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Micro-crystalline hydroxyapatite. Preparation and investigation

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The aim of this study was to establish the conditions to synthesize hydroxyapatite (HAP) with composition and crystalline degree, comparable to bone mineral tissue, able to enssure a good adhesion to dental tissue. We used, in our experiments, two systems of experimental conditions for future technological applications. A significant decrease of the size of micro-crystalline particulates was obtained.

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1. Introduction

Hydroxyapatite is chemically similar to the mineral component of bone and hard tissues in mammals. It is one of few materials that are classed as bioactive, meaning that it will support bone ingrowth and ossteointegration when used in orthopedic, dental and maxillofacial applications. The chemical nature of hydroxyapatite lends itself to substitution, meaning that it is not uncommon for non-stoichiometric hydroxyapatites to exist [1-2]. The most common substitutions involve carbonate, fluoride and chloride substitutions for hydroxyl groups, while "defects" can also exist resulting in deficient calcium hydroxyapatites. Hydroxyapatite powders exhibit a high degree of chemical variability. The crystal lattice quite readily undergoes substitutions, with ions such as CO_3^{2-}, HPO_4^{2-} and F^- often found in place of stoichiometric hydroxyapatite components [3]. Also, calcium to phosphorous molar ratios often vary in hydroxyapatites from the stoichiometric ratio of Ca/P = 1.67. For this reason, it is not uncommon to synthesise the own powders. Substitutions within the hydroxyapatite lattice can also induce changes in properties. For instance, fluoride substituting for hydroxide, producing fluorapatite, results in a more chemically stable compound. Property changes have inspired researchers to deliberately substitute ions to try and modify the properties and behavior of apatites, such as bone bonding. Sintered titanium-hydroxyapatite implant materials have been prepared and investigated [4]. In vitro hydroxyapatite growth on patterned surfaces has been achieved [5]. Hydroxyapatite powders can be synthesised via numerous preparation routes, using a range of different reactants. Some processing techniques include:

-Wet chemical methods (precipitation)
- Hydrothermal techniques
- Hydrolysis of other calcium phosphates
- Sol-gel.

Wet chemical methods suppose an acido/basic reaction; a pH greater than 9, assure the formation of apatitic structure. Reaction temperatures of between 25 and 90°C are common, the higher temperature producing a higher crystalised product. Another precipitation method used for producing hydroxyapatite involves calciumnitrate, diammonium hydrogen phosphate [3]. This method results in a faster production rate, with ammonium hydroxide being added to maintain a constant pH. Compared to the previous method, this approach requires washing of the precipitate to remove nitrates and ammonium hydroxide. In taking these factors into account, the production rate of these two techniques is similar. Continued stirring and ageing are usually carried out after the reactants have been combined as the calcium is slowly incorporated into the apatite's structure. This process also helps the material to approach stoichiometric Ca/P ratios. A morphological change from needle-like crystals to more "blocky" crystals is associated with the maturation process. Processing at pH's lower than 9 can also result in the production of a calcium deficient hydroxyapatite.

2. Experimental procedure

In the present study, submicron hydroxyapatite powders synthesized in our laboratory were used. Hydroxyapatite (HAP) was obtained by wet chemical methods (precipitation), by two ways, from calcium hydroxide [$Ca(OH)_2$] and orthophosphoric acid [H_3PO_4], respectively from $CaCl_2$ and Na_2HPO_4 as raw materials. In the first way (sample1 and sample2) $Ca(OH)_2$ and H_3PO_4, in necessary proportions, were used as raw materials. Orthophosphoric acid solution was added in a drop wise manner to a dilute solution/suspension of calcium hydroxide. The acid is added at a controlled rate, with stirring being maintained throughout the process. The precipitation reaction is slow. The solutions were heated at 80 °C-90 °C (sample1) and vigorously stirred for 3 hours; the suspension was maintained at room temperature 70 hours, for maturation. In the case of sample 2, the raw materials are the same as for the sample 1, but the reaction takes place at room temperature and the resulted suspension was maintained for maturation, at room temperature, for eight weeks.

$$10Ca(OH)_2 + 6H_3PO_4 = Ca_{10}(PO_4)_6(OH)_2 + 18H_2O$$

The precipitates that formed were recovered from the supernatant by filtering and washed five times with de-ionized water. The filtrates were dried at 120 °C, overnight. Filtered and dried powders, HAP precursors, were thermally treated at 900 °C for 2 hours.

The samples 3 and 4 were synthesized from $CaCl_2$ and Na_2HPO_4 as raw materials. Precipitation reactions take place at the room temperature:

$$10CaCl_2 + 6 Na_2HPO_4 + H_2O = Ca_{10}(PO_4)_6(OH)_2 + 12NaCl + 8HCl$$

The resulted precipitates were maintained in the suspension at the ambient temperature, in the presence of crystallization admixtures, for 24 hours (sample3) respectively 40 hours (sample 4), then filtered, washed with de-ionized water, dried at 120 °C and thermally treated at 900 °C for 2 hours.

Sample characterization: the powders obtained by precipitation, following procedure mentioned earlier, were characterized by X-ray diffraction (DRON-3M powderdiffractometer),IRspectroscopy(FTIRSpectrophotometer JASCO–610 mark), and scanning electron microscopy (Vega TESCAN).

3. Results and discussion

Using IR spectroscopy the specific bands for HAP were found and interpreted in accordance with the references date (Fig. 1 and Fig. 2). The X-ray diffraction analysis was carried out by means of standard DRON powder diffractometer. The mineralogical composition of the synthesized powders was determined by X-ray diffraction. The morphology and microstructure of the obtained powders were studied by SEM electron microscopy.

Starting from $Ca(OH)_2$ and H_3PO_4, the IR spectra of the powders synthesized in the conditions as described earlier, shown the presence of specific bands for hydroxyapatite. After heating 2 hours at 900 °C, the main component of the calcined powder is HAP and a higher crystalline degree was found for sample 1 (Fig. 1). The powder - sample 1- had an average grain size up to 0.5 µm. The samples 2-4 revealed powders with an average size under 0.1 µm, which are substantially smaller than the grain size of sample 1. The sample 2 revealed homogeneous HAP micro-crystalls formation.

The crystalline structure can be changed by varying the conditions of synthesis, as well as the maturation conditions (time and temperature). When nucleation and growth of crystals process take place in calcium phosphate solutions at room temperature, homogenous and uniform micro crystals are obtained.

The IR spectrum of synthesized powders, shown in Fig. 2, presents the characteristic bands for calcium hydroxyapatite.

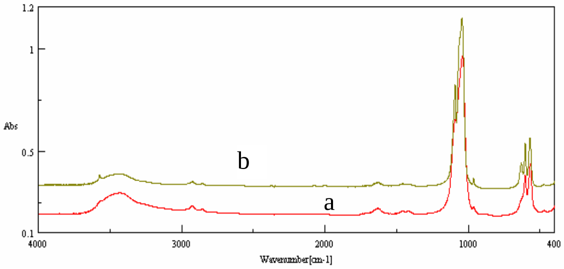

Fig. 1. IR spectra of HAP- sample 1- before heating (a) and after heating 2h-900 °C (b).

The IR spectra for samples 3 and sample 4, presents the specific bands for hydroxyapatite but there were observed secondary phases (amorphous calcium phosphate, crystalline octacalciumphosphate) resulted from carbonating or intermediate reactions. The IR spectrum exhibited carbonate bands at 1417.4 cm^{-1} respective 873.6 cm^{-1}. The position of these carbonate bands indicated that carbonate groups replace phosphate and hydroxyl groups in the hydroxyapatite lattice. Bands in region of 1190 to 975cm^{-1} are due to v_3 vibrational mode of phosphate group. Phosphate v_1 band is present near 962 cm^{-1}. The two sharp P-O bands at 561.2 cm^{-1} and 601.7 cm^{-1}(P-O deformation in PO_4), indicated a crystallized octacalcium phosphate structure. Phosphate vibration v_2 is observed in the form of two peaks at 471 and 433 cm^{-1}. The OH vibrations at about 3500 cm^{-1} (stretching) and 634 (bending) cm^{-1} are well defined. The band in the region of 1640-1600 cm^{-1} is due to H-O-H deformation. Atmospheric CO_2 is detected by the band at 2349 cm^{-1}.

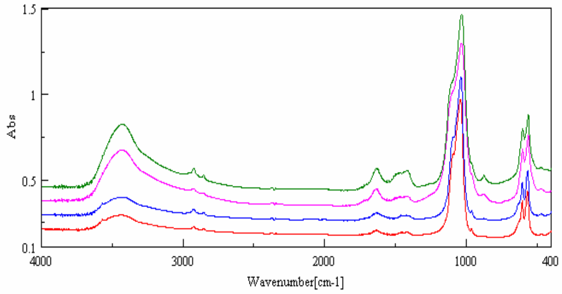

Fig. 2. IR spectra of powders: HAPsample 1(red) HAPsample 2(blue), HAPsample 3(magenta), HAPsample 4(green).

Experiences shown that the hydroxyapatites precipitated rapidly are non-stoichiometric. Maturing non-stoichiometric hydroxyapatites in an aqueous medium leads, by means of a surface phenomenon, to maturing of the apatite and a change towards an increasingly stoichiometric and decreasingly soluble phase. This non-stoichiometry results in:

- the presence of gaps in the cationic site and OH - an OH^- content of less than 2 per circuit
- a lower state of crystallinity the further away the hydroxyapatite is from the stoichiometry - increasing solubility the further away from the stoichiometry.

Ca/P ratios in the experimental powder samples were found to be 1.625-1.657. These can be regarded as Ca deficient, when compared with the same ratio of 1.667 in stoichiometric HAP. However, the Ca/P ratios of dental enamel and human bones are known to be 1.62 and 1.65, respectively [6].

Scanning electron micrographs (SEM micrograph) (Fig. 3), show the morphological aspects of hydroxyapatite powders. One observes the small grain of HAP (samples 2, 3, and 4), in contrast to the more large particulates of HAP (sample 1). The samples present agglomerated grain because they adhere due to hydrogen bonds, fact also confirmed by IR spectra.

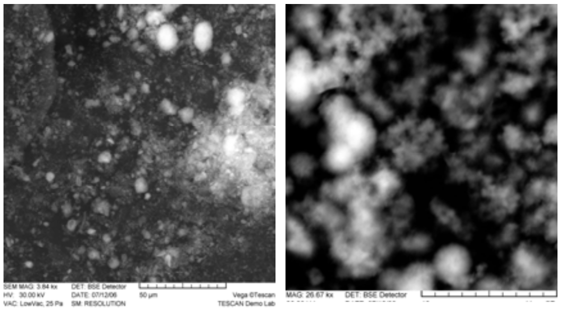

Fig. 3. SEM imagines of the powders HAP (samples 1-2).

The current study revealed that crystalline structure can be changed by manipulation of the calcium/phosphate ratio, as well as the content of carbonate.

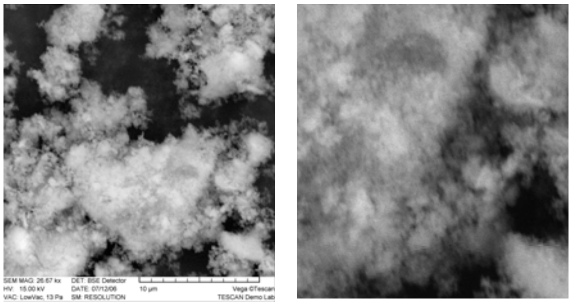

Fig. 3. SEM imagines of the powders HAP (samples 3-4).

Hydroxyapatite, formula $Ca_{10}(PO_4)_6(OH)_2$, forms part of the crystallographic family of apatites, isomorphic compounds with the same hexagonal structure. This is the calcium phosphate compound most commonly used for biomaterial. Hydroxyapatite crystallises in a hexagonal system (spatial group $=P_{6\ 3/m}$) with the following crystallographic parameters: a=9,418Å c=6,881Å; b=120° [7]. HAP's crystallographic structure consists of a quasi-compact packing of phosphate groups, which form two types of tunnels parallel to axis c, in which the Ca^{2+} ions are located. One of the apatitic structure's main characteristics is to allow a large number of substitutions, which leave the crystallographic structure unchanged [8,9]. The crystal lattice quite readily undergoes substitutions, with ions such as CO_3^{2-}, HPO_4^{2-} and F^- often found in place of stoichiometric hydroxyapatite. The results show some of the possible substitutions, taking into account the following general chemical formula: $Me_{10}(XO_4)_6(Y)_2$. Divalent cations such as Si^{2+}, Ba^{2+}, Mg^{2+},can replace Ca^{2+}. It has been confirmed that the experimental produced crystals cannot be regarded as "pure" calcium-hydroxyapatite and their chemical formula $(Ca_{10-x}Me_x)(PO_4)_6(OH)_2$ may be. Human bones and tooth enamel are also known to contain significant quantities of alkali and alkali earth cations, such as Na^+,K^+,Mg^{2+} [6], so that the composition of the experimented powders is very close to those. The presence of magnesium in the samples, in range under 0.7 wt%, is expected to substitute the Ca^{2+} sites of HAP lattice.

The existence of tunnels, in which the Y- ions are located, gives the apatites certain properties close to those of zeolites. The tunnels can also accommodate small molecules such as H_2O [10].

RDX analyses have shown that bone-like carbonated apatite and octacalciumphosphate consists of small micro-crystals can be obtained by chemical synthesis experimented by us. X-ray diffractograms show that there is an increase in crystalline phase with increasing temperature and evidence the trace of tricalciumphosphate, octacalciumphosphate and carbonate-apatite in the case of samples 3 and 4. In the case of sample 4, the precipitation of smaller apatite crystallites associated with the surface of the HAP, suggests that a mediated mechanism of mineralization may be involved in the formation of these phases. X-ray diffractogram (Fig. 4) seems to be much the same as that of the powder of bone mineral tissue (Fig. 5).

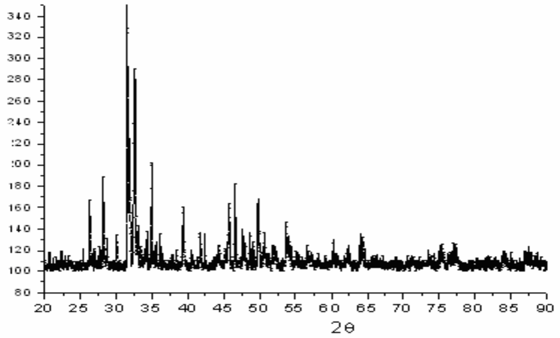

Fig. 4. RDX diffractogram of sample HAP 4.

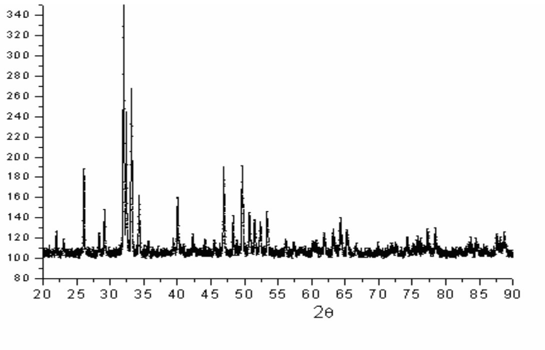

Fig. 5 .X-ray diffractogram of bone mineral tissue.

The results are comparable with the X-ray diffraction diagrame of HAP presented in literature dates [9].

4. Conclusions

In conclusion, two ways of obtaining HAP like-bone mineral were studied, resulting different microstructure powders. The process of nucleation and growth of crystals that take place at room temperature ensure, a uniform crystalline phase and composition. When the process of nucleation and growth of crystals takes place in calcium phosphate solutions at the ambient temperature, homogeneous and uniform crystalline phase can be obtained. The chemical composition of the samples, the crystal size and the degree of crystalline phase are more comparable to bone and dental mineral tissues. The powder obtained by the reaction of precipitation from $CaCl_2$ and Na_2HPO_4 at the room temperature and in the presence of some crystallization admixtures has the crystalline and mineralogical composition comparable to bone mineral tissue, fact that could enssure a good adhesion to dentine when this powder is used as filler in dentinal adhesives.

This study led us to conclude that variation in mineralogical structures and crystalline size of particulates hydroxyapatite could have specific effects on their bonding capacity, to develop chemical bonding of adhesives to dental tissues. This aspect will be studied in our future research works.

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