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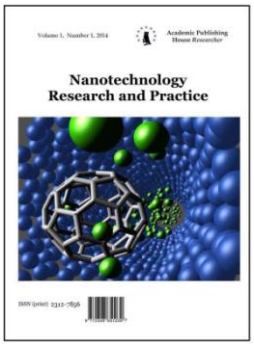

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Synthesis, Characterization and Antimicrobial Activity of Nano Hydroxyapatite Via a Novel Sol Gel Method

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Abstract

A novel sol gel method for the preparation of nano hydroxyapatite [HAP], a widely used bone and teeth substitute reputed for its bio compatibility and bio activity is reported in the present study. X-ray diffraction (XRD) studies of its crystalline phase, SEM, EDS and FTIR investigation are also reported. Invitro behavioral study of S.aureus and E- coli bacteria in the presence of nano HAP is also presented.

Keywords: Hydroxyapatite; Sol Gel method; XRD; SEM; antimicrobial activity.

Introduction

Calcium Phosphate with its predominant existence in human bones has been well known for its high levels of biocompatibility, bioactivity, osteoconductivity and nontoxicity to the human body [1, 2]. It has been known to exhibit a variety of crystal structure forms based on Calcium/Phosphor (Ca/P) ratio during its synthesis, like Hydroxyapatite (HAP), Octacalcium Phosphate, Tricalcium Phosphate, Dicalcium Phosphate Dehydrate and Dicalcium Phosphate [3]. Hydroxyapatite with chemical formula $Ca_{10}(PO_4)_6(OH)_2$ has been known for its wide spread applications in Orthopedic cases, tissue repair and replacement as well as an implantable material for bone cavity filler/ coating [4]. An important point to note is that the main constituent of the human teeth is nano hydroxyapatite rods of size less than 100nm arranged lamellarly and bound to collagen [5].

Several nano synthesis techniques have been reported for HAP [6, 7]. To name a few, Sol Gel method [8], Hydrothermal method [9],Co- precipitation method [10], Mechano Chemical method [11], Microwave Irradiation method [12] and Ultrasonic Irradiation method [13]. Of the above method, Sol Gel technique is a well-defined uncomplicated method for the preparation of homogeneous and highly pure forms of nano Hydroxyapatite [14].

In the present work, a unique customized version of the sol gel method has been reported for the synthesis of nano HAP. Precursors starting from Calcium hydroxide, Orthophosphoric acid and Ammonia have been used in this method.

Experimental Procedure

Calcium hydroxide $Ca(OH)_2$ and Orthophosphoric acid H_3PO_4 were used as beginning Calcium and Phosphor precursors. 1M of calcium hydroxide and 0.6M of Orthophosphoric acid were taken with a Ca/P ratio 1.67. Calcium Hydroxide solution was added drop by drop to Orthophosphoric acid solution with continuous stirring for 1hr. Subsequently Ammonia was summated to maintain the pH as 11. The novel idea that was implemented in this method was ageing the solution for 22hrs on ice, which finally separated neatly the required nano HAP gel from other constituents. The reason for choosing ice as aging media was to get a fine nano sized particle. The resulting solution was washed with double distilled water several times to remove impurities, if any.

The gel solution was then centrifuged and dried at 100°C using hot air oven to obtain the final resultant nano powder of the sample. The summary of the method is represented in the block diagram. Fig 1.

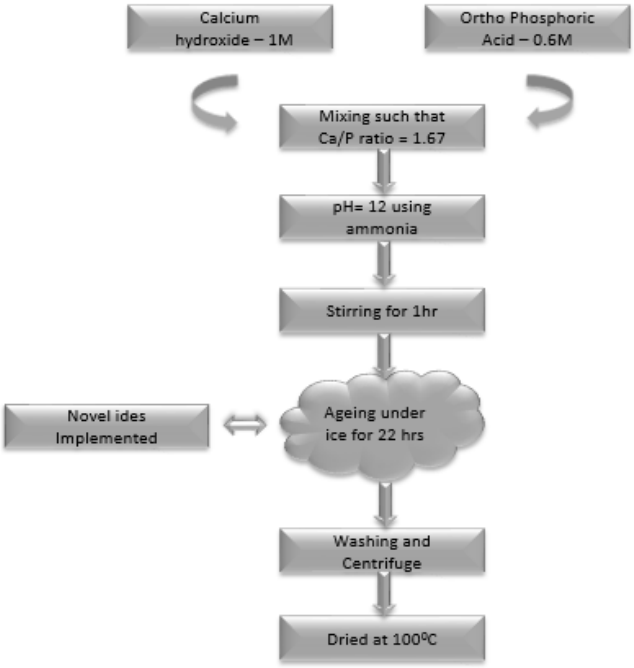

Fig 1: Block diagram of Preparation method of nano HAP

The sample was then characterized by X- ray diffraction (XRD), Fourier transform Infra-red spectroscopy (FTIR), Scanning electron microscopy (SEM), Energy dispersive spectroscopy (EDS) and antimicrobial activity.

Characterization and Discussion of Results

1. X ray diffraction (XRD)

Phase analysis was studied by X-ray diffraction. The mean crystallite size (D) of the particle was calculated from XRD line broadening measurement from the Debye Scherer equation [15]

$$D = 0.89\lambda / \beta\cos\theta \longrightarrow \quad [1]$$

Where λ is the wavelength of the CuKα line, β is the full width at the half maximum of the hydroxyapatite line and θ is the diffraction angle.

The lattice parameter 'a' and 'c' of the HAP nano particle and volume of the hexagonal structure HAP were calculated using the standard equation [16]

$$1/d^2_{hkl} = 4/3[h^2+hk+l^2]\, a^2 + l^2/c^2 \longrightarrow \quad [2]$$

$$V = 2.589a^2c \longrightarrow [3]$$

Figure 2 shows the XRD pattern of the prepared nano HAP powder. The obtained XRD data matches well with the standard JCPDS file no: 09-0432. The crystallite size calculated from Debye Scherrer formula was 29nm. The lattice parameter a is 9.378Å and c is 6.8124 Å with c/a ratio as 0.7264.

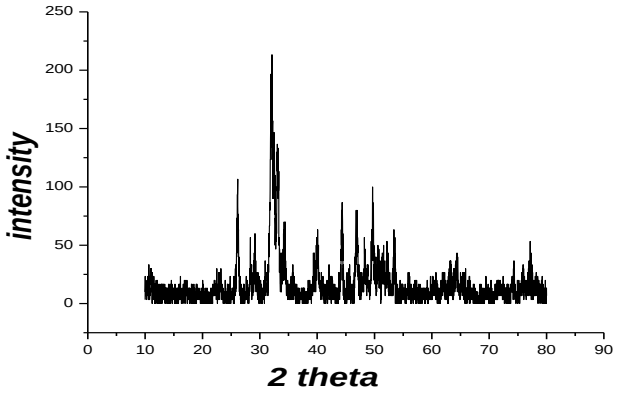

Figure 2: XRD pattern of HAP nano powder

These value were found to be matching well with the standard value. The unit cell volume is obtained as $1551.14X10^{-30}$ m^3. From the above data it was concluded that the obtained sample has a hexagonal structure.

2. Fourier Transform Infrared (FTIR) spectroscopy

Functional groups were investigated by FTIR spectrometer [17] in the range from 4000 – 400cm^{-1} at the resolution of 4cm^{-1}. Spectra were measured in the transmission mode.

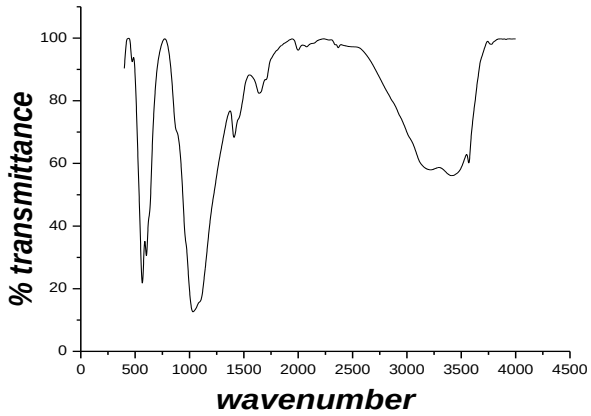

Figure 3: FTIR pattern of nano HAP

Figure 3 shows the FTIR spectra of prepared nano HAP. The bands at 1650cm^{-1} corresponds to the presence of hydroxyl groups OH^-. The presence of phosphate group PO_4^{3-} stretching modes were confirmed by 1033 cm^{-1} and 1090 cm^{-1}. The bands at 563 cm^{-1} and 470 cm^{-1} corresponds to PO_3 groups. Medium peaks numbers two at 633 cm^{-1} and 3565 cm^{-1} were attributed to the O-H bending deformation mode and structural OH mode respectively. The absence of other calcium phosphate groups in the spectra confirmed the presence of HAP.

3. Scanning Electron Microscope

The surface morphology and crystallite morphology of the synthesized particles were investigated by scanning electron microscope (SEM) [18] operating at an accelerating voltage of 20KV.

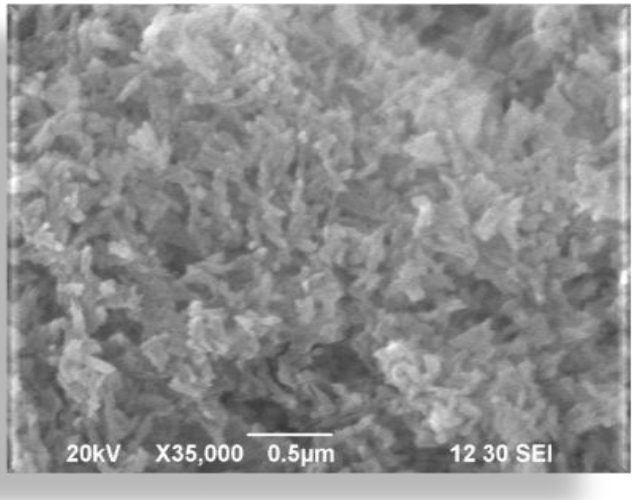

Figure 4: SEM image for nano HAP

Figure 4 shows the SEM image of nano HAP. SEM micrograph shows rod like structure similar to that of human teeth morphology with nano rod size ranges between 45-70nm

4. Energy Dispersive Spectroscopy (EDS)

The quantitative analysis of Ca, P, O were investigated by EDS studies [19]. The samples chemical constituents were substantiated by EDS. Figure 5 shows the EDS composition of the sample. The composition of Ca is 30.78wt%. P is 16.88wt% and O is 52.34wt%. The Ca/P ratio (1.84) is close to the expected value (1.67). The small difference in the value can be attributed to impurities in the chemicals.

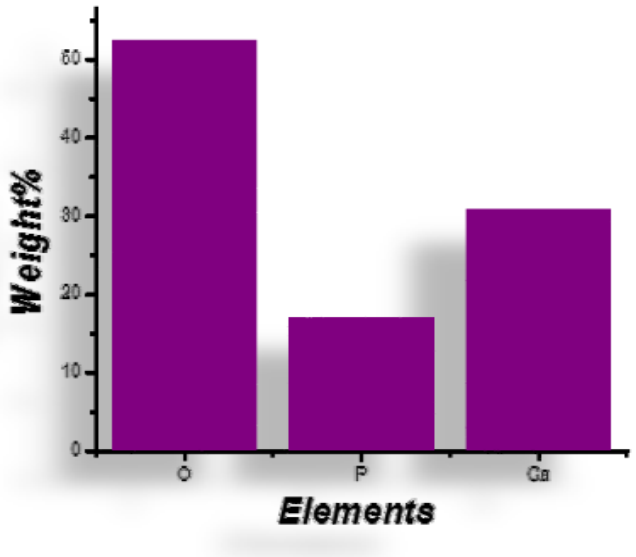

Figure 5: EDS spectra of nano HAP

5. Antimicrobial activity

The antimicrobial activity of the HAP was tested against S.aureus gram positive bacteria and E coli gram negative bacteria through Agar diffusion method [20]. The inoculums of all micro organisms were prepared from fresh overnight bath culture that were incubated at 37°C.

The diffusion technique was carried out by pouring agar on the Petri dish for thickness of 4mm and allowed to solidify for 10 minutes. Then the testing microorganism were spread over the agar solution. Petri plates were left to dry for 10 minutes at air and after that the sample powder diluted in DMSO solution was poured in a hole made on the inoculums. The prepared Petri dish was finally incubated for 24 hours at 37°C. Readings of results were carried out by measuring the width of zone of inhibition in mm.

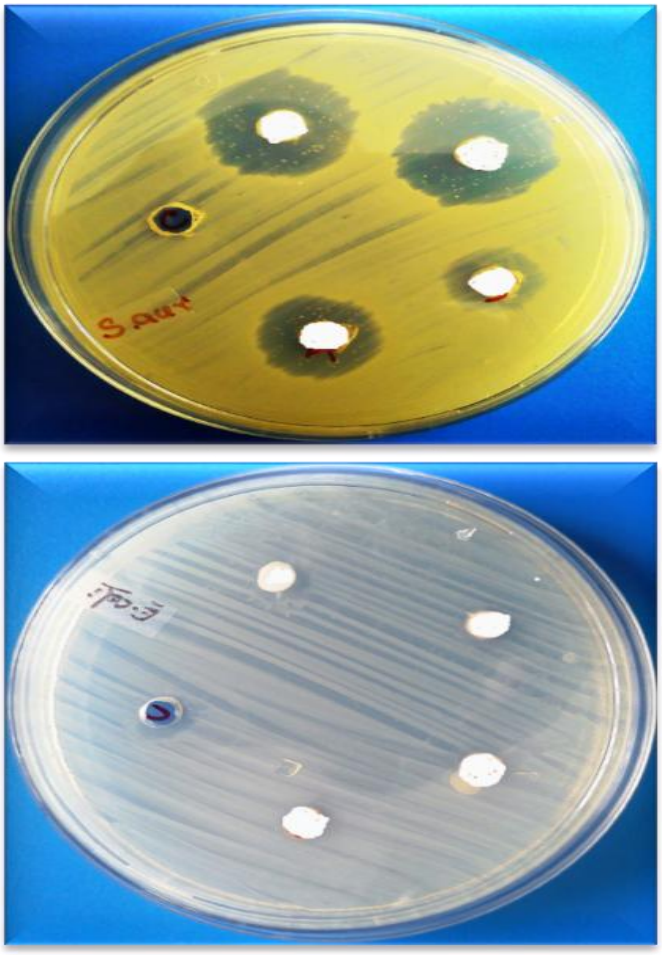

Figure 6: Photograph of antimicrobial test result of HAP sample against S.aureus and E-Coli bacteria

The result of antimicrobial disk diffusion test against S.aureus gram positive bacteria and E Coli gram negative bacteria are shown in figure 5. There is no zone of inhibition against E.coli bacteria and a good result was obtained for S.aureus bacteria with zone of inhibition of 25.6 mm.

Conclusion

Nano HAP was synthesized employing a novel idea through sol- gel method. The obtained sample was found to possess a homogeneous hexagonal structure containing nano sized crystal of size 29nm as per the prescribed standard specifications. All experimental confirmations like XRD, FTIR, SEM and EDS were carried out systematically. In addition antimicrobial activity of the sample was also studied against S.aureus and E.Coil bacteria.

These experimental confirmations reveal that the obtained sample has all characteristics as that of structured HAP. Moreover, sustained antimicrobial activity was found to be confined to S.aureus bacteria only. The obtained results thus point to a novel method of obtaining nano HAP which is better bio compatible than conventional HAP.

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