Microwave Synthesis of Au/Ag Doped Nano Hydroxyapatite from Dead Mussel Shell

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ABSTRACT: In the present study, the Au/Ag doped nano sized hydroxyapatite was synthesize from the combination of low cost dead Mussel shell (*Mollusk*) as calcium source with di-ammonium hydrogen phosphate $(NH_4)_2HPO_4$ as a phosphate source, at the same time as maintain the pH at 10 using ammonium hydroxide (NH_4OH) , followed by microwave irradiation for 30 min. The deposit was collected and calcined at 900°C for 2h, after the decomposition of the organic residue and carbonate phases. Synthetically this procedure contains right of way than other events for the reason that it is more facile and suitable method concerning such a biological ravage material of remaining mussel-shell. The functional groups, structural, morphological studies and the elemental analysis of the entire yield were characterized by different systematic techniques.

KEYWORDS: Mussel shell, Hydroxyapatite, Microwave irradiation, Calcium phosphate, Bone substitute.

1. INTRODUCTION

In the modern years, Hydroxyapatite $(Ca_{10} (PO_4)_6 (OH)_2)$ is one of the major part of essential bio-material which has been broadly used for substituting the injured segment of the human body, primarily for bones, tooth and cracked or broken parts, form a collection of inflexible tissues etc [1,2]. especially in recent times, it has been proved that the usages of calcium phosphates in biomedical field is an brilliant perform which is highlighting plentiful applications on biomaterial sector as dentistry, bone exchange, bone renovate resources and drug release systems [3].

To be sure, the main component of apatite calcium phosphate of bone mineral is made up of calcium carbonate. Due to this connection, the synthesized Au/Ag co-doped nano HAP originates to have better bio-activity and biocompatibility to the relevant as expected calcium phosphate. In addition, the prevalent applicability of synthesized nano HAP is paid to their agreeable chemical interaction through hard and host tissues [4]. Owing to these reasons, HAP has been required to be commonly used in medical field as implants and as coating or as bone substantial material. HAP synthesis including precipitation, sol-gel, combustion synthesis and plasma etc.,[5]. However, most of the predictable methods such as hydrothermal, micro emulsion and mechanochemical process regularly [6] are engaged to formation of HAP nano particles. The production of nano sized HAP with exact size, shape and clarity is relatively complicated [7]. For convenient applications in the field of medicine, the better densification of HAP is essential [8-10]. The overall rewards behind the nano sized HAP offers a great deal of significance to search a novel synthesis method for achieve engineered nano-HAP. The method associated with microwave-irradiation is one of

the best techniques to produce nano size HAP, which covers shorter reaction time and lesser-energy utilization and etc. However, most of the techniques involve the combination of both predictable and microwave irradiation technique for a period of time. In the earlier period, there have been establishing many information which deal the production of nano sized HAP through microwave-irradiation [11-16].

In addition, the effectiveness of biogenic substance for the preparations of HAP is also a advantaged process because of its precious conflict on biological and inexpensive yield. However, this system needs to be more optimistic in order to develop the biological ravage and manage over the pollution. In addition, to the best our understanding, the microwave -assisted synthesis of nanosized HAP from mussel-shell has been less explored. Concerning this view, we are fascinated to focus on the microwave -assisted synthesis of Nano sized HAP from dead mussel shell (mollusk). Since it is extremely enriched with CaCO₃ and also it is simply obtainable from natural sources. Thus, the mussel- shell, a ravage material after use of flesh, has been used as calcium source to synthesize highly clean and reasonable hydroxyapatite using di-ammonium hydrogen phosphate under microwave irradiation condition. The synthesized powder was characterized by XRD, FTIR, FE-SEM, EDAX and FE-TEM analytical techniques.

2. MATERIALS AND METHODS 2.1 SYNTHESIS OF HAP

The skeletons of mussel shells were collected and washed with tap water, followed by distilled water to get rid of excess deposits and muds. They were dried in direct sun light [17,18] for 24h and crushed by pestle and mortar, 200 size mesh used to sieve then obtain fine powder [19].The mussel-shell powder was dissolved in dilute nitric acid and

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the mixture was added to a solution of di-ammonium hydrogen phosphate. AuCl₃ and AgNO₃ each 0.15gm dissolved in 10 ml of de-ionized water were added to the solution prepared before and the pH was maintaining at 10 using ammonium hydroxide. The mixed solution was stirred for 1hr and quickly transferred to a domestic microwave oven (LG, India) and irradiated at 800W energy of frequency employing 2.45 GHZ for 30 min continuously. After the irradiation, the residue was washed with de-ionized water three times and then dried in a vacuum air oven at 60° C for 12h [20]

The phase analysis of the as-synthesized nano sized hydroxyapatite was analyzed by X-ray powder diffraction technique using Cu-k α radiation and the powder morphology was observed by FE- SEM (JEOL JSM 6701-F USA) fitted with EDAX (INCA, oxford instrument, UK) and FE-TEM (JEOL 2100 F JAPAN) techniques. The FTIR spectroscopy (RXI Perkin Elmer) was used to recognize the functional groups of as-synthesized hydroxyapatite.

3. RESULTS AND DISCUSSION

3.1 PHASE ANALYSIS

The XRD diffraction patterns of the as-synthesized HAP powder and the calcined powders at 900° C were shown in Fig 1. The result shows that all the observed major peaks assign the presence of nano sized HAP in all the samples. The broadening of the peak in Fig. 1(a) designate that the assynthesized undoped HAP powder and Fig.1(b) Au/Ag doped HAP powder both were dried at 60° C after microwave irradiation smaller in size with less crystallinity. To confirm the result of calcinations, the powders were calcined at 900° C and examine the phases [21]. As the temperature increases 900° C. The sharpening of the peak at 25.76°, 28.10°, 30.3°, 31.78°, 32.40°, 34.28° and 38.20° occurs related to the planes (002), (102), (312), (211), (112), (202) and (220), which represents the high crystallinity of the calcined HAP. The XRD pattern possessed a strong peak at 31.78 related to (211) plane to confirm the formation of hydroxyapatite. Clearly, these outcomes were good in agreement with standard JCPDS Card No.09-0432 and JCPDS Card No-89-6437.

Table.1Calculation of crystallite size of Au/Ag doped Nano
sized HAP powders

S.No.	Sample	Crystallite Size (nm)
1	Undoped HAP	10.33
2	Au /Ag doped HAP	10.59
3	Au /Ag doped Calcined HAP	10.86

 $D = k \lambda / \beta \cos \theta$.

Where β = FWHM × $\pi/180$, K=0.94, λ =1.5406 Å

additionally, the crystallite size was calculated for assynthesized and calcined powders from the XRD pattern by Debye- Scherer formula, which discloses that the typical crystallite size of synthesized undoped HAP was 10.33 nm, 10.59 nm for Au / Ag doped nano sized HAP, 10.86 nm for the Au/ Ag doped calcined HAPs, hence the better crystallite sizes of the calcined HAPs are predictable to the temperature 900° C as depicted in the table 1 [22].



Fig. 1 XRD pattern of HAP powders. (a) Un doped HAP (b) Au/Ag doped HAP (c). Au/Ag doped calcined HAP

3.2 CHEMICAL COMPOSITION

The FTIR spectrum of as-synthesized undoped HAP, Au/ Ag doped HAP and Au/Ag doped calcined HAP are shown in the Fig 2. The typical peaks at 1037.76 cm⁻¹ and the peak at 561.04 cm⁻¹ are match up to stretching vibration of PO₄³⁻ ions and the deformation of PO₄³⁻ ions correspondingly. The broad OH stretching band in the region of 2364.27 cm⁻¹ and 3428.15 cm⁻¹ proves the adsorption for H₂O molecules. A region of peak at 1454.45 cm⁻¹ assigns the survival of carbonate in trace level. The development of apatite was established by the form of doublet at around 605.28 cm⁻¹ - 561.04 cm⁻¹, which denotes the bending mode of P- O bonds in phosphate ions. Further, the peaks at 1454.45 cm⁻¹, 1596.49 cm⁻¹ are consistent for stretching mode of CO₃²⁻, this may be associated with weak bonding relations involving carbon and oxygen in the current study [23-25].



Fig. 2 FTIR Spectrum of HAP powders. (a) Un doped HAP (b) Au/Ag doped HAP (c). Au/Ag doped calcined HAP

3.3 MORPHOLOGICAL STUDIES

Fig.3 shows the powder morphology of as-synthesized (a) Au /Ag doped HAP (b-d) Au /Ag doped calcined HAP. Fig.3(a) reveals that the as synthesized Au/Ag doped HAP has solid apatite, excellent crystallite like structure morphology with less crystallite of highly agglomerated powders [21]. The changes in the morphology of the Au/Ag doped calcined nano sized HAPs are due to the increase in the crystallinity of the powder incorporated with the calcined temperatures. The Au/Ag doped calcined HAP powder achieve the small rod like morphology with homogenous microstructure, as the temperature increases, aggregation increases which results inaccurate sphereshaped particle with extensive size distribution with typical size of 1-0.2 μ m. This is due to the decreasing of the chance of applicable growths in grains and the improvement of densification of the particles [23]. Fig. 4 shows the EDAX Spectrum taken over the region in fig. 3(b-d) .The presents of Ca, P, O, Au and Ag to confirm the formation of Au/Ag doped apatite [26].



Fig. 3 FE-SEM images of nano sized HAP powders:





Fig.4 EDAX Analysis of Au /Ag doped HAP

Table. 2 Elemental Analysis of Au/Ag doped HAP

Element	Weight % Sigma	Atomic %
0 K	2.23	64.76
P K	0.70	13.82
Ca K	1.23	19.68
Ag L	0.41	0.97
Au M	0.60	0.76
Totals 100.00		

3.4 TEM ANALYSIS

TEM analysis was carried out to study the shape and size of Au/Ag doped HAP particles. Fig. (5) Shows the images of assynthesized (Fig. 5a and 5b) and calcined (Fig. 5c and 5d) HAP particles, respectively. The as synthesized particles seem to be more of roundish morphology containing rod-like crystal structure. This result was more hopeful with the grain size calculated by Debye- Scherer formula [27,28]. After calcinations, the crystallinity increases and the particles have typical hexagonal shape with platelet morphology (Fig. 5c and 5d).



Fig.5 FE- TEM images of HAP powders: (a) and (b) Au/Ag doped as- synthesized HAP powder (c) and (d) Au/Ag doped calcined HAP powder.

4. CONCLUSIONS

In this study we conclude Au/Ag doped hydroxyapatite hexagonal shape of nano platelet were successfully synthesized assisted by microwave irradiation using dead mussel shell powder as calcium source with di-ammonium hydrogen phosphate as phosphate source. The synthesized HAP powder was characterized by different systematic techniques to prove the formation of apatite. These synthesized hydroxyapatite powder may be more valuable for handling of bone defects in evaluation with expected HAP

and most successful as a bone substitute material to assist bone formation.

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