EFFECT OF TEMPERATURE AND REACTANT ADDITION TIME ON THE SYNTHESIS OF OCTACALCIUM PHOSPHATE AS A PRECURSOR ON THE CRYSTALLISATION OF HYDROXYAPATITE AS BIOMATERIAL

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Abstract The purpose of this work was to study the conditions of synthesis of octacalcium phosphate. During the process of synthesis, reactant addition time of the calcium acetate solution to the ammonium dihydrogenophosphate solution as well as the reaction temperature were found to be determinant on the composition of the final solid. Synthesis performed with pH 5 and at a constant temperature of 60°C, DCPD ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) was obtained for addition times of the calcium solution between 30 min and 1h30min and OCP ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$) was obtained for addition times superior to 2h without exceeding 5h. Synthesis performed with pH 5 and at a constant addition time of the calcium solution (2h45min), DCPD ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) was obtained at temperatures below 60°C, OCP ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$) was found at temperatures in the range of 60 to 80°C, and HAp ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) was obtained above 80°C.

INTRODUCTION

Three calcium phosphates can be found in nature - monetite (CaHPO_4), brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), and hydroxyapatite ($\text{Ca}_5(\text{PO}_4)_3(\text{OH})$) - being brushite metastable in relation to monetite. However brushite crystals have been identified in samples of recent dental calculi and in several types of pathological calcifications^{1, 2}. Hydroxyapatite is one of the fundamental constituents of bones and teeth.

Octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$) has been proposed as the solid phase, with a well defined composition, constituent of some pathological calcifications, such as renal and dental calculi, normally coexisting with whitlockite ($\text{Ca}_9(\text{Mg,Fe}^{2+})\text{H}(\text{PO}_4)_7$) and/or hydroxyapatite.

The numerous *in vitro* studies about the mechanism of transformation of octacalcium phosphate into hydroxyapatite and the factors that control this transformation have contributed to confirm the fact that octacalcium phosphate occurs

as precursor of many biomaterials. This theory is strongly supported by the structural similarities between these two solids^{3, 4}. However, octacalcium phosphate is not detected as enamel, dental or bone constituent, what can be explained by its rapid transformation into hydroxyapatite in biological systems^{1, 5}.

On the synthesis of octacalcium phosphates, the solid phase obtained - brushite, octacalcium phosphate or hydroxyapatite - depends on the temperature, pH, addition time of solutions, addition order and ageing time of the precipitate. This work is part of a larger one in which the conditions of crystallisation of the OCP solid will be verified. The influence of the variation of temperature and of the addition time of the calcium acetate solution on the synthesis of calcium phosphates keeping the pH of the solution and the ageing time constant has been studied. Being octacalcium phosphate a metastable solid in relation to hydroxyapatite, it can be foreseen that the addition time of the calcium solution may have an important role on the characteristics of the final product.

EXPERIMENTAL PROCEDURE

a) Addition time of calcium solution

All the solids were synthesised according to published methods⁶. 100 cm³ of aqueous 0.1 mol dm⁻³ ammonium dihydrogenophosphate solution was diluted to 750 cm³ and the pH adjust to the values of 5 with concentrated ammonia. A Delta Ohm pH meter HD8705 with combined glass electrode was used to measure the initial pH value of the phosphate solution.

The reaction was performed in a three necked round bottom flask containing the phosphate solution immersed in a glycerine thermostat bath. One neck had a dropper funnel, with a CO₂ remover, containing 250 cm³ of 0.4 mol dm⁻³ calcium acetate aqueous solution; the other two necks had a thermometer and a reflux condenser, respectively.

The aqueous solution of calcium acetate was dropwise added to the warmed solution of phosphate (at 60° C) with times varying between 30 min and 5 h. The ageing time was 60 min, with stirring, to the temperature of 60° C⁶.

The pH of the reaction was kept around 5 by the action of the acetic acid/acetate buffer formed in the course of the reaction.

b) Variation with the temperature

In order to study the effect of temperature on the synthesis of OCP, a set of synthesis was done in which all variables were kept constant, except for the temperature. The temperature of synthesis was varied in the range of 20 to 90°C, with intervals of 10°C between each synthesis. The method of synthesis of the solids was similar to that described above, keeping the addition time of the calcium acetate solution constant (2h45min). This value resulted from the observation that at the temperature of 60°C OCP was obtained for addition times above 2h.

Presented in the table are the results obtained from the attempts of synthesis of octacalcium phosphate for several reactant addition times (samples labelled E) and for several temperatures (samples labelled T), in which the initial molar ratio of calcium acetate and of ammonium dihydrogenophosphate is 1:1, the pH of the phosphate solution is 5 and the ageing time is 60min.

TABLE Types of solids obtained in the experiments performed varying the addition time of the calcium solution (E samples) and the temperature (T samples)

Sample	Time of addition	Temperatur	Ca/P (in solid)	Solids
E281	30min	60° C	1.02	DCPD
E282	30min	60° C	1.08	DCPD
E25	1h	"	1.07	DCPD
E251	1h	"	1.04	DCPD
E29	1h30min	"	1.30	DCPD+OCP
E291	1h30min	"	1.15	DCPD
E292	1h30min	"	1.34	OCP
E32	2h	"	1.39	OCP
E31	3h	"	1.39	OCP
E26	4h	"	1.33	OCP
E30	5h	"	1.38	OCP
T20	2h45min	20° C	-----	DCPD
T30	"	30° C	1.02	DCPD
T40	"	40° C	1.01	DCPD
T50	"	50° C	1.03	DCPD
T60	"	60°C	1.36	OCP
T70	"	70° C	1.37	OCP
T80	"	80° C	1.41	OCP+HAp
T90	"	90° C	1.52	HAp

CHARACTERISATION

The analytical determination of calcium was made by complexometric titration with ethylenediaminetetraacetate ion (EDTA), using Merck tablets as indicator. The chemical analysis of phosphorus was made by ICP in a Jobin Yvon 70Plus spectrometer.

Infrared spectra of the compounds obtained in the range of 4000 to 400 cm^{-1} , were registered in a Mattson 7000 spectrophotometer with Fourier transforms (FTIR) and are showed in figure 1.

The analysis by X-ray diffraction were obtained using a Rigaku Geigerflex Dmax-C X-ray diffractometer with $\text{CuK}\alpha$ radiation equipped with a graphite monocromator and are showed in figure 2.

FIGURE 1 Infrared spectra of a) DCPD, b) OCP, and c) HAp

FIGURE 2 Powder X-ray diffraction of a) DCPD, b) OCP, and c) HAp

RESULTS AND DISCUSSION

a) Addition time of calcium solution

The elemental chemical analysis of the solids obtained in the experiments at pH 5, temperature of 60°C and with different addition times of the calcium acetate solution,

showed that the Ca/P molar ratio of the solids increased with variation of addition times of the calcium solution from 30m to 3h. For addition times of the calcium solution between 3 and 5h, there were not significant changes in the Ca/P molar ratio of the products obtained.

Molar ratios between 1 and 1.1 showed the formation of solids with chemical composition different from that of OCP. The theoretical Ca/P molar ratio equal 1 exists in solids with a CaHPO_4 chemical composition which can crystallise with the structure of monetite (CaHPO_4) or of brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$). The infrared absorption spectra and the X-ray diffractograms of E281, E282, E25, E251, and E291 solids show characteristic peaks of DCPD with the crystalline structure of brushite. The infrared spectra of E29, E292, E32, E31, E26, and E30 solids are similar to the OCP spectrum referred by Racquel LeGeros³. Two phases were observed in the E29 solid diffractogram, DCPD and OCP. E292, E32, E31, E26, and E30 solids showed OCP characteristic X-ray diffractogram.

b) Variation with the temperature

The theoretic Ca/P molar ratio of DCPD is 1.00, of OCP is 1.33, and of HAp is 1.67. By analysis of the values of Ca/P molar ratios of the different calcium phosphates presented in the table, it can be concluded that at temperatures below 60°C, and with these conditions of synthesis, DCPD is formed and only higher temperatures OCP and HAp are obtained. This conclusion is confirmed by the analysis of the infrared absorption spectra and of the X-ray diffractograms of the several compounds. T60 and T70 compounds originated an X-ray diffractogram were one crystalline phase can be observed, OCP, but in the T80 solid diffractogram two crystalline phases were observed, OCP and HAp. The infrared absorption spectrum as well as the X-ray diffractogram of the T90 compound are characteristic of HAp. The results of the T20 compound Ca/P molar ratio were not presented because the amount of powder obtained was insufficient to perform elemental chemical analysis of calcium and phosphorus.

CONCLUSIONS

This work shows that the addition time of the calcium solution to the phosphate

solution can be determinant to the type of phosphate obtained. The absence of reference of this value in published work about octacalcium phosphate may contribute to the failure of the OCP synthesis. DCPD was obtained for addition times equal or superior to 1h, for addition times of 1h30m DCPD, mixtures of DCPD and OCP or OCP were obtained, showing this to be a transition time. For addition times superior to 2h, OCP was always obtained.

Where the variation of temperature on the final product was studied, it was noticed that for $T \leq 50^{\circ}\text{C}$ DCPD was always obtained with the structure of brushite, for $T = 60$ and 70°C OCP was obtained and for $T > 80^{\circ}\text{C}$ HAp with calcium deficiency.

Since the addition time of the calcium salt solution influences the chemical composition of the final product, it may be possible to obtain different solids for some of the temperatures studied, simply by increasing the addition times.

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