials and methods

The cement is formed by a mixture of tetracalcium phosphate $Ca_4(PO_4)_2O$ (TTCP), b-calcium phosphate $Ca_3(PO_4)_2$ (b-TCP), monocalcium phosphate monohydrate $Ca(H_2PO_4)_2.H_2O$ (MCP) [3]. A mixture of these reagents with water forms a hydroxyapatite following the reaction:

$$a\mathrm{Ca(H_2PO_4)_2.H_2O} + b\mathrm{Ca_3(PO_4)_2} + c\mathrm{Ca_4(PO_4)_2O} \xrightarrow{-\mathrm{H_2O}} \mathrm{Ca_{10}(PO_4)_6(OH)_2}$$

with $b = 2 - 3a$ and $c = 1 + 2a$

The addition of pure water to this mixture induces very rapid setting and the formation of a curdled heterogeneous mixture. In order to obtain a more convenient setting time and a homogeneous paste some additives or treatments are needed. First, the solid mixture is previously treated with a solution of water and ethanol, then heated to 150 °C. Secondly, phosphoric acid and sodium glycerophosphate are added to the water to prepare the reactive liquid.

The properties of the cement depend on many factors: the pre-treatment with the water/ethanol solution, the values of the stoichiometric coefficients a, b, c, the volume percentage of phosphoric acid, the liquid/solid ratio, the grain size of the solid phase, and of course the temperature of the reaction.

In order to determine the effects of these different factors on the setting time and hardness, orthogonal central composite plans were set up.

The final hardening of the cement was determined by the resistance to the penetration into the cement surface of a needle of 1 mm^2. It was possible to plot the curve of the resistance value versus time and to determine the setting kinetic. The setting limit would be considered to be reached when the resistance value was equal to 300 g/mm^2. In order to define and compare the final hardness, the compressive force was

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TABLE I Working ranges and variation steps of factors influencing the setting time

	Coded variables X_i	Level					Step
	$X_1, X_2, X_3, X_4, X_5, X_6$	-2	-1	0	1	2	Dx_i
Natural variables x_i	$x_1 = Pv$ (%)	0	1	2	3	4	1
	$x_2 = Te$ (°C)	20	25	30	35	40	5
	$x_3 = Cs$	0.1	0.225	0.35	0.475	0.6	0.125
	$x_4 = R_1$ ($cm^3 g^{-1}$	0.35	0.40	0.45	0.50	0.55	0.05
	$x_5 = Sg$ μm)	20	55	90	125	160	35
	$x_6 = Hu$ (%)	12	14	16	18	20	2

$X_1 = Pv$ = volume percentage of phosphoric acid
$X_2 = Te$ = temperature
$X_3 = Cs$ = stoichiometric coefficient of MCPM
$X_4 = R_1$ = liquid/solid ratio
$X_5 = Sg$ = grain size of TTCP
$X_6 = Hu$ = humidification level
$X_i = (x_i - x_i^0)/Dx_i$ X_i = coded variable
x_i = natural variable
x_i^0 = value of the ith natural variable at the centre of the range
Dx_i = variation step of the natural variable

measured after 10 days of hardening on a cement cylinder of diameter 4 mm and length 8 mm.

The characterization of the samples was carried out by X-ray diffraction (XRD) (CPS 120 INEL instrument and IR spectroscopy (IRS) (FTIR PE 7700).

3. Optimization of the cement

3.1. Optimization of the setting time

In order to optimize the cement according to its setting time an orthogonal central composite plan was set up taking in account the six variables previously mentioned and listed in Table I [4,5]. The glycerophosphate percentage was fixed at 0.6 g/ml. The experimental matrix was formed by 50 experiments randomly carried out.

At the level of confidence of 90%, the model equation which describes the variation of the setting time versus the different factors can be represented by the following relationship:

$$\widehat{st} = 138.69 - 18.53X_1 - 36.93X_2 + 40.83X_3 + 25.83X_4 + 14.19\,X_1X_1 - 19.43X_3X_3 - 16.31X_5X_5 - 12.53X_2X_3$$

The variables: stoichiometric coefficient of monocalcium phosphate monohydrate (Cs) and liquid/solid ratio ($R1$) have a confidence level of 99.9%; they both have a positive effect on the response, i.e. the setting time increases with the amount of monocalcium phosphate in the solid mixture and with the amount of liquid added to the solid.

The temperature (Te) and the volume percentage of phosphoric acid (Pv) also have a confidence level of 99.9% and have a negative effect on the response: when the temperature and/or the amount of phosphoric acid in the liquid increases the setting time decreases.

The square terms (stoichiometric coefficient of monocalcium phosphate)^2 and (grain-size of tetracalcium phosphate)^2 have a level of confidence of 95% and a negative effect on the response. The fact that the grain size of TTCP (Sg) does not have a significant effect on the seting time, while its square does, means that the effect of this factor is not linear.

TABLE II The optimum values of the variables

Variable	Experimental range
Percentage of phosphoric acid (%)	2–4
Temperature (°C)	30–40
Stoichiometric coefficient of MCPM	$\leqslant 0.35$
Liquid/solid ratio ($cm^3 g^{-1}$)	$\leqslant 0.4$
Grain size of TTCP (μm)	90
Humidification level (%)	16

The square terms (volume percentage of H_3PO_4)^2 and the interaction (temperature × stoichiometric coefficient of monocalcium phosphate) have a confidence level of 90% and, respectively, positive and negative effects. The variables grain size and humidification level do not present any effect on the setting time and their average values were fixed at 90 μm and 16%, respectively. The optimum range to obtain a convenient setting time is reported in Table II.

3.2. Optimization of the hardness

Previous experiments had shown that only three variables influenced the final hardness of the cement: they are listed with their working range in Table III. An orthogonal composite plan was used to optimize the final hardness. For such a plan with three factors 20 experiments were carried out. At the level of confidence of 90%, the model equation which describes the variation of the final hardness versus the three factors can be represented by the following relationship:

$$\hat{y}: 17.26 + 5.26X_2 - 1.89X_3 - 2.39X_1X_2$$

where $\hat{y}$ represents the hardness (MPa). The final hardness is mainly influenced by the proportions of

TABLE III Working ranges and variation steps of the factors influencing the setting time

	Coded variables X_i	Level					Step
	X_1, X_2, X_3	− 1.68	− 1	0	1	+ 1.68	Δx_i
Natural variables	$x_1 = Pv$ (%)	0.3	1	2	3	3.7	1
x_i	$x_2 = Cs$	0.14	0.225	0.35	0.475	0.56	0.125
	$x_3 = R_1$ ($cm^3 g^{-1}$)	0.37	0.40	0.45	0.50	0.53	0.05

$X_1 = Pv$ = volume percentage of phosphoric acid
$X_2 = Cs$ = stoichiometric coefficient of MCPM
$X_3 = R_1$ = liquid/solid ratio

TABLE IV The optimum values of the variables

Variable	Experimental range
Percentage of phosphoric acid (%)	0.3–2
Stoichiometric coefficient of MCPM	0.475 0.57
Liquid/solid ratio ($cm^3 g^{-1}$)	0.4–0.45

the three initial calcium phosphates (X_2) and also, but to a lesser extent, by the percentage of phosphoric acid (X_1) and the liquid/solid ratio (X_3). The range giving the optimum hardness is reported in Table IV.

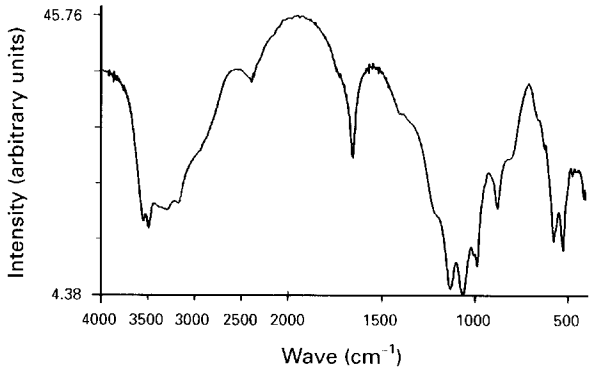

Figure 1 Infrared spectroscopy of formed DCPD.

4. Mechanisms of formation of the hydroxyapatite

Two main mechanisms were evidenced: at the beginning, dicalcium phosphate dihydrate ($Ca(HPO_4).2H_2O$, DCPD) is formed according to the two reactions:

$$Ca(H_2PO_4)_2.H_2O + Ca_3(PO_4)_2 \rightarrow 4Ca(HPO_4).2H_2O$$

$$Ca_4(PO_4)_2O + H_3PO_4 \longrightarrow 4Ca(HPO_4).2H_2O$$

The DCPD was detected either by X-ray diffraction or infrared spectroscopy as shown in Fig. 1

In the following stage, tetracalcium phosphate reacts with the previously formed DCPD and with tricalcium phosphate to give a final product with an apatite composition according to the reactions:

$$Ca_4(PO_4)_2O + Ca(HPO_4).2H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2$$
$$Ca_4(PO_4)_2O + Ca_3(PO_4)_2 \longrightarrow Ca_{10}(PO_4)_6(OH)_2$$

The reactions in the formation of DCPD in the first stage are very rapid and correspond to the setting stage. The reactions in the formation of the apatitic phase are quite slow and correspond to the second stage: the hardening. The two stages can be correlated with the general shape of the curve which plots setting (resistance to penetration of a needle into the surface of the cement) versus time. This curve can be considered as the superimposition of two curves corresponding to the two main reactions occurring during formation of DCPD and formation of apatite (Fig. 2).

Dicalcium phosphate does not evolve directly into apatite. The formation of an intermediate octacalcium phosphate ($Ca_8(HPO_4)_2(PO_4)_2.5H_2O$) certainly occurs. The octacalcium phosphate structure consists of alternating layers of PO_4^{3-} groups interspersed with Ca^{2+}

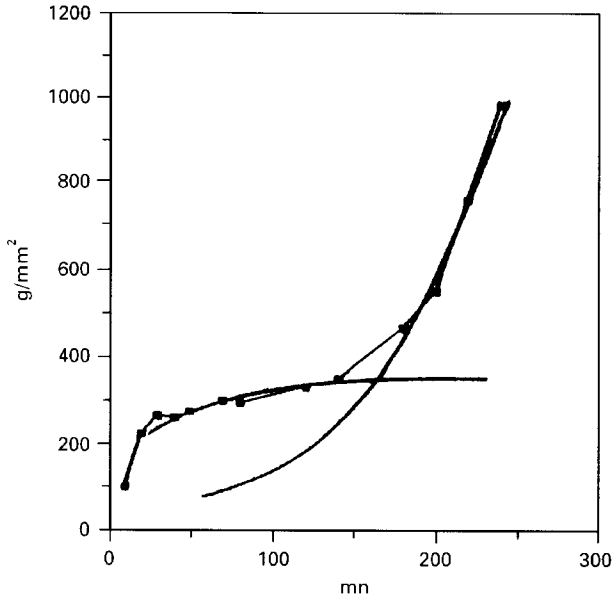

Figure 2 General shape of the curve: setting and hardening of the cement versus time:

ions, as in apatite (called "the apatitic layer") and layers of more widely spaced PO_4^{3-} and Ca^{2+} with H_2O interspersed (called the "hydrated layer")[6]. The formation of octacalcium phosphate is followed by its hydrolysis into apatite. The octacalcium phase is not easy to detect by X-rays. The presence of this hydrated phase has been proposed during the formation of hydroxyapatite: the addition to the cement of large amounts of fluorine, which hinders the formation of octacalcium phosphate and favours the direct formation of apatite, limits the hardening. So the

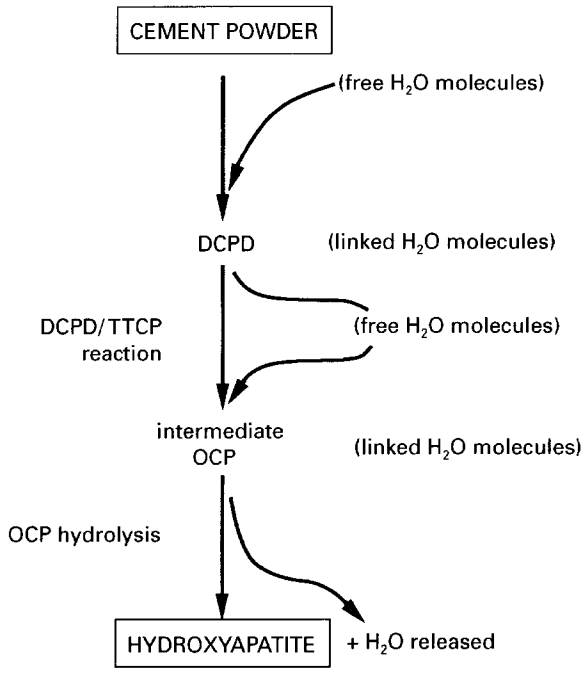

Figure 3 Mechanism of cement hydration.

intermediate formation of octacalcium phosphate is certainly necessary to obtain good hardening.

As in the general case of building cements, water plays important and different roles. First it is involved as a reactional medium to dissolve the calcium phosphates, quickly or slowly depending on the compound; secondly it is also involved as a crystallization molecule for the formation of dicalcium or octacalcium phosphate. Nevertheless, it is important to note that the quantity of water actually needed by the overall formation of the cement is small. The mechanism of hydratation is described in Fig. 3.

During the pre-treatment of the powder by the ethanol/water mixture a first stage of hydration occurs which involves the partial transformation of monocalcium into dicalcium phosphate. After setting in this first stage, the cement appears to be dried. Nevertheless the reaction continues with the intracrystalline water. If the cement is completely dried by lyophilization, all the reactions stop. This cement is a hydraulic cement which needs a humid medium to set and harden properly.

5. Conclusion

A mixture of monocalcium phosphate, tricalcium phosphate and tetracalcium phosphate, in convenient proportions and with an overall atomic calcium/phosphorus ratio equal to 1.67, with added liquid constitutes a self-setting cement which forms hydroxyapatite.

An orthogonal central composite plan was defined in order to determine the influence of various factors such as volume percentage of phosphoric acid, grain size, liquid/solid ratio temperature, stoichiometric coefficient and humidification level on both setting time and final hardness of the cement. Empirical models were set up. This allows suitable cements to be prepared for various uses.

Different stages of cristallization occur and intermediate compounds such as dicalcium phosphate are formed, evidenced by X-ray diffraction, and octacalcium phosphate is only suggested. Water plays an important role both as a reaction medium for dissolution and precipitation, and also as bound molecules in crystallized compounds.

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