Ceramics International xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Ceramics International

journal homepage: www.elsevier.com/locate/ceramint

Effect of the phosphorous content and synthesis method on the *in vitro* bioactivity and mechanical properties of calcium aluminate cements

B.A. Sánchez-Escobedo*, J.C. Escobedo-Bocardo, D.A. Cortés-Hernández, J.M. Almanza-Robles, G. García-Álvarez

CINVESTAV-IPN Unidad Saltillo, Ave. Industria Metalúrgica No. 1062, Parque Industrial Saltillo-Ramos Arizpe, Ramos Arizpe, Coah. CP 25900, Mexico

ARTICLE INFO

Keywords:
Calcium aluminate cement
Bioactive cement
Compressive strength
Phosphorous additions
Pechini method

ABSTRACT

The effect of the synthesis method and the phosphorous content on the *in vitro* bioactivity, compressive strength and setting time of phosphorous-containing calcium monoaluminate cements (CACs) was studied. In order to obtain pure monoclinic calcium aluminate (CA), two methods were used: Pechini and solid state reaction, incorporating the phosphorous to CA during synthesis. After 7 days of immersion in a simulated body fluid (SBF), a Ca-P rich compound was formed on the surface of all the CACs. The amount of this compound was increased as phosphorous content and immersion time were increased and its morphology was finer for the CACs obtained from CAs synthetized by Pechini method. Phosphorous-containing CACs showed a higher compressive strength than those with no P after 21 days of immersion in SBF. Final compressive strength of CACs elaborated with CA synthetized by solid state reaction was higher than that of CACs elaborated with CA synthetized by Pechini method due to the smaller particles obtained by this advanced method which promote a rapid hydration. Setting times decreased as phosphorous content was increased, being shorter in cements elaborated with CA synthetized by Pechini method due to the smaller particle size which has a strong effect on the hydration kinetics.

1. Introduction

Materials science faces a challenge in the design of a new generation of biomaterials for the replacement and regeneration of diverse parts of the human body, which exceed the expectations of the current long term implants [1].

Nowadays there are problems related to degenerative diseases such as osteoporosis or bone lesions leading to the search for biomaterials that allow regeneration and/or replacement of bone tissue, hence the idea of developing bioactive materials. In order to assess materials bioactivity, it is necessary to develop *in vitro* tests that predict the *in vivo* bioactivity [2]. One of the most common methods to evaluate the formation of hydroxyapatite (HA) on the surface of a material is through the use of a simulated physiological fluid (SBF) [3].

Calcium aluminate cement (CAC) shows characteristics that make it attractive for uses beyond the dental, due to their good physical and mechanical properties, biocompatibility and bioactivity, resulting in a wide range of possibilities for the use of this material [4–7].

The bioactivity of CAC was assessed using both phosphate buffer saline solution (PBS) and SBF, observing the formation of a thin Ca-P rich layer and small Ca-P rich particles on the surface of the cement, respectively [7,8]. A study performed on dorsal subcutaneous rat tissue shows satisfactory results on the biocompatibility of this cement, since no inflammatory reactions were observed [9]. Exposure of CAC to osteogenic cell cultures was evaluated and it was demonstrated that CAC supports the growth of osteoblasts [10].

Phosphorus-containing compounds (phosphoric acid, sodium phosphates, phosphorus pentoxide, aluminum dihydrogen phosphate or sodium polyphosphates) has been added to CAC improving mechanical properties, reducing degradation of the mechanical strength with time and shortening setting times [11–15]. In general, it was found that additions of polyphosphates to CACs form, during hydration, amorphous calcium phosphates and alumina gel that act as a binding matrix between the partially reacted and unreacted CAC particles, being this fact the responsible for high strength development.

A modified hydraulic calcium aluminate was obtained by adding P_2O_5 (0.49, 0.120 and 0.152 mol fraction) during the sol-gel synthesis [11]. They reported that P_2O_5 promoted the formation of a solid solution phase of calcium phosphor-aluminate, low crystallinity of modified CA and a high amount of glassy phase. The mechanical compressive strength of modified calcium aluminate pastes was as high as 134 MPa at 360 day hydration. However, the effect of the phosphor-

* Corresponding author.
E-mail address: brisa9730@gmail.com (B.A. Sánchez-Escobedo).

http://dx.doi.org/10.1016/j.ceramint.2017.07.068
Received 27 June 2017; Received in revised form 7 July 2017; Accepted 10 July 2017

ous-containing calcium monoaluminate (CA) synthesis method on the mechanical properties and bioactivity of the cement has not been studied.

In this work, phosphorous-containing calcium monoaluminates were obtained by two different methods: Pechini and solid state reaction, and the mechanical properties and bioactivity of the prepared cements were evaluated and compared. The synthesis conditions were selected to assure the obtaining a single monoclinic calcium aluminate phase since this phase is the responsible of the hydraulic properties of this kind of cements.

2. Materials and methods

2.1. Phosphorous-added calcium monoaluminate synthesis

Pechini method: The synthesis was performed according to the procedure described by Moore and Sang [16] with some changes. Aluminum nitrate ($AlN_3O_9{\cdot}9H_2O$) and calcium nitrate ($CaN_2O_6{\cdot}4H_2O$) were weighed at a molar ratio 1.93:1 and dissolved into water. Appropriate amounts of citric acid and ethylene glycol were then added to the solution. In this stage, the corresponding amount of phosphorous (P) was added as $(NH_4)_2HPO_4$ (Table 1, P added). Small P additions were selected for two reasons: i) to promote bioactivity and, ii) to evaluate the effect of P on the mechanical properties of the cement. In preliminary experiments, it was found that more than 0.75%wt of phosphorous promotes the formation of a second phase (tricalcium phosphate), detectable by XRD.

The components were thoroughly mixed and heated up to 80 °C to achieve complete dissolution and form a gel. This gel was heated up to 300 °C to promote polyesterification and remove the excess of water obtaining a solid polymeric resin. The product was ground and sieved (−100 mesh) and then discs were obtained by uniaxial pressing of powders at 100 MPa. The discs were heat treated at 1300 °C for 10 h and later they were milled and sieved (−325 mesh).

Solid state reaction: Stoichiometric amounts of calcium carbonate ($CaCO_3$) and alumina (Al_2O_3) powders were mixed with the selected amount of P, added as $(NH_4)_2HPO_4$ (Table 1, P added). The mixture was homogenized in acetone for 4 h and dried at 80 °C for 12 h. Thereafter, the resultant agglomerates were milled and then discs were obtained by uniaxial pressing of the powder at 100 MPa. The discs were heat treated at 1450 °C for 4 h and later they were milled and sieved (−325 mesh).

The obtained powders by both methods were characterized by inductively coupled plasma analysis (ICP; Optima 8300 Perkin Elmer), X-ray diffraction (XRD; Philips Xpert 3040e), Transmission electron microscopy (TEM; FEI-TITAN 80–300 kV), X-ray photoelectron spectroscopy (XPS; Perkin-Elmer PHI 560/ESCA-SAM) and infrared spectroscopy (FT-IR; Avatar 5360).

2.2. Cement sample preparation

Cement samples were prepared by mixing phosphorous-containing calcium monoaluminate and deionized water (w/c ratio of 0.4). The resulting pastes were poured into two different nylamid molds in order to obtain discs (10 mm in diameter and 2 mm in height) for the *in vitro* bioactivity assessment and cylinders (6 mm in diameter and 12 mm in height) for the measurement of the compressive strength. All samples were set for 24 h.

Table 1
Identification of the different CAs and CACs, phosphorous additions to the CAs and average particle size of CAs.

Synthesis route	CA (clinker)	CAC (hydrated CA)	P added (wt%)	P obtained (wt%)	Average particle size (μm)
Pechini method	M1P	M1PH	0	0	19.51
	M2P	M2PH	0.36	0.36	19.59
	M3P	M3PH	0.73	0.73	17.42
Solid state reaction	M1	M1H	0	0	39.85
	M2	M2H	0.36	0.34	40.48
	M3	M3H	0.73	0.74	38.15

2.3. In vitro bioactivity assessment

The simulated body fluid (SBF) used in these tests was prepared following the procedure described by Kokubo et al. [3]. After setting, each sample was immersed in SBF (the SBF volume was calculated according to the ISO 23317:2014(E) standard) [17] and placed into an incubator at 36.5 °C. The selected immersion times were 7, 14 or 21 days. After immersion, samples were stored in a desiccator. The surface of the samples was analyzed before and after immersion in SBF by scanning electron microscopy (SEM; Philips, XL30 ESEM), energy dispersive spectroscopy (EDS; EDAX, Pegasus) and X-ray diffraction (XRD; Philips, Xpert). To evaluate the amount of P, Ca and Al released from the cement samples, the remaining solutions were analyzed by ICP.

2.4. Compressive strength testing

Before compressive strength evaluation, samples were immersed in SBF for 7, 14 or 21 days at 36.5 °C. The compressive load was applied at a crosshead speed of 0.5 mm/min in (hydraulic MTS press QTEST/100, 100 kN load cell). The mechanical strength evaluation was performed according to the ASTM F 451-95 [18], testing 5 samples for each cement.

2.5. Setting times measurement

The setting times of cements were determined by the method described by Liu et al. and Mahshim et al. [19,20]. The paste was poured into a cylindric mold (15 mm in diameter and 10 mm in height) and the upper and lower surfaces of the mold were covered with two glass plates. Subsequently, the molds were placed in a chamber with controlled temperature and humidity (37 °C and 90% relative humidity). The initial and final setting times of cements were evaluated using a Vicat needle (Model Controls 63-L0028/1). In order to determine the maximum temperature generated by the cements setting, a digital thermometer was placed in the samples and the temperature was recorded every minute.

3. Results and discussions

3.1. Calcium aluminates

The phosphorous analyses obtained by ICP of the different CAs prepared are shown in Table 1 (P obtained). No significant differences were observed between P added and P obtained values. Fig. 1 shows the XRD patterns of CAs; in all the cases a single phase, corresponding to monoclinic calcium monoaluminate, was detected. No secondary phases were detected after adding $(NH_4)_2HPO_4$. Not a noticeable change was observed in the XRD peaks position; however, peaks are sharper in patterns corresponding to CAs obtained by solid state reaction indicating a higher crystallinity. Additionally, the height of the peaks slightly decreases and its width slightly increases as a function of P content for both CAs.

Table 1 shows the average particle size of CAs obtained by laser diffraction. As observed, the materials obtained by Pechini method show smaller particle size than those obtained by solid state reaction. Considering that the milling process was standardized for all CAs, this can be attributed to the fact that the CAs obtained by Pechini method were sintered at lower temperature (T = 1300 °C) than those obtained