



Short Communication

## A Facile Route for the Preparation of Phosphonic Acid Grafted Nanosized Hydroxyapatite Biomaterial

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### Abstract

The present research effort consists in the development of phosphonic acid grafted nanosized hydroxyapatite (n-HAp) through a straightforward chemical methodology. Fourier transform infrared absorption spectroscopy (FTIR) and electron diffraction X-ray (EDX) studies show the stoichiometric creation of HAp particles. The existence of phosphonic acid on the hydroxyapatite particle surface was investigated through FTIR study also. Transmission electron microscopy (TEM) analysis divulges needle like acicular crystals of HAp particles with inexact size of 25-33 nm in diameter by 70-85 nm in length. The prepared hydroxyapatite material may be used in building a biocomposite with high interfacial bonding.

**Keywords:** Biocomposite, nanosized hydroxyapatite, transmission electron microscopy.

### Introduction

Hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] (HAp) is an interesting biomaterial with potential orthopedic, dental and maxillofacial applications because of its excellent biocompatibility<sup>1</sup>. HAp, being structurally similar to the inorganic component of bone, enamel and dentin has received considerable attention from the biologist and bio-material scientists. HAp has been successfully used as bone fillers, aesthetic restorative, coating of orthopedic implants, filler of inorganic/polymer composites, cell-culture carriers and so on. It is however well-known that the application of pure HAp is restricted due to its brittleness. In modern years, the progress of bioactive ceramic-polymer biocomposites has gained a phenomenal impetus in the orthopedic field for their bone analog design in addition to good biological and mechanical performances to meet specific clinical requirements<sup>2-3</sup>.

The mechanical properties of a biocomposite can be considerably enhanced by controlling the interfacial bonding between matrix and the reinforcement. Various methodologies have been developed to improve the interfacial bonding between the same, such as, zirconyl salt  $\text{ZrO}(\text{CH}_3)_2$  absorption<sup>4</sup>, polyacid RCOOH adsorption<sup>5</sup>, and isocyanate NCO grafting<sup>6</sup>. On the other hand, the use of thoughtfully chosen coupling agents has been proved as a dependable method to strengthen the interfacial bonding between the filler and polymer matrix with an considerable improvement of the compatibility by making the chemical bridges between the same. Organosilane and organotitanate based coupling agents have previously been reported to tailor the particle surface properties by lowering their specific surface energy<sup>7</sup>. Nevertheless, these coupling agents are not suitable for the preparation of composites because

of their pitiable stability in an aqueous environment. Grafting of organophosphorus coupling agents (OPCA) on preformed inorganic supports or in-situ formation of the inorganic part in attendance of organophosphorus reagents offers a potential alternative to the silicon or titanate based coupling agents<sup>8</sup>.

The present research attempt consists in the development of novel phosphonic acid grafted nanosized hydroxyapatite (n-HAp) powders. 2-Carboxyethylphosphonic acid (CEPA), a nontoxic, highly bioactive and biocompatible OPCA has been used as the coupling agent<sup>9</sup>. The phosphonic acid group ( $-\text{PO}(\text{OH})_2$ ) of CEPA can powerfully anchor on apatite surface. We account here the synthesis of n-HAp in presence of CEPA through a simple chemical methodology. We have characterized the material through XRD, FTIR and TEM studies.

### Material and Methods

**Chemicals:** Calcium nitrate (99%) and diammonium hydrogen phosphate (DAHP) (99%) were procured from Merck, Mumbai, India. 2-Carboxyethylphosphonic acid was procured from Sigma-Aldrich, Spruce Street, St. Louis, USA. Ammonia solution (25%) was obtained from Merck, Mumbai, India.

**Preparation of phosphonic acid grafted nano hydroxyapatite (n-HAp):** 0.5 M stock solution each of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $(\text{NH}_4)_2\text{HPO}_4$  was prepared in distilled water. Both the solutions were taken in such extent that Ca: P molar ratio was maintained at 1.67. 2-Carboxyethylphosphonic acid was mixed with  $(\text{NH}_4)_2\text{HPO}_4$  solution in 1:1 molar ratio. The pH of both calcium nitrate and DAHP solutions was maintained at ~11 - 12. Then the mixture of phosphonic acid and  $(\text{NH}_4)_2\text{HPO}_4$  was added drop-wise to the  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$

solution and energetically stirred at room temperature using a mechanical stirrer (2000 rpm). The pH of the reacting mixture was also maintained in the range of 11 to 12 by adding  $\text{NH}_4\text{OH}$  solution gradually. The process was continued up to 6 h. The gelatinous precipitate thus obtained was filtered by a centrifugal filtration method. The precipitate was washed with water thoroughly and dried at  $90^\circ\text{C}$  for 15 h.

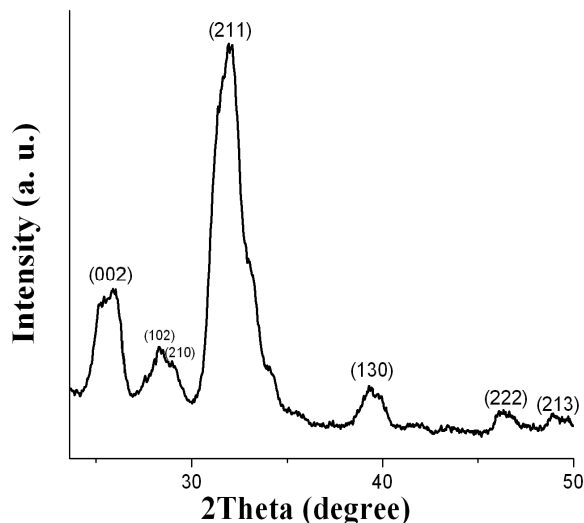
**Characterization:** The recognition of functional groups in the phosphonic acid grafted hydroxyapatite was analyzed by FTIR analysis (Model NEXUS870, FT-IR, Thermo Nicolet, USA) within the scanning range  $4000$  to  $400\text{ cm}^{-1}$ . The phase analysis of the HAp powders was done by XRD (Model PW 1729, Philips, Holland) using 35 milliamps, and 40 kV current, with a monochromatic  $\text{CuK}_\alpha$  (target) radiation ( $\lambda=1.5405\text{ \AA}$ ) with a step size of  $0.04^\circ 2\theta$ , a scan rate of  $0.02^\circ 2\theta/\text{s}$  and a scan range from  $2\theta = 20$  to  $50^\circ$ . The morphology, particle size of HAp powders were observed through a Phillips CM 200 transmission electron microscope (TEM) with an speeding up voltage 200 kV. Calcium-phosphorous molar ratio of hydroxyapatite powder was calculated by EDX analysis (Model ISIS300, Oxford, USA).

## Results and Discussion

The X-ray diffraction pattern of the synthesized apatite powder has been offered in figure 1. The d-values correspond to that of calcium hydroxyapatite [ $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ] (JCPDS card no. 09-0432). The broadening of XRD peaks indicates nanocrystalline nature of the synthesized apatite powder. It is evident from the observed outcome that no characteristic diffraction angles from other calcium phosphate phases are detected. The main crystalline peaks observed for the HAp at diffraction angles  $25.89^\circ$ ,  $31.91^\circ$ ,  $39.85^\circ$ , and  $46.71^\circ$  respectively represent the hydroxyapatite phase with d-spacing 3.44, 2.80, 2.26, and 1.94  $\text{\AA}$  likewise. The mean crystallite size has been calculated using Scherrer's equation i.e.  $D = \frac{0.9\lambda}{\beta_{\text{sample}} \cos \theta}$ , where D is the average

crystallite size in  $\text{\AA}$ ,  $\beta_{\text{sample}}$  represents the crystallite size contribution to the peak broadening of the diffraction line for the sample measured at half of its highest intensity in "radian",  $\lambda$  is the wavelength of X-rays, and  $\theta$  is the Bragg's diffraction angle. The peak width  $\beta_{\text{sample}}$  is calculated using the equation  $\beta^2_{\text{sample}} = \beta^2_{\text{exp}} - \beta^2_{\text{inst}}$ , where  $\beta_{\text{exp}}$  is the experimentally measured full width of the peak at half of the maximum intensity and  $\beta_{\text{inst}}$  is the instrumental broadening contribution determined from a silicon standard. The average crystallite size for the synthesized HAp powder is found to be 33 nm. The relationship between lattice constant (a and c), Miller's indices (h,k,l) and lattice spacing (d) is used to compute lattice parameter values i.e.

$$\frac{1}{d^2} = \frac{4}{3} \left( \frac{h^2 + hk + k^2}{a^2} \right) + \frac{l^2}{c^2}$$



**Figure-1**  
X-ray diffraction pattern of phosphonic acid grafted hydroxyapatite powders

The lattice parameters are found to be  $a=b=9.42\text{ \AA}$  and  $c=6.88\text{ \AA}$ . Therefore, the wide angle XRD represents that the synthesized material is pure hydroxyapatite phase. The result is also supported by the energy dispersive X-ray analysis (EDX), which designates the material is stoichiometric with Ca: P mole ratio 1.66 (theoretical value 1.67). The crystallinity (Xc) of the pure phosphonic acid grafted HAp composite is determined by an empirical relation between Xc and  $\beta_{002}$  i.e.  $\beta_{002} \times \sqrt[3]{Xc} = K_A$  [31], where Xc is the crystallinity degree,  $\beta_{002}$  is full width of the peak at half intensity of (002) plane in degree- $2\theta$ ,  $K_A$  is a constant (0.24)<sup>10</sup>. The crystallinity (Xc) for HAp powder is calculated to be 0.21. For biomedical purposes, samples containing nanoapatites with low crystallinity are wanted for their high 'in vivo' restorability rate<sup>11</sup>.

FTIR spectrum (figure 2) shows the existence of structural OH ( $632$  and  $3571\text{ cm}^{-1}$ ),  $\text{H}_2\text{O}$  ( $3416$  and  $1641\text{ cm}^{-1}$ ) and  $\text{PO}_4^{3-}$  ( $1093$ ,  $1027$  and  $604\text{ cm}^{-1}$ ) in the n-HAp crystals<sup>12</sup>. The characteristic peak at  $1726\text{ cm}^{-1}$  (C=O) and a number of peaks in the range of  $1200$ - $1250\text{ cm}^{-1}$  (C-H bending), peaks at  $986$ ,  $1045\text{ cm}^{-1}$  (P-OH) as well as the peaks at around  $1100$ - $1200\text{ cm}^{-1}$  (P-O, P=O) in the figure indicate the existence of phosphonic acid on the surface of n-HAp particle. The presence of phosphonic acid on the particle surface is recognized to the chemical bonding interaction between phosphonic acid and calcium ion of the HAp particle. The morphology and the particle size of synthesized hydroxyapatite powder are exposed by TEM micrograph. Figure 3 shows the TEM micrograph of as synthesized n-HAp powder. The micrograph depicts the acicular needle like crystals of HAp powder in nanometer range, having 25-33 nm in diameter by 70-85 nm in length.

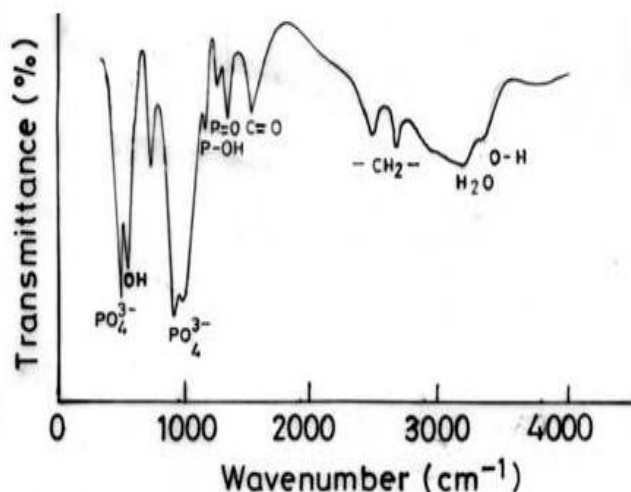


Figure-2  
FTIR spectral signature of phosphonic acid grafted hydroxyapatite powders

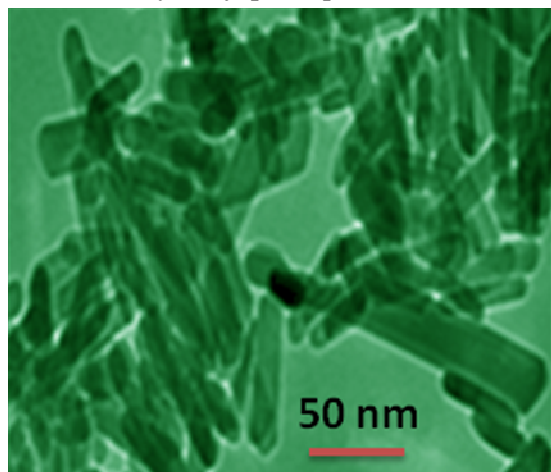


Figure-3  
HRTEM image of synthesized phosphonic acid grafted hydroxyapatite powders

## Conclusion

2-Carboxyethylphosphonic acid grafted n-HAp particles have been synthesized effectively following a solution-based chemical methodology. XRD study confirms that the synthesized material restrains single phase pure hydroxyapatite. EDX study shows that the material is stoichiometric with Ca:P mole ratio of 1.66. TEM analysis exposed the morphology of HAp powders with dimensions of 25-33 nm in diameter by 70-85 nm in length. The developed phosphonic acid grafted hydroxyapatite material may have applications in making a biocomposite with elevated interfacial bonding.

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