# Rapid synthesis, characterization, and studies of hydroxyapatite nanoparticles.

# Sharanabasava V Ganachari\*, Anjali A Bevinakatti, Jayachandra S Yaradoddi, Nagaraj R Banapurmath, Anand M Hunashyal, Ashok S Shettar

Centre for Material Science, BV Bhoomaraddi College of Engineering and Technology, Hubballi, India

# Abstract

Hydroxyapatite (HAp) is a calcium phosphate that is similar to the human rigid tissues and is a form of calcium apatite. It constitutes, the main component of bone and enamel and can be synthesized artificially via various methods and the method proposed in this paper is based on wet chemical precipitation. An attempt has been made to synthesize nano-structured hydroxyapatite using improved surface morphology when compared to other methods. Porosity is another very important property that can be enhanced using various materials. Calcium hydroxide  $(Ca(OH)_2)$  and ortho phosphoric acid  $(H_3PO_4)$  were used in the present study. Experiments were conducted under controlled environment maintaining required pH, concentration of solutions and temperature as well. The variation in any of these parameters gives a change in the resultant obtained. The present report emphases on the synthesis and characterizing the nano-structured Hap powder. Fourier-transformed infrared spectroscopy (FTIR), X-ray diffraction (XRD), and Scanning electron microscopy (SEM) for the surface, size and dimensions analysis of the Hap were carried out. From the work carried out it was found that the HAp was in the range of 35 nm to 90 nm and is a desirable output.

Keywords: Hydroxyapatite, Nanoparticle, Wet chemical method, X-ray diffraction, Fourier-transformed infrared spectroscopy.

#### Introduction

Seventy percent of bone is made up of the mineral hydroxyapatite, which is (HAp)  $[Ca_{10} (PO_4)_6 (OH)_2]$  an inorganic compound. The crystal size of HAp in natural bone is in nano-range. The fundamental elements of hydroxyapatite are primarily calcium and phosphorus, with a stoichiometries calcium and phosphate ratio of 1.667. Hydroxide ions are eliminated at elevated temperatures in this chemical synthesis method. Although numerous approaches investigated ways of making these nanocrystals, controlling the size, shape and crystallinity and many parameters affect the size and shape of such materials and this still needs further research [1-4]. Synthetic ceramic based on calcium phosphates particularly in the composition of tricalcium phosphate and hydroxyapatite have extensively been studied and clinically used. The biomaterials research field focuses on the synthesis of these materials for three decades to applications in orthopedics and dentistry [5-7].

Hydroxyapatite is the utmost widely accepted biomaterial for the repair and reconstruction of bone tissue imperfections. It has the characteristic features of biomaterials, such as biocompatible, bio-active, osteoconductive, non-toxic, noninflammatory and non-immunogenic properties [6-10]. Several researchers reported that HAp bioceramics were prepared by citrate gel pyrolysis method [11]. Biocompatible scaffolds find applications in bone tissue engineering, medical application, *in vitro* study, cytotoxicity in fibroblasts, HAp for bone regeneration etc. [12-30].

In the present study, synthesis and characterization of hydroxyapatites were carried out which include nanodimensional and nano-crystalline hydroxyapatite. The method proposed in this paper is based on the wet chemical method. Accepted on December 05, 2016

The hydroxyapatite powders are incurred from the reaction of inorganic oxide chemical solutions. Wet Chemical coprecipitation involves the use of aqueous solutions. This method leads to obtaining a hydroxyapatite characterized by high precise superficial area and small particle size distribution. In most cases difference from the stoichiometry will occur, at the same time, the amorphous nature is obtained [11-15]. These processes depend on a number of influences such as reactants involved in synthesis, concentration and preliminary pH of solutions, reaction temperature etc. [8]. Wet chemical methods emerged as potential in contrast to conventional and solid-state synthesis approaches of compounds and materials that are widely used in ceramics manufacturing. Different methods of powder and material production (liquid phase sol-gel process, spray drying, hydrothermal synthesis, Pechini method, aerosol spray pyrolysis, cryochemical synthesis, etc.) using liquid phase as one of the process phases are reported. The main difference between wet chemistry and similar products of solid-phase synthesis is that much smaller grains (crystallites) and, usually, lower temperature and shorter duration of phase formation occurs in the former [28-35]. Wet chemical methods involved in synthesis of hydroxyapatite led to products obtained that are characterized by a large degree of crystallization, depending on the circumstances of synthesis. These approaches are based on two types of reaction: neutralization (acid-base) or reaction between the two compounds [9]. In the case of hydroxyapatite synthesized by chemical precipitation, the final product resulting is characterized by high purity and crystalline that further depends on a number of process parameters, such as synthesis temperature, the starting concentration of reactants involved in the synthesis reaction, the pH of the environment reaction, the rate of acid addition, the stirring speed of the chemical

*Citation:* Ganachari SV, Bevinakatti AA, Yaradoddi JS, et al. Rapid synthesis, characterization, and studies of hydroxyapatite nanoparticles. Adv Mater Sci Res. 2016;1(1):9-13.

substance and the conditions of thermal treatment applied to as dried hydroxyapatite powders.

The objective of this work is to obtain hydroxyapatite powders by co-precipitation method, using different experimental conditions. The thermally treated powder was characterized by Fourier-transformed infrared spectroscopy (FTIR) study, X-ray diffraction (XRD) study, and scanning electron microscopy (SEM) in order to identify the phase composition, functionality, crystallinity and crystallite size.

#### **Materials and Methods**

The starting materials used in this process were calcium hydroxide and ortho phosphoric acid. Ammonium hydroxide is used as a pH regulating agent. Figure 1 shows an experimental setup for hydroxyapatite synthesis, beaker (600 ml) contains calcium hydroxide, burette contains ortho phosphoric acid, magnetic stirrer with heating facility for reaction mixture continues mixing at a higher temperature, pH meter, thermometer and weighing equipment were used.

Figure 2 Shows the flow chart of the experimental steps involved in wet chemical synthesis of hydroxyapatite. Figure 3 shows the graphical representation of the reaction mechanism of wet chemical synthesis of Hydroxyapatite in reaction showing how calcium ions (Ca<sup>2+</sup>) and phosphate ions (PO<sub>4</sub><sup>3-</sup>) takes place in the formation of nanocrystalline HAp. Figure 3 shows HAp nanoparticles were held together by impartially weak forces and can be simply dispersed by ultrasonication. In contrast, it can be found that the HAp NP aggregates are held together by stronger, covalent bonds. Particle agglomeration is influenced by a number of factors, including primary particle characteristics as well as the properties of the medium. The particles are suspended in the stability of HAp NPs in suspension and depends on the sum of the attractive and repulsive forces between the particles nanocrystalline HAp particles formed large agglomerate particles i.e. clearly shown in SEM images.



*Figure 1. Experimental setup for hydroxyapatite synthesis. Adv Mater Sci Res 2016 Volume 1 Issue 1* 

# Experimental

#### Hydroxyapatite synthesis

The synthesis flowchart of the hydroxyapatite is illustrated in Figure 2. Ca/P ratio is maintained in the stoichiometric during the experiment and the basic medium is maintained for the comparison of the results obtained. Aqueous solutions of 0.3 M ortho phosphoric acid and 0.5 M calcium hydroxide were prepared. The Ortho phosphoric acid solution is filled in the burette and calcium hydroxide solution taken in a flask and was mixed well to ensure that all the lime dissolves well in water.

The solution from burette is added drop by drop to the calcium hydroxide solution. Accordingly, the pH is determined using the pH meter and is maintained basic by adding ammonium hydroxide. The experiment is conducted at a temperature between 40°C to 45°C and pH of 8.5+, while continuously stirring the solution in the flask. The solution becomes milky; it is then kept for aging for 24 hours. Later a precipitate is obtained as in Figure 4.

#### Characterization techniques

Characterization of the obtained precipitate was carried out using SEM coupled EDAX Model-JEOI-SEM 6360 with a high resolution of 3.0 nm at 30 kV, X-ray diffraction (XRD) Phillips-3710 powder X-ray diffractometer in the 20 range



Nucleation of Hap at  $\leq 8.5$  at  $45^{\circ}$ C



SEM of HAp

### PO43-

*Figure 3.* Graphical representation of wet chemical synthesis of nano hydroxyapatite (HAp).

10° to 100° using CuK $\alpha$ 1 radiation ( $\lambda$ =1.54056 Å) and FTIR spectrophotometer 4000 cm<sup>-1</sup> to 450 cm<sup>-1</sup> Perkin Elmer spectrum one were undertaken at Shivaji University, Kolhapur. The analysis of the results is presented in the following section.

#### **Results and Discussion**

#### SEM analysis

Figure 5 shows the SEM images of hydroxyapatite and from the figure it was found that the particle size varied from 35 nm to 90 nm and that some particles were agglomerated to the larger size of 150 nm to 300 nm. Also it can be seen in the images that the synthesized hydroxyapatite is porous in nature. This porous nature is desirable property and can have a positive impact



Figure 4. Synthesized hydroxyapatite powder.



Figure 5. SEM images of hydroxyapatite.

when used into the implant as it facilitates interaction between the implant and the biological environment [12].

#### XRD analysis

XRD pattern of hydroxyapatite obtained is as in Figure 6 and is similar to the XRD pattern of Calcite (CaCO<sub>3</sub>). The unit structure obtained is Rhombohedral. The peaks match well with the primary reference 09-432 (JCPDS reference file) and gives proof for the presence of calcium. The particles are rhombohedral in shape. And the density is 2.71 g/cm<sup>3</sup> and this is in good agreement with those published in the literature and it can be concluded that the precipitate has calcium content [4].

#### FTIR analysis

FTIR pattern curve of hydroxyapatite is presented in Figure 7. The point with wavenumber 3132 cm<sup>-1</sup> shows the presence of NH<sup>+4</sup> functional group, and shows for a given range from 3300cm<sup>-1</sup> - 3030 cm<sup>-1</sup>. The curve from 2500 cm<sup>-1</sup> to 3300 cm<sup>-1</sup> shows the growth of -O-H- functional group. Wavenumber 1420 cm<sup>-1</sup> to 1050 cm<sup>-1</sup> denotes  $\delta$ OH in plane variable position. Band of 1900 cm<sup>-1</sup> to 1200 cm<sup>-1</sup> is the stretching mode of PO<sub>4</sub><sup>3-</sup> group. Bands 545.75 cm<sup>-1</sup> and 432.12 shows the presence of PO<sub>4</sub><sup>3-</sup>. The large separation of the bands shows the presence of crystalline phase. A weak band of CO<sub>3</sub><sup>-2</sup> was detected in the region around 1527.39 cm<sup>-1</sup> [5,6]. The broad band at 3132 cm<sup>-1</sup> and 1724.61 cm<sup>-1</sup> were attributable to absorbed water [17]. Therefore, according to these explanations, it is obvious that the synthesized powder is certainly hydroxyapatite.

#### Conclusion

Hydroxyapatite was successfully synthesized using the most easily available materials. The experimental conditions used were easy to maintain and no extreme environment was essential. And as per the characterization, it is found that the particles are nano in size. From the SEM analysis, it is found that the pores were obtained on the surface of the material and these pores support the osteoblasts to interact and grow over its surface [9,12]. Accordingly, it can be concluded that the synthesized hydroxyapatite is Osteoconductive in nature. The XRD pattern



*Figure 6. XRD of hydroxyapatite.* 

*Citation:* Ganachari SV, Bevinakatti AA, Yaradoddi JS, et al. Rapid synthesis, characterization, and studies of hydroxyapatite nanoparticles. Adv Mater Sci Res. 2016;1(1):9-13.



Figure 7. FTIR pattern of hydroxyapatite.

with reference to JCPDS number 09-432 assures the presence of calcium in the material. Through the FTIR analysis the presence of -O-H-, NH<sup>+4</sup> and PO<sub>4</sub><sup>3-</sup> can be confirmed. Thus, by these various characterizations conducted it can be conclude that Hydroxyapatite exists in the prepared sample. The future scope of the project would be to rigorously carry out the various bio compatibility tests.

#### Acknowledgement

This work is fully supported by B.V. Bhoomaraddi College of Engineering and Technology under "Capacity Building Projects" grant Ref 2014-15 Sl. No 14. Main Author Sharanabasava V Ganachari thanks KLE Society and BVBCET, Hubli for providing funding and support.

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# \*Correspondence to:

Sharanabasava V Ganachari Centre for Material Science B.V. Bhoomaraddi College of Engineering and Technology Hubballi India Tel: +91- 08362378413 (O), +91-9480203231 (M) E-mail: sharanu14@gmail.com