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Effect of Some Physical–Chemical Variables in The Synthesis of Hydroxyapatite by the Precipitation Route

C. García[1,a], C. Paucar[1,b], J. Gaviria[1,c], A. Durán[2,d]

[1] Universidad Nacional de Colombia Sede Medellín Facultad de Ciencias, Escuelas de Física y Química, A.A. 3840 Medellín, Colombia

[2] Inst. Cerámica y Vidrio (CSIC) Campus de Cantoblanco, 28049 Madrid, Spain

[a] cpgarcia@unalmed.edu.co, [b]cgpaucar@unalmed.edu.co, [c]jgarango@unalmed.edu.co, [d]aduran@icv.csic.es

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Abstract. The synthetic hydroxyapatite is a very useful material for numerous applications in medicine as a biomaterial. One of the most economic manufacturing process is the precipitation route. In the present work, synthetic hydroxyapatite was prepared using the precipitation route, starting with aqueous solutions of calcium nitrate ($Ca(NO_3).4H_2O$) and ammonium phosphate ($H_2(PO_4)NH_4$). The effects of physical-chemical variables such as pH, temperature, time of agitation, ageing time and heat treatment of the mixture were evaluated. The characterization of the samples obtained in different conditions made possible to conclude about the optimal values of the studied variables for the synthesis of this material in laboratory conditions.

Introduction

The repair of defects in hard tissues is a permanent challenge in orthopaedic and dental applications[1]. The hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) has been widely used as a bone substitute, taking into account its biocompatibility, as its mineral components are similar to those of the bones and teeth of the human body. Synthetic hydroxyapatite is an important biomaterial used in many medicine applications in bulk, as a coating, or as a component of a composite [2-5]. Many routes have been developed to synthesize the hydroxyapatite using hydrolysis, precipitation or hydrothermal methods[4]. Among these methods, the precipitation route is the most simple and cheap and, moreover, it has industrial application possibilities [1].
In the present work, hydroxyapatite was obtained by a chemical synthesis route, using aqueous solutions of ammonium phosphate and calcium nitrate and controlling parameters such as pH, order of addition of the reagents, temperature of the mixture, ageing time and heat treatment. In order to evaluate the effect of these parameters, several syntheses were carried out, changing a variable in each test.
The intermediate and final results were evaluated by means of X-ray diffraction (XRD) and infrared Fourier transformed spectroscopy (FTIR).

Experimental

The initial aqueous solutions were prepared using tetra-hydrated calcium nitrate ($Ca(NO_3).4H_2O$) (Aldrich) and ammonic phosphate ($H_2(PO_4)NH_4$) (Aldrich) in concentrations 1M and 0.48M respectively.
In order to evaluate the influence of pH on the final product, in the first series of tests, the pH of the different solutions was adjusted to 10-11 before mixing, using ammonium hydroxide (Aldrich), whereas, in those corresponding the second series, the solutions were used with their characteristic pH (6 for calcium nitrate and 8 for phosphate of ammonium) and the pH turned out to be 5 after mixture. Once the aqueous solutions were prepared and adjusted for pH, they were mixed following a predetermined order (variable according to the test)

dropping slowly a product onto the other placed in a flat bottom flask with permanent agitation of 240 rpm, continued during 3 to 9 hours. Later, the mixture was aged, either at room temperature or at 40ºC, during different times. After ageing, the precipitates were collected by filtration, washed with distilled water and dried slowly at 60ºC. The dried powders were treated at 1050ºC during variable times and the crystalline phases in the final product were measured by XRD with a Siemens equipment (Difrac 5000 model).
With the purpose of evaluating the kinetic of the reactions and the effect of the values set for the different variables in the hydroxyapatite formation, a mixture was done in order to recover a sample in every stage of the process. The phosphate was added first in the flat bottom flask. Then, the nitrate was added slowly and immediately after the end of the nitrate addition, one section of this mixture was taken (0 hours mix) and after 9 hours mixing (9 hours mix); also, samples of the precipitate formed with different ageing periods at 40ºC (24, 48, 72 and 115 hours) and samples of these precipitates after washing and heating at 1050ºC during 7 hours. These samples were analysed by FTIR spectroscopy (Perkin Elmer model 1760) in the interval of 4000 – 400 cm^{-1}. For solids samples, 2 mg of powder sample was thoroughly mixed with 200 mg of KBr using a mortar and pestle, following pressing at 5 MPa to form pellets. For liquid samples, a AgCl holder was employed.

Results and discussion

For initial solutions adjusted to pH 10, addition of phosphate of ammonium to calcium nitrate, a period of agitation of 3 hours at 40ºC, an ageing period of 6 days and a heat treatment of 7 hours at 1050ºC, an incipient formation of hydroxyapatite appears with a high percentage of amorphous phase. With a much longer period of heat treatment (15 hours) well crystallized hydroxyapatite was obtained as the only phase in the reaction product. The pH plays a fundamental role in the formation of crystalline hydroxyapatite. When the pH of the mixture was not adjusted with the addition of ammonium hydroxide, and therefore, the synthesis was made at pH of 5, the result was a mixture of somewhat amorphous calcium phosphates. In spite of that the samples were put under a heat treatment of 1050ºC during 15 hours, there is no presence of hydroxyapatite in the final product; on the contrary, it continues the formation of an amorphous phase in an important amount. In basic pH, the solubility decreases and the OH^- presence favours the precipitation of hydroxilated calcium phosphate. For a synthesis made with initial solutions adjusted to pH 10, addition of calcium nitrate to phosphate of ammonium, a stirring time of 9 hours, an ageing period of 3 days at 40ºC and a heat treatment of 7 hours to 1050ºC, results in the presence of an additional crystalline phase (tricalcium phosphate[8]). For the initial solutions adjusted to pH 10, addition of calcium nitrate to ammonium phosphate, period of agitation of 3 hours and ageing period of 5 days at room temperature, a heat treatment of 1 hour to 1050ºC was enough to obtain well crystallized hydroxyapatite as the only phase.
According these results, the stirring time and the stirring and ageing temperature do not have a marked influence on the synthesis for obtaining well crystalline hydroxyapatite. The basic pH of the initial solutions and the final pH of the mixture, the order of addition of the reagents (the phosphate first) and a long period of ageing, are the more relevant parameters in the formation of well crystalline hydroxyapatite.
The nucleation and the crystal growth rates determine the appearing of crystalline phases in the synthesis of a material. Each mineral species has a characteristic kinetics of nucleation and growth that depends on the time that the reagents are allowed to play their role in the reaction. If this time is not reached, it is possible that other species (not in equilibrium) be formed, because the reactions that give origin to them are not complete. In the particular case of the hydroxyapatite, a period of ageing of 5 days was needed to obtain complete reactions in the formation and good development of the crystalline phase. In the reaction of the calcium nitrate with the ammonium phosphate to obtain hydroxyapatite, it is necessary to obtain first an equilibrium of the reagents in the aqueous media. Therefore, it is necessary to stabilize

them before the reaction. The PO_4^{3-} has low solubility, and this can be the reason why, when the ammonium phosphate is added to the calcium nitrate, a good amount of the reagent precipitates, and this effect does not allow the reaction with calcium for the hydroxyapatite formation. When the order of the addition of the reagents is reversed, the reaction is favoured by the high solubility of calcium nitrate.

Fig. 1 shows the FTIR for a liquid sample corresponding to the mixture at 9 hours and for solids samples corresponding to the precipitated ones after a period of ageing of 24 hours and 115 hours, to the sample after been heated at 1050ºC during 7 hours and for the commercial hydroxyapatite (CEROS 80, USA).

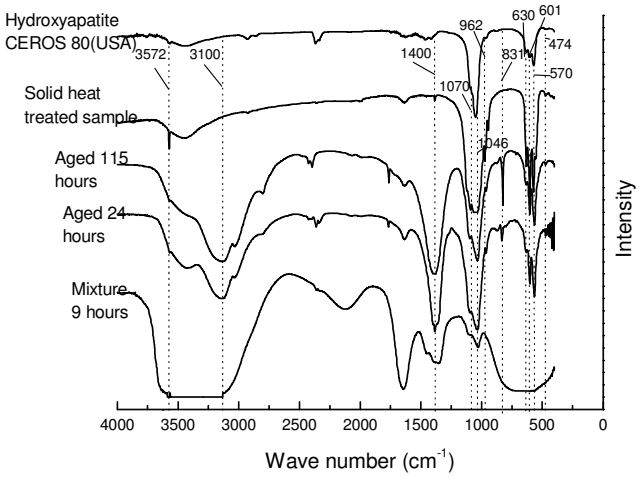

Figure 1. FTIR spectra for the liquid sample corresponding to the mixture at 9 hours and for solid samples corresponding to the precipitated ones after a period of ageing of 24 hours and 115 hours, to the sample after been heated at 1050ºC during 7 hours and for the commercial hydroxyapatite (CEROS 80, USA)

Table 1 summarizes the FTIR frequencies and assignments for Hydroxyapatite[11], those corresponding to commercial hydroxyapatite (CEROS 80, USA) and for all the stages of preparation of the studied sample.

Table 1. Frequencies (cm^{-1}) and assignments for hydroxyapatite [11], Commercial hydroxyapatite (ceros80, USA), and for all the stages of preparation of the studied sample.

λ (cm^{-1})	Assignment	Hydroxyapatite[11]	CEROS 80 (USA)	Mixture 9 hours	Sample precipitated after 24 hours of ageing	Sample precipitated after 115 hour of ageing	Sample after a heat treatment of 1050ºC during 7 hours
3572	OH^- stretch	X	X				X
3100	OH^- group			X	X	X	
1400	NO_3^- group			X	X	X	
1087	$\nu_3PO_4^{3-}$	X	X	X	X	X	X
~ 1072							
1046							
~ 1032							
962	$\nu_1PO_4^{3-}$	X	X				X
831	NO_3^- group			X	X	X	
630	OH^- libration	X	X	Broad Band	X (Shoulder)	X (Shoulder)	X
601	$\nu_4PO_4^{3-}$	X	X		X	X	X
571							
474	$\nu_2PO_4^{3-}$	X	X			X	X
~ 462		(shoulder)	(shoulder)				

From an ageing time of 24 hours, well defined bands are observed at 570 cm^{-1}, 600 cm^{-1}, 1046 cm^{-1} and 1090 cm^{-1} as well as a small shoulder at 630 cm^{-1}. According to the assignments, these bands correspond to absorption modes PO_4 groups [9] in hydroxyapatite and the shoulder at 630 cm^{-1} corresponds to a vibrational OH^- band in the same material [4,9]. The presence of these bands in samples subjected to different ageing times, indicate that hydroxyapatite structure is formed from the beginning of ageing process. The small band at 474 cm^{-1} and the better definition of the PO_4 groups bands for an ageing time of 115 hours and for the solid heat-treated sample show the relevance of both factors in the synthesis. A

long period of ageing and the appropriate heat treatment, make the bonds PO_4 better defined in the structure of hydroxyapatite. The bands appearing at 831 cm^{-1} and at 1400 cm^{-1} on samples aged during 24 hours and 115 hours are due to NO_3 groups [9] and consequently disappear with the heat treatment.

Conclusions

- The mixture pH is a decisive factor in the crystallization of hydroxyapatite. A basic pH (10-11) benefits in the formation of hydroxyapatite under the studied conditions of precipitation and must be controlled during the whole process .
- The order of addition of the reagents is very important. Calcium nitrate must be added slowly to the ammonium phosphate. Otherwise, the formation of pure and crystalline hydroxyapatite becomes very difficult.
- The ageing period plays an important role in the synthesis of pure hydroxyapatite. For times of ageing shorter than 5 days, three-calcium phosphate and some other calcium phosphates other not well defined amorphous phases were formed in addition to hydroxyapatite.
- The synthesis temperature does not seem to play a preponderant role in the hydroxyapatite formation. It is possible to produce pure and well crystallized hydroxyapatite from room temperature up to 40ºC.
- The heat treatment is crucial at the moment of defining the crystalline phases present in the mixture. Under the ideal conditions, it is enough to heat treat the mixture during 1 hour to synthesize pure and well crystalline hydroxyapatite. Under other conditions, it is necessary to increase the heat treatment up to 15 hours in order to obtain the desired effect.

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