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Synthesis and Characterization of Hydroxyapatite Powder with Alanine and Silica Gel for Biomedical Applications

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Abstract

Hydroxyapatite (HAP) is a widely used bio ceramic material due to its excellent similarity to natural bone. In this research work, HAP powder was synthesized by hydrothermal method using alanine and silica gel as additives. Alanine was used to regulate crystal growth and promote bioactivity through organic–inorganic interactions, while silica gel served as a silicate source to improve osteoconductivity. The synthesized powders were characterized by XRD, FTIR and SEM. The results shows that the presence of alanine and silica gel influenced HAP crystallinity, morphology and surface area. The results obtained suggest that the combined use of alanine and silica gel provides a promising route to develop HAP powder for bio medical applications.

Keywords: Hydroxyapatite, Alanine, Silica gel, Bio-ceramics, Biocompatibility

1. Introduction

Hydroxyapatite (HAP), represented by the chemical formula Ca10(PO4)6(OH)2 is a calcium phosphate bio-ceramic that closely associates with the mineral constituents of human bone and teeth. Hydroxyapatite (HAP) is extensively utilized as an implant material owing to its superior biocompatibility, excellent bio affinity and resemblance to natural bone properties, thereby gaining significant importance in orthopaedics and dental material development. [1] HAP exhibits excellent biocompatibility, being non-toxic, non-irritant, non-allergenic, non-mutagenic and non-carcinogenic. Moreover, nano-hydroxyapatite can actively interact with bone tissue, promoting chemical bonding that enhances osteoconductivity [2]. Different types of synthesis techniques have been developed to synthesize nano-hydroxyapatite, such as ultrasonic assisted irradiation [3], precipitation [4,5], polymer assisted synthesis [6,7] solgel [8], microemulsion [9,10] microwave method, [11,12]. However, many of these synthesis methods are often associated with inherent limitations, such as inadequate control over particle size, broad size distribution, inconsistent morphology and particle agglomeration. These challenges can be effectively addressed through the hydrothermal synthesis process. This technique, which operates under elevated temperature and pressure conditions, offers significant advantages by enhancing the crystallinity and purity of hydroxyapatite (HAP) [13], thereby making it a highly efficient and reliable method for the production of nanomaterials. However, hydroxyapatite (HAP) alone is not suitable for the use as a bone substitute in load-bearing applications or in cases involving extensive bone defects due to its low flexural strength, limited elastic properties, and high brittleness.

These mechanical limitations prevent it from replacing the functional requirements of natural bone tissue. [14,15]. To overcome these drawbacks, various methods have been employed to modify HAP through the assistance of additives to improve the physicochemical and biological properties of HAP. Amino acids, such as alanine, have gained significant role as organic modifiers due to their

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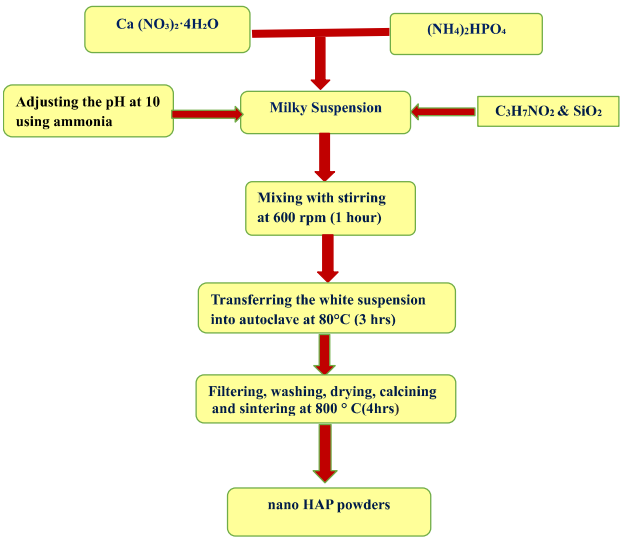
potential to affect calcium and phosphate ions during HAP formation. These interactions can improve nucleation, crystal growth, morphology and surface area of the resulting HAP [16]. Alanine a non-polar and biocompatible amino acid that has been known to enhance the biological performance of hydroxyapatite (HAP)-based materials by promoting protein adsorption and facilitating cellular attachment [17]. Silica gel, a readily available source of silicate ions, offers a cost-effective and efficient approach for silicon incorporation in the synthesis of hydroxyapatite (HAP). The substitution of silicate ions within the HAP lattice has been shown to stimulate osteoblastic activity, enhance bone mineralization, and accelerate in vivo degradation, thereby establishing its significance in the modification and functionalization of HAP-based biomaterials.[18]. In this study, nano-hydroxyapatite (HAP) powder assisted with alanine and silica gel was synthesized using a hydrothermal method. This synthesis approach effectively produced nano-HAP with uniform surface morphology and high crystallinity, highlighting its potential for successful application as a biomaterial. This biomaterial could serve as a promising candidate in orthopaedic applications.

2. Experimental methods

2.1. Chemicals and Reagents

Hydroxyapatite (HAP) was synthesized using calcium nitrate tetrahydrate [$Ca(NO_3)2.4H_2O$] and diammonium hydrogen phosphate ($(NH4)_2HPO_4$) as precursor materials. Ammonia (NH_3) was employed to adjust the pH of the solution. All reagents, including alanine and silicon dioxide (SiO_2) used as additives, were procured from Merck, India, and were of analytical reagent (AR) grade. Double distilled water was used throughout the experimental procedures.



Scheme 1. Flow chart of HAP synthesis with $C_3H_7NO_2$ & SiO_2

2.2 Synthesis of Hydroxyapatite Powder

In a standard experiment, 0.04 M of calcium nitrate tetrahydrate was dissolved in double distilled water and 0.024 M of diammonium hydrogen phosphate dissolved in water was added dropwise under continuous mechanical stirring to maintain a 1.67 Ca/P ratio. Alanine was dissolved in double distilled water and DMF (Dimethylformamide) was used to dissolve SiO2, while ammonia was employed in order to maintain the pH at 7-8. The obtained mixture was vigorously stirred for an hour at 600 rpm using a mechanical stirrer. The resulting calcium phosphate solution, alanine and SiO2 reaction mixture was transferred to a Teflon-lined autoclave and heated to 80°C for three hours. The precipitate was then separated by centrifugation and subjected to washing with water-ethanol (1:1) to remove any remaining unreacted components. Finally, the precipitate was dried in an oven and allowed to calcinate for 2 hours followed by sintering at 800°C for 4 hours to produce pure HAP.

2.3 Characterization

Perkin-Elmer Fourier transform infrared spectroscopy (FTIR) (India) was employed to find the functional groups

in hydroxyapatite, specifically the phosphate and hydroxyl groups. The analysis was conducted using the potassium bromide disk technique with 200 mg of KBr compressed with 2 mg of hydroxyapatite granules under hydraulic pressure. FTIR spectra were recorded in the range of 4000 to 400 cm-1. The X-ray diffraction (XRD) method (Bruker, Germany) was used to identify the crystal phases in synthesized hydroxyapatite sample. Powder X-ray diffraction (XRD) patterns were scanned over 2θ range of 10-80°.The widely recognized Debye-Scherrer equation was used to calculate the particle's size. The surface morphology of the hydroxyapatite was examined using scanning electron microscopy (A Carl Zeiss EVO 18).

3. Results and discussion

3.1 X Ray Diffractions (XRD) Studies

X-ray diffraction (XRD) analysis is a significant method to confirm the crystallographic structure and purity of developed HAP. The XRD patterns of hydroxyapatite powder without and with additives are displayed in Fig.1(a-d) and the peaks obtained are in good match with those of standard nano HAP (ICDD NO.09-0432) [19]. Fig.1(c) shows that the prominent diffraction peaks at 2θ degree of 25.84 ,28.96,31.80, 34.08,39.54,46.39,49.55,53.11 are able to be indexed as (002), (210), (211), (202), (212), (213), (312) and (004) shows the presence of distinctive HAP phase peaks, confirming the development of HAP structure in the composites. The peaks observed indicate that the synthesized HAP has nanoscale crystallinity. The particle size of the alanine and silicon dioxide assisted hydroxyapatite powder is calculated by using Scherer's Eq. Table.1 displays the grain size of blank HAP and additive assisted HAP. Finally, the obtained result shows that the development of pure phase of alanine and silica gel HAP.

D = Kλ / (β cos θ) Where λ is wavelength of X-ray (1.5406°A), b is FWHM in radian, and θ is Bragg's angle in degree

Table -1

The calculated average grain size of HAP powders using Scherer's equation

Sample code (nm)	hkl values	Average grain size
HAP	(002), (211), (212), (312),(222)	32
Alanine & SiO_2 (0.4g) (0.1g)	(002), (211), (300), (212), (213)	33
Alanine & SiO_2 (0.2g) (0.05 g)	(002), (211), (300), (222), (213)	30
Alanine & SiO_2 (0.2g) (0.2 g)	(002), (211), (300),(212),(222)	38

Fig. 1. XRD pattern of (a) Blank HAP (b) 0.4 g Alanine & 0.1 g SiO2 (c) 0.2 g Alanine & 0.05 g SiO_2 (d) 0.2 g Alanine & 0.2 g SiO_2.

3.2. FTIR Spectroscopic Studies

Fourier transform infrared spectroscopy was employed to identify the functional groups present in hydroxyapatite (HAP), within the spectral range of 400 to 4000 cm−1 All the peaks in the FTIR spectra of hydroxyapatite samples are attributed to vibrations from the phosphate and hydroxyl groups of HAP and the resulting powders with and without additives are presented in Fig.2 (a–d) [20]. The peak shown at 949–967 cm−1 can be assigned to symmetric stretching mode of PO43- group, ν1, the highest IR absorption band in the region of 1042–1079 cm−1 can be assigned due to the ν3 asymmetric stretching vibration of phosphate group. The observed peaks at 573–629 cm−1 is due to asymmetric bending mode PO43- ν4 [21,22]. The bending mode of H2O is represented by the absorption band at 1626 cm^{-1}, while adsorbed water is indicated by the large peak at 3416 cm^{-1}. The stretching and bending modes of OH are linked to peaks at 3448 -3569 cm^{-1} respectively [23–24]. Carbonate groups, which are created when atmospheric carbon dioxide is captured during the stirring and calcination processes, are responsible for the peaks in the

1437–1450 cm^{-1} ranges. Thus, the purity of the resulting powder was confirmed by the FT-IR spectra, which show no signs of impurities. All the peaks in FTIR spectra for the samples of hydroxyapatite are assigned to vibrations owing to the phosphate group and OH group of HAP.

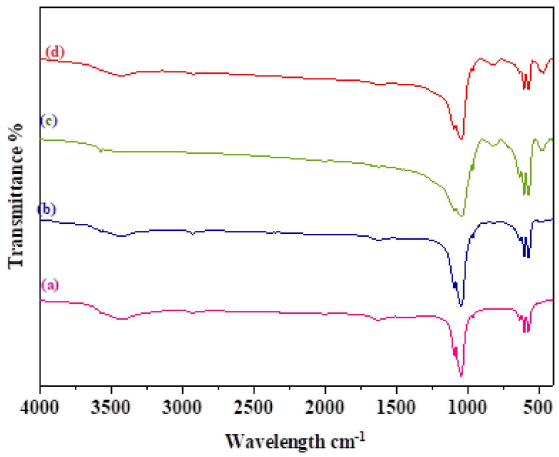


Fig. 2. FTIR pattern of (a) Blank HAP (b) 0.4 g Alanine & 0.1 g SiO_2 (c) 0.2 g Alanine &0.05 g SiO_2 (d) 0.2 g Alanine & 0.2 g SiO_2.

3.3 Scanning Electron Microscopic Studies (SEM)

The SEM images indicate that the powders synthesized in the presence of alanine and SiO2 demonstrate improved structural properties. It is noticed that HAP synthesised with alanine and SiO2 has contributed improved spherical morphology as seen in Fig. 3 (b). Hydroxyapatite powder without additives as shown in Fig. 3 (a) lacks uniformity, more agglomerated particles with larger size. But the addition of alanine and SiO2 has effect on the morphology of synthesised hydroxyapatite particles with uniform morphology, smaller particle size, with decreased agglomeration and with better dispersion. The images reveal that the synthesized alanine and silica gel assisted hydroxyapatite powders exhibit a spherical morphology. The spherical morphology of the synthesized hydroxyapatite (HAP) nanoparticles is considered advantageous for biomedical applications, as it can enhance the interaction between the implant material and the surrounding biological environment. This favourable morphology is attributed to the presence of alanine, which not only facilitates and promotes HAP formation but also acts as a filling agent during the synthesis process, contributing to the development of uniform nano-HAP powder.

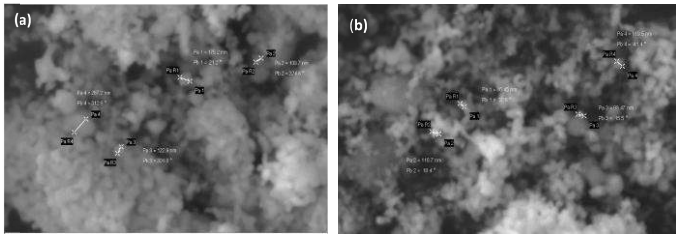


Fig. 3. SEM micrographs of HAP powders (a) Blank HAP (b) 0.2 g Alanine &0.05 g SiO_2

4. Conclusion

Nano HAP powders synthesised by hydrothermal method produced spherical elongated nano HAP with less agglomeration, uniformity and good morphology owing to the additives used in the synthesis. The formation of nano HAP was confirmed by FTIR which shows characteristic peaks with no impurities. XRD analysis confirmed, good crystallinity and elongated spherical shape of developed HAP. Compared to pure hydroxyapatite (HAP), amino acid-modified hydroxyapatite exhibits reduced particle size and increased specific surface area, making it a promising candidate for use as a drug delivery carrier in bone repair treatment. These properties highlight its dual functionality in promoting bone regeneration while providing therapeutic effects. Thus, it serves as a favourable material for biomedical applications.

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