

# Green Synthesis and Characterization of Nano Silver Doped Nano Hydroxyapatite (nAg-nHAP) using Ocimum Tenuiflorum Leaf Extract and Sea Shell - A New Approach

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**Abstract:** In this work Calcium Oxide (CaO), a prime material for hydroxyapatite (HAP) synthesis, was derived from sea shells by calcination at 1200°C for 2 hours. The calcined CaO was treated with phosphoric acid with ammonium buffer (pH 10) and Nano hydroxyapatite (nHAP) was synthesized. The colloidal dopant solution of nanosilver was green synthesized from silver nitrate solution using ocimum tenuiflorum leaf extract. Colloidal nanosilver (nAg) was added to nHAP and the obtained nanosilver doped nano hydroxyapatite (nAg-nHAP) was calcined at 700 °C for 2 hours. The XRD results of prepared nAg-nHAP powder showed essential peaks of standard nHAP JCPDS file No. 09-0432. The Ca/P molar ratio of synthesised nAg-nHAP observed as 1.71, showed a minimum deviation from standard nHAP due lattice disturbance on nAg doping. The nAg-nHAP nanoparticles synthesized in the range 22-44.2 nm. The FTIR, UV-Vis, XRD, SEM and EDAX analysis substantiated the confirmation of hydroxyapatite structure with the incorporation of nanosilver (nAg) along with calcium (Ca), phosphorous (P) and oxygen (O).

**Keywords:** Silver nanoparticles; nano hydroxyapatite; nano silver doped nano hydroxyapatite; sea shells; ocimum tenuiflorum leaf extract.

## 1. Introduction

Nanotechnology is the emerging research area and nanoparticles find unbelievable benefits due to their enhanced surface to volume ratio and band gap. Particularly metal nanoparticles capture the wide attention in industrial and medical field [1]. Among all, Silver Nanoparticle stamp a significant signature. The recent literature study reveals that plant-mediated biological synthesis of nanoparticles is attaining much more focus due to its easy handling and eco-friendliness. Green synthesis of silver nanoparticles using plants like limon leaf [2], aloe vera [3], piper betle [4] were already reported. In this study ocimum tenuiflorum, a herbal plant well known for its remedy for cough, asthma, kidney stone in traditional medicine very commonly found in tropical areas is used in the nano silver synthesis.

In recent days, the utilization of naturally existing eco-friendly materials to synthesize biomaterials like Hydroxyapatite ( $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ) the predominant inorganic constituent of human bones and teeth, gain prime importance because of their non-toxic nature to the environment. Lot of researchers reported the HAP synthesis from natural material such as sea shell [5], animal bones [6]

and eggshell [7] as they contain large amount of calcium carbonate and used them as a calcium precursor in the synthesis. Efforts are continuously made to enhance the properties of HAP as a biomaterial by synthesizing in nano form (nHAP) along with action based dopant materials. Among various dopants, silver nanoparticles dominate more in recent research due to their antibacterial and antimicrobial activities.

In order to minimize the release of residual pollutants from synthesis to the environment, a new trial has been made in the present study to synthesize HAP in nano form with the addition of nano silver particles to impart antibacterial and antimicrobial property to the biomaterial. This paper focuses on the synthesise of nano Hydroxyapatite using sea shells collected from sea shore and doping HAP with green synthesized nanosilver with the usage of minimum chemical compounds.

The synthesised nAg-nHap particles were characterised by UV-Vis, FTIR, XRD, SEM and EDAX analyses to confirm the formation nHAP structure, Ca/P molar ratio, nano size and effective inclusion of nAg in to nHAP lattice.

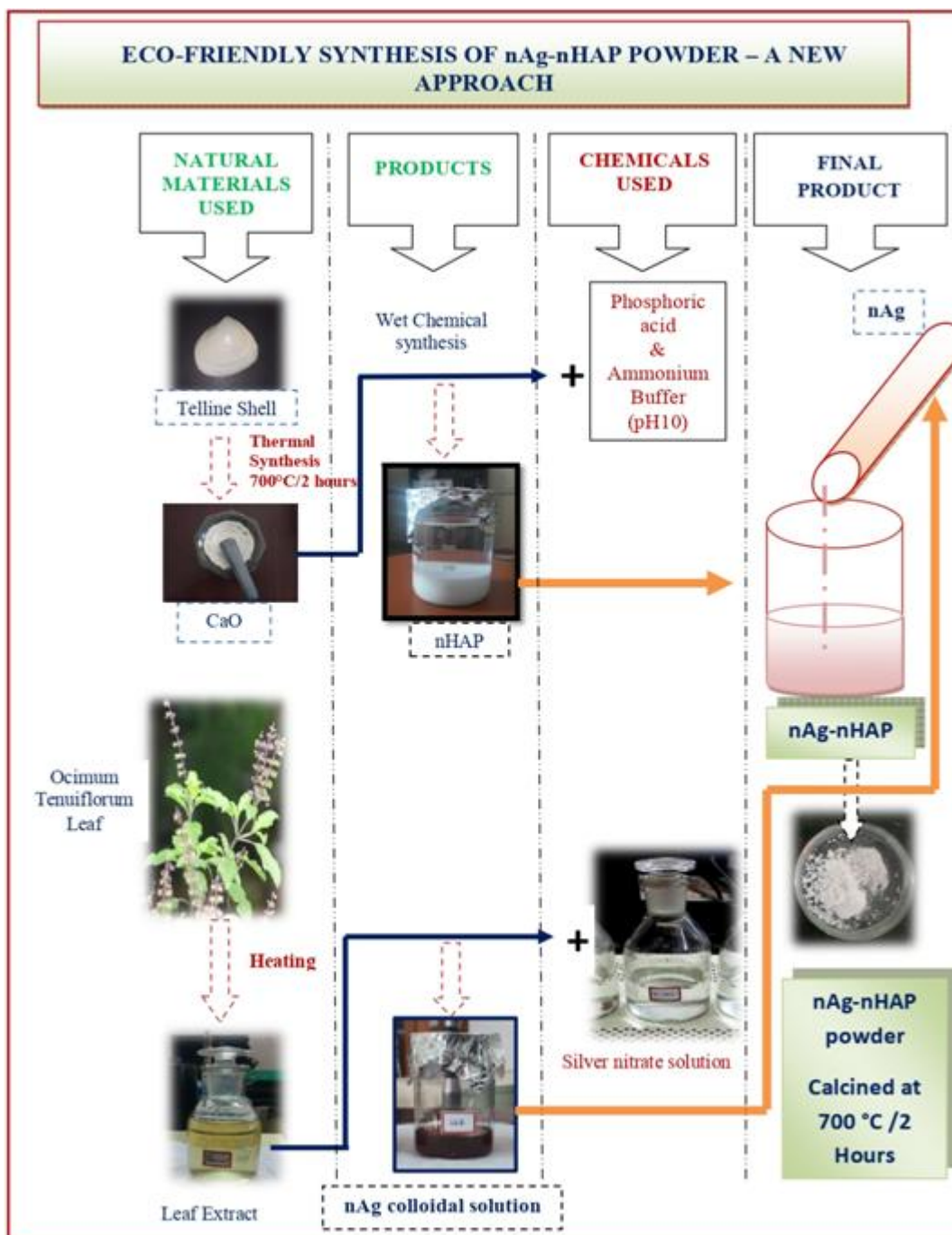


Figure 1: Synthesis of nAg-nHAP Particles

## Laboratory Procedures

### 2. Methods of synthesis

#### 2.1 Preparation of nano-silver colloidal solution

The fresh leaves of *Ocimumtenuiflorum* (Thulasi) leaf of 20g were taken and boiled for 1 hour in a 100 ml of distilled

water and it is filtered. This extract is used as reducing and stabilizing agent. Aqueous solution of silver nitrate is prepared at the concentration of 0.002M. 5ml of leaf extract is added in 20 ml of silver nitrate solution. A change in the colour of solution from yellow to dark brown/ red colour indicates and confirms the formation of silver nanoparticles [8].

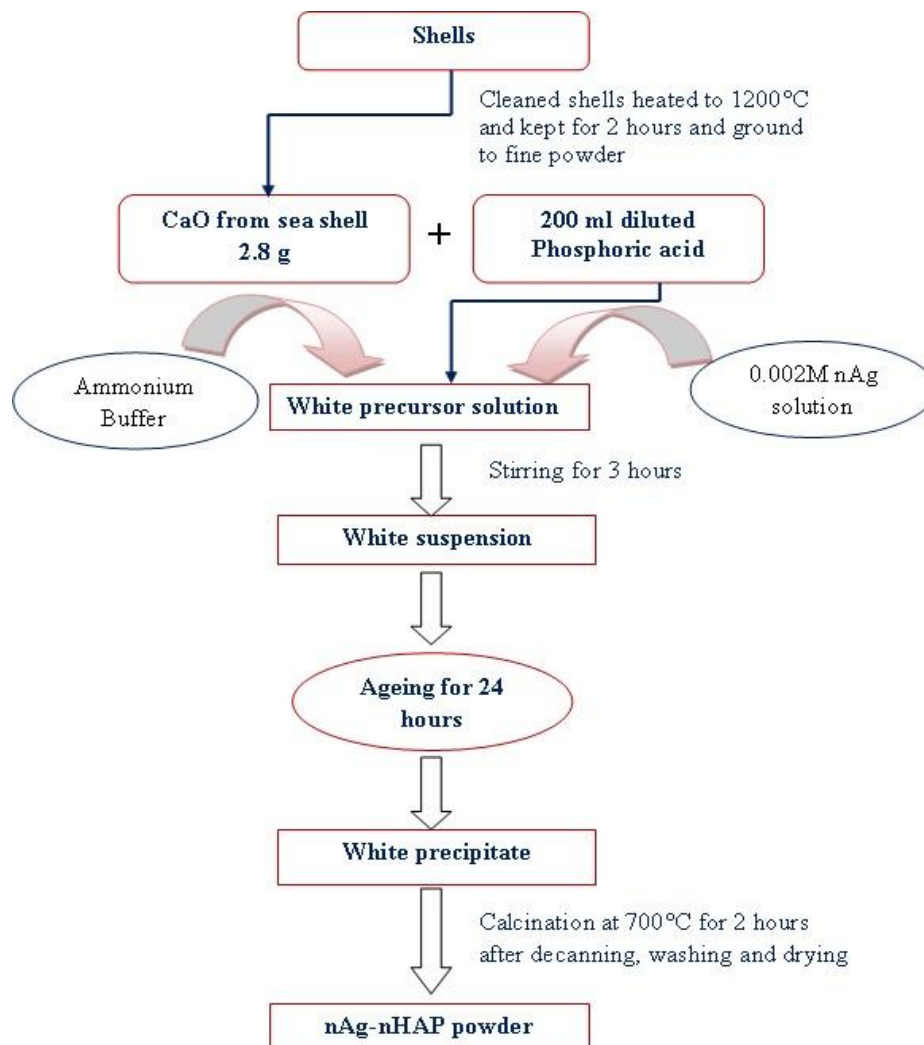
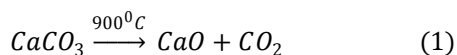


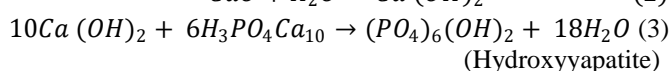
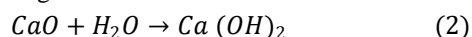
Figure 2: Flow chart for the synthesis of nAg-nHAP

## 2.2. Synthesis of nanosilver doped hydroxyapatite nanopowder

The Sea Shells (precursor of CaO) were collected from the sea shore of the coastal town of Karaikal, TamilNadu, South India. These shells were cleaned and heated in a furnace at 1200°C. The decomposition  $\text{CaCO}_3$  into  $\text{CaO}$  yielded amorphous shells of calcium oxide which were further powdered using mortar and pestle [9].



Calcium hydroxide and Orthophosphoric acid are the main precursors for calcium and phosphorous. 2.8 g of CaO was dissolved in 200 ml of distilled water to prepare 0.25M solution of calcium hydroxide and to which 0.15M of phosphoric acid (1.7 ml of phosphoric acid dissolved in 200 ml of distilled water) was added drop-wise at the rate of 1ml /minute and stirred continuously at room temperature. The above combination normally gives a Ca:P molar ratio to 1.67 through the following reaction



With the resulting product of above reaction, 5ml of nano silver solution(0.002 M) mixed with Ocimumtenuiflorum extract was added drop wise and stirred for 3 hours. Continuous stirring resulted in a gluey precipitate. The pH was maintained at 10 [10]. The solution was held undisturbed for 24 hours. The precipitate was decanted and washed with distilled water and dried in a furnace at 80°C for 6 hours. The dry sample obtained was powdered well using mortar and pestle. The powder further calcined at 700°C for 2 hours and labelled as nAg-nHAP.

## 3. Result and Discussion

The synthesized undoped pure nanohydroxyapatite (Pure nHAP) and nAg-nHAP was characterised by UV-Vis, FTIR, XRD, SEM and EDAX analyses.

### 3.1 UV-Vis spectra of nHAP and nAg-nHAP nanoparticle

The UV spectra of nAg-nHAP clearly shows a red shift in comparison with the UV spectra of nHAP nanoparticles.

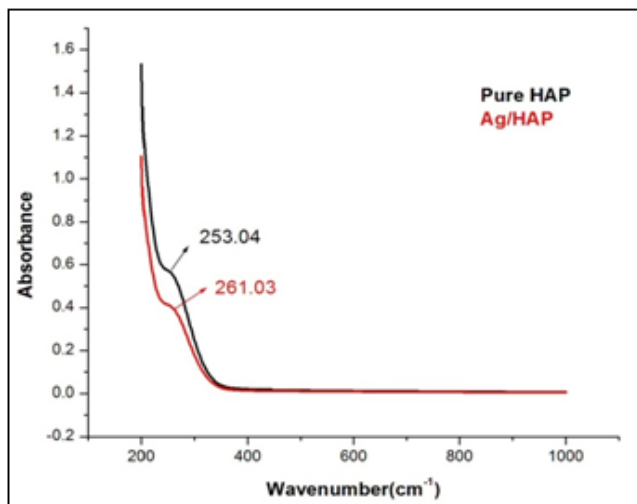


Figure 3 (a): UV-Vis Spectra of nHAP and nAg-nHAP

The UV-Vis analysis was carried out using UV-Vis spectrophotometer Analytikjena at SCSVMV deemed

University research laboratory, Kancheepuram, Tamilnadu, India. The surface plasmon resonance (SPR) for nanosilver normally occurs in the wavelength range about 400nm and in present study no SPR peaks is observed at 400nm for nAg/nHAP. When compared to nHAP which has a peak at 253nm, a red shift is observed for nAg-nHAP at 261nm[11]. This shows the inclusion of nano silver with hydroxyapatite Nanoparticles. The red shift may be due to the lattice deformation of nHAP due to the inclusion of nanosilver in to the lattice.

3.2 FTIR analysis of nHAP and nAg-nHAP nanoparticles

The functional groups of synthesized nAg-nHAP were analyzed by FT-IR spectroscopy. The FTIR analysis was carried out using FTIR Spectrophotometer Bruker alpha -T at SCSVMV deemed University research laboratory, Kancheepuram, Tamilnadu, India.

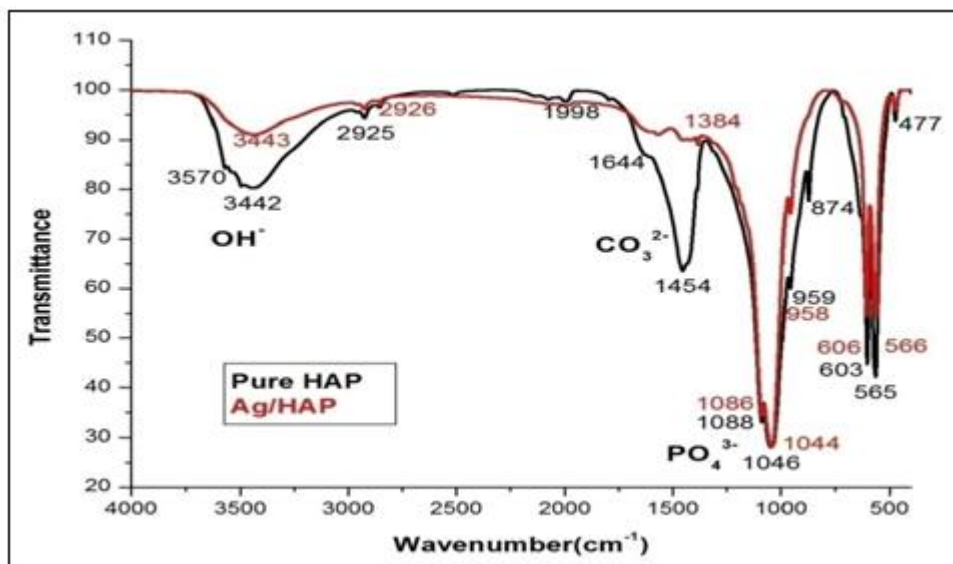


Figure 3 (b): FTIR Spectra nHAP and nAg-nHAP

In the spectra of nHAP and nAg-nHAP, the peaks observed at 565, 603, 959 cm<sup>-1</sup> and 1000-1100 cm<sup>-1</sup> correspond to PO<sub>4</sub><sup>3-</sup> groups and the peaks observed at 874 cm<sup>-1</sup> correspond to HPO<sub>4</sub><sup>2-</sup> ions. The peak at 1046 cm<sup>-1</sup> corresponds to asymmetric P-O stretching and 1086 cm<sup>-1</sup> corresponds to PO<sub>4</sub><sup>3-</sup> groups revealed the exact formation of nHAP [14]. In spectra the small band at 1384 cm<sup>-1</sup> corresponds to vibration of CO<sub>3</sub><sup>2-</sup>. The regions 1600-1700 and 3200-3600 cm<sup>-1</sup> correspond to lattice water due to H-O-H vibrations [12].

The peak at 3570 cm<sup>-1</sup> for O-H group and 1454 cm<sup>-1</sup> for CO<sub>3</sub><sup>2-</sup> [13] was observed in the nHAP and it was not observed in the nAg-nHAP [fig3. (b)]. Further nAg-nHAP structure show a overall reduction in the intensity of absorption bands to a little extent compared to nHAP. The observed changes in absorption bands of nAg-nHAP may be due to the lattice deformation in doped structure due to the inclusion of Nano silver in to the lattice. These results

confirm the doping of nAg in to the nHAP lattice.

Table.3.1: Functional group present in hydroxyapatite nanoparticles[14]

| Wavenumber cm-1 | Stretching mode              | Functional group              |
|-----------------|------------------------------|-------------------------------|
| 3570            | Ion Stretching               | OH-                           |
| 1454            | Asymmetric stretching        | CO <sub>3</sub> <sup>2-</sup> |
| 1046            | Asymmetric stretching        | PO <sub>4</sub> <sup>3-</sup> |
| 959             | Symmetric stretching         | PO <sub>4</sub> <sup>3-</sup> |
| 565             | Asymmetric bending vibration | PO <sub>4</sub> <sup>3-</sup> |

3.3 XRD analysis

One of the main characteristics of nHAP is its bioabsorption which is directly related to crystal size and crystallinity [12]. The XRD analysis was carried out using Powder X-Ray Diffractometer Bruker D8 ADVANCE at Department of Chemistry, IITM, Chennai, India.

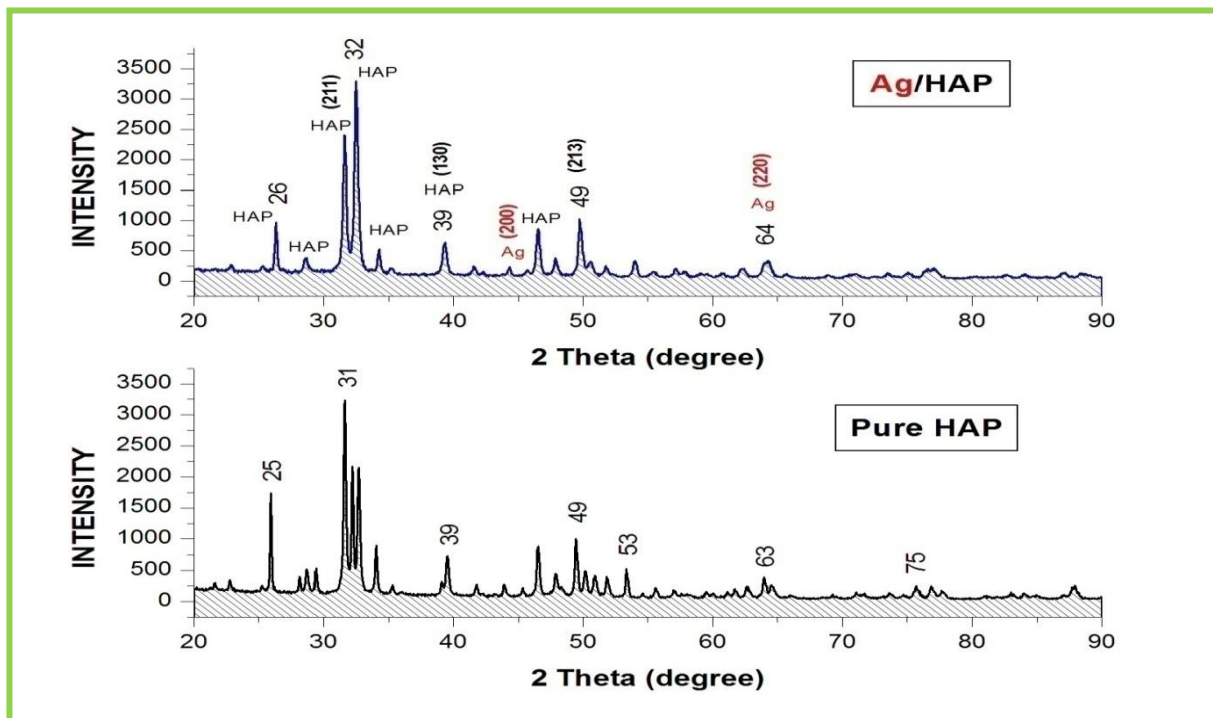


Figure 3 (C): XRD Spectra of nHAP and nAg-nHAP

The characteristic peaks for Hydroxyapatite ( $2\theta = 26^\circ, 28^\circ, 32^\circ, 39^\circ$  and  $49^\circ$ ) are well observed in the nAg-nHAP and this confirms the formation of nAg-nHAP crystals. The XRD results of nAg-nHAP nanoparticle can be indexed with the standard one with JCPDS card no. 09-0432 [9]. When compare to nHAP the intensity of the nAg-nHAP peak is less due to the presence of silver doped in to the nHAP lattice. There is no great difference in the diffraction peaks. The characteristic peaks of silver ( $2\theta = 44.2^\circ, 64.2^\circ$ ) shows the formation of nAg doped nHAP. Since the addition of nAg in

to the nHAP is in very small quantity, considerable peaks are not available and that cannot be detected by the XRD system.

### 3.4. SEM/EDAX analysis

The morphology and average particle size of nAg-nHAP were investigated by a scanning electron microscope. SEM/EDX analysis was carried out at SAIF centre at IITM, Chennai, India.

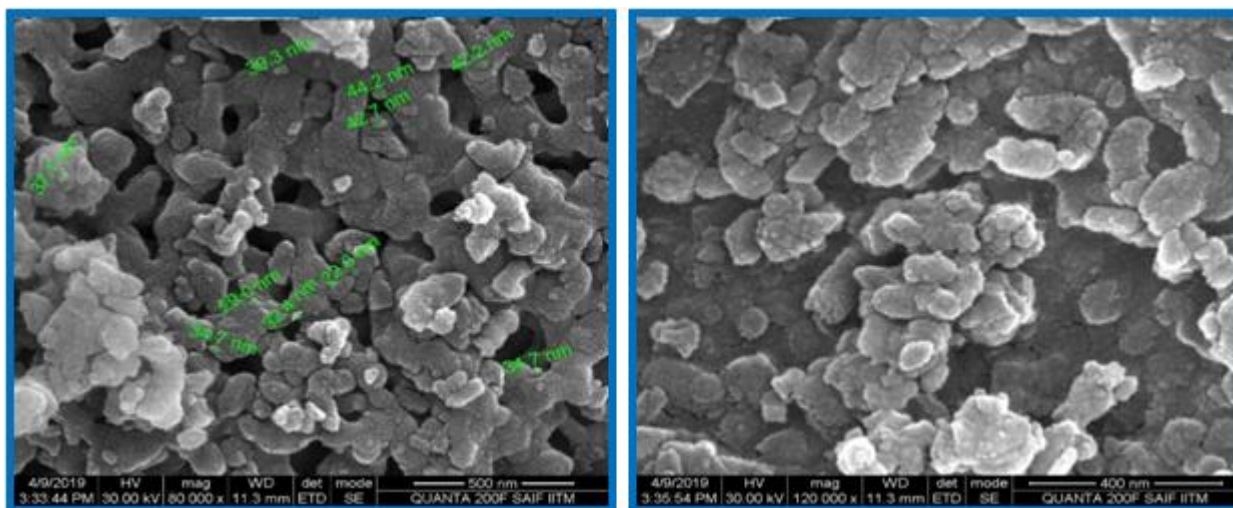


Figure 3(d): SEM images of nAg-nHAP nanoparticles

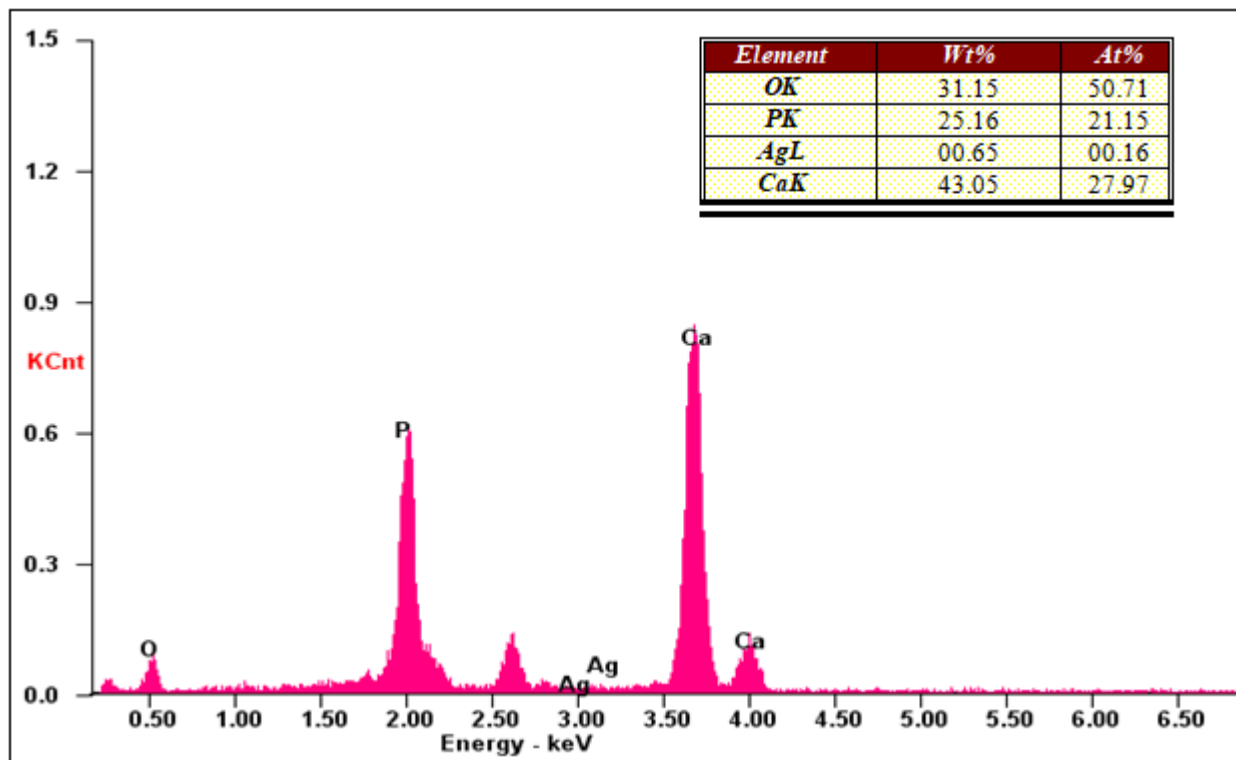


Figure 3(e): EDAX Spectra of nAg-nHAP nanoparticles and elemental composition

The SEM image shows the nanoparticle size ranging from 22nm to 44.2nm. The shape of nanoparticle displayed in the SEM image is partially spherical and agglomerated in to small aggregates. The EDAX Spectra was used for quantitative elemental analysis of the nAg-nHAP nanoparticles. The peaks in EDAX spectra of nAg-nHAP confirms the presence of essential elements calcium(Ca), phosphor(P), Oxygen(O) of HAP and in stoichiometry ratio along with doped nano Silver(nAg.) [9,12].

The standard Ca/P molar ratios for nHAP is 1.67. The EDAX spectra of nAg-nHAP shows the Ca/P ratio is 1.71, this is due to the nano silver added in to the lattice structure of nHAP. EDAX confirms the presence of nanosilver with nano hydroxyapatite. Thus the image obtained from the SEM proved the formation of nAg-nHAP nanoparticle.

#### 4. Conclusion

In this study nAg-nHAP was synthesized through green method using sea shells and Ocimum Tenuiflorum Leaf Extract with the usage of limited chemical compounds. The synthesized nAg-nHAP nanoparticles may be a better choice for a good bio material with antibacterial properties and the suggested green method may be considered for a green mass production of the biomaterial from the available natural materials.

The FT-IR spectra confirmed the functional groups of nHAP and showed very mild variations in absorption peaks for nAg-nHAP indicating its doped nature. The XRD results confirmed the formation nHAP particle and also confirmed the inclusion of nAg in to nHAP lattice [15]. The spherical and agglomerated nano formation nAg-nHAP nanoparticle was confirmed by SEM analysis in the range 22nm to 44.2nm. The EDX Spectra confirmed the elemental

composition of nHAP (Ca, O, P) and also confirmed the inclusion of nAg in to nHAP lattice [15,16]. The small variation observed in Ca/P ratio between nHAP (1.67) and nAg-nHAP (1.71) may be due to the inclusion nAg in to nHAP lattice. The UV-Vis Spectrum analysis indicated the inclusion of nAg in to the nHAP lattice by a red shift in SPR peak for nAg-nHAP [15]. The synthesized nanomaterial nAg-nHAP may be a wonderful candidate for further studies in bone substitute medical sciences applications.

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