Experimental studies on caffeine mediated synthesis of hydroxyapatite nanorods and their characterization

R Subramanian 1,2, P Murugan 3, G Chinnadurai 1, K Ponmurugan 1 and Naif Abdullah Al-Dhabi 4

1 Department of Chemistry, Sun Arts and Science College, Tiruvannamalai 600675, Tamil Nadu, India
2 Department of Chemistry, K.S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode 637 215, Tamil Nadu, India
3 Department of Chemistry, Sacred Heart Arts and Science College, Perani, Villupuram 605652, Tamil Nadu, India
4 Department of Botany and Microbiology, College of Science, King Saud University, PO Box 2455, Riyadh, 11451, Saudi Arabia

E-mail: subu_m1@yahoo.com

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Abstract
Hydroxyapatite nanorods (HA) were fabricated using caffeine as a modifier at room temperature. Various concentrations of caffeine were utilized to synthesize the HA nanoparticles to analyze its effect on size, shape and morphology. The formation of phosphate ion was confirmed by Fourier Transform spectrophotometer (FTIR). Crystalline structure and phase of the nanoparticles was confirmed by x-ray diffraction study (XRD). The formation of HA nanorods with diversified morphology was confirmed by Transmission Electron Microscope (TEM) images. The results acquired in this study reveals that the HA nanoparticles synthesized using caffeine are uniform and evenly scattered nanorods. The average size of the nanorods obtained from the XRD study was in the range of 20–30 nm. The TEM and SAED investigation revealed the shape, morphology and arrangement of HA nanorods with higher crystallinity. This investigation claims that caffeine is a natural modifier to produce HA nanorods.

1. Introduction
Hydroxyapatite (Ca10(PO4)6(OH)2) is composed 70% apatite calcium phosphate and remaining 30% of natural materials [1]. The structure and properties of hydroxyapatite nanoparticles are similar to the human bones. It has been widely used in orthopaedic applications such as dental inserts, bone tissue materials and fillings [2, 3]. The properties and morphology of HAp are interlinked with each other. Effect of pH, temperature and organic modifiers influenced the morphology of HAp NPs [4]. The morphology parameter of hydroxyapatite very much influenced by chemical substances as surfactants such carboxymethyl cellulose (CMC) and ethylenediammine tetraacetic acid (EDTA) [5, 6]. Previous reports have shown that the CTAB, Sodium dodecyl sulfate (SDS) and mixtures of surfactant CTAB are used as surfactants [7–9]. Plant extracts mediated green synthesis of metal nanoparticles becomes popular nowadays due to the eco-friendly methods. Green synthesis of HA got extensive scope because of its eco-friendly. Reducing, capping and stabilizing properties of many plant extracts such as Moringa oleifera leaf, gum-arabic, grape seed, banana, tamarind extract, Coccinia grandis and Azadirachta indica, orange, and potato peel, papaya leaf and calendula flower [10–16]. When an aqueous plant extract is used a green reducing agent during the synthesis of metal or metal oxide nanoparticles, explaining the role of a particular phytochemical is not an easy one. To explore the role of organic modifier or surfactant, many researchers have synthesized metal and metal oxide nanoparticles and revealed their functions in the formation and morphology. The role of organic modifiers in the nucleation of HA nanoparticles explained by many researchers [17, 18]. Recently, molecular modeling of carbonated-HA, titanium/carbonated HA nanobiocomposites, carbonated HA and Carbonated-Fluoroapatite have been prepared by researcher and explored their applications [19–22].

Caffeine is commonly nitrogen containing heterocyclic compound present in tea leaves (Camellia sinensis). It involves around 4%–5% of the dry weight of tea takes off. Tea is common beverages consumed in India and around the globe. Caffeine is focal apprehensive stimulant [19]. Caffeine is in all likelihood the most generally
ingested pharmacologically dynamic stuff on the planet [20]. Caffeine, also affecting intellectual execution, improves the affectability of sharpness and attentiveness [21] and at times incites nervousness [22, 23]. Caffeine is demonstrated to incite nervousness [24, 25]. Caffeine is critical stimulants, consumed in daily life in the form of tea and coffee beverages. Caffeine is a strong bactericidal property against pathogenic bacteria [26]. Caffeine is one more acknowledged medication utilized for the incitement of focal apprehensive and cardiovascular framework and gastric corrosive discharge [27]. Thinking about the therapeutic properties of caffeine, an exertion is made to analyze its part in the combination of hydroxyapatite. In the present work, an attempt has been made to study the role of caffeine on the formation of HA nanoparticles synthesized via chemical precipitation method.

2. Materials and methods

2.1. Reagents

The following chemicals were purchased from Loba Chemie, Mumbai, India: Calcium nitratetetrahydrate (Ca(NO₃)₂H₂O), diammonium hydrogen phosphate (NH₄)₂HPO₄), ammonium hydroxide (NH₄OH, 25%) and dichloromethane. The purchased chemicals samples were used without any further purification. Double distilled water was used as the carrier solvent to make the metal salt solutions.

2.2. Isolation of caffeine

Commercial tea dust purchased from the local market for the isolation of caffeine. Around, 50 g of tea dust was thoroughly boiled with distilled water. After cooling, the filtrate was filtered using the muslin cloth followed by filter paper. The filtrate was poured into 250 ml separating flask and to this 50 ml of dichloromethane was added. After shaking well, the organic layer was removed and the same process was repeated for few times for better yield. The organic layer was left at Laboratory at room temperature to yield the caffeine. A similar process was repeated for three times to get enough amount of caffeine [28].

2.3. Synthesis procedure

Calcium nitrate (1.0 M) and diammonium hydrogen phosphate (0.6 M) (NH₄)₂HPO₄ solution were prepared separately and their pH values were adjusted to 12 using NH₄OH solution. Various concentrations of caffeine, 5, 10 and 20 mg were added into calcium nitrate solution and ensured the dissolution of the caffeine in aqueous calcium nitrate solution. The hydroxyapatite samples synthesized without caffeine, with 5, 10 and 20 mg of caffeine was labeled as HA, HA5, HA10 and HA20, respectively. The caffeine containing calcium nitrate solution was slowly mixed diammonium hydrogen phosphate solution with constant stirring for 12 h at room temperature. The precipitate formed was thoroughly washed with distilled water and centrifuged to obtain the precipitate. A pure HA sample was prepared without using caffeine for the purpose of comparison. The prepared samples were dried at 110°C for 48 h before characterization. A blank sample (without caffeine) was prepared for comparison.

2.4. Characterization

FTIR spectra of HA nanoparticles were taken by Perkin Elmer (Model: Spectrum RX I) FTIR spectrophotometer using a KBr pellet method. X-ray diffraction pattern of pure HA and HA synthesized using caffeine were recorded by PANalytical XPERT-PRO diffractometer system. The instrument was operated at 40 kV with 30 mA voltage and current setting. The x-ray diffraction pattern was recorded in the range 20 to 80° at with Cu Kα radiation as the x-ray sourced (1.5406 Å). TEM pictures were recorded by Jeol/JEM 2100 Transmission Electron Microscope (TEM) to find out the morphological aspects samples at varying magnification.

3. Results and discussion

3.1. FTIR studies

Caffeine, 5, 10 and 20 mg were used as an organic modifier to study the formation of the HA nanoparticles (Figure 1). The FTIR spectrum was used to identify the functional groups present in the samples. Figure 2(a)–(d) represents the FTIR spectra of HA, HA5, HA10 and HA20. Two broad peaks around at 3242 and 3044 cm⁻¹ assigned to the absorbed moisture content on HA. The characteristic peak for OH group vibration on HA was not clearly observed in the spectra. The intensity of the peak at 3242 cm⁻¹ decreased after the heat treatment of the sample at 800°C for 3 h [29, 30]. Besides, the weak peaks observed at 1419 and 1326 cm⁻¹ attributed to the absorption bands of CO₂²⁻, signifying the existence of carbonate ions due to atmospheric CO₂ [31, 32]. A weak band noticed at 2140 cm⁻¹ in all the samples was assigned to (v₃) asymmetric stretching of carbonate CO₃²⁻, ion impurity [5]. The small peaks located at 1025, 827 and 714 cm⁻¹ attributed to the PO₄³⁻.
3.2. XRD analysis

The x-ray diffraction study of HA nanorods fabricated using caffeine are shown in Figure 3(a)–(d). The XRD analysis shows that the HA nanorods fabricated using caffeine are highly crystalline in nature. A sharp, small peak noticed at 25.84° indicates the (002) Miller’s plane, which shows the nucleation of crystals initiated along the c-axis of the HA nanorods \[32\]. The existence of corresponding diffraction peaks at lattice planes (002), (211), (300) and (213) confirmed the presence of hydroxyapatite with a hexagonal phase structure, which matched with the standard (JCPDS No.9–0432).

The XRD peaks found to be notably sharper, which suggest that the nanorods synthesized using caffeine is nanosized. It was noticed that the diffraction peaks belong to the planes (002) and (300) are unchanged in blank HA, HA5, HA10 and HA20. The average crystallite size of blank HA, HA5, HA10 and HA20 calculated using the Scherrer’s formula was 25, 28, 32 and 35 nm, respectively. The size of the crystallites slightly found to be increased with increasing concentrations of caffeine from 5 mg to 20 mg.
Figure 3. XRD pattern of HA nanorods (a) Pure HA; (b) HA5, (c) HA10 and (d) HA20 synthesized with 5, 10 and 20 mg of caffeine.

Figure 4. Signify the TEM images of hydroxyapatite nanoparticles. HA (i), HA (ii)- TEM image at 20 nm and SEAD pattern of HA synthesized without caffeine. HA5 (i), HA (ii)- TEM image at 20 nm and SEAD pattern of HA synthesized without caffeine.
3.3. TEM pictures analysis

Figure 4 (HA-HA20) shows the TEM morphographs of HA synthesized with and without caffeine. The HA synthesized with and without caffeine compared with each other in terms of size, shape, and morphology. Figure 4 HA5 (i) represents the TEM images of the HA produced using 5 mg of caffeine. The TEM micrographs of the HA synthesized without caffeine is shown in Figure4 HA (pure HA (i)). The blank HA nanorods formed without caffeine was found to be aggregated. The shape and orientation of the HA nanorods was unclear as seen in the images. The selected area electron diffraction pattern (SAED) further provides evidence to poor crystallinity of HA synthesized without caffeine HA (ii).

Figure 5 HA10 and HA20 (i) reveals the TEM images of HA nanorods produced using 10 and 20 mg of caffeine. The shapes of the particles found to be improved in both samples. The morphology of the both samples (HA10 and HA20) found to be similar. By closely watching the TEM images, it is realized that the HA nanorods synthesized using 20 mg of caffeine found to be more crystalline than pure HA, HA5 and HA10.

The size of particles found in TEM images approximately matched with the size of the samples calculated from the XRD pattern of the respective samples. As the concentration of caffeine increased from 5 to 20 mg, appreciable changes observed in size, shape, and morphology of HA nanorods formed. It is understood that the HA nanorods started to grow into crystals when caffeine was used as an organic modifier. The HA nanorods synthesized using 10 and 20 mg of caffeine are more crystalline than that of blank HA. Apart from improving the crystallinity, caffeine also improves its shape along with size. The SEAD pattern of HA without caffeine show diffused ring with very smaller spots, which reveals the less crystalline nature of the HA. HA5 did not make a difference from HA without caffeine. Whereas, HA synthesized with 20 mg of caffeine show bright spots than HA, HA5 and HA10. The ring formed by bright spots reveals the more crystalline nature of the HA synthesized.
using Caffeine. From the results of TEM images, it is confirmed that the caffeine prevents the aggregation of HA nanorods. In similar manner, effect of citric acid and EDTA on the morphology of HA nanoparticle have been proved. They also prevented agglomeration of nanoparticles as stabilizing agent [5]. As an organic modifier, caffeine acted produces new surfaces by breaking the bond between the neighboring atoms which lead to the development of smaller particles by controlling the driving force between the nanoparticles [33].

It follows that at higher concentrations, caffeine plays a key role in the development of nanostructures, shape and size. Some researchers stated that the chelating agent is not compulsory for the creation of nanostructures of HA [34]. In our findings, we emphasize that the formation of well separated HA nanorods in particularly at two concentrations, 10 and 20 mg are directly controlled by the driving force. We come out with a reason; the strong interaction between the caffeine and the HA crystallites have caused the formation smaller rods [17]. Since caffeine is a water soluble molecule, it easily produces a homogeneous solution and initiates the nucleation process during the synthesis. The formation of uniform shaped nanorods obtained using caffeine revealed its role in this synthesis. The size and shape of HA nanoparticles seem to be varied by increasing the concentration of caffeine. The edges of the HA nanorods produced without caffeine found to be unclear. Whereas, the HA produced using 10 of caffeine found to be nanorods with clear in shape and size.

4. Conclusions

Hydroxyapatite was successfully synthesized using three different concentrations of caffeine. The synthesized HA nanorods were characterized by FTIR, XRD and TEM-SAED studies and confirmed the formation of nanoparticles. The present study reveals that the HA nanorods can be synthesized using caffeine as an organic modifier. FT-IR, x-ray diffraction studies and TEM image analysis revealed the formation of HA nanorods with high crystallinity with well defined shapes. The crystallinity of HA improved with increasing the concentrations of the caffeine content. The result derived from this study confirms that the caffeine evidently improves the shape and morphology of HA nanorods. The average size of HA nanorods was in the range of 25 to 35 nm are extremely useful to produce a large surface area and volume. Hence, caffeine could be used as modifier to design and develop different shape and size of hydroxyapatite nanoparticles.

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ORCID iDs

R Subramanian @ https://orcid.org/0000-0002-1983-1838

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