DEVELOPMENT OF HYDROXYAPATITES AND THEIR APPLICATION IN MEDICINE

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ABSTRACT

This study concerns the results of the development of hydroxyapatites, in the field of Biomaterials, we mainly encounter ceramics, especially the uses and developments of bioactive ceramics based on calcium phosphate: hydroxyapatite (HAP), there are therefore uses of hydroxyapatite (HAP) in dental implants and filling materials and in orthopedic surgery. The objective of this work was initially, it was a new field of research that deals with more advanced ceramic materials used in Biomaterials and Nanotechnologies, the second objective was to develop hydroxyapatite of high purity from natural resources for their application. We can consider that this double objective has been achieved since on the one hand we have characterized these hydroxyapatites; on the other hand, this material is synthesized from several routes. Several techniques were used; including X-ray diffraction (XRD), scanning electron microscopy coupled with EDX microanalysis (SEM-EDX), differential thermal and gravimetric analyzes (DTA-TGA) and finally the Fourier transform infrared (FTIR) and X fluorescence (XRF).

KEYWORDS

Hydroxyapatite, DRX, IR, Synthesis, Characterization, Porosity, Osteoconduction.

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1. INTRODUCTION

Bioceramics are considered one of the best biomaterials, since they are well tolerated by the biological system of the organism. This material has the advantage of being osteoconductive. Hydroxyapatite which is one of the most common forms of calcium phosphate with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, denoted (HAP), the same found in bone tissue (Ca^{2+}) and (PO_4^{3-}) are major players in the regulation of the body's phosphocalcic metabolism [3]. The most important minerals are [4]: -Calcium: the skeleton contains 99% of the body's calcium (1100 to 1200g); -Phosphorus: the skeleton contains 90% of the total the body (about 600g); phosphorus of -Sodium: 18g for an adult skeleton; -Potassium: 6g; -Magnesium: 3g; The inorganic matrix gives the bone its mechanical properties of rigidity, hardness and resistance to compression, but also its role in its functions metabolic and homeostatic [5]. It is synthesized in the laboratory by different methods. However, the high cost of the different precursors used for the synthesis prompted researchers to find an alternative method to prepare HAP that can mimic human bone apatites. Given their osteoconduction and bioactivity, hydroxyapatite (HA) coatings promote bone tissue development and contribute to bone cell adhesion and proliferation while preserving the mechanical integrity of the metal prosthetic device. In this study we will focus on the synthesis of HAP by neutralization for its coating on prostheses.

Hydroxyapatite (HAp) is a well-known calcium phosphate material, chemically identical to the mineral phase of the bone and the hard tissues of mammals. The most interesting property of this ceramic material is its ability to interact with living bone tissue, forming strong bonds with the bone, without causing toxicity or inflammatory response. It is commonly used for orthopedic, dental, and maxillofacial applications, either as a coating material for metal implants or as a bone filler [25]. Hydroxyapatite in various forms, such as powder, porous blocks, or pearls, can be used to fill bone defects and free spaces in the bone [22].

Therefore, various methods for synthesizing Hap, with tailored properties, have been investigated. These can be classified as dry methods (solid-state and mechanochemical), wet methods (chemical precipitation, hydrolysis, sol-gel, hydrothermal, emulsion, polymer assisted routing, synthesis via biological tissue, ultrasonic spray freeze-drying, microwave irradiation, and sonochemical procedures), and high temperature processes (combustion and pyrolysis). [22-24].

2. MATERIALS, METHODS AND RESULTS

2.1 Materials

The hydroxyapatites (HAP) $[Ca_{10}(PO_4)_6(OH)_2]$ are materials very important inorganic substances in biology and chemistry. Biological HAP are the most common crystalline calcium phosphates, the first constituent mineral of bones, tooth enamel and dentin [8].It seems to be the most suitable ceramic material for the construction artificial bone tissue thanks to its excellent biocompatibility properties [9].

2.1.1 Crystallographic structure

Hydroxyapatite (HAP) belongs to the family of apatites which are mineral compounds whose main characteristic is their ability to admit a large number of substitutions and ionic vacancies. Apatites are generally represented by the chemical formula [1]: $Me_{10}(XO_4)_6Y_2$ - Me is a divalent cation, XO_4 a trivalent anion and Y a monovalent anion. The phosphocalcic apatites which enter into the constitution of the calcified tissues can be described from the HAP. Stoichiometric hydroxyapatite is therefore represented by the formula: $Ca_{10}(PO_4)_6(OH)_2$ It crystallizes in a hexagonal structure. The lattice parameters are: a = b = 9.432Å; c = 6.881 Å. Its cell contains 10 calcium atoms, 6 (PO_4) tetrahedra and two hydroxyl groups (Figure 1) [2.9].

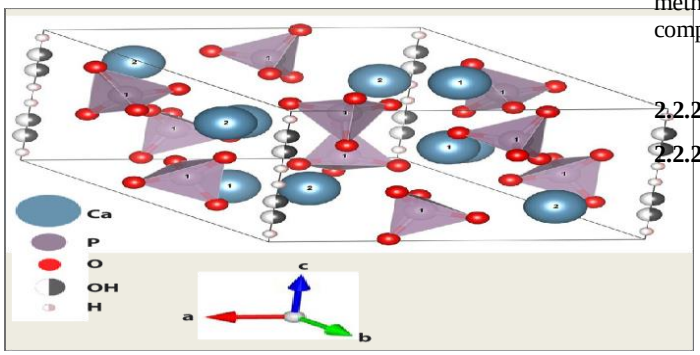

Figure 1.Schematic representation of the unit cell of the HAP [2].

The mineral phase of the bone presents a non-stoichiometric HAP with many ion substitutions in the crystal lattice.
HAP deserves great attention due to its chemical composition very close to that of the mineral phase of natural biological apatites as well than its great ability to chemically bind to bone tissue. The structure of HAP is highly crystalline, but it exhibits resorbability and solubility low in the human body [7].

2.2 Methods and Results

2.2.1. Synthesis by neutralization method

It consists in neutralizing a solution of calcium hydroxide $Ca(OH)_2$ commonly called milk of lime, by adding a solution of phosphate monoammonium or dihydrogen ammonium phosphate [11,12].

The reaction involved between the two solutions is the following:

$$10Ca(OH)_2 + 6NH_4H_2PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + (OH)_2 \quad \textbf{(1)}$$

This reaction **(1)** makes it possible to quickly obtain large quantities of HAP calcium phosphate with little material [12,13]. After 48 hours, the reaction mixture is filtered in order to recover the white precipitate. The solid is then washed several times with a mixture (water + ethanol) and dried at 80°C in an oven for 24 hours. The material thus obtained is crushed and sieved desired grain size [14].

The hydroxyapatite from coprecipitation and sol-gel methods showed a significant degree of crystallinity compared with that of the microwave route.[21].

2.2.2. Characterization method

2.2.2.1 XRD (X-ray)

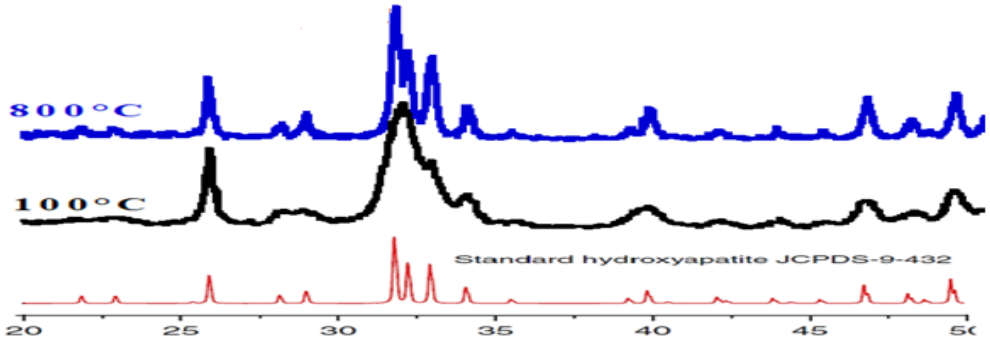

Figure.2.RX Diffractogram of the HAP.

The X-ray analysis was performed by a diffractometer (45kv, 40mA in the range 2θ = 4 ÷ 74◦, and 2θ step of 0.03◦, whose technical characteristics are as follows: Type configuration PW3064, Goniometer type PW3050/60; rotating sample holder (spinner) type PW3064, using either a copper or a cobalt anticathode. The thermograms were realized by a XPERT-PRO operating under the following conditions: heating rate = 10°C/min, sample weight = 40 mg, atmosphere: air.
The phase identification was completed with the help of the DIFFRAC.EVA release 2019 program from the DIFFRAC.SUITE.EVA software package and the ICDD PDF 9-432 database.
The figure 2 presents the diffractogram of a calcined HA powder. Only the peaks of the apatitic phase (PDF 9-432) are detected and this regardless of the heat treatment applied.

By comparing the diffractograms of the RX spectra of the two hydroxyapatites (standard and synthesized) it can be deduced that we have synthesized a high quality hydroxyapatite with good crystallinity:
-Heating to 100°C resulted in broad diffraction peaks which correspond to poor crystalline apatite.
-Increasing the heating temperature to 800°C results in sharper and narrower diffraction peaks which indicate the increase in crystallinity.

2.2.2.2 Spectroscopy Fourier Transform Infrared (FTIR)

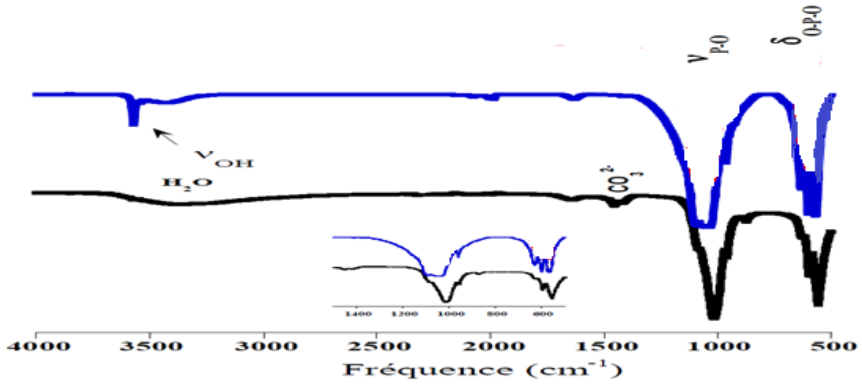

Figure 3.FTIR spectre of the HAP.

The Fourier transform infrared spectra (FTIR) of samples were obtained on a spectrometer with a DTGS detector and a KBr beam splitter, the KBr pressed-disk technique (1 mg of samples and 200 mg of KBr) was used, the spectra were recorded in the region 4000- 400 cm^{-1} .
.From figure 3. There is a symmetric and antisymmetric absorption bands attributable to (PO_4^3) groups in the 1100 and 900 cm^{-1} range and those deformation between 600 and 500 cm^{-1}. For the parboiled product, a wide band between 3000-3650 cm^{-1} and another of low intensity at 1649 cm^{-1} corresponding to the stretching vibration of the OH groups of the water molecule. Others bands at 1420 and 870 cm^{-1} were detected characteristic of the presence of carbonates in the powder. Moreover, the characteristic absorption bands to the vibrations of the OH^- hydroxyls of the apatitic lattice are located around 3560 and 630 cm^{-1} are visualized only in the case of hydroxyapatite calcined at 800°C. On these IR spectra, we observe that the bands related to carbonates disappear, which explains the total decomposition of the carbonate residues.

2.2.2.3 Elemental Chemical analysis Scanning Electronic Microscope

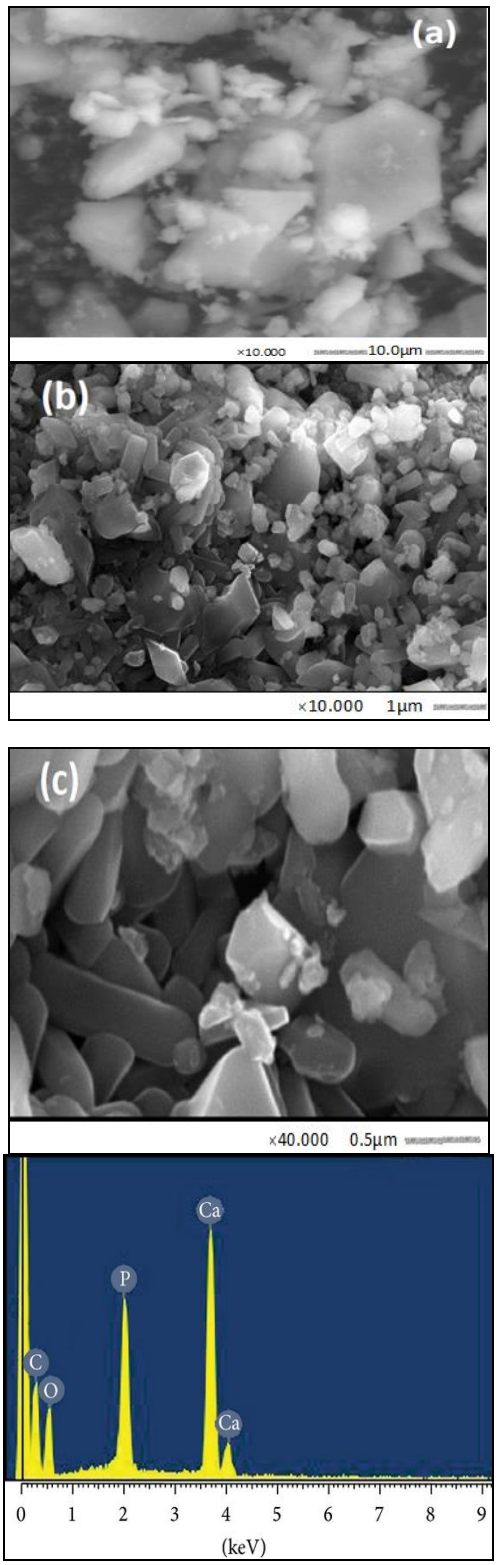

Figure.4.SEM micrographs and EDX analysis of hydroxyapatite obtained by scanning electron microscopy of the product baked at 100°C (a) and powder calcined at 800°C (two magnifications (x10,000 (b),x40,000 (c)) and Energy dispersive X-ray (d).

The morphology (a), (b) and (c) shows an irregularity of the particles forming the aggregate of hydroxyapatite (HAP) with the major elements of phosphorus and calcium proven by EDX energy spectra (d).

Table 1. Characterization by X Fluorescence of HAP.

Element	Ca/P	CaO	PO_4
HAP (Wt %)	1.67	39.6	18.5

The elemental chemical analysis of the hydroxyapatite (HAP) (Table 1) and EDX shows that there is a significant percentage of CaO (39.6%), PO_4 (18.5%), which proves that HAP is rich in calcium and phosphorus.

According to the Ca/P molar ratio in the apatitic structure, the composition chemistry of HAP may vary. A HAP having a Ca/P atomic ratio of the order of 1.67 is said to be stoichiometric whereas the other apatites ($1.5 < Ca/P < 1.67$) are called deficient apatites. [7].

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2.2.2.4 Thermal analysis: ATD / ATG

The method involves measuring the temperature difference ΔT between the sample to be studied and a reference inert sample, both s the same heating rate of 10 ° C / min in a tempe ge from 25 ° C to 1000 ° C. The reference sample is alumina. This difference is related to the amount of heat released or absorbed by the studied material, and ΔT is recorded as a function of temperature. This allows the detection of endothermic and exothermic peak changes.

The idea is to continuously monitor the change in the sample weight as a function of temperature. The sample, placed in an alumina boat suspended from the beam of a balance, is located in a chamber at controlled temperature. The equilibrium of the balance is provided by an electromagnetic compensation system. The change in weight, given by rebalancing the system, is recorded as function of the temperature rise.

The purpose of thermogravimetric analysis (TGA) is to continuously record the mass variations of a sample during a thermal cycle. This technique is coupled with differential thermal analysis (DTA), which is based on the study of the energy released or absorbed by the material studied when it undergoes physical or chemical transformations. The thermal evolution of the PAH powder by (ATG) and (ATD) from room temperature to 800°C under a stream of argon-air mixture, with a temperature rise rate of 10°C/min.

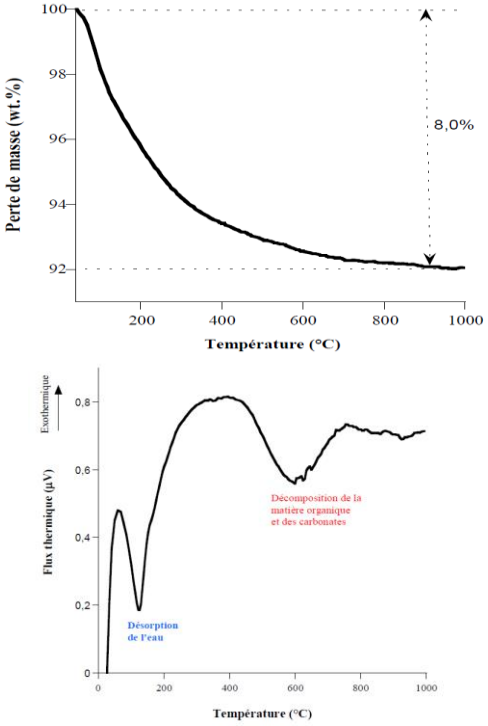

Figure.5. Curves of differential and gravimetric thermal analyses of HAP.

The figure 5 shows a continuous mass loss between room temperature and 800°C.
The first loss of 7.5% in total weight of the material occurs between ambient temperature and 600°C.
=> produced by the increase in temperature is attributed to the desorption of water.
The 2nd loss (> 600°C), of the order of 0.5% by weight, may correspond to the decomposition of residual carbonates, which are detected by infrared spectroscopy.
The absence of endothermic peaks up to 800-1000°C, showing that the hydroxyapatite powder is pure and does not undergo any decomposition phenomenon.

2.2.2.5 Plasma projection torch

The material to be sprayed: HAP Calcium phosphate for validation defined by: chemical composition, cristallinity, ratio Ca/P, porosity, particle size and physico-chemical characteristics, thickness, roughness, adhesion.
As mentioned previously, thanks to the similarity with the component inorganic material of human hard tissue, hydroxyapatite is considered a material suitable for various applications frequently in the field of medicine and pharmacy [6,8].
HAPs are also frequently used as a "coating" on titanium prostheses to facilitate osteointegration [15] or to prevent wear due to oscillatory micro-movements at the interface between the implant and the bone sometimes even leading to rupture of the prosthesis [16].
A hydroxyapatite-based coating improves the fixation of the prosthesis on bone tissue and reduce the release of metal ions from the alloy of the implant to the living organism [17][18].
There are some examples of use as a cement used in facial surgery to replace autogenous bone grafts [19,20] and a increasing number of applications as a vector of drugs for the direction of the cells.
Thus, synthetic grafts based on hydroxyapatite fit into the human body without being rejected by the host tissue and they facilitate the formation of new bone tissue, by creating bonds with the tissue newly formed [6].
In all these applications, it is ultimately the surface reactivity of the HAP with the biological medium which is the key point.
The results obtained after validation of the coating conditions and clinical tests have shown great satisfaction and are in progress to move on to the application part.

3. CONCLUSION

In the fight to increase the lifespan of the human body, the biomaterial is the alternative most seriously considered by doctors.
The field of materials science is undergoing a great evolution and the field of biomedical science is therefore a field for which the requirements are going to be increasingly important in the race to increase the duration of life of human beings. Choosing the right materials is very important particular. We believed for a long time that it was necessary, above all, to seek materials with the highest possible resistance to deformation. Then, gradually, in many cases, efforts had to be made to seek biomaterials presenting a better compromise between their resistance and their biocompatibility or, more generally, their resistance to fatigue.
The tests must be carried out and interpreted by specialists according to the future clinical use of the biomaterial, this makes it possible to avoid rejecting biomaterials used successfully for a long time and which would not pass the tests imposed today on modern materials.

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