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Influence of particle size and morphology of chemically modified hydroxyapatite by sol-gel method

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Abstract : Hydroxyapatite (HAP) is an inorganic compound with the chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. It has been recognized as a substitute material for bone and teeth in orthopedics and dentistry due to their chemical and biological similarity to human hard tissue. Synthetic nano-sized HAP particles exhibit favorable biocompatibility and bioactivity and in order to better match the composition to natural HAP there is a great interest in producing a range of chemically modified hydroxyapatite powders. In this study, three different powders have been synthesized via an ethanol-based sol-gel method. Calcium nitrate tetra hydrate and P_2O_5 were employed as calcium and phosphorus precursors respectively. An aqueous solution of Ammonium hydroxide was added as dispersing agents to the reaction mixture. Separately, sodium hydroxide was added as a dispersing agent and the pH of the solution was modified as 10.5. Subsequent powder calcinations have been carried out within the temperature range of 900 °C and the resultant powders were characterized by AAS, Powder-XRD, FT-IR and SEM analysis.

Keywords: Hydroxyapatite, Glass ceramic, SEM, particle size, dispersing agent.

Introduction

The hydroxyapatite crystallites in human bone, enamel and dentin are extremely small in size and can be considered as nanostructured materials¹. HAP is the prototype of biological apatites and is known to accelerate bone growth, enhance bone fixation and extensive efforts have been made to produce synthetic nano HAP materials². The stoichiometric molar ratio of hydroxyapatite is 1.67; however, this is not the value observed in the organism because small amounts of other materials are incorporated (carbonates, fluorine, chlorine, magnesium ions etc.)³. However, the calcium phosphate presence in bone is in the form of nanometer-sized needle-like crystals of approximately 5-20 nm width by 60 nm length, with a poorly crystallized and non-stoichiometric nature. Despite the chemical resemblance to the mineral component of bone, the peculiar brittleness and low fracture toughness of HAP has restricted its usage in applications such as high load-bearing implants^{4,5}. Due to these diverse applications, the materials should be tailored for real world application. Hence researchers have tried to customize its properties such as bioactivity, mechanical strength, solubility and sinterability by controlling its composition, morphology and particle size⁶⁻⁸.

Ceramic powders can be prepared by several techniques such as ball milling, sol-gel processing, plasma arcing and chemical vapor deposition process. Of which, the sol-gel technique is an elective method for the preparation of highly pure ceramic due to the possibility of a strict control of the process. It has been widely used in the preparation of various inorganic materials because of the homogeneity due to atomic level mixing,

easy formation of crystalline films at relatively low temperature, which include heat treatment for calcination, sintering and crystallization, offering the possibility to tailor microstructures and its convenience for complex shape coatings^{9,10}.

Moreover, the high reactivity of the sol-gel powder allows a reduction of the processing temperature (refluxing) and of any degradation phenomena occurring during sintering. The major limitation to sol-gel technique application is linked to the possible hydrolysis of phosphates and the high cost of the raw materials¹¹. Both problems are solved in the present paper where a preparation of nano HAP powder via the sol-gel route is presented. The present paper focuses with the specific aim of producing calcium phosphate material of the required phase (hydroxyapatite) and suitable morphology (mono disperse, non-aggregating particles <100 nm). The synthesized powder was characterized by FT-IR, XRD, SEM, AAS analysis. It was found that by fine-tuning of the synthesis parameters and incorporating the dispersing agent phase-pure nano-sized particles less than 100 nm were attained, which may be suitable for clinical applications in bone regeneration.

Experimental

Preparation of Various Ceramic Materials by Sol-Gel Technique

All chemicals are purchased from Sdfine chemicals and used without purification for the synthesis of HAP by sol gel technique. Hydroxyapatite (HAP) powders were synthesized using a calcium nitrate tetrahydrate and phosphorous pentoxide as calcium and phosphorus precursors, respectively. Ethanol was used as a diluting medium for HAP sol preparation and sodium hydroxide and ammonium hydroxide was used separately to adjust the pH of the solution. In the first process, calcium phosphate synthesis involves a drop wise addition of 0.6M phosphorus precursor into a 1M calcium precursor and the pH of the solution was modified with NH₄OH. After addition, the sol was kept in a water bath to evaporate ethanol and the resultant gel was dried at 105 °C for 24 hrs. Finally, the powder was sintered at 900 °C for the duration of 2 hrs.

In the second method, the above process is repeated with same calcium and phosphorus precursors, but the pH of the solution was modified with NaOH. The resultant gel was washed with plenty of water to avoid the contamination of the powder with sodium ions. The obtained powders were compared for its crystallinity, high purity and its particle size determination.

Characterization of powder samples

The functional characteristics of the powders were performed by Fourier transform infrared spectroscopy (FT-IR) in the range of 400–4000 cm⁻¹ using a SHIMADZU model 8300 spectrophotometer by using a KBr pellet technique (0.1 weight%). The XRD patterns were recorded in an X-ray diffractometer (D8 model, BRUKER, Germany) with a step size of 0.02° and a scan rate of 10°/min with Cu K α radiation ($\lambda = 1.54056\text{\AA}$). The powders were subjected to SEM analysis for the structural and morphological behavior occurred on the surface. The instrument Philips 501 scanning electron microscopy was used for the analysis. The amount of Calcium and phosphorous was determined using Atomic Absorption Spectroscopy (AAS).

Results and discussion

Aging is an important factor for the formation of phase pure apatite. During aging there is an interaction between calcium and phosphorous precursors which will lead to the intermediate complex responsible for the development of pure apatite¹². The development of hydroxyapatite using sol-gel route is based on the hydrolysis and poly condensation reactions between the calcium and phosphorus precursors. Hence, the present study aims the development of different composition of ceramic materials without ageing in an oil bath. Figure 1. represents the flow chart for the preparation of ceramic materials.

Fourier Transform Infrared Spectroscopy (FT-IR)

Figure 2a. shows the FT-IR spectra of the powder sintered at 900°C for 2 hrs. The presence of bands at 3444 cm⁻¹ and 1654 cm⁻¹ has clearly seen in all the spectrum which indicates the presence of physically adsorbed water on the powder sample. The characteristic bands for P₂O₇⁴⁻ appeared in 450, 542, 682, 1008 cm⁻¹ respectively. Also, the main bands at 730 and 1218 cm⁻¹ indicates the presence of symmetric and asymmetric stretching of P-O-P vibrations in P₂O₇⁴⁻ ions¹³. So this spectrum confirms the formation of calcium pyrophosphate glass ceramic without any chemical modification. This is due to the incomplete reaction between the precursors and during sintering the incomplete residue decomposes into calcium pyrophosphate.

Figure 2b.represents the FT-IR spectra of the sample modified by NH_4OH . The results indicated the presence of well resolved peaks for HAP and $\text{P}_2\text{O}_7^{4-}$. The quartet peak with well resolved bands at 1218, 1096, 1085 and 1056 cm^{-1} identical to phosphate bands (P_2O_7) was observed. In addition to that the hydroxyapatite peaks at 571, 602 and 633 cm^{-1} corresponds to the triply degenerate γ_4 bending vibrations and the band at 472 cm^{-1} are attributed to γ_2 bending vibration, led to conclude that the sample has the accompanying phase of HAP.

Figure 2c.shows the FT-IR spectra of the sample prepared using NaOH as pH modifier. A significant increase in the intensity of the bands at about 1046 and 1090 cm^{-1} was observed. This fundamental mode corresponds to the triply regenerate asymmetric P-O stretching modes and the band at 960 cm^{-1} corresponds to the symmetric P-O stretching vibrations of the PO_4^{3-} ions. The distinguishable presence of these bands together with the bands at about 602 and 566 cm^{-1} corresponding to the triply degenerate γ_4 bending vibrations of the PO_4^{3-} ions indicate the increased quality of HAP at higher sintering temperatures¹⁴. Also, the results indicated the presence of OH^- , H_2O , PO_4^{3-} and CO_3^{2-} ions in the sample. The well resolved sharp peaks at 3641, 3567 cm^{-1} corresponds to the stretching vibration of the lattice OH^- ions. Hence, the same calcium and phosphorus precursors with and without the presence of pH modifiers was found to produce different nature of powders such as Calcium Pyrophosphate, Hydroxyapatite and combination of both phases respectively.

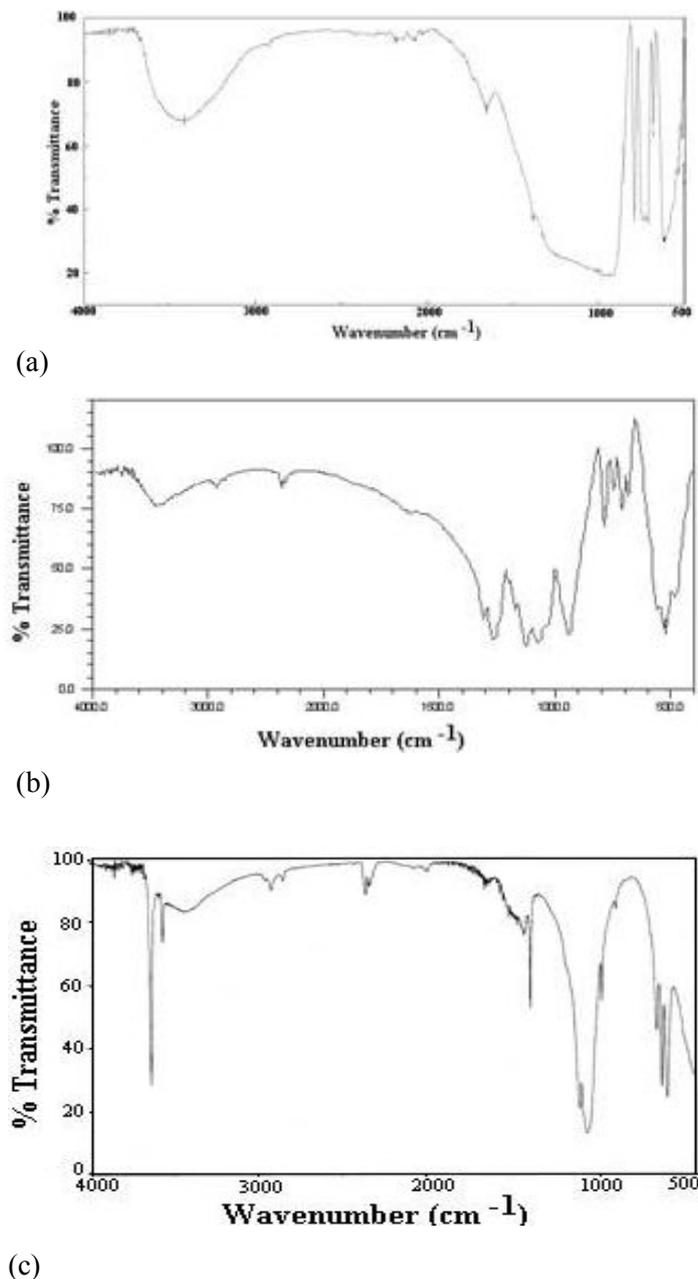


Figure 2 FT-IR Spectra of sol-gel powders modified (a) without modifiers (b) with ammonia (c) with sodium hydroxide

Powder X-Ray Diffraction Analysis (XRD)

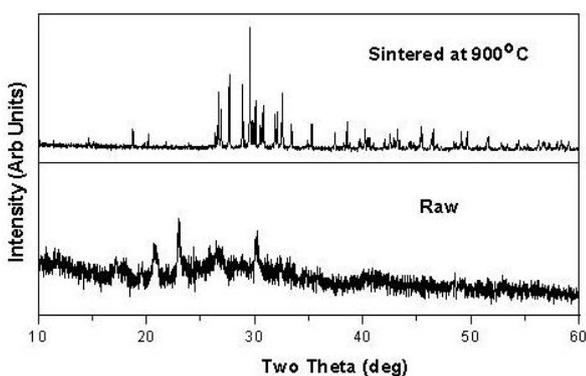
Figure 3. shows the XRD spectra for the raw powder sintered at 900 °C for 2hrs. The as dried powder was completely amorphous and the proportion of amorphous phase decreases with the increase in heat treatment temperature. As evident from the FT-IR analysis, the XRD spectra for the powder sample without any modification (Figure 3a.) have shown strong peaks such as 29.554, 28.870 and 27.681 respectively, which confirm the formation of β - $\text{Ca}_2\text{P}_2\text{O}_7$. All the other pyrophosphate peaks are clearly seen from the spectrum which exactly matches with the software. Figure 3b. shows the XRD pattern for the sample modified by NH_4OH , and the results indicated the presence of well-crystallized HAP and $\text{Ca}_2\text{P}_2\text{O}_7$ phases. The presence of $\text{Ca}_2\text{P}_2\text{O}_7$ peak at 27.681 and a strong peak at 29.554 together with the apatite peaks at 31.7331 and 32.1961 confirms the presence of combination of $\text{Ca}_2\text{P}_2\text{O}_7$ and HAP phases¹⁵.

Figure 3c. shows the XRD spectra for the powder modified with NaOH. A strong XRD peak at 31.7331 corresponding to the strongest intensity reflection and two peaks having equal intensities at 32.1961 and 32.9021 was observed for the samples sintered at 900 °C¹⁶. The XRD spectrum confirms the formation of well resolved HAP peaks for the sample modified with NaOH. This shows the stability of the powder prepared from different modifiers. pH plays an important role in the adsorption process by affecting the degree of ionization. Thus, the particles may well disperse and adsorbed by the calcium precursor and thereby increase the reactivity between the precursors. The adsorption capacity of bases (Ammonia or NaOH) decreased with poor reactivity with the precursors.

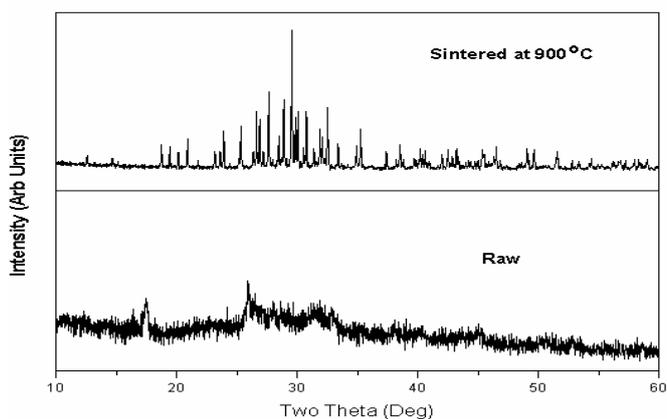
Finally, the powder prepared from NaOH was taken to determine the thermal stability at higher temperatures. It was found that the sample sintered at 1200 °C does not show any new crystalline phases, instead there is a gradual increase in the intensity with enhanced resolution and all the HAP diffraction lines were observed. According to Zhou et al., Phase transformation of HAP in to Oxy apatite has occurred between 1200 °C and 1400 °C¹⁷.

It can be concluded that the adsorption capacity of NaOH with the precursors was much higher than that of NH_4OH . Hydroxyapatite is a polar inorganic material, which had a relatively high affinity to polar organic molecules and might form chemical bondings with polar molecules^{18,19}. The ability of the formation of HAP depends on its polarity and the coordination ability with surface calcium ions. So, the other base like ammonia would be repulsed by HAP inorganic molecule, and the affinity towards calcium was lower than NaOH base. Furthermore, it could be seen from the Figure 4. the particle size was about five times lesser than that of other particles prepared from with and without ammonium hydroxide. Moreover, it was generally accepted that the adsorption ability of HAP towards NaOH was controlled by surface area, which showed direct dependence on particle size. HAP prepared by a sol - gel method using NaOH have smaller particle size with spherically aligned morphological behavior.

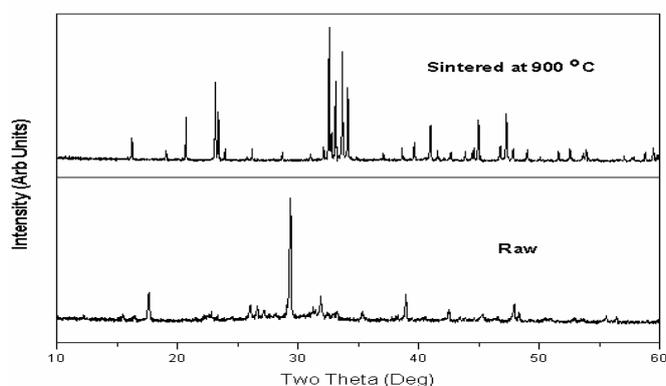
All the solutions have been prepared by ethanol with the same solute concentrations. Strong bases can produce solutions of pH greater than 7 than weak bases. During evaporation of the sol, the pH of the solution was found to be maintained at 11 for the NaOH based sol, whereas, the pH was drastically reduced to 9 for the NH_4OH based sol. Both NH_4OH and NaOH are bases, but, NaOH is a stronger base than NH_4OH so that the NaOH solution would have the highest pH followed by the NH_4OH solution. Hence, the sol modified with NaOH produced pure HAP without any other phases because of the fast reaction with calcium precursor as well as with spherically aligned particle size.



(a)



(b)



(c)

Figure 3 XRD Spectra of sol-gel powders modified (a) without modifiers (b) with ammonia (c) with sodium hydroxide

Atomic Absorption Spectroscopy (AAS)

The variations of Ca/P ratio with respect to different reactions are investigated by AAS studies. The Ca/P ratio was found to be 1.1 for the sample prepared without ageing treatment, whereas, the Ca/P ratio was changed into 1.56 for the sample prepared from NH_4OH treatment. The sample treated with NaOH reached a Ca/P ratio of 1.677 which is exactly equal to the stoichiometric hydroxyapatite. This result is in accordance with those described in the literature²⁰. The calcium-deficient hydroxyapatite (DAP, $\text{Ca}_{10-z}(\text{HPO}_4)_z(\text{PO}_4)_{6-z}(\text{OH})_{2-z} \cdot n\text{H}_2\text{O}$, $0 < z < 1$) gradually forms at a ratio of 1.56 and the stable HAP ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$), was formed at a Ca/P ratio of 1.67). Glass ceramic was composed of the radicals of OH^- and HPO_4^{2-} , and DAP had radicals of OH^- and HPO_4^{2-} , while HAP had no radicals of HPO_4^{2-} , but the peak of OH^- was higher than that of DAP. This study showed that high purity HAP with varying degrees of crystallinity could be obtained using this simple technique.

Scanning Electron Microscopy Analysis (SEM)

An interesting behavior was noted on the preparation of solutions with the same concentration and modified with different bases have produced different particle size and shape. Figure 4. represents the SEM images for the powder sample sintered at 900 °C for the duration of 2 hrs. From the figure (Figure 4a.), it was found that the shape of the powder without any modifiers have produced chunky shaped particles with micron sized particles. The sample prepared with NH_4OH (Figure 4b.) have shown the particles with nano-micron sized particles, with rod like appearance, whereas, the same sample prepared with NaOH (Figure 4c.) have shown nano sized particles with a spherical shape. Hence, the same solution with and without pH modifiers have produced interesting results with different particles alignment and size.

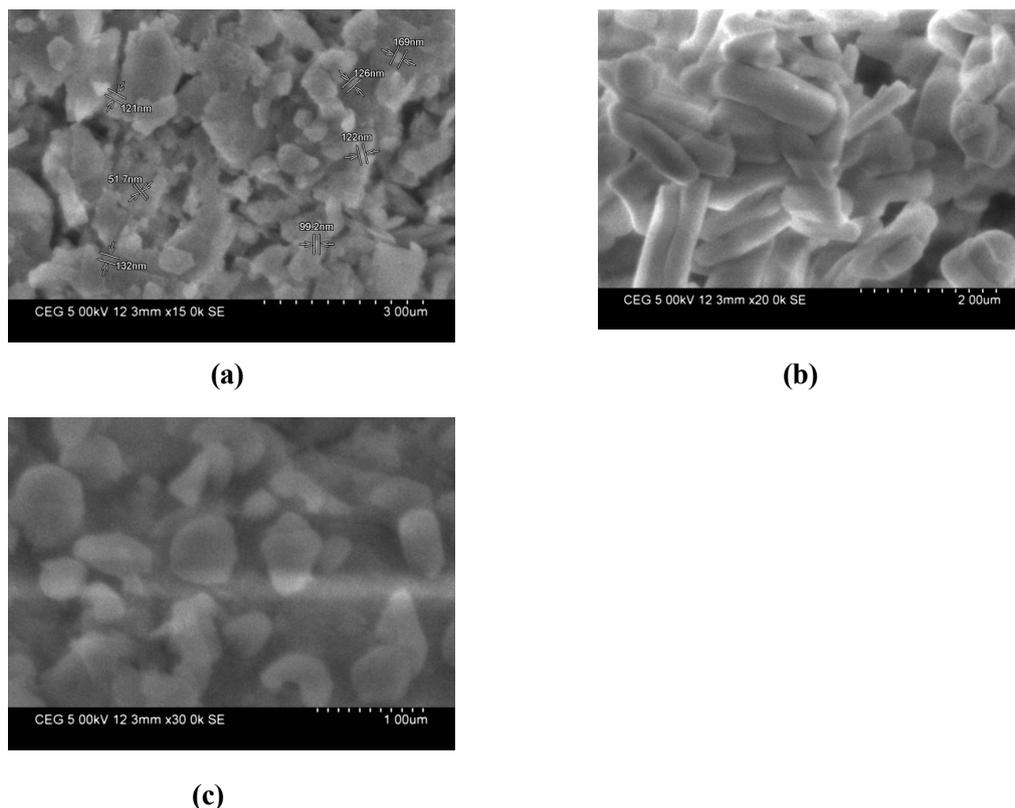


Figure 4. SEM Spectra of sol-gel powders modified (a) without modifiers (b) with ammonia (c) with sodium hydroxide

Conclusion

The formation of HAP from aqueous solution at pH 9-11 with two different modifiers such as NH_4OH and NaOH was investigated. The results showed that the reaction undergoes the following process: transformation from glass ceramic for the sample without any treatment such as aging, refluxing etc. calcium-deficient hydroxyapatite with glassy pyrophosphate was obtained for the powder prepared from NH_4OH , which has showed a Ca/P ratio of 1.56 and confirms the need of ageing to some extent. The powder prepared from NaOH has shown an excellent advantage of producing HAP without any ageing with a ratio of 1.677. The pure-phase HAP with desired morphology could be prepared by adjusting reaction conditions, pH, reaction temperatures etc. This paper deals with the advantage of producing different powders with different morphological arrangements such as chunky, rod and spherical shaped with micron to nano sized particles and further work is going on to predict the advantages of HAP produced from NaOH .

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