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Influence of Ultrasound Irradiation on Ascorbic Acid-Mediated Hydroxyapatite

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Abstract

Bio ceramic materials like hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$, or HAP) are both bioactive and biocompatible, allowing them to chemically bond with bone tissue. Hydroxyapatite-assisted ascorbic acid nanoparticles were synthesized through ultrasonic irradiation at various concentrations of ascorbic acid using $Ca(NO_3)_2{\cdot}4H_2O$ and KH_2PO_4 as the calcium and phosphorus sources, respectively. The obtained nano hydroxyapatite powders were characterized by Fourier transform infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and scanning electron microscopic (SEM) techniques. The results have showed that the nano hydroxyapatite powders synthesized by ultrasonic method showed outstanding reduction in the particle size when compared with Ascorbic acid and hence these powders could be used as a coating material in biomedical applications.

Keywords: Biomaterials, Hydroxyapatite, Nanoparticle, Ascorbic acid, Ultrasonic

1 Introduction

In recent years, hydroxyapatite (HAP) has developed as a highly valued bio ceramic material due to excellent bioactivity, biocompatibility, and osteoconductive properties. These characteristics make HAP particularly appropriate for a wide range of biomedical applications, including orthopaedics, dentistry and maxillofacial surgery [1–4]. Outside the medical field, HAP is also used in various industrial and technological domains, such as catalysis in chromatography, gas sensing [5], water purification, fertilizer production, and drug delivery systems [6]. The performance and functionality of HAP are significantly influenced by factors like its crystallite size, morphology, stoichiometry, purity and crystal structure [7,8]. Among calcium phosphate compounds, HAP is especially favoured because its chemical composition, mineral composition and structural and functional properties are closely resembled those of natural bone and dental tissues.

Since the wide applications of HAP in biomedical fields, numerous hydroxyapatite synthesis methods have been developed. These include variety of methods have been employed to prepare hydroxyapatite including solid-state reactions [9,10], co-precipitation [11], sol-gel techniques [12,13], hydrothermal synthesis [14], ultrasonic irradiation [15] and microwave [16]. Among the ultrasound method is considered a technically feasible, simple, and environmentally friendly approach for treating biowaste prior to the extraction of hydroxyapatite.

Ascorbic acid, or vitamin C, is soluble in slightly acidic water and is a natural antioxidant. It is used in pharmacy and nutrition as a food supplement and also in certain therapeutic applications. Ascorbic acid plays a significance role in the synthesis and regulation of hydroxyapatite especially in the context of bone and dental tissue engineering. Ascorbic acid would be useful for biomedical applications including bone tissue engineering and drug delivery systems [17].

In the present work, nano-HAP with spherical structure were prepared using Ascorbic acid by Ultrasound technique using calcium nitrate and Potassium dihydrogen phosphate as calcium and phosphorous precursors. The results showed that, the synthesis of Ascorbic ac-

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id assisted HAP powder has a well-defined crystallinity and do not show more agglomeration and even spherical morphology was attained through Ultrasound method. The as-achieved hydroxyapatite powders were characterized by different techniques.

2. Experimental Method

2.1. Chemicals and reagents

The starting materials include; Calcium Nitrate tetrahydrate (Ca $(NO_3)_2.4H_2O$), Potassium dihydrogen phosphate (KH_2PO_4), Ascorbic acid and ethanol solution were purchased from Merck, India. All the chemicals were of analytical grade and used without further purification. Deionized water (DI) was used throughout the experimental process.

2.2. Synthesis of Hydroxyapatite

Hydroxyapatite nanoparticles in the presence and absence of ascorbic acid as an additive was synthesized by Ultrasound method according to the following procedure: 0.5 M Ca $(NO_3)_2{\cdot}4H_2O$ and ascorbic acid with concentration of 0.5 g with constant stirring for 30 minutes and 0.3 M of KH_2PO_4 solution was dissolved in deionized water. The Ca $(NO_3)_2{\cdot}4H_2O$ with ascorbic solutions was after added drop by drop into KH_2PO_4 solution. The obtained white suspensions were strongly stirred for 1h till a transparent solution was obtained while the pH of the solution was adjusted to 10 using Ammonium Hydroxide solution. Then the resulting suspension was allowed under constant stirring for 1h and then shifted into sonicator and heated at 60 °C for 40 min. The obtained white precipitate was centrifuged and washed several times with double distilled water and ethanol followed by drying in an oven at 100 °C for 3h. The resulting residue was calcined and sintered finally to obtain pure nano-HAP crystal. The synthesis of hydroxyapatite was repeated with other two concentration of AA such as 1 g and 1.5 g and without the addition of AA.

2.3. Fourier Transform Infrared Spectroscopy (FTIR)

The IR spectra of the samples were collected using a PerkinElmer Spectrum One spectrophotometer with KBr pellets. FTIR measurements were conducted over the range of 4000–400 cm^{-1} with a resolution of 4 cm^{-1} to identify organic and inorganic species present in the precipitates.

2.4. X-ray diffraction (XRD)

The XRD patterns of the synthesized hydroxyapatite samples were obtained using a Bruker D8 Advance instrument with Cu-Kα radiation. The study was attained over a 2θ range of 20°–60° with a step size of 0.02° to assess the crystallinity and phase purity of the crystals.

2.5. Scanning Electron Microscopy (SEM)

The morphology and particle size of the synthesized hydroxyapatite crystals were analyzed using a JEOL SEM (Japan). SEM micrographs were captured for hydroxyapatite particles synthesized with and without ascorbic acid to compare their morphological differences and particle sizes.

3. Results and Discussion

3.1. Fourier transform infrared (FTIR) spectroscopic Results

The IR spectrum of the synthesized hydroxyapatite provides information about the functional groups like phosphate and hydroxyl (-OH) group present in the sample. The FTIR spectra of obtained hydroxyapatite powder and Ascorbic acid assisted HAP powder are presented in **Fig. 1 (a-d).** The peak at 977 cm^{-1} is assigned to symmetric stretching mode (ν_1) of phosphate group, a strong band in the region of 1041–1129 cm^{-1} is assigned to asymmetric stretching mode (ν_3) of phosphate group [18,19]. The lowest bending mode appears at (ν_2; 447 cm1) and the peak at 540 and 595 cm^{-1} is attributed to asymmetric bending mode (ν_4) of phosphate group [20, 21]. The sharp peaks detected in the region of 3500–3600 cm^{-1} correspond to the stretching vibrations of lattice OH^- ions, the broad band at 3435 cm^{-1} is attributed to hydroxyl group of hydroxyapatites [22]. The IR spectral assignments of the functional groups are shown in **Table 1.**

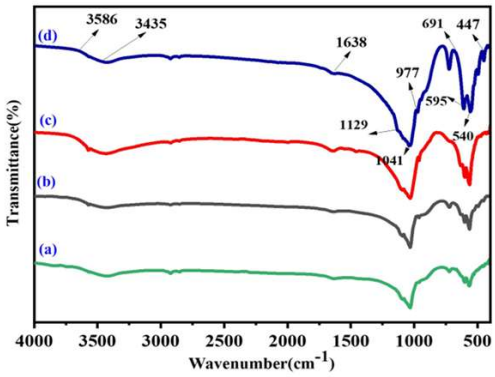

Fig.1. IR spectra of hydroxyapatite crystal prepared without and with different concentration of Ascorbic acid: (a) 0 g AA, (b) 0.5 g AA (c) 1 g AA and (d)1.5 g

Table 1. IR spectral assignment of the functional groups

3.2. X-ray diffraction (XRD) studies

The formation of crystalline hydroxyapatite phase both in the absence and presence of Ascorbic acid was confirmed by XRD technique. In Fig. 2(a–d), the synthesized spherical HAP powder attained from ultrasound technique with various amounts of (AA) concentration (0.5g, 1g, and 1.5 g) respectively. All the four samples showed, the major characteristic peaks detected at 2θ values of 25.73, 28.86, 30.86, 32.35, 34.32, 46.77 and 52.88 corresponding to (002), (210), (211), (112), (202), (222) and (004) planes of hexagonal HAP) [23]. It is evident that all major diffraction peaks correspond to the hydroxyapatite structure, with no additional crystalline phases detected. The sharp and distinct peaks indicate a high degree of crystallinity in the synthesized HAP. Furthermore, the observed diffraction pattern closely matches the standard JCPDS data (09–0432) [24]. The particle size (D) of the hydroxyapatite powder synthesized with 1.5 g of ascorbic acid was found to be approximately 28 nm, which is smaller compared to the other samples. The particle sizes were calculated using Scherrer's Equation (1). The grain sizes of both pure HAP and ascorbic acid-assisted HAP powders are summarized in Table 2.

$$D = K\lambda / (\beta \cos \theta)$$

Eq. (1)

where D is the crystallite size (nm); λ the wavelength of the monochromatic X-ray beam (nm) (λ=1.5418 Å for Cu Kα radiation); β is the full-width half maximum (FWHM) for the diffraction peak under consideration; and Θ the diffraction angle [25].

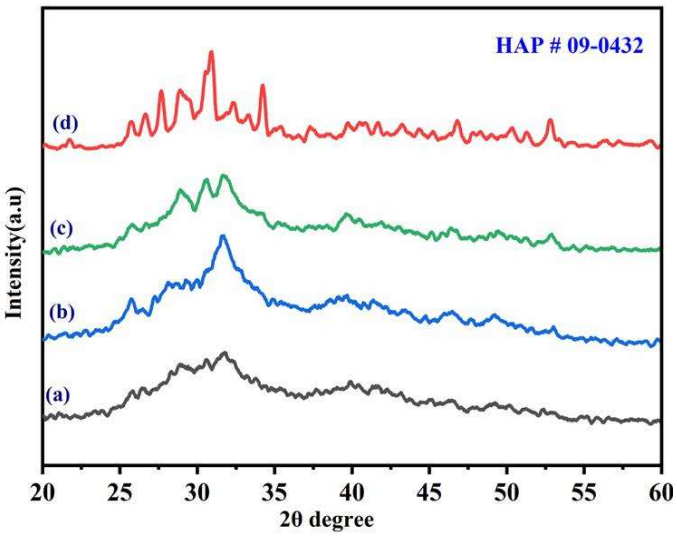

Fig.2. XRD pattern of hydroxyapatite crystal prepared at various concentration of Ascorbic acid: (a) 0 g AA, (b) 0.5 g AA (c) 1 g AA and (d)1.5 g AA

Table: 2 parameters of the as synthesized nano HAP

Sample code	**Average grain size (nm)**
HAP	45
HAP with AA (0.5g)	39
HAP with AA (1g)	34
HAP with AA (1.5g)	28

3.3. Scanning electron microscopic studies

Fig. 3 (a-d) shows the surface morphology of synthesized spherical HAP powder using ultrasound method with different amounts of (AA) concentration (0.5g, 1g, 1.5g respectively). The hydroxyapatite powder obtained without the addition of Ascorbic acid and lower concentration of AA presented less uniformity in size with the particles combined together resultant in high numbers of disordered and aggregated particles with irregular morphologies **(Fig. 3 (a, b)).** As presented in **Fig. 3(c, d),** the effect of Ascorbic acid on HAP particles was found to be very useful as it resulted in an approximately homogeneous powder with even morphology, a smaller sized particle with better spreading when compared to hydroxyapatite samples achieved without additive and lower concentration of AA [26,27]. By calcination, the impurities are removed from the above powder, so the calcium phosphate is converted to pure HAP [28]. Finally, the SEM image showed that the AA concentration of 1.5g were optimal for the preparation of uniform spherical morphology of HAP powder.

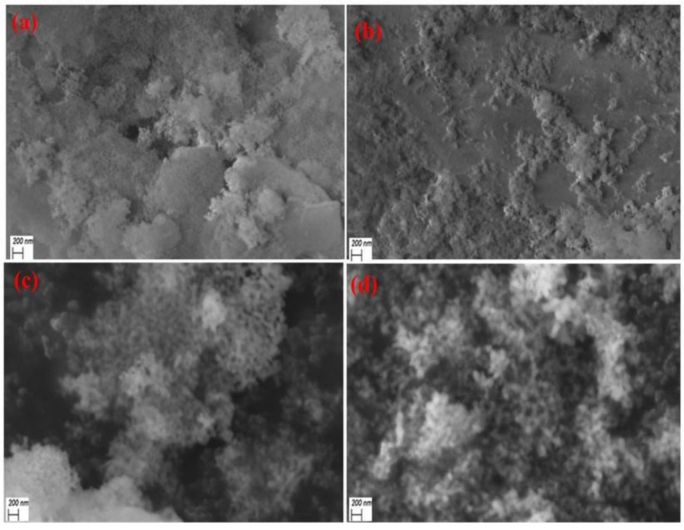

Fig. 3 SEM images of the HAP crystal prepared at various Ascorbic acid concentrations: (a) 0g AA, (b) 0.5 g AA (c) 1 g AA and (d)1.5 g AA

4. Conclusions

In the current work, HAP crystal has been successfully synthesized by ultrasound method with different concentration of ascorbic acid. By this route, the characteristic peak of phosphate and hydroxyl group occurred in FTIR spectra and no impurity peeks was detected. The XRD studies have revealed that, the AA-hydroxyapatite powders are well crystallized with hexagonal structure with no impurity phases. Ascorbic acid plays a main role in formation of hydroxyapatite particles because it controls the morphology and reduce the particle size. The SEM results showed that the decrease of particle size and morphology control was attained resulting in hydroxyapatite powder with spherical shape. The attained AA-HAP crystal could well serve as promising applicant in biomedical applications and hence this technique could be a novel approach to synthesis of hydroxyapatite powder.

Conflict of interest

The authors declare that they have no conflict of interest.

Availability of Data

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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