Novel one-step synthesis and characterization of bone-like carbonated apatite from calcium carbonate, calcium hydroxide and orthophosphoric acid as economical starting materials

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ABSTRACT

There is growing interest in the development of substituted calcium hydroxyapatite (Ca-HA) for biomedical applications. Carbonated apatite (CAP) appears as an important substituted Ca-HA because of its better biocompatibility compared to pure Ca-HA. This paper reports a novel pressure-mediated one-step synthesis of CAP starting from orthophosphoric acid, calcite and calcium hydroxide as available and high-purity starting materials. Under moderate synthesis conditions (80 °C and <13 bar), CAP with different carbonate contents could be obtained. The ratio of calcite/calcium hydroxide mixture played a crucial role for both the advancement of reaction and the carbonate content inserted in CAP's structure. At 80 °C, the total decomposition of calcite required a calcite/calcium hydroxide mixture containing at least a half of calcite. CAPs containing 2.25–4.17 wt% of carbonate inserted in its structure were obtained as a function of the composition of calcite/calcium hydroxide mixture. The results open a simple but effective way for the synthesis of high quality biomimetic CAP.

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1. Introduction

Calcium hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, Ca-HA) is found to be the main mineral phase of human hard tissues [1]. However, pure Ca-HA never occurs in any biological system but co-exists with its derivatives [2]. These are formed by partial replacement of calcium (Ca^{2+}), orthophosphate (PO_4^{3-}) or hydroxide (OH^-) groups by other species such as Mg^{2+}, Na^+, K^+, F^-, Cl^-, and CO_3^{2-} [3,4]. Among them, carbonated apatite (CAP) is the closest biomimetic solid resembling the minerals in calcified tissues [4–6]. The amount of carbonate present in human bone mineral reaches typically 4–8 wt% [3,7]. Recent studies showed that substituted Ca-HA, such as CAP, were more effective than pure Ca-HA for skeletal implants [7–9]. This leads to a growing interest in the development of such substituted materials.

Many hydroxyapatite types have been synthesized, characterized and examined for use as implantable bone-compatible biomaterials [10–12]. In synthesis procedures for pure Ca-HA, atmospheric carbon dioxide can be incorporated in the precipitated solids which lead to phosphate substitution with carbonate ions. This yields Ca-HA with low carbonate content and non-stoichiometric Ca/P ratios [13,14]. Only the more closely stoichiometric Ca-HA withstands the high temperature related to ceramic sintering or plasma-spraying, without decomposition into lime or tricalcium phosphate (TCP). CAP containing higher carbonate amounts can be synthesized by multi-step synthesis procedures including the precipitation of soluble calcium salts (Ca^{2+}) with PO_4^{3-} in the presence of CO_3^{2-} (liquid route) [15] or the heating up to more than 1000 °C of a calcium phosphate under carbon dioxide flux (thermal route) [16]. These methods have disadvantages of added manipulations for the elimination of waste counter ions or heating step under controlled carbon dioxide. CAP starting from calcium carbonate as the source of both calcium cations (Ca^{2+}) and carbonate anions (CO_3^{2-}) and potassium dihydrogen orthophosphate (KH_2PO_4) under atmospheric pressure was briefly mentioned by Lomo [17]. This route has recently been investigated under higher pressures of hydrothermal conditions [15,18,19]. Despite the severe reaction conditions used (high temperature, long reaction time and water rinsing), the conversion of calcium carbonate was not complete [19]. So, it is of current interest to find simple synthesis procedures that lead to CAP with controlled carbonate contents.

In this study, the synthesis of CAP using calcium carbonate (calcite), calcium hydroxide and orthophosphoric acid was investigated under moderate conditions. Orthophosphoric acid was chosen because of its strongest acidity among the available orthophosphate sources and the absence of alkali cations in the final CAP product.

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Table 1
Composition of the starting reactant mixtures and designation of solid products; other condition: synthesis temperature of 80 °C; stirring rate of 800 rpm; initial water of 45 g.

Calcium source		Phosphate source	Solid product designation
$CaCO_3$ powder, mmol	$Ca(OH)_2$ powder, mmol	H_3PO_4, mmol	
100	0	66.7	CAP100/0
75	25	66.7	CAP75/25
50	50	66.7	CAP50/50
25	75	66.7	CAP25/75

Table 2
Results of elemental analysis using ICP–AES technique; Ca^{Liq} and P^{Liq}: amount of calcium and phosphorus remained in the liquid phase; Ca/P: molar ratio of bulk solid products.

Solid	Ca^{Liq} (%)	P^{Liq} (%)	Ca/P
CAP100/0	1.4	1.1	1.88
CAP75/25	0.9	1.2	1.84
CAP50/50	1.4	1.2	1.82
CAP25/75	1.3	1.2	1.74

Various molar ratios of calcium carbonate to calcium hydroxide were used in order to control the amount of carbonate inserted in the apatitic structure. The results showed that CAP with different carbonate contents could be obtained in a one-step synthesis at 80 °C and pressures below 13 atm, without a washing step.

2. Materials and methods

Calcite powder ($CaCO_3$, 98 wt%) from Fisher Scientific, calcium hydroxide powder ($Ca(OH)_2$, 98 wt%) from Acros Organics and orthophosphoric acid (H_3PO_4, 85 wt% in water) from Merck were used as received. CAP synthesis was carried out in a 250 mL stainless steel reactor (Top Industrial) which was equipped with an electrical heating jacket and a magnetic stirrer. For each reaction, calcite or mixture of calcite/calcium hydroxide containing 100 mmol of calcium and 45 mL of water were fed into the reactor. After closing, 66.7 mmol of orthophosphoric acid were quickly injected into the reactor. This led to the starting molar ratio of calcium to phosphorus of 1.67 (Table 1). During the reaction, the stirring rate was set at 800 rpm and the reactor was thermostated at 80 °C for 48 h. This reaction temperature was chosen because supplementary experiments showed that the temperatures lower than 80 °C were not sufficient for a good decomposition of calcite particles. After 48 h of reaction, the reactor was freely cooled down to room temperature. Solid products were separated from liquid phase by filtration using 0.45 μm filter paper. Then they were dried at 50 °C for 48 h before further characterizations.

Both solid and liquid phases obtained from the filtration step were analyzed using different analysis and characterization techniques. Elemental analysis was carried out using inductively coupled plasma atomic emission spectroscopy (ICP-AES, HORIBA Jobin Yvon Ultima 2 apparatus). Thermogravimetry (TG) was performed in a TA Instruments SDTQ600 analyzer with a heating rate of 5 °C min^{-1} under air flux (100 mL min^{-1}). Thermo-mechanical analysis (TMA) was carried out in a SETARAM SetSys 16/18 analyzer with 5 g constant load on the powder sample. X-ray diffraction (XRD) data of the solids collected using a Phillips Panalytical X'pert Pro MPD diffractometer with a Cu Kα (1.543 Å) radiation source. Fourier transform infrared (FTIR) spectroscopy was performed using a Shimadzu 8400S spectrometer. Particle size distribution was determined by laser scattering in a Mastersizer 2000 (Malvern Instruments Ltd., Malvern, UK) in the particle size range of 0.020–2000 μm. Scanning electron microscopy (SEM) was carried out on a Philips XL30 ESEM apparatus (FEI Company) which was coupled with an energy-dispersive X-ray spectroscopy (EDX analysis).

3. Results

3.1. Elemental analysis

The liquid separated from the filtration of the reaction mixture was acidified with concentrated nitric acid to avoid all further re-precipitation. Solid products were mineralized using concentrated nitric acid. The resulting homogeneous solutions were analyzed by ICP-AES technique and results are presented in Table 2. In all cases, the contents of soluble calcium and phosphorus in the liquid phase were lower than 1.4% of the initial quantities of calcium and phosphorus introduced in the reactor. So, the precipitation of orthophosphate species was quite complete and most of the calcium existed in solid phases after 48 h of reaction. Because of the absence of any counter ions in the liquid phase, no washing step was required for the purification of the final solid products. This brings a significant advantage of the present synthesis process in comparison with the synthesis using soluble calcium salts and/or alkali orthophosphates, since the washing step is usually arduous when small particles are formed.

The molar ratios of the bulk solids were higher than that of the stoichiometric Ca-HA (1.67). This result is discussed in more details in the section of FTIR analysis.

3.2. Decomposition of calcium carbonate

The reaction of calcite with orthophosphoric acid led to the formation of carbon dioxide which can exist in both gas and soluble states. Pressure in the reactor increased with the formation of carbon dioxide in gas phase. Taking into account the volume of gas phase in the reactor, the partial pressure of water vapor at 80 °C and using the ideal gas law, the quantity of carbon dioxide in gas phase could be calculated from the final pressure at 48 h of reaction. Fig. 1 indicates the relative advancement of the reaction via the calculated amounts of carbon dioxide. The decomposition of calcite reached at least 43, 89, 83 and 88% in the syntheses using 25, 50, 75 and 100% of calcite as calcium source, respectively,
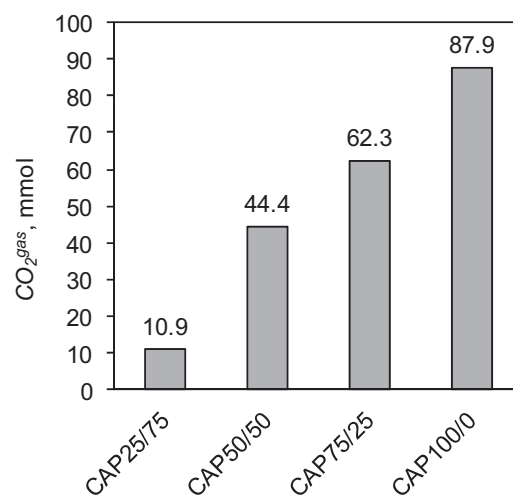

Fig. 1. Quantity of carbon dioxide in gas phase (CO_2^{gas}) calculated from the final reaction pressure as a function of the molar percentage of calcite in the initial mixture of calcite and calcium hydroxide.