Green Synthesis of Hydroxyapatite Nano Rods Using Camellia Sinesis (White Tea Extract)

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Abstract— **This study presents the physicochemical characterization of the additive-assisted size control of hydroxyapatite (HAP) nanorods for bone tissue engineering (BTE). Rod-Shaped HAP nanoparticles were synthesized through a simple route by hydrothermal treatment and with the assistance of the Tea extract. Powder X-ray diffraction (XRD), Fourier Transform Infrared Spectroscopy(FTIR), and Energy-Dispersive X-ray spectroscopy(EDAX) were used to characterize the structure and composition of the HAP samples. Results of the synthesized TEA extract assisted HAP showed regular rod-shaped HAP nanoparticles while observing its morphological features using FESEM and HRTEM. The results also revealed HAP with a mean length of 40 nm and a mean width of 15 nm was successfully produced by this method. TEA extract possesses an excellent antioxidant property as evident from UV-Studies.**
Keywords-component; Hydroxyapatite , Nanorods , CAMELLIA SINESIS

I. INTRODUCTION

The chemical composition of hydroxyl apatite (HAP) is very close to that of human hard tissues, such as bone and teeth and has been widely used in various biomedical fields [1,2].Due to similarity in chemical composition, the HAP ceramic has ability to bond directly with the bone tissues. It also possess excellent biocompatibility, bioactivity and osteoconductivity. These properties render HAP as an ideal material for dentistry, orthopedics, maxillofacial surgery and also as drug delivery agents. [3,4]. Due to the nano size, the HAP acquire a large surface area which favours the resorption by osteoclasts [5-8]. Different shapes of HAP nanoparticles can be synthesized by a variety of methods such as co-precipitation [9], hydrothermal [10], sol–gel [11–13], freezing [14,15], ultrasonic irradiation [16] and reverse microemulsion [17] etc,. Recently, the template assisted synthesis is gaining importance for the synthesis of nano particles. [18,19]. However, the toxicity of most of the organic templating agents, researchers has a special interest towards the economic and eco friendly green synthesis [20-25]. Currently, synthesis of HAP nano particles using natural products has gained much attention and emerged to be an active research area in the field of nanotechnology.

Therefore, in this work we report a plant extract mediated green synthetic approach for HAP nanoparticles by using extracts from fresh leaf buds of tea (Camellia Sinensis) which can act as a reducing, stabilizing and capping agent [26. This synthesis is a simple, low cost, stable for long time, and suitable for large scale commercial production. Camellia Sinensis, commonly known as white tea is the species of plant whose leaves and leaf buds are used for production of Chinese tea [26]. Tea leaves contain many compounds such as polysaccarides, volatile oils, vitamins, minerals, purines, xanthine alkaloids (e.g. caffeine, theophylline, theobromine) and polyphenols of the flavonoid type such as theaflavins, catechins [26]. Most of these compounds are reported for their ability to produce nano particles..

II. MATERIALS AND METHODS

A. Chemicals and Materials

Reagent grade calcium nitrate tetra hydrate ($Ca(NO_3)_2.4H_2O$), ammonium dihydrogen ortho phosphate, liquid Ammonia,from Aldrich chemicals were used and white tea extract from symrise chemicals were used.

B. Synthesis of HAP

In hydrothermal synthesis, a solution of 1M calcium nitrate and 0.67M of $NH_4H_2PO_4$ solution was prepared by using distilled water and the pH was maintained at 11.0 using aq.ammonia. Camellia sinesis extract was used as

an additive. Varying quantity of tea extract (1,5,10,25mL) was added to the 1M ($Ca(NO_3)_2.4H_2O$) and the solution was continuously stirred for 1 hour followed by drop wise addition of 0.67 M of $NH_4H_2PO_4$ (pH 11.0). The aqueous solution was continuously stirred for about 2 hours. The white precipitate obtained was placed in an autoclave for hydrothermal treatment at 180^0c for 2hrs. The product was filtered washed and dried in an oven at 80ºC for 24 hr. The HAP powder thus obtained were ground with a mortar and pestleand calcined at 800ºC in a conventional furnace under an air atmosphere for 2 hr.

C. Characterization

A Schimadzu FT-IR 8300 series was used for recording IR spectrum for HAp an polymer composites. The samples were scanned at the range of 4000 to 400 cm-1 by KBr pellet technique. Phase analysis was performed using BRUKER D8 advance X-ray Diffractometer (XRD).Synthesized HAp powder were characterized using XRD to determine the fraction of crystallinity, crystallite size, specific surface area. The crystallite size of the sample was calculated from the Scherrer's equation [12],

$$Xs = 0.9\lambda / \beta\cos\theta$$

Where, λ is the wavelength CuKα radiation source (λ=1.54A°)
β is the full width half maximum,
θ is the angle of diffraction
The morphology of the sample was investigated using FESEM (HITACHI SU6600) and HRTEM (HRTEM-FEI,TECHNAI G2,30s-twin D905).

III. RESULTS AND DISCUSSIONS

A. FTIR analysis

A Schimadzu FT-IR 8300 series FT-IR spectrophotometer is used to determine functional groups. The FTIR spectra of HAP and WTE-HAP were shown in Fig.3.1. FTIR spectral pattern indicated that both fresh and calcined samples correspond to HAP. The bands at 3572 cm^{-1} is characteristic of structural OH of HAP. The stretching vibrational modes of $PO4^{3-}$ group expected at 1093,1036 cm^{-1} were merged together to form a broad peak and a minor peak at 960cm^{-1} shows the presence of phosphapte group. The peak at 1624cm^{-1} was related to the presence of adsorbed water, present only in fresh samples. However, the bands at 1479 and 876cm^{-1} were related to the presence of carbonate ions and may be due to the handling of the substrate in the atmospheric air. The peak observed at 1560cm^{-1} can be matched with the organic moieties present in WHITE TEA EXTRACT.

B. Raman Analysis :

The Raman spectrums of hydrothermally treated samples were shown in Fig.3.2. The band at 960 cm^{-1} was assigned to the strongest v_1, P–O stretching mode of HAP. Bands observed in the region 1,000–1,150 cm^{-1} were attributed to v_3, P–O stretching mode. The splitting of peaks at 864 and 881 cm^{-1} corresponds to HPO_4^{2-} [27].The bands at 426, 527 and 554 cm^{-1} were assigned to O–P–O doubly degenerated bending mode. The peaks at 588 and 615 cm^{-1} were attributed to v_4, O–P–O triply degenerated bending mode. The splitting of peaks at 1026, 1044, 1067, 1097 and 1112 cm-1 suggested the co-existence of HAP ,α- and β-TCP [27].It is inferred that the increase in quantity of WTE enhances the induced fluorescence effect of the as-prepared sample while in case of sintered sample the intensity of the peak decreases with the increase in concentration of the WTE.

C. X-Ray Diffraction:

The as-prepared and additive used samples were subjected to XRPD. The data (Fig.3.3) on the hydrothermally treated samples revealed a mixture α-TCP and β-TCP. β-TCP produced a characteristic shoulder peak which was observed in all the hydrothermally treated samples of HAP [27] . The XRD data of synthesized samples were matched with JCPDS-ICDD # (09-0432 corresponding to hexagonal phase ie., a=9.432 ,c=6.881,space group p63/m. All the synthesized samples with varying quantities of extract were in good agreement with ICDD values showing hexagonal phase which leads to the pure phase of HAP.There was no evidence for the presence of other phases except crystalline phase of HAP and TCP.
The intensity of all the peaks observed with samples analyzed before sintering were found to decrease with increase in quantity of extract and also even after dried at 110^0C the same pattern persist, which signifies the stability. The observed narrow peaks represents there is good agreement for crystallinity in the samples of HAP. However, the presence of additives reduces the crystallinity, this is due to the suppressing act by high concentration of the additives. Lesser quantity of bio-resorbable materials like α and β-tri-calcium phosphate are supportive for the formation of strong and fast bonding of the contrived bones. However, enormous amount of β-TCP will boot extravagantly resorption in human body [27]. The presence of trace levels of these compounds in the present synthetic approach is commendable.

D. FESEM and HRTEM:

The morphologies observed with FE-SEM and HR-TEM for as prepared and sintered HAP samples synthesized with varying quantities of white tea extract were illustrated in Fig. 3.4. The FESEM results revealed agglomerated particles with varying dimensions for both HAP synthesized without tea extract. The tea extract additions aided samples showed agglomerated cloudlike morphology for all the samples before sintering. However, sintering of these samples resulted in formation of nanorods. Higher quantity of the extract resulted in a well defined nano rods with orderly arrangement. This may be attributed to the influence of tea extract in the synthesis procedure. The HR-TEM results also showed uneven particles in micro scale range for HAP synthesized and sintered without extract, whereas the sintered HAP prepared with higher quantity of tea extract produced well defined orderly nano rods.

E. INDENTIFICATION OF REDUCING PROPERTY OF TEA EXTRACT:

The reducing ability of tea extract was ascertained treating it with an oxidizing agent,$KMnO_4$ using UV-Vis spectroscopic technique. The results were shown in Fig. 3.5, which indicated an absorption peak at 266 nm for extract alone, which may due to the presence of organic moieties. A similar study was carriedout with 0.01% KMnO4 alone, which indicated two peaks at 551nm and 526 nm corresponding to respective Mn^{7+} and Mn^{5+} oxidation states. Further, the equal volume of 1% extract and 0.01% of $KMnO_4$ were mixed together and the mixture is subjected to UV-Vis analysis. The results of this investigation revealed substantial decrease in intensity of peak corresponding to 266 nm and absence of peaks at 551 and 526 nm. This may be concluded that the Camellia sinesis has the high reducing property.

Conclusion

In this study HAP nanorods has been synthesised by Hydrothermal method with the help of white tea exttract. Reducing property of the tea extract is examined by spectroscopic analysis.Functional group analysis has been done for both white tea extract HAP and sintered sample. Raman analysis revealed that the HAP mixed with tea extract exhibit the induced fluorescence effect, such an effect was missing with sintered HAP sample.Morhological studies for the samples showed that HAP synthesised without tea extract shows the particles are irregular shape, whereas the tea extract aidded HAP produced a regular orderly arranged nanorods. Moreover, this study is a positive addition to the ongoing research on the preparation of HAP nanostructures using plant extract to reduce the particle size and also headed for the development of biocompatible composite scaffolds for BTE applications.

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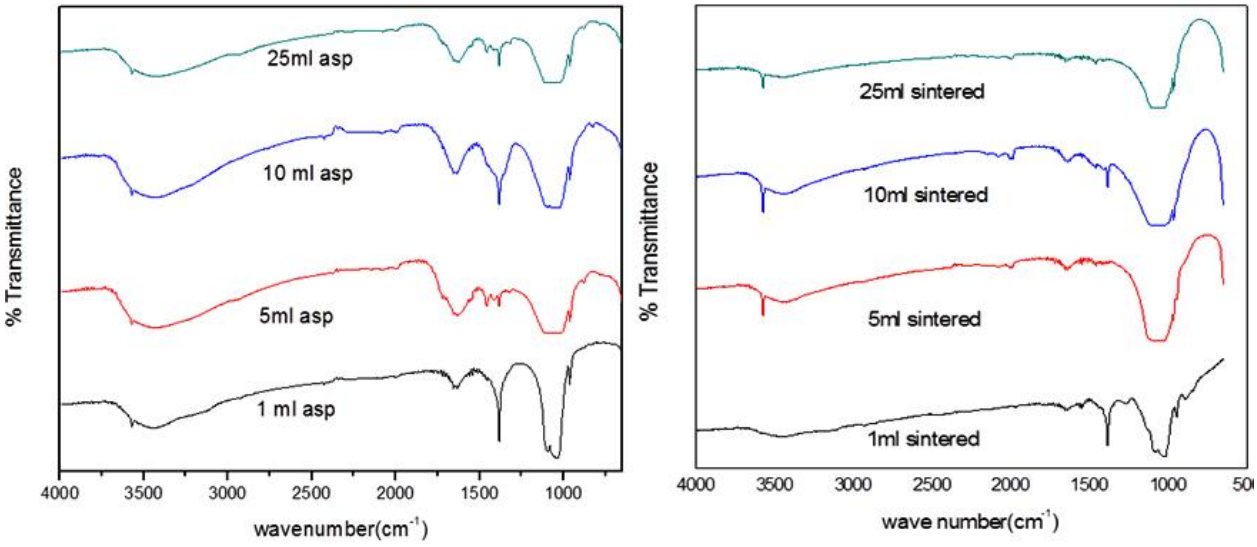


FIGURE -1

FIG.3.1.REPRESENTS FTIR ANALYSIS OF WTE-HAP AND SINTERED HAP

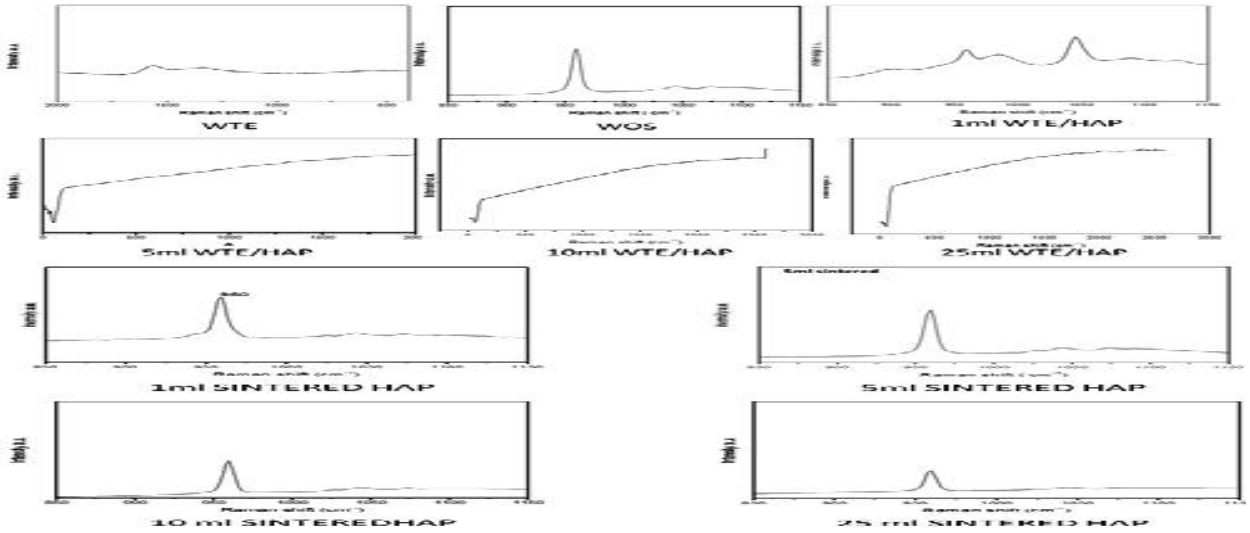


Fig.3.2.REPRESENTS THE RAMAN ANALYSIS OF THE SAMPLES

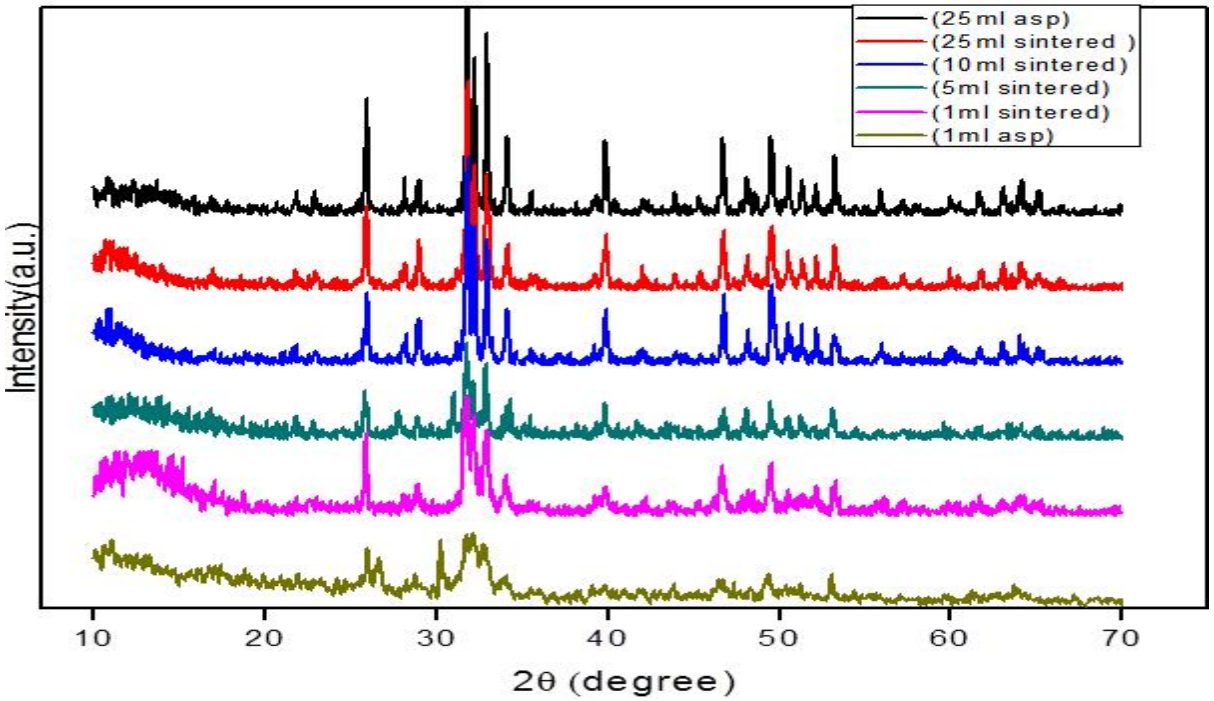
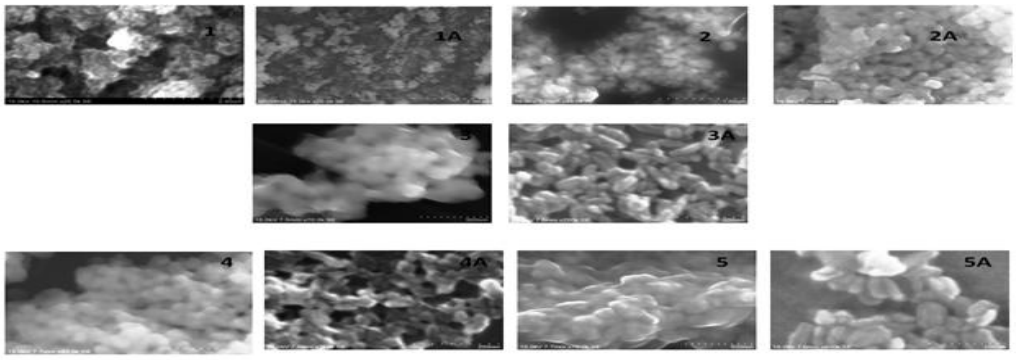
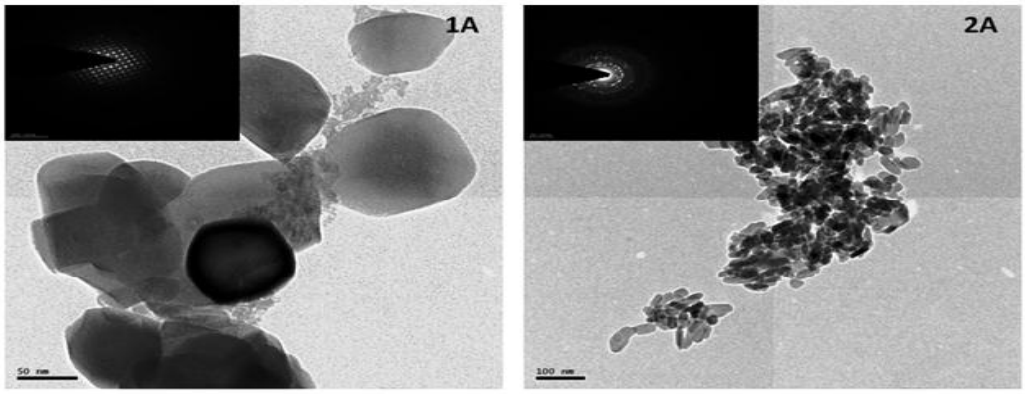


FIG:3.3.X-RAY DIFFRACTION ANALYSIS:



(1)-WOS, (2)-1ml WTE/HAP, (3) -5ml WTE/HAP, (4) -10ml WTE/HAP, (5)-25ml WTE/HAP , A-ALL SINTERED SAMPLES



HRTEM 1A-WITHOUT WTE SINTERED SAMPLE 2A-WITH WTE SINTERED SAMPLE

FIG.3.4. FESEM AND HRTEM IMAGES OF HAP SYNTHESISEDWITH AND WITHOUT WHITE TEA EXTRACT:

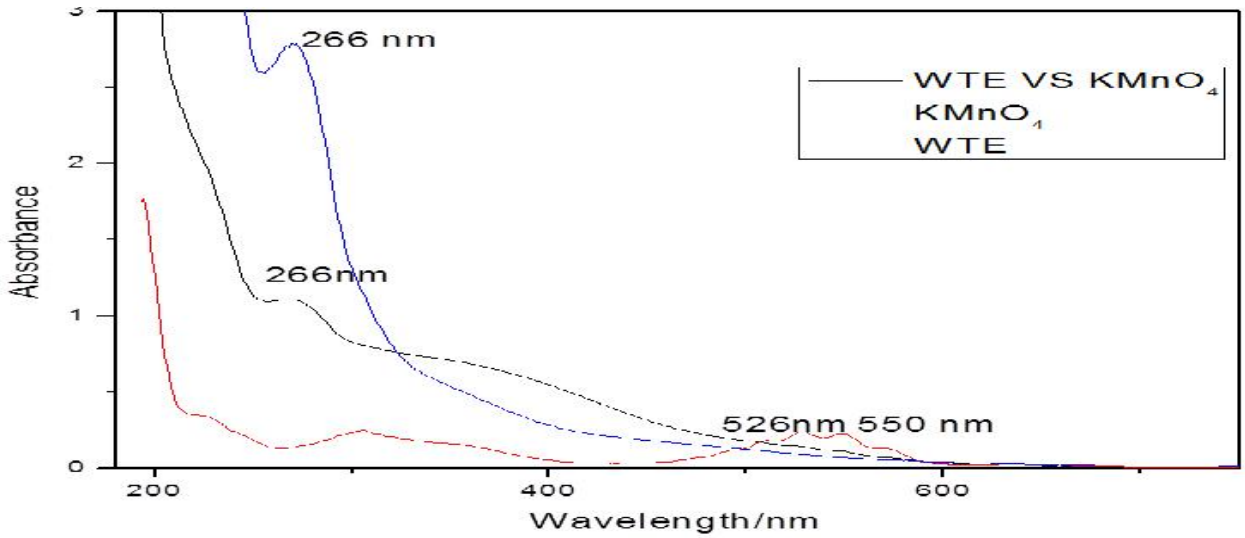


FIG:3.5. REDUCING PROPERTY FOR WTE:

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