Preparation and characteristics of synthesized hydroxyapatite from bovine bones and by co-precipitation method

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Abstract

Hydroxyapatite (HA, $(Ca_{10}(PO_4)_6(OH)_2)$ is a widely studied bioceramic due to its biocompatibility, bioactivity, and chemical similarity to the mineral component of bone. Generally, hydroxyapatite can be made from several natural and synthetic sources. The objective of this study is to prepare hydroxyapatite powders from different precursors (natural or chemical). Hydroxyapatite was synthetized by co-precipitation method, the chemical precursors of which are $[Ca(NO_3)_2.4H_2O$, $(NH_4)_2HPO_4]$ and the natural source was bovine bone. Bovine hydroxyapatite (BHA) was extracted from the bovine bone bio-waste via thermal method and milling process. Synthesized HA (SHA) was prepared by co-precipitation method with the pH 10.0 of mother liquor. The prepared powders were characterized using various analytical techniques such as XRD, FTIR spectroscopy, thermogravimetry (TG), and scanning electron microscope (SEM). These techniques provide information about the structural, chemical, morphological and physicochemical of each of the prepared powders. The use of co-precipitation method produced a low crystallinity of HA while the thermal method increased crystallinity. On the other hand, the results showed that the Ca / P ratio of synthetic hydroxyapatite (SHA) as well as that of bovine bone source (BHA) was also stoichiometric.

Keywords
Hydroxyapatite, Bovine bone, Synthesis, Co-precipitation, thermal decomposition.

1. Introduction

Bioceramics based on calcium phosphates (CaP) have developed considerably in recent decades due to their excellent biocompatibility and bioactivity. Hydroxyapatite (HA) is a mineral species of the phosphate family, the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ (Elliott et al. 1994), with a molar ratio equal to 1.67 (HA stoichiometric). However, Hydroxyapatite (HA), a calcium phosphate bioceramic, is the primary material for applications in bone replacement. It is a material that exhibits an absence of local and systemic toxicity, an absence of inflammatory responses and an apparent capacity to bind to the host tissue (Silva et al. 2012). It has been widely used for biomedical applications, including orthopedics, dentistry, and as a coating material for metal implants (Hendi 2017).

In recent decades, hydroxyapatite (HA) has received considerable attention as a biomaterial due to its similarity to human bone. The hydroxyapatite (HA) preparation for medical purposes must meet specific criteria in terms of repeatability, raw material composition, and finished product purity (Bernard et al. 1999). Generally, the physicochemical properties and morphology of hydroxyapatite (HA) depended on the origin and methods of preparation (Sobczak-Kupiec et al. 2012). Indeed, the physicochemical properties of materials depend mainly on their production processes. Due to the interesting properties of hydroxyapatite (HA), various techniques have been and are being developed to produce hydroxyapatite. There are two main ways to obtain hydroxyapatite; one is by inorganic synthesis (synthetic), and the other is to obtain hydroxyapatite (HA) from natural sources. Extensive attempts have

been made to create high-quality HA with appropriate biocompatibility while looking for cost-effective and nonpolluting technologies (Sadat-shojai et al. 2013, Huang et al. 2011). Indeed, some researchers have attempted to synthesize hydroxyapatite from biological materials. Corals (Manjubala et al. 2000), eggshells (Ibrahim et al. 2015) (Siva Rama Krishna et al. 2007) and ostrich eggshells (Dupoirieux et al. 1999), seashells (Shavandi et al. 2014), coral (Dorozhkin et al. 2009), nacre (Dorozhkin et al. 2009) and bovine bones (Herliansyah et al. 2009) have been used to produce hydroxyapatite.

However, a problem with natural hydroxyapatite (HA) is the transmission of disease when proper preparation is not followed to remove all proteins (Goller et al. 2006). Above time, various methods have been developed to synthesize hydroxyapatite (HA) suitable for different applications. Hydroxyapatite (HA) can be also manufactured synthetically by using number of different methods. The process for the preparation of synthetic hydroxyapatite comports reactions in solid state (Arita et al. 1995), co-precipitation (Gentile et al. 2015, Ignjatovic et al. 2004), sol-gel process (Jafari et al. 2018, Padmanabhan et al. 2009, Ramanan et al. 2004), microemulsion (Ma et al. 2016, Koumoulidis et al. 2003), and hydrothermal method (Ashok et al. 2007, Yoshimura et al. 2004). Nevertheless, the preparation and synthesis of HA from different sources with characteristics that are nearly identical to stoichiometric HA (Ca/P = 1.67) has not been understood fully, and research in this field is still wide open.

This work is part of the understanding of the synthesis, shaping and structural characterization of hydroxyapatite composition. Variations in the parameters which affect synthesis are necessary in order to produce hydroxyapatite (HA) particles of the required size, crystallinity, and composition. This research is of particular interest for the understanding of hydroxyapatite preparation (quantity and crystalline structure) under controlled physico-chemical conditions. One of the major objectives lies in the preparation of hydroxyapatite powders and their control by a simple and reproducible process.

2. Experimental Procedure

2.1. Synthesis of HA powders

In this study, to prepare hydroxyapatite (HA) two sources (natural and synthetic) were used. The natural source is bovine cortical bone because it is morphologically and structurally similar to human bone. In addition, this natural source is easy to obtain, inexpensively and available in unlimited quantities. The second aspect relates in particular to the synthesis of hydroxyapatite by co-precipitation route in order to optimize the synthesis protocol and to develop the purest possible ceramics for their characterization.

2.1.1 Synthetic hydroxyapatite (SHA) by co-precipitation route

The precipitation experiments were performed by mixing the solutions of calcium nitrate and diammonium hydrogen phosphate. Figure .1 shows an experimental setup for the preparation of synthetic hydroxyapatite. Figure 1 shows the typical steps for fabrication of HA powder using the chemical precipitation.

Synthetic hydroxyapatite (SHA) obtained by co-precipitation uses calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) salt with a purity of 98% and dibasic ammonium phosphate ($(NH_4)_2HPO_4$) as precursors of the reaction. Solutions of distilled water 0.5 M $Ca(NO_3)_2$ and 0.3 M $(NH_4)_2HPO_4$ (to meet the stoichiometric Ca / P = 1.67) were prepared. The phosphate solution ($(NH_4)_2HPO_4$) was added drop wise to the solution of calcium nitrate, with stirring at temperature (80 °C). The speed of adding the phosphate solution to the calcium solution must be low enough to limit the formation of calcite in the precipitates. In addition, the pH of the mixture was regulated to 10 by dropwise addition of 25% v / v ammonium hydroxide solution (NH_4OH), with continuous and vigorous magnetic stirring (500tr/min), giving an opaque suspension.
The reaction involved during this synthesis is designated by the equation below (1) (El-Kholy et al. 1998):

$$10\ Ca(NO_3)_2 + 6\ (NH_4)_2HPO_4 + 8\ NH_4OH \rightarrow Ca_{10}(Po_4)_6(OH)_2 + 20\ NH_4NO_3 + 6\ H_2O \qquad (1)$$

The precipitates formed were aged (maturation) overnight (24 hours) at room temperature. At the end of the maturation, the final precipitates collected were filtered, washed several times with distilled water to remove all the sub products. Then the precipitates were dried in an oven in air for 8 hours at 100 °C. The dried powders were crushed with a pestle mortar and the ground powders were calcined at the temperature 850 °C at a speed of 10 °C min^{-1} for 2

h before proceeding to a new characterization. This method of SHA preparation has been repeated several times and found to be reproducible.

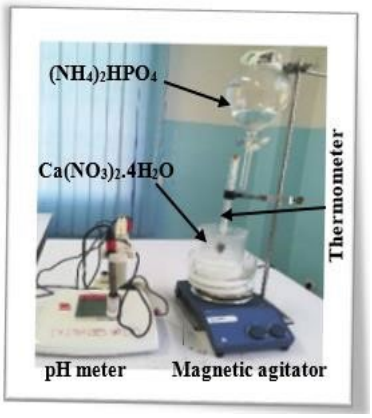


Figure 1. Preparation of synthetic Hydroxyapatite (SHA) by co-precipitation method

2.1.2 Extraction of HA from bovine bone by thermal decomposition

Hydroxyapatite (HA) can be obtained from biogenic sources such as bovine bone by means of calcination at high temperature. In this study may be used to extract hydroxyapatite from biowaste bovine bovine femur. In order to ensure the homogeneity of the material, a sufficient quantity of bovine femurs was collected. Initially, to eliminate the organic matter from the bovine femur (marrow, tissue), the bone has been submitted to a deproteinisation process. Figure 2. Photographs of natural bone condition after degreasing, deproteinization and pulverizing treatments.

Figure 2. Photographs of bovine femur: (a) Raw state; (b) washed and cleaned; (c) pulverized

Bovine bones (femur) were cleaned from the meat with a knife, boiled in distilled water to remove fat. The bone cooking treatment was performed in a domestic stainless steel pressure cooker with water covering the bones. Pressure cooking of the bones was performed in potable (tap) water, while degreasing and deproteinization treatments used distilled water. The bones, cleaned and dried in an oven at 100 °C, then fragmented into small pieces using a pestle mortar. Calcination of the powder at a temperature of 900 °C/3h to improve the crystallinity and to check the purity of natural bovine hydroxyapatite (BHA) powders. Before grinding of the powder using a planetary mill (Fritsch, Pulverisette 7), for a period of 4 hours at a speed of 350 rpm. finally, the sieving of the ground powder to obtain an adequate particle size fraction as required.

2.2.Physicochemical characterization of hydroxyapatite (HA) powders

Different investigative techniques were used in this study for the preparation of hydroxyapatite (HA): differential thermal analysis (DTA), X-ray diffraction (XRD) analysis was carried out, Fourier transform infrared (FTIR), scanning electron microscopy (SEM) coupled with energy dispersive x-ray spectroscopy (EDX), and a laser particle size analyser. The existing phase's analysis was performed by X-ray diffraction (XRD) with a Rigaku device, Ultima IV (CuKα radiation λ = 1,5406Å). The spectra were recorded in the 2θ interval of 10°-90° (0.05° step and time in 5s step). The microstructure and the morphology of prepared hydroxyapatite powder was examined using a scanning

electron microscope (SEM/EDS) type QUANTA 250 and Chemical analysis was carried out using an EDX at 20 kV. Analysis of the functional groups of the prepared powders were characterized utilizing an IRAfinitty-1S (Shimadzu) Fourier transform infrared (FTIR) spectrometer and the measurements were carried out in the transmission mode with wave numbers from 400 cm^{-1} to 4000 cm^{-1} with a resolution of 1 cm^{-1}, averaging 100 scans. TGA-DSC analysis (SDT Q600 equipment) was used to evaluate the thermal changes of bovine bone produced by temperature between 25 and 1200 °C, at a heating rate of 10 °C / min, in a nitrogen atmosphere. A laser particle size analyser (Partica LA960) was utilized to determine the particle size distribution of the prepared hydroxyapatite (HA) powder.

3. Results and Discussion

3.1. DSC /ATG analysis

Moreover, in order to evaluate the calcination temperature, sample of bovine femurs was investigated by thermogravimetric analysis. The thermal transformation process of bovine bone and the hydroxyapatite thermal stability were studied by using a TGA/DSC analyzer. The TG/DSC analysis of as-received bovine bone are shown in Figure 3. During treatment at different temperature, the percentage of weight loss of bovine change. The removal of the organic portion from bovine bone confirmed by TGA analysis. It can be see no weight loss was observed from room temperature to 600 °C (no decomposition), furthermore, at the temperature ranging from 600 to 800 °C, the extensive weight loss was perceived; this was related to exothermic reaction or the evaporation of carbon dioxide gas. After that, no weight loss was detected at temperatures ranging from 800 °C to higher, implying that the change was complete. So the calcination temperature should be set from 800 °C, and it was essential to note that percent weight loss was estimated to be 55 %. However, in several other articles (Chraïbi et al. 2016, Buasri et al. 2013), the calcination temperature was found to be higher than 800 °C.

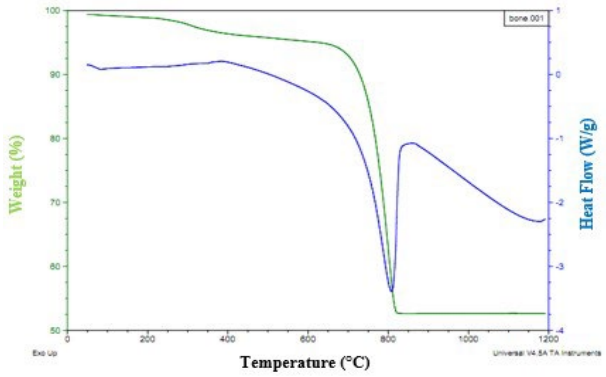


Figure 3. Differential thermal analysis of raw bone

3.2.XRD analysis

The phase composition and purity of the prepared hydroxyapatite (HA) were determined by X-ray diffraction (XRD). The XRD patterns obtained of the HA powder synthesized are identified by comparison with the references of the International Center for Diffraction Data - Powder Diffraction Files (ICDD-PDF) file (Figure 4). Powder diffraction patterns of HA powder prepared from bovine bone wastes and synthetic HA are illustrated in Figure 4. The as-prepared HA powders shows broad peaks and is identified as HA based on JCPDF 9-432. This is a typical characteristic of the HA. Figure 4a shows the diffraction patterns of the bovine bone waste. According to this figure, the natural bovine hydroxyapatite (BHA) exhibits higher crystallinity than the synthetic hydroxyapatite (SHA). By performing the calcination, the diffraction lines become more refined and become more and more resolute and intense. Analysis indicates that the crystallinity of apatite is only clear at a temperature of 850 °C.

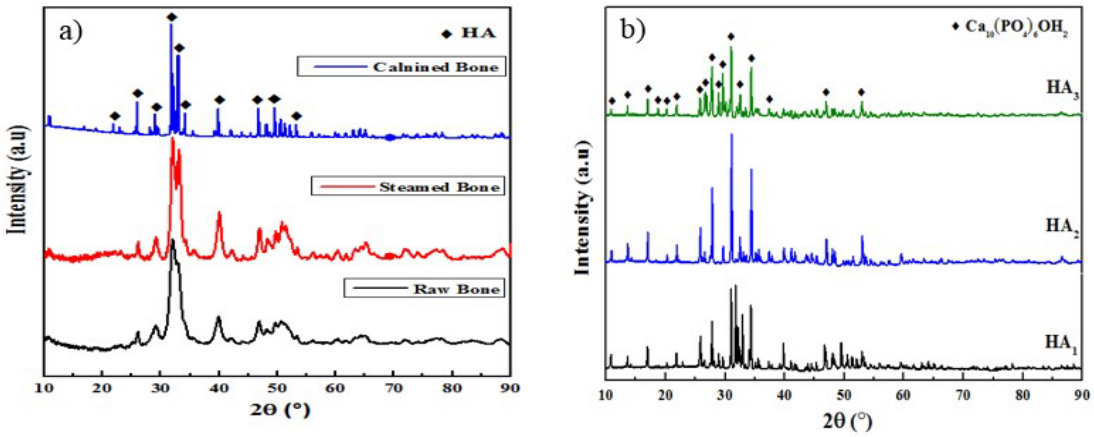


Figure 4. X-ray diffraction pattern of HA powder prepared from a) bovine bone wastes, b) synthetic

3.3.FTIR analysis

FTIR analysis was performed to obtain information about the functional groups of the prepared HA powders. Figure 5. Illustrate the FTIR spectra of hydroxyapatite synthesized from bovine bone and by co-precipitation route. The peaks for the $PO^3{}_4{}^-$and OH^-groups in the hydroxyapatite may be identified in the FTIR analysis in Figure 5. In these spectra, bands placed at 630 and 3600 cm^{-1} corresponding to the hydroxyl (OH^-) vibrational groups, can be identified. Furthermore, bands at 570, 582, 964, 1030 and 1068 cm^{-1} belonging to phosphate groups ($PO^3{}_4{}^-$) were found. These functional groups are characteristic of stoichiometric hydroxyapatite (Raya et al.2015).

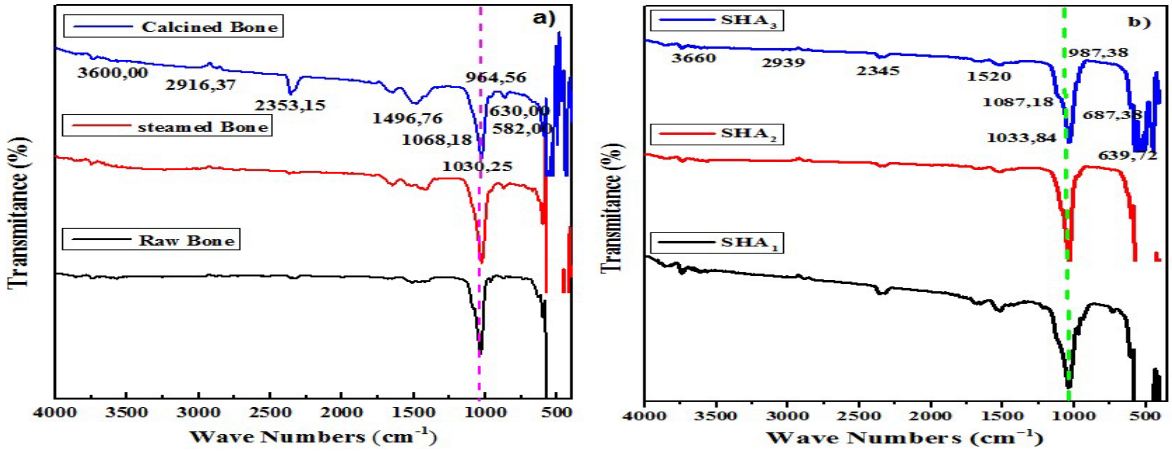


Figure 5. FTIR spectra of the HA prepared from a) Bovine bone, b) by Co-precipitation method

Figure 5 shows the transmittance intensity of the bands at 639 and 400 cm^{-1} belonging to OH^- that is lower in the case of the natural bovine hydroxyapatite (BHA) compared to synthetic hydroxyapatite (SHA). This difference can be explained by the fact that the natural bovine hydroxyapatite (BHA) was obtained by means of physical reactions (thermo decomposition) while the synthetic hydroxyapatite (SHA) was obtained by means of reactions between two solutions in aqueous medium thus allowing the formation of a large amount of such functional groups.

3.4.FESEM Characterizations

The morphology and texture of the hydroxyapatite powders are revealed by scanning electron microscopy (SEM). The morphology of the HA powder prepared from bovine bone, examined by SEM, is illustrated in Figure 6. Small and large pores can be found in raw bovine bone; however, the microstructure is often dense due to the presence of organic compounds impregnated with the mineral phase present in bovine bones. As can be seen from the particle morphologies, there is a distribution of small particles and large agglomerates. Grains display several sizes and shapes.

These agglomerates are made up of very fine particles welded together. So, unlike the raw bovine bone powder, which has a more compacted microsurface, the decomposed powder has numerous pores generated by the dissolution of the organic and mineral phases.

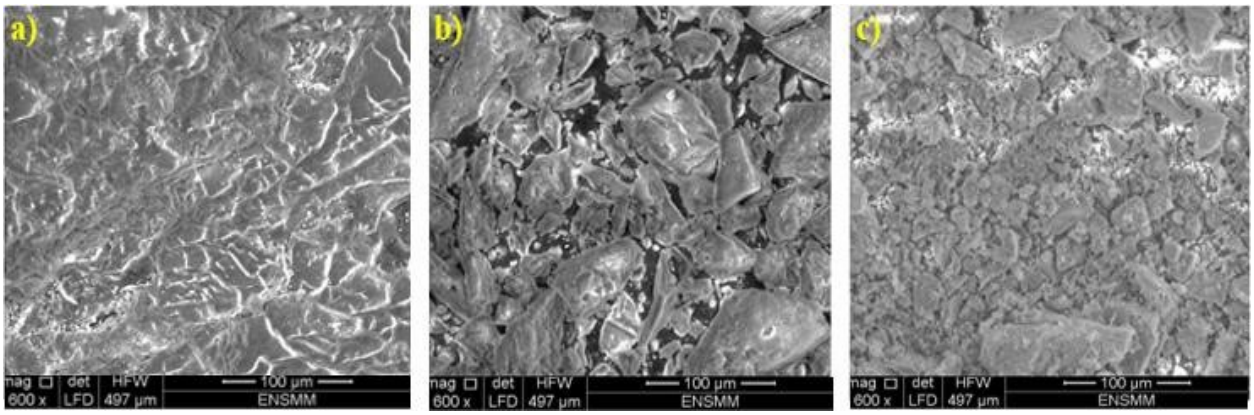


Figure 6. SEM micrographs of bovine bone: (a) Raw material; (b) Grounded; (c) Calcined at 900 °C/3h

The microstructure of synthetic hydroxyapatite (SHA) is shown in Figure 7. The presence of well-distributed macroporous through and microporous within the pore walls can be seen on the figure. The size, morphology, and interconnectivity of pores determine the quality of powders. Figure 7 shows SEM images corresponding to synthetic hydroxyapatite (SHA) at different magnifications [a) X1 000, b) X3 000, c) X12 000]. In these images, the grain distribution can be observed, show semi-spherical shapes, with more homogeneous sizes.

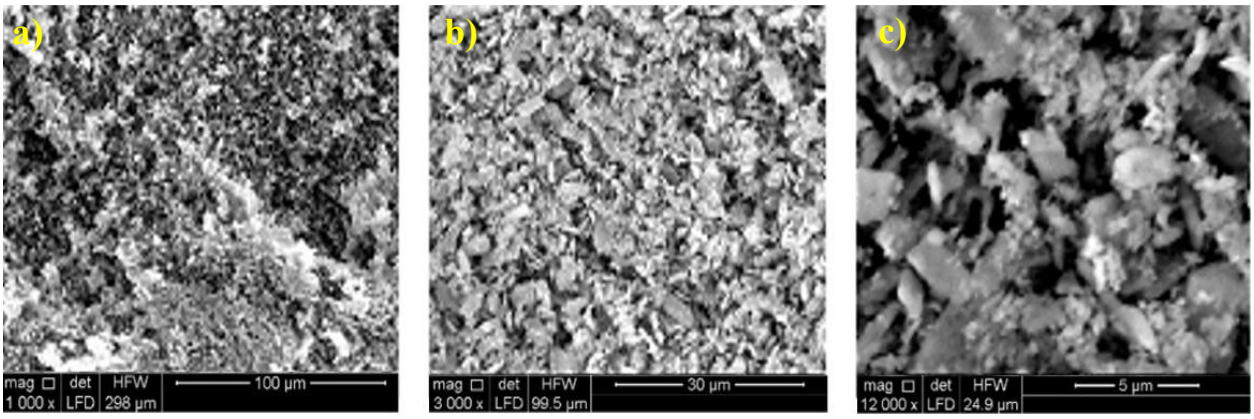
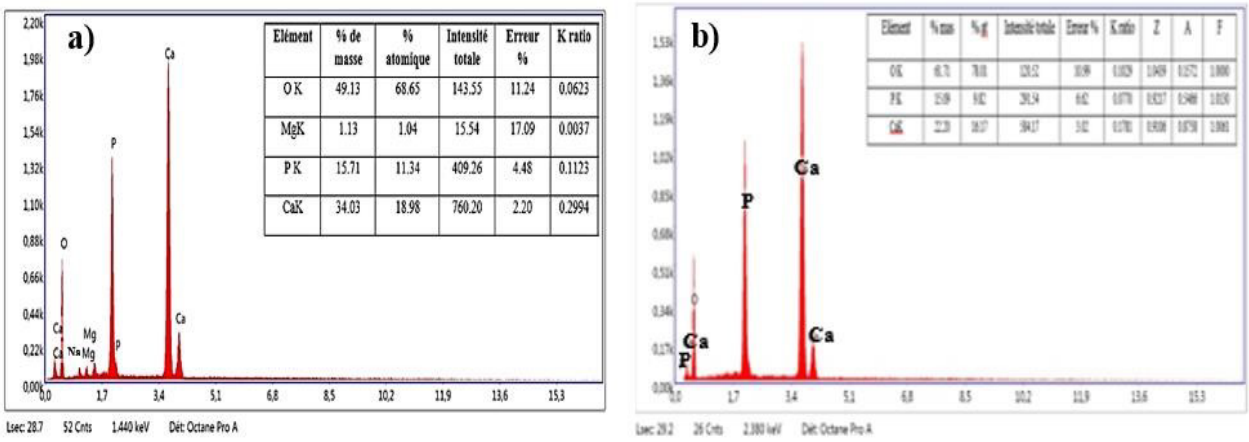


Figure 7. SEM images of SHA powders at different magnifications: a) X1 000, b) X3 000, c) X12 000

3.5.Energy dispersive X-ray spectroscopy (EDX)

Semi-quantitative chemical analysis of the powder to obtain the Ca / P ratio was performed using energy dispersive X-ray spectroscopy (EDX, Oxford, Aztec Energy X-Act). Also, the approximate values of Ca/P molar ratio for HA powders were calculated from the results of XRF analyses. The theoretical Ca/P molar ratio of pure HA is 1.67. The results of the EDS analysis showed that the Ca/P ratio (18,98 / 11,34) of BHA investigated in this study is about 1,673. This result is relatively close to 1.67 for the stoichiometric HA (Milovac et al. 2014, Huang et al. 2011).



Elément	% de masse	% atomique	Intensité totale	Erreur %	K ratio
O K	49.13	68.65	143.55	11.24	0.0623
MgK	1.13	1.04	15.54	17.09	0.0037
P K	15.71	11.34	409.26	4.48	0.1123
CaK	34.03	18.98	760.20	2.20	0.2994

Figure 8. EDS analysis of of the HA prepared from a) Bovine bone, b) by co-precipitation method

According to the EDS results, the predominant elements in both prepared powders are P, Ca and O; furthermore, the spectrum of natural bovine hydroxyapatite (BHA) reveals the presence of impurity traces of Mg and Na. These impurities are not present in the synthetic hydroxyapatite (SHA).

3.6. Particle size analysis

The particle-size distribution (PSD) of the powder was determined by the Laser scattering particle size distribution analyzer. Figure 9 shows the particle size distribution of SHA powder calcined at 850 °C for 2 hours. The average diameter of the hydroxyapatite powder produced by co-precipitation was estimated to be 20.52 µm.

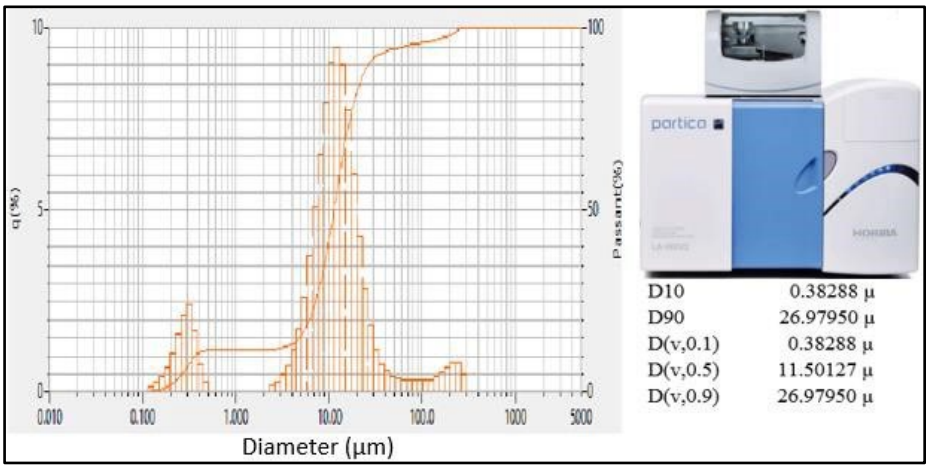


Figure 9. Particle size analysis of SHA calcined at a temperature of 850 °C prepared by co-precipitation

4. Conclusion

According to the results obtained from the above analyzes, it was found that hydroxyapatite can be successfully produced by the co-precipitation process as well as from bovine bone by thermal decomposition but each route has advantages and disadvantages. The method of preparing the powders must be reproducible and, as far as possible, simple to implement. Varied processing characteristics of synthesizing HA particles can result in different morphologies. Thus, it can be concluded that:

- The nature of the precursors plays a very important role in obtaining hydroxyapatite.
- The main difference between SHA derived from the chemical processing route and that of natural-biological origin (bovine bone) is the presence of other minerals such as Na and Mg in BHA from natural source.

- Synthetic process parameters such as pH, temperature, calcination temperature and aging period are important factors that control the purity of the prepared SHA.
- Synthetic hydroxyapatite (SHA) is more commonly used because it is more readily available and free from disease transmission.
- Bovine bone can be considered a promising biomaterial to be used to produce hydroxyapatite. As, there is an economic and environmental incentive due to the sheer volume of agricultural waste or other types of bone available to find value-added applications for these materials in biomaterials.
- According to the method, reagents and variables adopted, it is possible to obtain with diverse characteristics and properties on the intended application.

Acknowledgements

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