

MICROWAVE ASSISTED SYNTHESIS AND CHARACTERIZATION OF SILVER AND ZINC DOPED HYDROXYAPATITE NANORODS FROM MUSSEL SHELL (MOLLUSK)

G. NEDUNCHEZHIAN*, D. BENNY ANBURAJ[#], B. GOKULAKUMAR *,
S. JOHNSON JEYAKUMAR**

*PG & Research Department of Physics, T.V.K Government Arts College, Tiruvarur-610003, India,

[#]bennyburaj@rediffmail.com, + 91-9944573126

** PG & Research Department of Physics, T. B. M. L College, Porayar-609307, India

Abstract. In this study, we report the synthesis of silver and zinc co-doped nanosized hydroxyapatite (Ag-Zn-HAP) from the mixture of cheaply available calcium precursor of mussel shell (mollusk) with di-ammonium hydrogen phosphate $(\text{NH}_4)_2\text{HPO}_4$ assisted by microwave irradiation. The grained powder of mussel shell was dissolved in diluted hydrochloric acid and di-ammonium hydrogen phosphate solution was slowly added to the mixture while maintaining the pH at 10 using ammonium hydroxide (NH_4OH) . Stoichiometric amount of Silver nitrate and zinc chloride was added followed by microwave irradiation for 30 min. The residue was collected and calcined at 900 °C for 2 h, after the decomposition of the organic fragments and carbonate phases; the expected silver and zinc doped hydroxyapatite was afforded as nanorod of 20–50 nm scale with quantitative yield. Synthetically this protocol would have more precedence than other procedures since it is a more facile and convenient method involving such a biological waste material of discarded mussel shell. The final products were characterized by X-Ray Diffraction Spectroscopy (XRD), Fourier Transform Infrared Spectroscopy (FT-IR), Field Emission Scanning Electron Microscopy (FE-SEM), The Energy Dispersive Analysis of X-rays (EDAX), and Field Emission Transmission Electron Microscopy (FE-TEM) analysis.

Key words: Mussel shell, hydroxyapatite, microwave irradiation, calcium phosphate, bone substitute.

INTRODUCTION

In the recent years, considerable attention that has been put forward towards the application of bio-ceramics in medicinal field is due to their potent rectification resistances, better compressive strength and comparatively lower density and weight. As a successful result of bio-physics exploration the damaged bones and

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tooth can be easily replaced by various bio-ceramics materials including hydroxyapatite and its analogues. In this view, hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is one of the most important bio-materials which have been extensively used for substituting the injured part of the human body, especially for bones, teeth and fractured or damaged form of various hard tissues, etc. Very recently, it has been proved that the usage of calcium phosphates in biomedical field is an emerging practice which emphasizes innumerable applications as dentistry, bone substitute, bone repair materials and drug delivery systems [10, 16, 17]. Indeed, the main constituent of apatite calcium phosphate of bone mineral is made up of carbonate and a small amount of magnesium and trace of other elements [21]. Artificially prepared HAP was found to have enhanced bio-activity and bio-compatibility to the respective naturally occurring calcium phosphate. For example, in the field of orthopedics, as implants HAP is used as a coating or bone filling material. Though, the preparation of HAP encountered with some difficulties, there are several literatures which describe the various synthetic methods including precipitation, sol-gel, combustion synthesis and plasma, etc. [1, 4, 24]. However, most of the conventional methods are employed through hydrothermal, micro emulsion and mechanochemical process [26] respectively. At the outset, the overall advantage behind the nano sized HAP offers a great deal of interest to search a novel synthetic protocol for accomplishing engineered nano-HAP [3]. The protocol associated with microwave-irradiation is one of the finest methods to synthesize nanosized HAP, which covers shorter reaction time and lesser-energy consumption, etc. However, most of the protocols designate these methods by combining both conventional and microwave irradiation technique under heating or room temperature for a period of time [13, 27]. In the past few years, there have been found many reports which deal with the fabrication of nano sized HAP through microwave-irradiation using the surfactants (CTAB) and chelating (EDTA) agent to furnish the particles size of 5–50 nm scale [7, 9, 15, 22]. In addition, the usefulness of biogenic material for the preparations of HAP is also a privileged process because of its valuable impact on environmental and economical benefits. However, these methods need to be more encouraged in order to utilize the biological waste and control over the pollution. Moreover, to the best of our knowledge, the microwave-assisted synthesis of nano-sized HAP from mussel shell has been less explored. Connecting this view, we are interested to focus on the microwave-assisted synthesis of nano-sized HAP from mussel shell (Mollusk), since it is highly enriched with CaCO_3 and also it is easily available from natural sources.

Thus, the mussel shell, a waste material after use of flesh, has been used as calcium originator to synthesize highly pure and inexpensive hydroxyapatite using

di-ammonium hydrogen phosphate under microwave irradiation condition. The synthesized powder was characterized by various analytical techniques.

MATERIAL AND METHODS

SYNTHESIS OF HAP

The skeletons of mussel shells were collected and washed with tap water, followed by distilled water to get rid of unwanted deposits and muds. They were dried in direct sun light for 24 h and crushed by pestle and mortar to obtain powdered mass of 200 size mesh used to sieve. The stoichiometric amount of mussel-shell powder was dissolved in dilute hydrochloric acid and the mixture was added to a solution of di-ammonium hydrogen phosphate at pH 10 using aqueous ammonia. Then stoichiometric amounts of silver nitrate and zinc chloride were added. The resulting mixture was stirred for 1 h and immediately transferred to a domestic microwave oven and irradiated at 800 W energy of frequency employing 2.45 GHz for 30 min continuously. After the irradiation, the residue was washed thrice with de-ionized water and then dried in a vacuum oven at 60 °C for 12 h.

EVALUATION AND MEASUREMENT

The phase purity of the as-synthesized nano sized hydroxyapatite was analyzed by X-ray powder diffraction using Cu- α radiation and the surface morphology was determined by FE-SEM (JEOL JSM 6701-F USA) EDAX (INCA, Oxford instrument, UK), FE-TEM (JEOL 2100 F JAPAN) and The FTIR spectroscopy (RXI Perkin Elmer) was used to identify the functional groups of as-synthesized hydroxyapatite.

RESULTS AND DISCUSSION

X-RAY DIFFRACTION SPECTROSCOPY

In order to check the crystallinity, the as-synthesized silver and zinc doped HAP powder and the calcined silver and zinc doped HAP powders were subjected to XRD analysis using Cu- α radiation. In the plot (a), the broadening of the peak indicates that the particles are small in size and less crystallinity of the as-synthesized Silver and zinc doped HAP powder dried at 60 °C after microwave irradiation as shown in Figure 1. To check the effect of calcinations, the powder was heated at 900 °C and the phases analyzed. This result shows that all the observed major peaks designate the presence of nano sized HAP. As the

temperature increases, the sharpening of the peak at 21.35° , 25.87° , 27.81° , 31.72° and 38.11° increases corresponding to the planes 311, 002, 021, 211 and 111 which represents the strong improvement in the crystallinity of the calcined nano sized HAP, 38.11° indicates the presence of metallic silver located in the 111 plane and 31.72 indicates the presence of zinc containing beta tri-calcium phosphate observed in the 211 plane [12]. Evidently, these results were good in agreement with standard JCPDS Card No. 09-0432, JCPDS Card No. 35-0495 and JCPDS Card No. 04-0783.

Table 1

Crystallite size of nanosized HAP powders

Serial No.	Samples	Crystallite size (nm)
1	Ag/Zn doped HAP	12.66
2	Zn/Ag doped calcined HAP	19.11

Using Scherer formula, the crystallite sizes were calculated:

$$D = K \lambda / \beta \cos \theta, \quad (1)$$

where $\beta = FWHM \times \pi / 180$, $K = 0.94$, $\lambda = 1.5406 \text{ \AA}$.

Further, the size of the crystallite was calculated for as-synthesized and calcined powders by Scherer formula from the XRD pattern, which discloses that the crystallite size was apparently 12.66 nm for as-synthesized Zn-Ag doped nanosized HAP and 19.11 nm for the calcined Zn-Ag doped HAPs, consequently, the increased crystallite sizes of the calcined Zn-Ag doped HAPs are attributed to the temperatures 900°C [20] as depicted in Table 1.

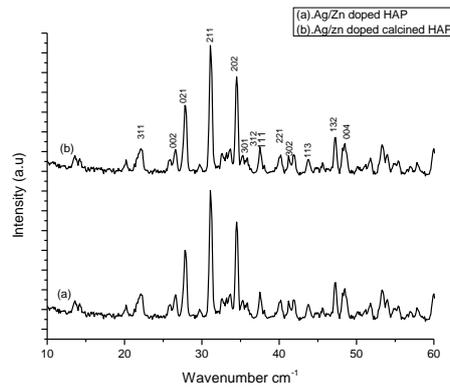


Fig. 1. XRD-analysis of hydroxyapatite powder. (a) Zn/Ag doped HAP; (b) Zn/Ag doped calcined HAP.

FOURIER TRANSFORM INFRARED SPECTROSCOPY

The FTIR spectrum of the undoped HAP, as-synthesized silver and zinc doped HAP, silver and zinc doped calcined nano-HAP at 900 °C temperature are shown in Fig. 2. The characteristic peaks corresponding to stretching vibration of PO_4^{3-} ions at 1035.03 cm^{-1} , and the peaks at 565.17 cm^{-1} , are assigned to the deformation of PO_4^{3-} ions [5, 18]. The broad OH stretching band around 2366.26 cm^{-1} – 3435.50 cm^{-1} proves the adsorption for H_2O molecules. A region of peak at 1405.84 cm^{-1} designates the existence of carbonate in trace level. The formation of apatite was confirmed by the appeared doublet at around 604.09 cm^{-1} – 565.17 cm^{-1} , which denotes the bending mode of P–O bonds in phosphate ions. Further, the peaks at 1405.84 cm^{-1} , 1596.23 cm^{-1} are responsible for stretching mode of CO_3^{2-} [2, 6, 23, 25] and this may be associated with weak bonding interaction between carbon and oxygen in the present study.

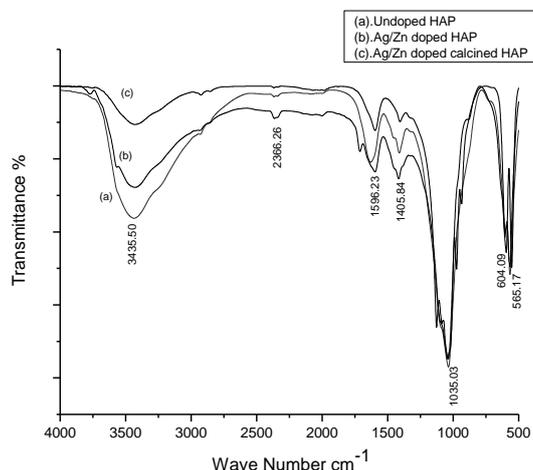


Fig. 2. FTIR Spectrum of HAP powders. (a) Undoped HAP, (b) Zn/Ag doped HAP, (c) Zn/Ag doped calcined HAP.

FIELD EMISSION SCANNING ELECTRON MICROSCOPY

Figures 3(a–b) show the FE-SEM morphology of as-synthesized silver and zinc doped HAP with different magnification. The synthesized hydroxyapatite is highly agglomerated. After calcination at 900 °C the agglomeration is reduced. The gradual changes in the morphology of the calcined nanosized HAPs are due to the increase in the crystallinity of the powder incorporated with the sintering temperatures. The calcined HAP possess rod-like homogeneous microstructure, as the temperature increases, aggregation increases which results in distorted spherical and rod-like shape with average size of 0.2–1 μm . This is due to the decreasing of

the probability of respective growths in grains and the improvement of densification of the particles [8, 19]. The energy dispersive analysis of X-rays (EDAX analysis) of silver and zinc doped HAP composites revealed that P, Ca, O, Ag and Zn peaks indicate and confirm the presence of apatite formation. The presented elements with atomic and weight percentage were shown in Table 2.

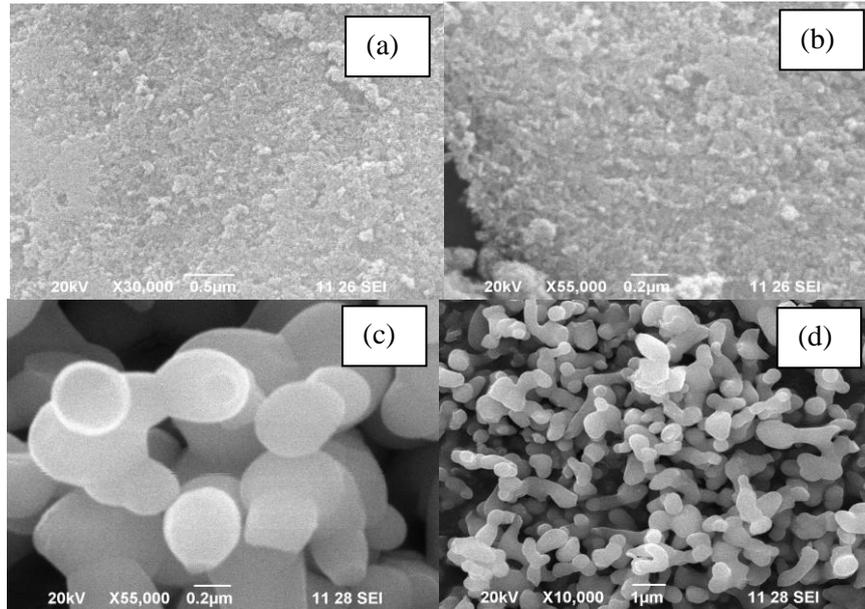


Fig. 3. FE-SEM images of nanosized HAP powders: (a–b) synthesized Ag/Zn doped HAP; (c–d) Ag/Zn doped calcined HAP.

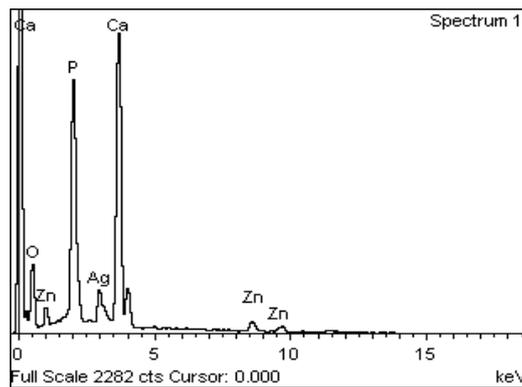


Fig. 4. EDAX Analysis of Ag/Zn doped HAP.

Table 2

EDAX Analysis of Ag –Zn doped HAP

Element	Weight %	Atomic %
O K	37.21	60.41
P K	17.41	14.60
Ca K	32.23	20.88
Zn K	6.00	2.39
Ag L	7.15	1.72
Total: 100.00		

FIELD EMISSION TRANSMISSION ELECTRON MICROSCOPY

To further confirm the shape and size of the silver and zinc doped and calcined nano-HAP crystallite, they were investigated by FE-TEM analysis. Figure 5 demonstrates the micrograph of the as-synthesized HAP powder obtained after microwave irradiation with calcinations.

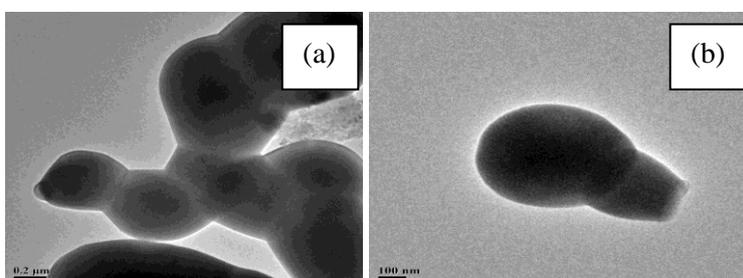


Fig. 5(a-b). FE-TEM images of calcined Ag/Zn doped HAP with spherical rod shape.

Figures 5(a-b) show spherical like structure nano-HAP powder with different magnification and displays spherical rod like structure of silver and zinc doped nano-HAP powder. From the micrograph, it has been observed that after calcinations of silver and zinc doped nano HAP the particle shape changed to spherical rod like structure. This result was more promising with the grain size calculated by Scherer formula [11, 14].

CONCLUSIONS

In conclusion, by a more facile and convenient method, silver and zinc doped HAP nano-rods were prepared cheaply available dead mussel shell (mollusk) as a calcium precursor with di-ammonium hydrogen phosphate as a phosphate precursor. The purity of the prepared Ag-Zn doped nano HAP powder was verified by various analytical techniques to confirm the formation of apatite. The HAP-nano rods may be used as a bone substitute for filling bone deficiencies.

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