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Simple Route Synthesis of Hydroxyapatite – Gelatin Nanocomposite and its Characterization

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Abstract
Hydroxyapatite (HAP) is a bioactive inorganic material, major ingredient of vertebrate bones. Hydroxyapatite (HAP) nanoparticles were synthesized successfully by sol-gel method. The synthesized HAP-Gel nano powders was characterized by UV-visible spectrophotometer, X-ray diffraction, Fourier transform infrared spectroscopy, Raman spectroscopy and Scanning electron microscopy. The results obtained from the characterization specify that the formed HAP-Gel nano particles have uniform morphology and controlled size.

Keywords: Sol-Gel method, Hydroxyapatite, Nanoparticle, FT-IR spectroscopy, SEM.

Introduction

Bone related disorders are most significant problem in modern orthopedic surgery due to increased life style, trauma and accidental injuries in early and middle age populations with end of life. Calcium phosphates based ceramics are mostly used as orthopedic implant alternative in hard tissue regeneration due to its similar chemical composition, size, crystallinity, morphology to bone inorganic component and excellent biocompatibility [1-2].

Hydroxyapatite (HAP) chemical formula [$Ca_{10}(PO_4)_6(OH)_2$] has excellent biocompatibility, bioactivity and bio-resorbability, so it is used in repair of damaged bone or tooth [3]. Along with HAP, collagen is also one of the most important constituents of bone. It contributes up to 89 % of the organic matrix and 32 % of the volumetric composition of bone [4]. HAP has shown great potential for diverse applications, such as catalytic agent in chromatography and gas sensor [5] water purification, fertilizer manufacturing and as drug delivery agent [6].

Some recent reports also suggest that, HAP nano-particles obstructs the progression of different types of the cancer cells [7, 8].

Unfortunately, due to poor mechanical strength of HAP in wet environment, it could not be used for major load bearing applications [9]

HAP-Gel nanoparticles can be synthesized by different chemical-processing methods such as chemical co-precipitation method [10], hydrothermal method [11], Ultasonic Assisted Irradiation method [12], Mechano chemical method [13], Microwave irradiation method [14] and sol–gel method [15]. The products obtained by these methods are mostly irregular. But, the traditional sol-gel method has ability to produce homogeneous nano-sized particles easily as compared to other methods [12]. Organic constituent (Gelatin) controls the seeding of the inorganic (HAP) materials by geometric, electrostatic and stereo chemical complementarities [13, 14].

In this present study, HAP-Gel nanoparticles with consonant morphology and restrained size have been synthesized using sol-gel method. Gelatin (Gel) is used as template which coordinates the nucleation of HAP and crystal growth.

Methods

The Hap–Gel nanocomposite was synthesized using the method described by Yadav. et. al. 2010 [15]. In short, HAP-Gel was coalescenced by dissolving 1.85 g calcium hydroxide [$Ca(OH)_2$ (0.1M Ca2++) in diluted glacial acetic acid [CH_3COOH] solution containing 2.71 mL . After that, 0.67 g of Gel was added to above solution with constant magnetic stirring at 60˚C for 2 hrs to ensure a homogeneous distribution. Meanwhile, In a second flask, 0.1M

orthophosphoric acid [H_3PO_4] solution was prepared by dissolving 0.93 mL [H_3PO_4] in dilute ammonia solution with a pH of about 11–12. The calcium and phosphate solutions were added slowly to the third flask with a rate of about 5 mL/min and 3.5 mL/min, with continuous vigorous stirring for 12 hrs. After that gray colored slurry was obtained. The slurry was kept in a water-bath at 60˚C for about 8 h. The flask was kept to precipitate slurry and remaining part at the top was discarded, and the precipitate was washed three times with distilled water with a series of incubation at water bath at 60˚C followed by decantation. Finally, the precipitates were centrifuged at 2503g for 5 min to obtain consolidated nanocomposites of desired composition and store for future experiments.

Fig. 1: Schematic representation of for the synthesis of HAP.

Characterization

In order to phase identification of the HAP crystal was accomplished by the X-ray diffraction (XRD) technique, using a Philips TW 1710 diffractometer with Cu-Kα incident radiation regulated at 40 kVand 30 mA. The data was collected at room temperature over the 2θ range of 20–70°, with scanning rate 3˚/min. The X-ray diffraction pattern obtained from synthesized HAP was compared to standard data (JCPDS file No.24-0033) for identification of phase formation.

Fourier transform infrared spectroscopy (FTIR) was performed to predict the molecular structure and inter/intra molecular bonding of synthesized HAP using a Thermo Scientific FTIR (Thermo Nicolet-6700) spectrophotometer. Test samples were recorded in the range 4000–400 cm^{-1}.

The surface morphology and microstructure feature of synthesized HAP was evaluated by scanning electron microscope (JEOL JSM-6400).

Raman spectroscopy was performed to confirm the various groups present on HAP. The excitation source was 514.5 nm lines of Ar laser.

Results

The X-ray diffraction pattern of formed HAP allows us to evaluate the degree of crystallinity. The diffraction pattern shows the occurrence of sharp and high intensity peaks exactly corresponding to JCPDS file No. 24-0033 with no additional peaks.

To evaluate the molecular structure of synthesized HAP, FT-IR was monitored. The strong absorbance band observed between 950 and 1230 cm^{-1} is because of asymmetric stretching bands of O-P-O in PO_4^{3-} of HAP. While the corresponding Raman band perceived at 961 cm^{-1} having a very sharp intensity. Peak observed near about 606 and 3425 cm^{-1} is due to vibrational stretching mode of water.

FT-IR and Raman characterization provide complimentary and supplementary information about synthesized HAP.

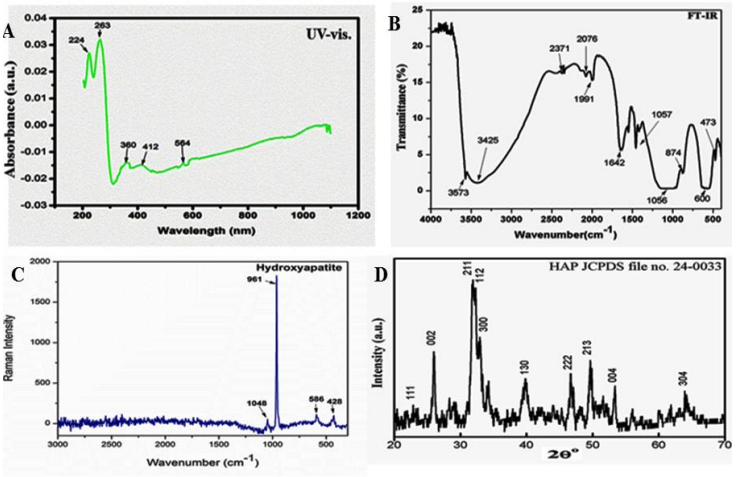

Figure 2: Characterization of HAP-Gel Powder. (A) UV-Vis. Absorption, (B) FT-IR Spectrum, (C) Raman Spectrum, (D) X-ray diffraction pattern.

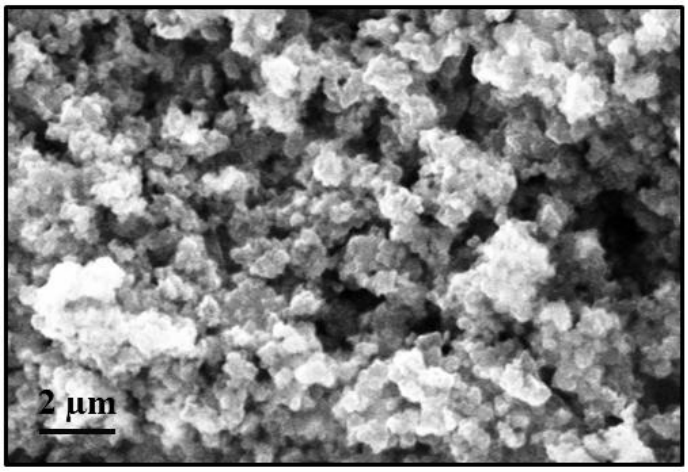

Figure 3: SEM image of HAP-Gel powder

Discussion

The X-ray diffraction pattern of synthesized HAP-Gel nano composite was compared with standard JCPDS file, which give good concession with high intensity and position of peaks. The XRD peaks of prepared HAP, presented in the figure 2, shows good concession with the data of JCPDS (File No. 24-0033). Data reveals that a synthesized Hap formed and no other specific peaks of impurities (calcium hydroxide [Ca(OH)2]) present in x-ray diffraction pattern.

The average crystallite or grain size of sol-gel synthesized HAP powder was measured by using Scherer formula of X-ray scattering.

$d=0:9\lambda/B\cos\Theta.$

where "d" is the mean grain size (nm), λ is the wavelength of x-ray tube, B is the width of the peak at half maximum expressed in radians and Θ is the Bragg's angle.

The FT-IR spectrum of prepared HAP-Gel nano-powder was obtained and wave numbers of all bands are presented in Fig. 2. Careful examination of FT-IR spectrum conceals that, the most of the observed bands attribute to one or the other vibrational mode of prepared HAP bonds matching to the several functional groups. The crews at 472 cm^{-1} is correspond to ν2 phosphate stretching mode [18]. A wide band at 3425 cm^{-1} is appeared in the IR spectrum of the synthesized HAP powder which may be due to the presence of water.

Raman band detected at 961 cm^{-1} with a very good intensity corresponds to ν1 symmetric P-O stretching vibration and band observed at 1046 cm-1 shows asymmetric P-O stretching vibration.

The SEM micrograph of HAP powder was taken after air oven drying at 80°C. The dried HAP powder appears as agglomerated due to different event occurring during drying. The overall morphology appears as very small particles/Nano beads having diameter around 1μm. The size of the HAP nanoparticles can be increases with the increasing the duration of sintering and temperature.

The nanometer size of HAP particles tophography and surface wettability are main feature of nano-ceramic material that have ability to promote vitro-nectin adsorption (osteoblast adhesion protein) and also alters the conformations, which enhances the functions osteoblast [19].

Conclusion

Hydroxyapatite nanoparticle was synthesized using [Ca $(OH)_2$] and [H_3PO_4] as precursor by simple sol- gel method, which shows uniform morphology and meticulous size.

The crystallographic and chemical composition of synthesized hydroxyapatite nanoparticle is analogous to natural bone apatite. So the prepared HAP nanoparticle can use in orthopedic tissue implants.

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