

Influence of synthetic methodologies on the particle size of Hydroxyapatite

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Abstract— Hydroxyapatite (HAP) is the major mineral constituent of bones and teeth. It has been well documented that HAP nanoparticles can significantly increase the biocompatibility and bioactivity of man-made biomaterials. Since last decade, the HAP nanoparticles have increasingly been in demand and extensive efforts have been devoted to develop many synthetic routes to synthesis it. Several investigations have also been made to determine how critical properties viz. crystallite size, shape, phase purity, mechanical strength of HAP can be effectively controlled by varying the processing parameters. With such a wide spectrum of methods available for the preparation of HAP nanoparticles, choosing a specific procedure to synthesize a optimum quality powder can be laborious. Hence, in our present investigation we try to evaluate the Hydroxyapatite crystals synthesized through three different methodologies. The methodologies adopted were hydrothermal, microwave and precipitation methods. The calcium and phosphorus precursors were chosen with molar ratio of 1: 0.6 to achieve the synthetic bone apatite. The prepared samples with different synthetic methods were characterized using XRD, SEM, EDX and FTIR to ascertain the crystalline size, phase structure, morphology, stoichiometric Ca/P ratio and the functional groups.

Keywords- Biomaterial, Hydroxyapatite, Nanoparticles

I. INTRODUCTION

Hydroxyapatite, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ is the principal inorganic constituent of bones and teeth and consequently has become a topic of extensive biological and physico-chemical investigations[1]. This calcium phosphate-based bioceramic is similar in structure to the natural bone mineral phase and accounting for 69% of the bone weight .It has excellent biocompatibility and osteo-conductivity to that of bone [2]. Hence, it is used widely as dental material, orthopedic applications as bone & bone graft substitutes and hard tissue paste.

Numerous processing routes have been developed for the synthesis of nano-HAP such as chemical precipitation, solid state combustion, emulsion, sol-gel, microwave, hydrothermal synthesis, etc. It is pertinent to develop an inexpensive HAP synthesis method, which facilitate the precise control of particle size, morphology and chemical composition [3]. However, nano HAP synthesis depends on controlled parameters such as pH and temperature of the solutions, nature and composition of the starting materials, reagent's concentration, addition rate, stirring technique and stirring speed, ageing and presence of impurities. [4-5].Hence, in the present work nano HAP has been aimed to synthesize by hydrothermal, microwave and chemical precipitation method and to compare their crystal size, phase purity, morphology and chemical composition.

II. MATERIAL AND METHODOLOGY

A. Materials

Reagent grade calcium nitrate tetra hydrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$), diammonium hydrogen phosphate ($(\text{NH}_4)_2\text{HPO}_4$) and liquid ammonia (NH_3) from Merck chemical were used.

B. Maintaining the Integrity of the Specifications

HAP were synthesized through hydrothermal method (M_1), microwave method (M_2) and precipitation method (M_3). The same precursors, $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and $(\text{NH}_4)_2\text{HPO}_4$ were used for all the methodologies. A solution of 0.6M $(\text{NH}_4)_2\text{HPO}_4$ was added drop wise to a 1M $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ solution under continuous stirring at room temperature. Using ammonia solution the final pH was adjusted to 11.0, followed by aging process. The chemical equation that describes the reaction is



In precipitation and microwave synthesis, the aging was done under continuous stirring in room temperature for 24hrs, whereas for hydrothermal synthesis the aging was in autoclave for 2hrs at 150°C. Then, suspension was allowed to cool naturally. The white precipitate obtained was then collected by centrifugation and washed repeatedly with distilled water.

The products thus obtained were dried in an air oven at 100°C for 24 hr. Thus obtained HAP crystals through precipitation and hydrothermal methods were ground with a mortar and pestle and calcinated at 800°C in a muffle furnace under an air atmosphere for 2 hr.

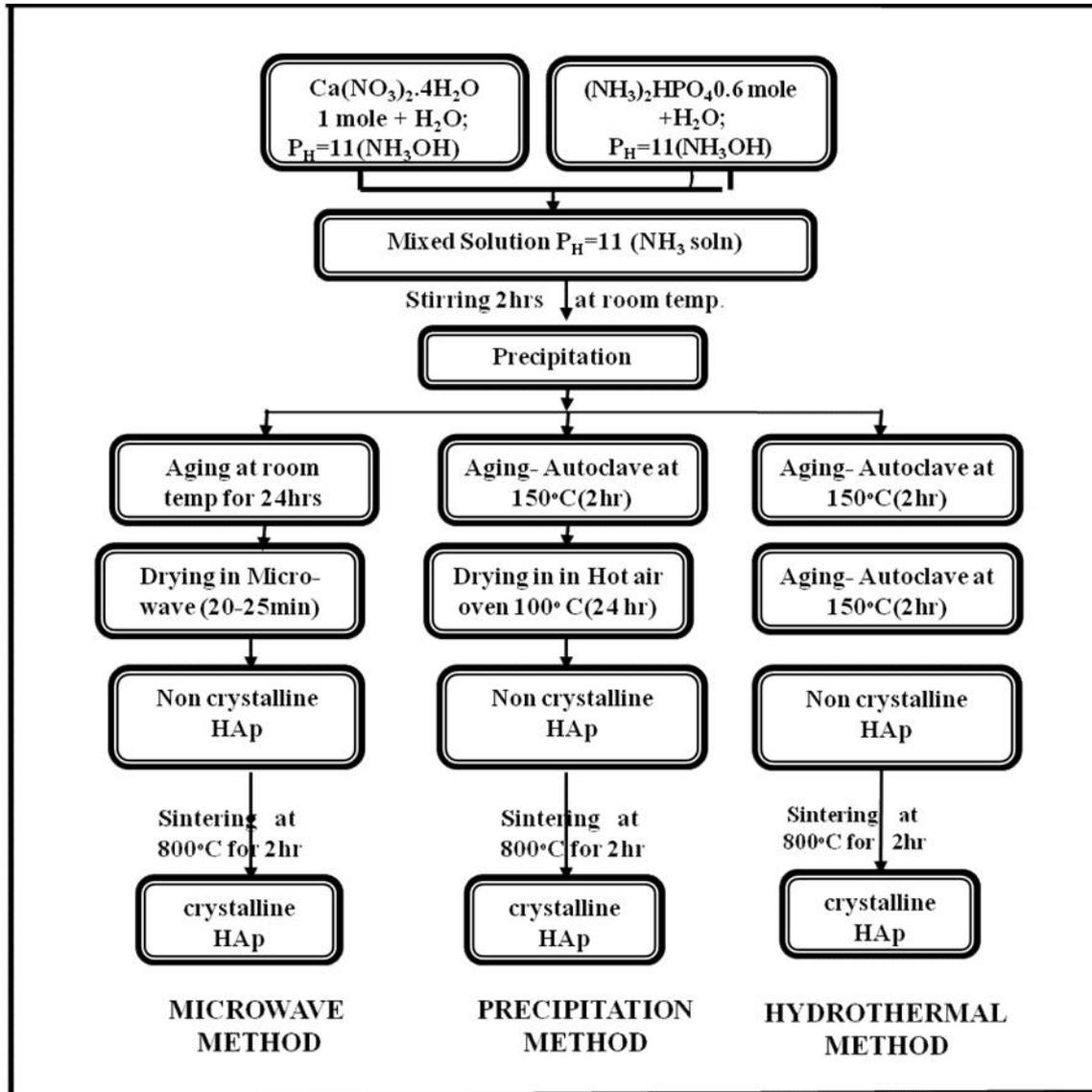


Figure 1: Flow chart for the synthesis of hydroxyapatite

In microwave synthesis after aging, the resultant suspension was exposed to microwave radiation for 20 -25 mins in a domestic microwave oven. The quantity of the solution was adjusted to avoid overflowing of the solution during heating, until the product is completely dry finally ground and calcinated.

C. Characterizations

1. Functional group Identification: FTIR measurements were carried out using a single beam Fourier transform infrared spectrometer (Agilent, Cary 630). The FTIR spectra of the samples were obtained in the spectral range from 4000 to 650 cm^{-1} . All samples were prepared using the KBr pellet technique. The samples are diluted to 1% with KBr (IR grade). The mixture was well mixed for 3 min and pressed as a pellet of 10 mm thickness by using a hydraulic pressing system at 10 ton.

2. Phase analysis: Phase analysis of thus prepared HAP were carried out by the X-ray diffraction (XRD) technique using a diffractometer (X'pertPro, Philips, The Netherlands)and monochromated Cu K radiation (wavelength =1.54060Å). The 2θ scanning range was from 10° - 80° with step time of 1sec and step size of $2\theta = 0.1$. The average particle size was calculated from XRD data using the Dyebye- Scherrer approximation.

$$D_{hkl} = K / \cos$$

Where D_{hkl} is the particle size, as calculated from (hkl) reflections, λ is the wavelength Cu K radiation (1.54060Å), β is the full width at half maximum for the diffraction peak under consideration (in radians), θ is the diffraction angle ($^\circ$) and K is the broadening constant chosen as 0.9. The degree of crystallinity(X_c) can be evaluated by the following equation.

$$X_c = (0.24 / \beta)^3$$

Where β is the full width at half maximum for the particular diffraction peak of miller's plane

3. Microstructural characterizations: The microstructures and morphologies of the HAP crystals were studied by field emission scanning electron microscope (FESEM:Supra VP35 Carl Zeiss, Germany) .The energy dispersive X-ray (EDX: X-Max, USA) spectra were obtained by a standard unit (Oxford Instruments, UK) attached to the FESEM.

III. RESULTS AND DISCUSSIONS

The FTIR spectra of the samples were shown in Fig. 1.0. It revealed the characteristic transmittance bands of hydroxyapatite from 4000 cm^{-1} to 650 cm^{-1} . The PO_4^{3-} bands appear at 1035 cm^{-1} (1 vibration mode of the phosphate group), 1085 and 968 cm^{-1} (1 vibration mode of the phosphate group) [7,8]. The two shoulder peaks at 3576 is typical stretching vibration modes confirming the presence of hydroxyl ion in the apatite lattice [6]. The XRD data for standard HAP and synthesized HAP by different route are presented in the table.1.

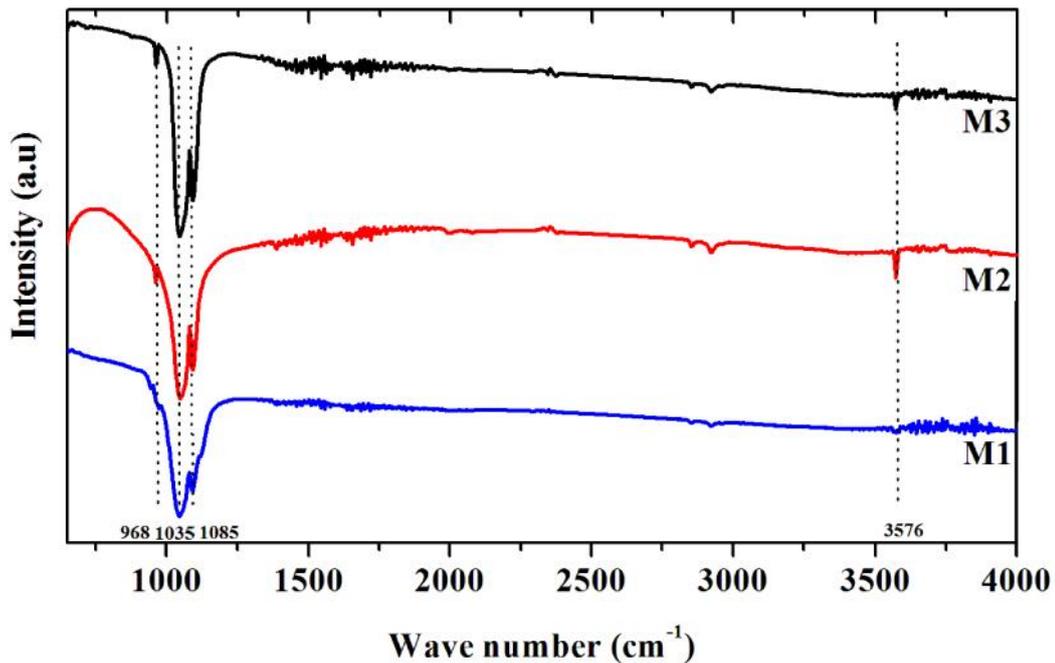


Figure 2: FT-IR Spectrum of Hydroxyapatite prepared by hydrothermal (M1), Microwave (M2) and Precipitation (M3) methods

The diffraction patterns show exclusively sharp clear reflections which confirm the phase purity and higher degree of crystallinity. The XRD pattern, with major diffraction peaks located in $2\theta = 25.9^\circ$ (002), $2\theta = 31.7^\circ$ (211), $2\theta = 32.1^\circ$ (112) and $2\theta = 32.8^\circ$ (300), $2\theta = 46.6^\circ$ (222), $2\theta = 49.4^\circ$ (213) matched with the data obtained with the ICDD - PDF2 card: 00-009-0432 and confirming the major phase as hydroxyapatite particles. Thus, the studied methods may be used to obtain hydroxyapatite nano crystals. The crystallite sizes obtained were in the range of 45 to 106 nm. The size was larger in M2 ($\approx 106\text{nm}$) when compared to M3 ($\approx 62\text{nm}$) and M1($\approx 45\text{nm}$).

Table 1: The XRD data and crystallite size for M1, M2 and M3

S.NO.	2 theta(degree)	Crystallite size(nm)
Standard	31.76	-
M1	31.75	45
M2	31.92	106
M3	31.73	63

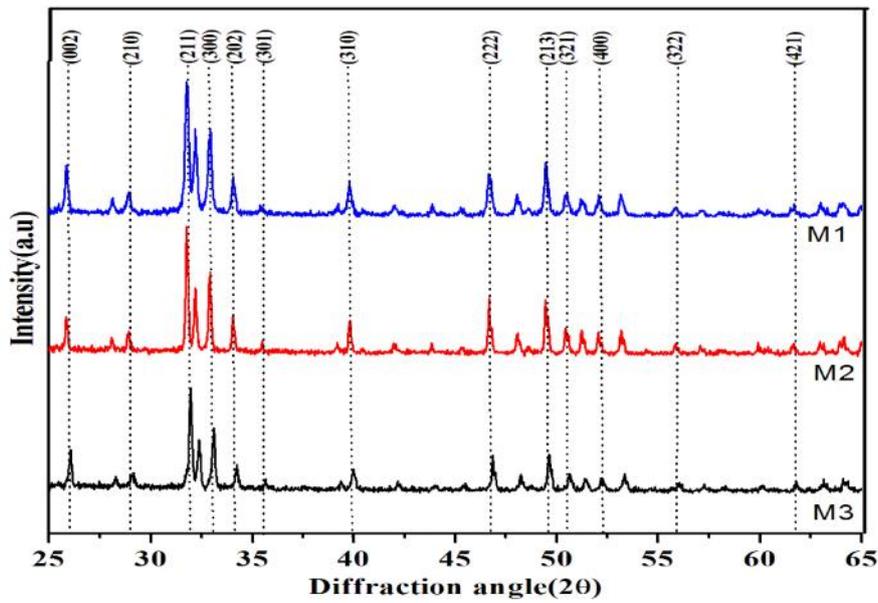


Figure 3: XRD studies of Hydroxyapatite prepared by hydrothermal (M1), Microwave (M2) and Precipitation (M3) methods

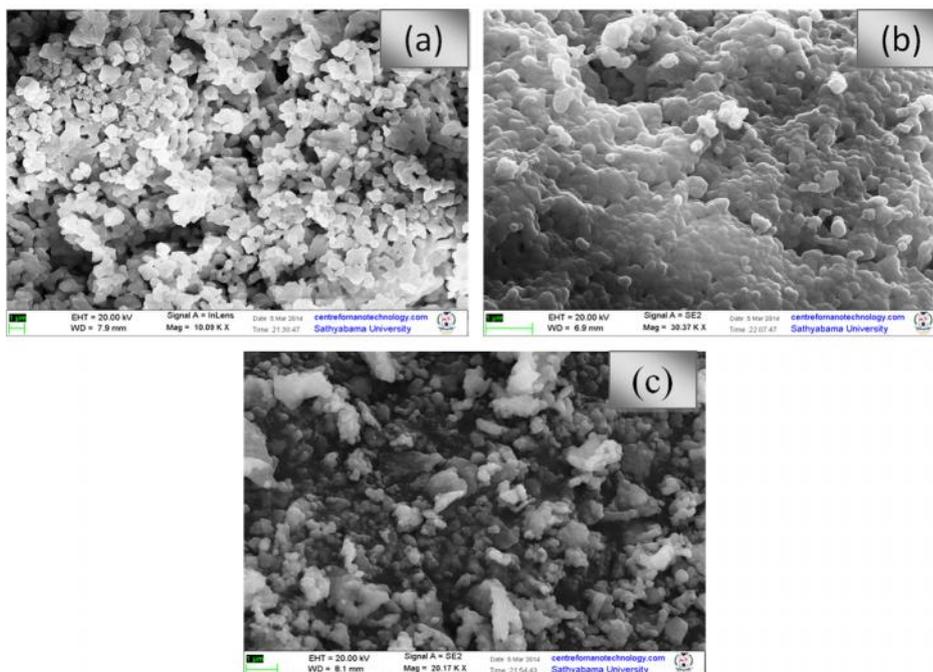


Figure 4: Scanning Electron micrograph of Hydroxyapatite prepared by hydrothermal (a), Microwave (b) and Precipitation (c) methods

The degree of crystallinity was in the order of $M1 > M3 > M2$. The higher crystallinity is due to the hydrothermal treatment of the sample and the crystallinity of the particle decreases with increasing in size i.e. $M1 < M3 < M2$. The XRD spectrum for M1, M2 and M3 were shown in Fig.3.

FESEM studies: FESEM morphology of the samples was shown in Fig.4. The typical microstructure of hydrothermally synthesized HAP sample (Fig.4.a) showed fine agglomerated crystals. The microwave irradiation method produced samples (Fig.4.b) are highly agglomerated uneven particles. The sample (Fig.4.c) exhibited homogeneous aggregations of nano rods in the size of 65 nm. The agglomeration is in the order of $M1 < M3 < M2$.

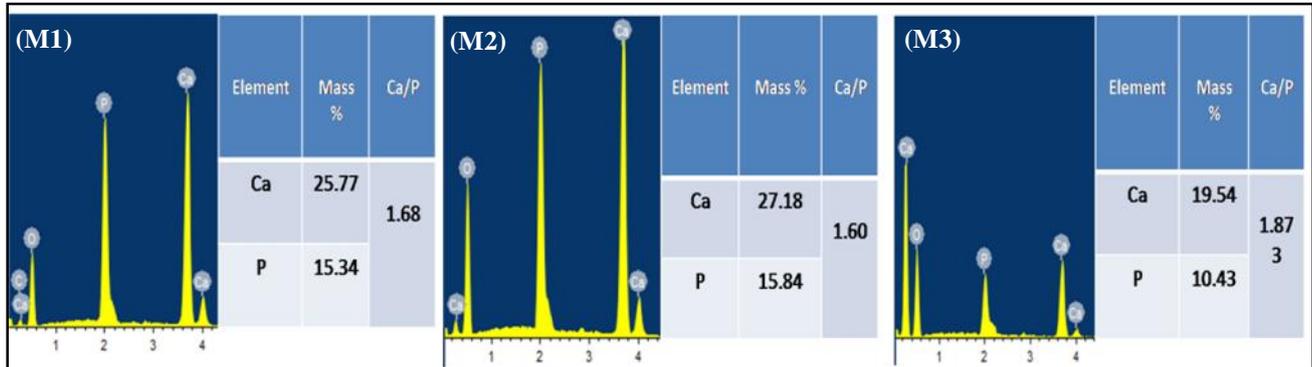


Figure 5: Energy Dispersive X-ray analysis (EDX) of Hydroxyapatite prepared by hydrothermal (M1), Microwave (M2) and Precipitation (M3) methods

The EDX spectrum of HAP samples were shown in Fig. 5. The data confirmed the presence of calcium and phosphate. The stoichiometric ratio of Ca/P found to vary from 1.60 to 1.87 in the order of $M3 < M1 < M2$. Thus, HAP produced hydrothermal method (1.68) proved to be more stoichiometric than M2 and M3.

IV CONCLUSION

The present work compares properties of HAP powders prepared under different synthetic methods, such as hydrothermal, microwave and precipitation. HAP synthesized by hydrothermal method revealed almost homogenous, smaller crystal with lesser agglomeration this may be due to the influence of temperature and pressure maintained throughout the reaction. High reaction temperature is advantageous to high-purity HAP formation. By comparing the size of synthesized nano HAP by experimental methods, HAP with higher crystallinity and smaller crystals besides high purity has been achieved by hydrothermal method. Therefore, the processing method played an important role in preparing nano HAP and its associated properties.

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